# JAHRESTAGUNG
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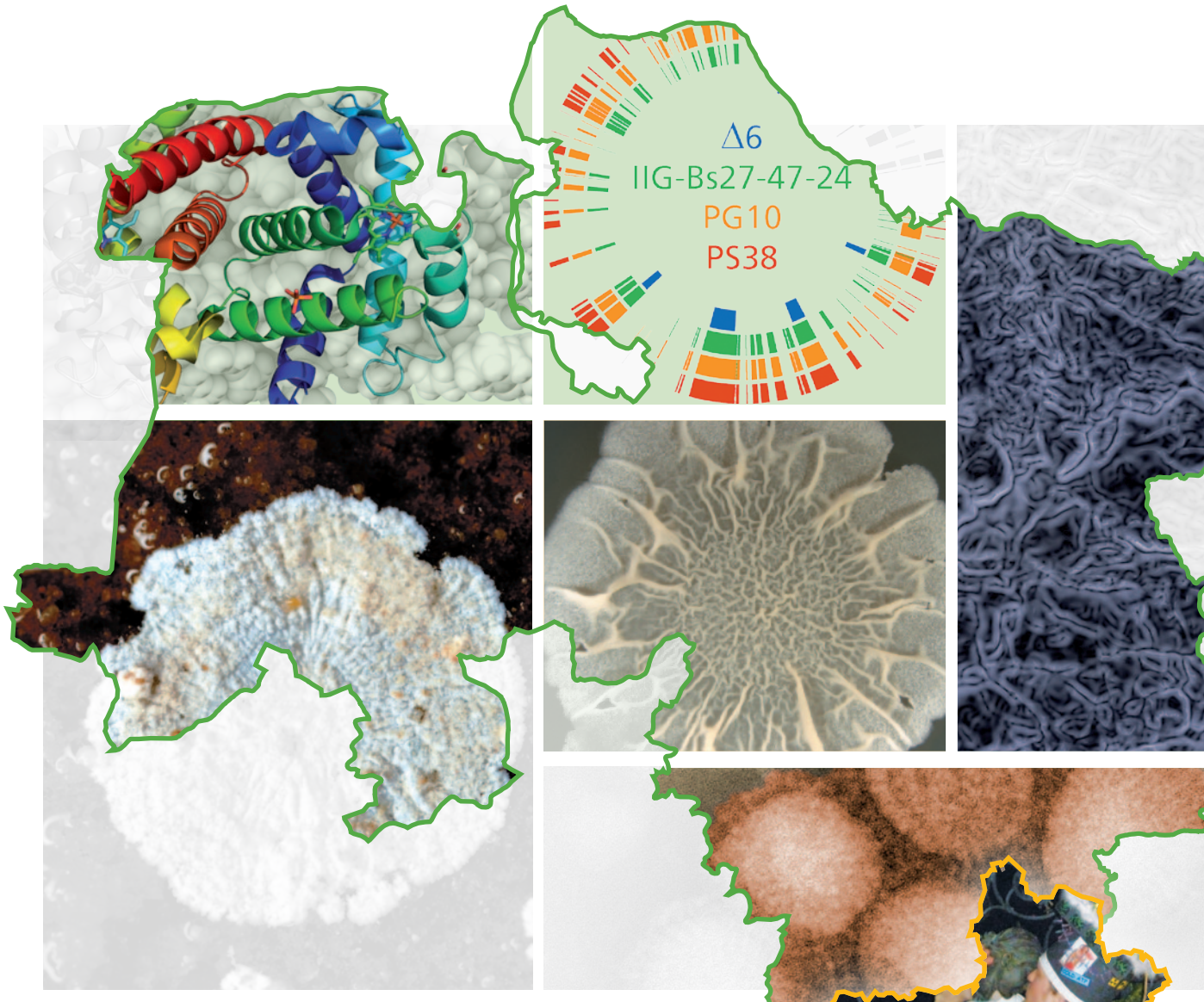




## 10–13 SEPTEMBER 2023
## GÖTTINGEN | GERMANY

## ABSTRACTS

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**IL03**

**Insights into the defense (immune) system of a mushroom**

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In their natural environment, fungi are engaged in antagonistic interactions with other organisms including competitors and micropredators. The main survival strategy of fungi in these regards is chemical defense. Accordingly, fungi are well known producers of a plethora of chemicals, including secondary metabolites, peptides and proteins, which are able to negatively affect the fitness of these antagonists. The regulation of the biosynthesis of these defense effectors (toxins) is poorly understood. In our laboratory, we take a reductionist and experimental approach aiming at the identification and characterization of novel fungal defense effectors as well as the molecular dissection of the regulatory mechanisms governing the biosynthesis of these chemicals. Our studies focus on the coprophile model mushroom Coprinopsis cinerea and its antagonistic interaction with bacteria and fungivorous nematodes and involve a variety of experimental tools. Some of the used tools and summaries of the hitherto obtained insights using these tools are presented

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**IL06**

**Little things that matter**

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Small proteins and small RNAs (sRNAs and CRISPR RNAs) carry out important cellular functions and are central players in the regulation of various reactions and pathways. Comparatively little is known about their function in archaea.

To identify the small proteome of the model archaeon Haloferax volcanii we combined small protein-optimised mass spectrometry and ribosome profiling. Annotation independent analysis resulted in the detection of 47 novel genes for small proteins in H. volcanii (Hadjeras, Bartel et al., 2023). Functional characterisation of the small protein CdrS revealed that this protein is a transcription factor that is involved in regulation of cell division (Liao, Vogel et al., 2021).

In addition to small proteins, we are interested in small RNAs. Just like bacteria, archaea encode many small RNAs and hitherto only for a few the function has been unravelled. We could show that the sRNA479 is involved in the regulation of zinc transporter genes. Additional analyses revealed that this sRNA is bound by the CRISPR-Cas complex Cascade linking CRISPR-Cas and small RNA pathways (Märkle, Maier et al., 2021).

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**IL08**

**Petri nets-based modeling techniques to explore xenophagic capturing of *Salmonella* and processes of *Salmonella* infection**

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We start with a brief introduction to Petri nets, focusing on invariant-based analysis techniques and in-silico knockouts. To illustrate research of a Petri net application, we consider *Salmonella enterica* serovar Typhimurium. Processes of infections with *Salmonella* and the host's response are still not fully understood. In the talk, we present analysis and simulation techniques based on the Petri net formalism. First, we explain a model of antibacterial autophagy at the molecular level, enabling the prediction of systems behavior without knowing the kinetic parameters. Second, we simulate processes of *Salmonella* infection using a stochastic Petri net model. Finally, we will discuss the combination of Petri nets with agent-based modeling as a future direction.

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**SPE01**

**The International Code of Nomenclature of Prokaryotes**

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The International Code of Nomenclature of Prokaryotes (ICNP) and its predecessors have governed the naming of bacteria and archaea for almost a century. The ICNP is published by the International Committee on Systematics of Prokaryotes (ICSP), a representative international body that is a subsidiary of the International Union of Microbiological Societies (IUMS). The ICNP covers all prokaryotes, both cultivated and uncultivated. A code of nomenclature does not prevent anyone from describing taxa or proposing names. A primary function of a code of nomenclature is to distinguish between other names and names that have standing. The ICNP calls the latter "validly published". Since 2001, the valid publication of the name of a prokaryotic species or subspecies requires the deposition of its nomenclatural type, the type strain, in two culture collections in two countries. Tens of thousands of names of previously uncultivated prokaryotic species have been validly published in this way after isolation, cultivation and deposition. For names of uncultivated prokaryotes, the ICNP uses the Candidatus concept. Tens of thousands of Candidatus names have been proposed in the literature, most with a genome sequence as the nomenclatural type. In 2020, the ICSP rejected a proposal to allow genome or other DNA sequences as the nomenclatural type of a prokaryotic species or subspecies with a validly published name. Among other criticisms, such as being in logical contradiction with other sections of the ICNP and significantly reducing the incentive to deposit cultivated prokaryotes, this proposal was widely seen as an attempt to lower scientific standards, leading to problems of quality and reproducibility. The ICSP is committed to continuing to serve the community by maintaining its high standards. The ICSP cooperates with the List of Prokaryotic Names with Standing in Nomenclature (LPSN), which provides accurate and comprehensive information in accordance with the ICNP.

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**AM01**

**Degradation of sulfoquinovose in *Faecalicatena* spp. via parallel 6-deoxy-sulfofructose transaldolase and 6-deoxy-sulfofructose transketolase pathways?**

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Sulfoquinovose (SQ; 6-deoxy-6-sulfoglucose) is the polar head group of sulfoquinovosyl diacylglycerols (SQDG) produced by most photosynthetic organisms [1, 2]. It is one of the most abundant sulfurcontaining compounds in nature, thus playing an important role in the biogeochemical sulfur and carbon cycles [3]. We investigated the degradation of SQ by a strictly anaerobic stable mixed culture, which was enriched from anoxic sediment of Lake Constance. It degrades SQ *via* isethionate (2-hydroxyethanesulfonic acid) and presumably sulfolactaldehyde (2-hydroxy-3-oxopropane-1-sulfonate) as transient intermediates to, ultimately, acetate and hydrogen sulfide. Through a metagenomic analysis of the anaerobic stable mixed culture, we identified the SQ degrader as *Faecalicatena* sp. A genome-sequenced representative from a culture collection, strain DSM22707,

also degraded SQ to isethionate. With transcriptomics and differential proteomics, we found that *Faecalicatena* sp. DSM22707 most likely employs a combination of the 6-deoxy-sulfofructose transaldolase [4] and 6-deoxy-sulfofructose transketolase pathway [5]. To validate this, we aim at an identification of key intermediates by LC/MS in cell-free extracts of SQ-grown *Faecalicatena* sp. DSM22707, and at a heterologous expression of the key enzymes for reconstruction of the parallel pathway *in vitro*.


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**AM02**

**Enzymatic benzene ring reduction at a 4Fe-4S-O cluster, a key reaction of aromatic degradation in anaerobes**


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Anaerobic bacterial degradation of monoaromatic compounds proceeds via the central intermediate benzoyl-CoA (BCoA), catabolized by dearomatising benzoyl-CoA reductases (BCRs). Class I BCRs couple the reduction of the substrate to cyclohexa-1,5-diene-1-carbonyl-CoA to a stoichiometric ATP hydrolysis to ADP and Pi[1]. A "Birch-like" reaction mechanism via radical intermediates was proposed to achieve substrate reduction at $E^{0'}$= −622 mV, one of the most negative redox potentials of a redox couple in biology[2].

The active site subunits of the ATP-dependent benzoyl-CoA reductase from the beta-proteobacterium *Azoarcus* sp. CIB[3] was heterologously produced in *Escherichia coli*. The crystal structure with the aromatic substrate bound was solved at 1.7 Å and revealed an active site [4Fe-4S] cluster coordinated by three cysteine residues and one hydroxy/water ligand. The structure suggests a hydrogen atom transfer-based mechanism via a neutral radical. Electron transfer to the active site is mediated by a double-cubane [8Fe-7S] cluster. The results reveal that nature uses metal-water complexes to achieve enzymatic Birch-like benzene ring reduction at the negative limit of the biological redox window.


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3. Barragan et al., 2004. *J.Bacteriol.* 186(17), 5762-5774


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**AM03**

**Novel substrate level phosphorylation catalyzed by an AMP-dependent phosphite dehydrogenase**


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Oxidation of phosphite ($HPO_3^{2-}$) to phosphate ($HPO_4^{2-}$) releases electrons at a very low redox potential ($E^{0'}$= -690 mV), which renders phosphite an excellent electron donor for microbial energy metabolism. Two pure cultures of strictly anaerobic bacteria have been isolated so far that run their energy metabolism on the basis of phosphite oxidation, the Gram-negative *Desulfotignum phosphitoxidans* and the Gram-positive *Phosphitispora fastidiosa*. In *P. fastidiosa*, a strongly expressed, cytoplasmic enzyme catalyzes phosphite oxidation only in the presence of adenosine monophosphate (AMP) and auxiliary enzymes to form adenosine diphosphate (ADP) concomitant with reduction of $NAD^+$ to NADH. This enzyme was heterologously produced in *Escherichia coli*. It is active under both oxic and anoxic conditions as judged by photometric enzyme assays monitoring NADH-production. The enzyme has a molecular mass of 35.2 kDa and a high affinity for phosphite and $NAD^+$. A similar enzyme was found in *D. phosphitoxidans* but it could not yet be heterologously produced. The genes of both enzymes are annotated to encode nucleoside-diphosphate-sugar epimerases, yet epimerase activity could not be detected *in-vitro*. In summary, these phosphite-oxidizing enzymes may catalyze a novel type of substrate-level phosphorylation that allows highly efficient energy conservation with concomitant, nearly complete electron assimilation into cell material. Microbial phosphite oxidation might be a remnant of early metabolic evolution when reduced phosphorus compounds were more common than today.

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**AM04**

**Novel citramalate lyase involved in glutamate fermentation of enterobacteria**


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The methylaspartate pathway is one of the main pathways used for glutamate fermentation in bacteria. Since it is more oxygen tolerant than the 2-hydroxyglutarate pathway, the methylaspartate pathway is widespread in soil bacteria [1]. Additionally, it functions in pathogens like *E. coli* O157:H7 (EHEC) [1 & 2]. Since glutamate and glutamine are abundant in the human intestinal tract, organisms able to use these substrates may have advantages as to their pathogenicity [3]. Recent studies showed that EHEC strains possess a gene cluster encoding the key enzymes of the methylaspartate pathway [2]. However, to date, no enzyme activity has been detected [2]. We found that the described gene cluster is present in *Roultella planticola* JCM 20069, in which activities of several enzymes of the methylaspartate pathway could be measured previously [4]. Indeed, we could measure the activities of all enzymes of the methylaspartate

pathway in *R. planticola*, and identify a novel citramalate lyase in this bacterium. This enzyme consists of two subunits and requires ATP to be active. The enzyme was structurally analyzed by X-ray crystallography and on this basis amino acid residues of the active site were exchanged to elucidate the catalytic mechanism. Nine variants with one amino acid exchange respectively were generated via site-directed mutagenesis and expressed in *Escherichia coli* BL21. In comparison to the wild type enzyme, the variants showed significantly lower activity, in agreement with the structural data. Homologs of the new citramalate lyase were identified in the genomes of other bacteria incapable of fermenting glutamate. Heterologously produced *Bacillus oleivorans* protein had no citramalate lyase activity but was active in the 2-methylisocitate lyase reaction. It showed high structural similarities with *R. planticola* citramalate lyase. Moreover, the AtuA protein of *Pseudomonas aeruginosa* involved in acyclic terpene degradation is a fusion protein combining the two subunits of *R. planticola* citramalate lyase [5]. Based on our analysis, we conclude that these three proteins belong to a novel family that possesses C-C-lyase activity with various substrates.

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**AM05**

**Overflow metabolism at the thermodynamic limit of life: How carboxydotrophic acetogens mitigate carbon monoxide toxicity**

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Introduction: Carboxydotrophic metabolism enables the conversion of carbon monoxide (CO)-rich waste streams to fuels and commodities. Acetogenic carboxydotrophs play a central role in current gas fermentation processes. In contrast to other energy-rich microbial substrates, CO is highly toxic, which makes it a challenging substrate to utilize. Instantaneous scavenging of CO upon entering the cell is required to mitigate toxicity. We pose that the reduction of acetate to ethanol is an effective mechanism to detoxify CO and will be employed increasingly with increasing CO stress.

Objectives: Our objective is to confirm the hypothesis that CO fermenting microorganisms employ overflow metabolism to help them deal with CO toxicity.

Materials and Methods: We address the theoretical case study of *Clostridium autoethanogenum* grown on CO and syngas mixtures to assess the relation between growth rate and ethanol formation based on literature data. Thermodynamic pathway analysis is used to generate a deeper understanding of this relation. Furthermore, pulse feeding experiments in a chemostat cultivation of *C. autoethanogenum* (DSM10061) are performed to establish the relationship between CO feeding rate and metabolic strategy.

Results: Literature data shows that elevated ethanol production occurs at increasing biomass-specific growth rates, both when CO is the only carbon-and electron source and when syngas mixtures are fed. Pulse feeding experiments confirm that increased CO feeding rates lead to increased ethanol formation rates at the expense of acetate. Thermodynamic pathway analysis reveals that more Gibbs free energy is dissipated when reducing acetate to ethanol than when converting CO to acetate. Conversion of CO to acetate has a higher ATP yield than acetate reduction to ethanol with CO. Yet, at high CO fluxes, the less energy-efficient pathway with higher energy dissipation, is preferred.

Conclusion: The increased allocation of electrons towards ethanol at higher growth rates strongly suggests that *C. autoethanogenum* employs a form of overflow metabolism to cope with varying CO influxes. We argue that this overflow branch enables acetogens to mitigate CO toxicity while still supporting growth.

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**AM06**

**Characterising electron transport to the Fe-nitrogenase in *Rhodobacter capsulatus***

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Nitrogenases are the only class of enzymes able to "fix" gaseous N2 to bioavailable NH3 and hence are essential for biological life. Catalysis by nitrogenases requires both a large amount of ATP and electrons, donated by a strongly reducing ferredoxin (Fd) or flavodoxin (Fld)[1]. There is limited knowledge about the mechanism of intermolecular electron transfer to nitrogenase, specifically electron transport to the Fe-nitrogenase had never been investigated. Here, we elucidated which electron carrier proteins are important for electron delivery to the Fe-nitrogenase in *Rhododobacter capsulatus*, hence providing targets for future bioengineering of electron delivery for nitrogen fixation.

Proteome analyses revealed 4 Fds were over-produced under nitrogen-fixing conditions via the Fe-nitrogenase compared to non-nitrogen-fixing conditions. Based on this information, *R. capsulatus* strains with genetic deletions of ferredoxin (*fdx*) and flavodoxin (*fld*) genes were constructed to investigate which genes were important for nitrogen-fixation via the Fe-nitrogenase. Δ*fdx* strains with phenotypes were then complemented with the missing *fdx* encoded on plasmids to evaluate the recovery of phenotypes. *R. capsulatus* strains were characterised by monitoring diazotrophic growth, measuring nitrogenase activity *in vivo* and performing whole proteome analysis.

Deletion of only 2 *fdx* genes produced phenotypes, these being slower growth and reduced nitrogenase activity; whilst deletion of both *fdx* genes together abolished diazotrophic growth. Proteome analyses of these Δ*fdx* strains revealed large over-production of electron transport proteins, specifically Fld, indicating an interruption in electron transfer to nitrogenase. Complementation of either *fdx* into the respective Δ*fdx* strains recovered phenotypes but to different extents between the two *fdx* genes. Differences in the proteomes of the Δ*fdx* strains and differing complementation behaviours, between the 2 Fds, indicated the 2 Fds were likely not redundant in function. Future research will involve purifying the two Fds to study interactions with the Fe-nitrogenase *in vitro*. Our findings will guide future bioengineering of electron transport systems to nitrogenase

in *R. capsulatus*, with the aim of increased electron flux and product formation.

References:

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**AM07**

**Allocation of cellular resources in *Thermoanaerobacter kivui* under catabolic and anabolic limitation**

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*Thermoanaerobacter kivui* is an anaerobic thermophilic acetogen, which uses $H_2$ and $CO_2$ via the Wood-Ljungdahl pathway (WLP), or carbohydrates, such as glucose, via glycolysis and the WLP. *T. kivui* is a candidate for biotechnological production of multi-carbon compounds from $CO_2$ or CO.

The fitness of a microbe requires efficient and coordinated allocation of proteomic resources between catabolic, anabolic, and maintenance processes. *Escherichia coli*, for example, shifts its catabolism during fast growth to shorter but less efficient pathways to accommodate a larger anabolic proteome (especially higher ribosome content) required for faster growth. In contrast, *Methanococcus maripaludis* does not change its proteome composition over a wide range of growth rates under energy-limitation, which is presumably more advantageous for this niche-specialized microorganism with limited metabolic versatility. As systems-level studies of growth rate-dependent resource allocation in microorganisms with different types of metabolism are rare, we studied *T. kivui* under catabolic and anabolic limitation to obtain more insights into the proteomic resource allocation strategy of an acetogen with biotechnological relevance.

*T. kivui* was grown in chemostats at different growth rates under catabolic (glucose) or anabolic (phosphate) limitation. Such chemostat-grown cells were analyzed for cellular composition, proteome, as well as metabolic parameters. Energy-limited cells showed decreased cell growth with decreasing flow rate, indicating a higher fraction of energy needed for maintenance, as expected. This was also observed in anabolically-limited cells, which degraded more substrate that was not used for biomass synthesis at lower growth rates. In contrast, fast-growing phosphate-limited cells metabolized more glucose while producing less acetate, which together with genomic analyses indicates synthesis of storage compounds. Initial proteome analyses of catabolically-limited cells showed higher relative abundance of ribosomes at higher growth rates, similar to the stringent response in *E. coli*. The fraction of glycolysis proteins also increased with higher growth rate, correlating with observed higher glucose degradation rates. In contrast, the catabolic proteome of the WLP was reduced.

The results indicate a substantial proteome re-allocation of *T. kivui* in response to growth rate and provide further systems-level insight into bacterial metabolism and ecophysiology.

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**AM08**

**High-ordered protein complex formation of the ferredoxin: $NAD^+$ oxidoreductase (Rnf) in *Clostridium ljungdahlii***

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The membrane-spanning ferredoxin:$NAD^+$ oxidoreductase (Rnf) complex of the acetogenic bacterium *Clostridium ljungdahlii* plays a vital role in chemiosmotic ion-gradient formation through an ion-pumping function. The ATP synthase uses the higher outer cell ion-gradient for ATP generation. In previous studies, an increased ATP gain was described under nitrate supplementation. Here, our main interest is the elucidation of an interplay between the Rnf complex and proteins involved in energy conversion. For this purpose, we established an *in-vivo* interactomic approach for *C. ljungdahlii* based on affinity purification of protein complexes coupled with mass spectrometry. A strep-tagged version of RnfC from a recombinant clostridial strain was used as a bait protein to capture relevant interaction partners under heterotrophic conditions. The wild-type strain with an empty vector underwent similar experimental conditions and was used as a column control. Furthermore, we performed a proteomic experiment to visualize all of the produced wild-type proteins before purification. Proteins involved in nitrogen and alcohol metabolism as well as in formate production interacted with the Rnf complex. Moreover, the similarly used interactomic approach in the presence of nitrate showed a rearrangement of interaction partners. Next, obtained Rnf complex interactions will be visualized by using polyclonal antibodies against the bait- and prey proteins in an immunogold labeling experiment *via* transmission electron microscope. Finally, our results showed a high-ordered Rnf complex formation in *C. ljungdahlii*, which provides an energetic benefit for the cell. A rearrangement of the Rnf supercomplex demonstrated the constant change of a dynamic bacterial cell system to the environment. Overall, the elucidation of energy generation processes in *C. ljungdahlii* forms the basis for genetic cell modification of this strain to produce an abundance of industry-relevant products.

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**AR01**

**Characterization of the molecular architecture of the archaeal CODH/ACS complex**

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Introduction: The Carbon-monoxide-dehydrogenase/ Acetyl-CoA-Synthase (CODH/ACS) complex is an important protein complex in the metabolism of many bacteria and archaea. It is an essential part of the Wood-Ljungdahl pathway (WLP), maybe the oldest metabolic pathway on our planet – the total synthesis of acetate from H2 and CO2. There, it catalyzes the last step, the formation of Acetyl-CoA from a methyl-group, a carbon-monoxide molecule and CoA. The well-studied bacterial CODH/ACS complex is stably purified and structurally characterized as a heterotetrametric complex consisting of a homodimeric CODH core, where each subunit is binding an ACS "wing". The remaining three vital methyltransferase subunits are ought to be transient

interactors. The archaeal CODH/ACS (archCODH/ACS) on the other hand is stably purified as a 1.6-2.4MDa megacomplex consisting of all five subunits including the methyltransferases, in a high and most likely even stoichiometry.

Objectives: The objective of this study is the characterization of the molecular architecture of the archCODH/ACS megacomplex to understand it"s mechanism and shine light on why it diverts so much from its bacterial homolog.

Materials & Methods: Anaerobic cultivation: archCODH/ACS is purified natively by established protocols from Methanosarcina.

Single-particle Cryo-EM: We use cryogenic transmission electron microscopy to solve the structure of archCODH/ACS in it"s native, anaerobic, and active state.

Integrative structural biology: We use native and crosslink mass-spectrometry and photometry to characterize subcomplex formation and interactions within the huge assembly.

Results: I want to present our recent results on the characterization of the architecture of archCODH/ACS natively purified, using cryo-EM, mass-spec and biochemical data.

Conclusion: In the bacterial WLP Acetyl-CoA is produced mainly to generate ATP and conserve energy. In Methanogens the WLP pathway branches also into the pathway of methane formation – methanogenesis and generally, methanogens produce acetyl-CoA via WLP only for biomass assimilation and the reverse process, the formation of methane from acetate is the main source of energy conservation. Thus, the enzymatic machinery responsible for this process, namely the archCODH/ACS complex has a unique architecture. We want to characterize it using the latest cryo-EM and other integrative structural techniques.

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**AR02**

**Complete Genome Assembly and Methylome Dissection of two Archaeal species *Methanococcus aeolicus* PL15/Hp and in *Methanobacterium wolfei* DSM 2970**

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Introduction: Although restriction-modification systems are found in both Eubacterial and Archaeal kingdoms, comparatively less is known about patterns of DNA methylation and genome defense systems in archaea.

Objective: Two Archaeal species, *Methanococcus aeolicus* PL15/Hp, a strain of the CO2-reducing methanogenic archaeon and a commercial source for MaeI, MaeII and MaeIII restriction endonucleases (Schmid, K. et.al., 1984) and *Methanobacterium wolfei* DSM 2970, the original source of the MwoI restriction endonuclease (Lunnen, K.D. et.al.,1989) were previously isolated, characterized and deposited in the German collection of archaea (Kendall, M.M et.al., 2006). However, the complete genome and methylome information for these two species was not available.

Material and Methods: The next generation sequencing platforms such as Single Molecule Real Time (SMRT), allow not only the sequence and assembly of genomes (Chin, C.-H. et.al., 2013), but also enable the determination of epigenetic modification patterns (Flusberg, B.A. et.al., 2010; Clark, T.A. et.al., 2012; Korlach, J. et.al., 2012). The combination of bioinformatics together with cloning and expression of candidate genes and MS-MS analysis confirmed all of the methyltransferase genes responsible for their modified motifs.

Results: The *M. aeolicus* PL15/Hp genome consists of a 1.68 megabase circular chromosome predicted to contain 1,615 protein coding genes and 38 tRNAs. The sequencing of *M. wolfei* DSM 2970 also revealed a small genome of 1.7 megabases with a circular chromosome, predicted to contain 1,817 protein coding genes and 39 tRNAs. In addition, a combination of SMRT sequencing, homology-based genome annotation, and recombinant gene expression identified five restriction-modification (RM) systems encoded by *M. aeolicus* (Fomenkov, A., et.al., 2023), including the methyltransferases and site-specific endonucleases of MaeI, MaeII, MaeIII and two RM systems including the methyltransferase and site-specific endonuclease, MwoI, for *M. wolfei* DSM 2970.

Conclusion: We report the complete closed genomes of *Methanococcus aeolicus* PL15/Hp and *Methanobacterium wolfei* DSM 2970. The sequencing data are publicly available from NCBI. In addition, the complete methylome of these two strains and the identified RM systems may help other investigators to develop novel genetic systems and create efficient transformation protocols for these archaeal species.

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**AR03**

**Unraveling archaeal tetramethylammonium degradation**

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Methanogenic archaea are major players in the global carbon cycle as well as main producers of the greenhouse gas methane. Methanogens, especially methylotrophic methanogens, can use a broad range of substrates for methane generation. Next to the rather common methanogenic substrates such as methanol, mono, di- and tri- methylamine, some marine methanogens were found to be able to use quaternary amines for methanogenesis. We enriched a methylotrophic methanogen belonging to the genus *Methanococcoides* from Black Sea sediments. The enrichment was shown to grow on tetramethylammonium, a compound that is present in marine environments, with ammonium as the main product. Comparative transcriptomic analysis of the enrichment culture grown on tetramethylammonium versus trimethylamine revealed that genes encoding specific corrinoid-dependent methyltransferase systems were upregulated with tetramethylammonium as the growth substrate. Genes encoding methyltransferases and the corresponding corrinoid proteins that were upregulated under growth on tetramethylammonium were heterologously expressed in *Escherichia coli*. Pyrrolysine-containing methyltransferases were purified from *Methanococcoides* cell-free extract by using a pulldown assay with the respective corrinoid protein. We hypothesize that tetramethylammonium is converted to trimethylamine with a methyltransferase system (Mtq) that consists of a methyl transferase MtqB and the respective corrinoid protein MtqC, that is activated by a methylamine methyltransferase activating protein (MAP) and transfers the methyl group to a coenzyme M methyltransferase (MtbA), which is assumed to transfer the methyl group to Coenzyme M. To confirm this hypothesis, we will use UV-vis-

spectroscopy and activity assays to measure the activity of the purified methyl transferases with tetramethylammonium and other amines as substrate. In this study we unravel which genes encode a methyltransferase system involved in the degradation of tetramethylammonium which will enable us to better predict which archaea can use tetramethylammonium as substrate.

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**AR04**

**Expanding the genetic toolbox for *Methanothermobacter thermautotrophicus* ΔH**

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*Methanothermobacter* spp. are thermophilic, chemolithotrophic methanogens with a long history in fundamental research as model microbes for methanogenesis. More recently, *Methanothermobacter* spp. have been adopted as biocatalysts in power-to-gas processes in which the microbes convert H2 from water electrolysis with surplus renewable electrical energy and CO2 from industrial off-gasses into methane. Further investigations of *Methanothermobacter* spp. and the expansion of the power-to-gas process to produce value-added chemicals have been hindered by the lack of genetic tools. This only changed recently with the development of a genetic system for *Methanothermobacter thermautotrophicus* ΔH in our lab (Fink et al., 2021).

After successfully establishing the genetic system, here, we focused on the development of additional genetic tools, such as selective markers and tools for genome editing.

We investigated several antibiotic resistance genes and the formate dehydrogenase (*fdh*) operon from *Methanothermobacter thermautotrophicus* Z-245 as selective markers in *M. thermautotrophicus* ΔH. Tools for genome editing are also under investigation.

We demonstrate that the formate dehydrogenase (*fdh*) operon from *M. thermautotrophicus* Z-245 enables *M. thermautotrophicus* ΔH to utilize formate as a growth substrate and that the *fdh* operon can also be used as a selective marker. We also illustrate how a CRISPR/Cas9 system based on a thermostable Cas9 from a *Geobacillus* strain could be used for genome editing in *M. thermautotrophicus* ΔH.

The results presented here are important additions to the genetic toolbox for *M. thermautotrophicus* ΔH that can be used to advance fundamental research and industrial applications.

Fink, C., Beblawy, S., Enkerlin, A. M., Mühling, L., Angenent, L. T., & Molitor, B. (2021). A Shuttle-Vector System Allows Heterologous Gene Expression in the Thermophilic Methanogen Methanothermobacter thermautotrophicus ΔH. *mBio*, *12*(6), e02766-02721. https://doi.org/doi:10.1128/mBio.02766-21

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**AR05**

**A trehalose deficient *Sulfolobus acidocaldarius* strain as platform for extremolyte production**

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Introduction: Compatible solutes including extremolytes - compatible solutes mainly or exclusively formed by extremophiles - are low molecular weight compounds that protect cell structures like proteins, membranes and DNA from damage under stress conditions including extremes of osmolarity, temperature, and pH. These outstanding properties offer great potential for a multitude of applications in medicine, cosmetics, and food industries.

Objective: Here, we present a comprehensive study on the role and metabolism of trehalose in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* and elucidated the possibility to substitute this compatible solute by the extremolyte mannosylglycerate.

Material & methods: To identify the trehalose synthesis pathways we used a combined approach of bioinformatics, growth experiments, as well as enzyme measurements. The functions of each of the identified pathways was confirmed by gene deletions, finally leading to a trehalose deficient *S. acidocaldarius* strain which was then complemented by mannosylglycerate synthesis genes.

Results: Beside the two known biosynthesis pathways (maltooligosyltrehalose-synthase/hydrolase and glycosyltransferring synthase pathway) we identified a third, novel trehalose synthesis pathway in *S. acidocaldarius* (trehalose-6-phosphate synthase/phosphatase pathway) [1]. The deletion of all three pathways resulted in a trehalose deficient strain which lost the ability to grow under high salt concentrations demonstrating the function of trehalose as (sole) compatible solute and its essential role under salt stress. By introducing the mannosylglycerate synthase gene from *Rhodothermus marinus* into the trehalose deficient mutant we also showed that mannosylglycerate can functionally substitute for trehalose restoring the ability to thrive under high salt conditions.

Conclusion: Our studies unraveled the complexity of trehalose metabolism and demonstrated its essential function in salt stress response in *S. acidocaldarius*. Additionally, the constructed trehalose deficient strain allows for the functional analyses of other compatible solutes and extremolytes and thus holds great potential as archaeal extremolyte production platform.

[1] Stracke, C., et al. (2020), *Appl Environ Microbiol*, ***86***(24), e01565-01520.

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**AR06**

**Genome validation and expansion of the family of borg archaeal extrachromosomal elements reveals a syntenous core genetic repertoire**

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Introduction: Borg linear extrachromosomal elements of up to 1.1 Mbp in length are associated with anaerobic methane-oxidizing archaea of the genus "Candidatus Methanoperedens" [1].

Objectives: The first objective of this work was to validate the overall Borg genome topology with long-read nanopore sequencing. The second objective was to discover new

Borgs, recover a complete Borg host genome, and shed light on the DNA methylation patterns.

Materials & Methods: Nanopore sequencing was performed on a subset of DNA samples from deep soil of a natural wetland where the majority of previously reported Borg genomes originate from. Methylation motifs and types of Borg DNA and host DNA were predicted using Nanodisco and Megalodon and Rerio models.

Results: Comparison of the nanopore assembled sequences to five published manually curated, Illumina-based Borg genomes validated their overall topologies. Nanopore sequences also provided a roadmap for the reconstruction of seven new Borg genomes. We find that all 17 Borg genomes have a largely syntenous core genome structure that comprises genes likely involved in replication, nucleotide processing, cell decoration and signaling. We also circularized and completed a 4 Mbp *Methanoperedens* genome inferred to be a Borg host and detect it has six distinct methylation motifs. Borg genomes generally have very distinct and pervasive methylation motifs composed of just two nucleotides.

Conclusion: This work demonstrates that Borgs possess a core set of genes that likely derived from a common ancestor. Phylogenomic analysis further uncovers a consistent separation of Borgs into two main clades. The DNA modifications observed in Borg and *Methanoperedens* DNA suggest that the replication and gene expression processes of each entity are distinctly regulated.

[1] Al-Shayeb, B., Schoelmerich, M.C., West-Roberts, J. et al. Borgs are giant genetic elements with potential to expand metabolic capacity. Nature 610, 731–736 (2022).

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**AR07**

**Structure-function probing of SECIS-dependent selenocysteine insertion in Archaea**

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Introduction: The mechanism of recoding a UGA stop codon for co-translational incorporation of selenocysteine (Sec) is not known in Archaea. A secondary structure on selenoprotein mRNAs, called selenocysteine insertion sequence (SECIS) element, is critical for Sec insertion. In Bacteria, it is located within the coding region, in Archaea (and Eukarya), it is found in the untranslated region.

Objective: To extend analysis of the SECIS by using an *in vivo* reporter to quantify SECIS-dependent Sec translation that was recently developed for the archaeal model organism *Methanococcus maripaludis.*

Materials and methods: The reporter system consists of the Sec-encoding gene for bacterial β-lactamase (*bla*). The activity of the resulting enzyme depends on the structural integrity of a SECIS element during Sec translation. We created various SECIS variants and measured Bla activity in cell lysates of *M. maripaludis* strains carrying these variants, allowing us to correlate structural changes in the SECIS to enzyme activity.

Results: Here we present further insights in the structure-function relations of the methanoarchaeal SECIS element by a) exchanging single and multiple nucleotides in an established SECIS, and by b) assessing the functionality of other presumed SECIS elements of *M. maripaludis*.

Conclusion: The activity of Sec-containing Bla correlates to the functional integrity of archaeal SECIS elements. The data gained from our *in vivo* analysis identifies critical positions and structural components of the methanoarchaeal SECIS element. We also confirmed the authenticity of other SECIS elements in *M. maripaludis*.

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**AR08**

**Small metal-binding proteins in *Methanosarcina mazei* are differentially expressed in response to various stress**

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Small proteins (<100 amino acids (aa)) often fulfill stress-related functions and are present in all domains of life. Comprehensive RNAseq data sets obtained from the methanogenic model *Methanosarcina mazei* under a range of different stress and growth conditions, lead to the prediction of 1,330 additional small open reading frames (sORFs). However, the detection, identification and characterization of translated sORF encoded proteins via mass spectrometry (MS) is particularly challenging due to a number of inherent limitations, e.g., low abundance or limited proteotypic peptides (reviewed in Weidenbach et al., 2022). As such, the applied methods have to be specifically tailored to target the small proteome. The combination of Riboseq and MS approaches is especially promising and provides the ability to cross-validate both methodologies. Today, only two of the identified *M. mazei* small proteins have been functionally characterized (reviewed in Weidenbach et al., 2022; unpublished data). To broaden this perspective, here we first performed an enrichment of the low molecular weight proteome of *M. mazei* under different stress conditions, followed by quantitative liquid chromatography-mass spectrometry (LC-MS) and further functional analysis of four small proteins. A total of 38 small proteins were identified in the LC-MS approach during the exponential or stationary growth phase, under high temperature, salt stress, or upon challenge with virus. Especially small proteins quantified in LC-MS with supporting Riboseq data are highly interesting candidates to have a function in *M. mazei*. We focused on four small proteins (51 to 67 aa), which significantly changed in abundance in response to salt stress or viral challenge. Based on structure predictions, they represent small zinc finger proteins or bind an iron-sulfur cluster. Spectra analyses and size exclusion chromatography of heterologously expressed A01453 revealed this small protein to bind iron-sulfur clusters while dimerizing. Further characterization will be presented and their physiological role proposed.

Weidenbach, K., Gutt, M., Cassidy, L., Chibani, C., Schmitz, R.A., 2022. Small Proteins in Archaea, a Mainly Unexplored World. J. Bacteriol. 204, e00313-21. https://doi.org/10.1128/JB.00313-21

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**BT01**

**Natural products from interacting microorganisms and ancient microbiomes**

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Introduction: Microbial natural products have been an indispensable source of novel therapeutic agents. The search for new bioactive natural products has prompted scientists to exploit environmental niches in which the production of these compounds can be anticipated.

Objectives: The aim is to investigate innovative approaches to identify novel natural products. One the one hand, we use microbial predator–prey interactions as a particularly rich source of natural products. One the other hand, we add the time dimension to natural product research by accessing ancient natural products from the DNA found in the dental calculus of Neanderthals.

Materials & methods: We use a combination of methods from the following fields to access novel natural products: bioinformatics, microbiology, molecular biology, analytical chemistry and synthetic chemistry.

Results: We identified a number of novel nonribosomal lipopetides that mediate microbial interactions.[1–3] Importantly, some of these natural products were modified within polymicrobial associations.[3,4] Using ancient dental calculus, we were able to reconstruct 100,000-year-old bacterial genomes and we could identify an undescribed biosynthetic gene cluster in a *Chlorobium* species. Reconstruction of the gene cluster allowed us to generate paleofuran A and B.[5]

Conclusion: Overall, we could show that understanding polymicrobial interactions allows for the identification of new natural products. Furthermore, we were able to add the time dimension to natural product research.

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[2] J. Arp, S. Götze, R. Mukherji, D. J. Mattern, M. García-Altares, M. Klapper, D. A. Brock, A. A. Brakhage, J. E. Strassmann, D. C. Queller, B Bardl, K Willing, G. Peschel, P. Stallforth, *Proc. Natl. Acad. Sci. USA.* **2018**, *115*, 3758–3763.

[3] S. Zhang+, R. Mukherji+, S. Chowdhury, L. Reimer, P. Stallforth, *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, e2013759118.

[4] S. Zhang, K. Schlabach, V. H. Pérez Carrillo, A. Ibrahim, A. Komor, R. Mukherji, S. Chowdhury, L. Reimer, C. Hertweck, U. A. Hellmich, P. Stallforth *submitted*

[5] M. Klapper, A. Hübner, A. Ibrahim, et. int., C. Warinner, P. Stallforth, *Science* **2023**, *First release DOI: 10.1126/science.adf5300*.

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**BT02**

**Development of a co-culture platform for natural product-targeted bioprocesses**

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Co-cultures of filamentous microorganisms are a potent tool to unlock the natural product biosynthetic machinery encoded in silent gene clusters. However, if bioactive compounds are to be produced biotechnologically, it is essential to move beyond an approach of arbitrary strain combinations, to one studying the dynamics of microbial partnership or competition, specifically, how such interactions can be exploited for bioprocesses. Therefore, a co-culture platform that makes use of crystalline cellulose as the main carbon source and two filamentous microorganisms is proposed. In this system, the fungus Trichoderma reesei RUT-C30 degrades cellulose into soluble sugars to support the growth of the natural-product producing bacterium Streptomyces coelicolor A3(2), which in monoculture is unable to utilize alpha-cellulose. This co-cultivation thus mimics a carbon-limited interaction scenario in nature. Additionally, in a bioprocess context, it can favor fed-batch-like conditions that stimulate production of bioactive compounds. Here, we investigated how the population dynamics affects metabolite production by combining high-throughput online fluorescence monitoring with high-resolution LC-MS/MS-based metabolomics. To that end, both species were fluorescently labeled and used to inoculate co-cultures at a wide range of strain ratios, either with spores or mycelia. To determine the metabolic profile of the cultures and to identify metabolites specifically induced during co-cultivation, both targeted and untargeted metabolomic analysis with specialized software (MZmine, SIRIUS) was used. Coupled analysis of growth ratios and metabolomic data indicated that the highest number of induced metabolites (compared to axenic cultures of both species) is observed in balanced co-cultures with fluorescence ratios of T. reesei to S. coelicolor around 3 RFU. Surprisingly, the steroidal lactone withanolide B was identified to be induced in co-cultures. Withanolides have so far not been described to be produced by any of the two species, thus highlighting the potential for the discovery of natural products. Additionally, we deciphered a possible biotransformation of dimerumic acid, produced by T. reesei, into eleutherazine B by S. coelicolor. The platform described here combines the search for new natural products with a biotechnological production strategy that can also be applied to other Streptomyces bacteria with an abundance of cryptic biosynthetic gene clusters.

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**BT03**

**In vitro evolution of a synthetic organelle inside a picoliter habitat**

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Introduction: The endosymbiotic theory states, that about one billion years ago a heterotrophic host organism incorporated a cyanobacterium, which subsequently evolved into the chloroplast we find in algae and plants today. Until today, few hypotheses concerning the molecular mechanisms underpinning endosymbiosis have been subject to direct experimental testing. We will use microfluidic cultivation technology to trap and observe single cells in picoliters of medium, mimicking the inclusion vacuole.

Objectives: Establish microfluidic single-cell cultivation of cyanobacteria as a method to test hypotheses about endosymbiosis.

Material & Methods: Disposable PDMS chips developed at IBG-1 are used, for microfluidic cultivation. Time-lapse imaging is performed to monitor cultured cyanobacteria. Image data is processed by in-house segmentation software to derive data on growth, morphology and autofluorescence. The cyanobacteria strain *S. elongatus* UTEX2973 is used as a model organism. It is known for its tolerance to high irradiance and low doubling times. BG11 media is used for growth studies.

Results: The developed "growth light" system consists of a lamp placed outside the microscope incubator that is capable of modulating any spectrum within the photosynthetic active range (PAR) of electromagnetic radiation. The light is transmitted through a waveguide with a circular ending, which can be fixed around the microscope condenser. This provides homogeneous illumination and efficient use of the limited space

A double-layer chip design is used, for carbon dioxide control. It consists of a thin bottom layer within witch the microfluidic cultivation is located. Gas is perfused trough a thick top layer. The thin bottom layer enables fast gas exchange while the top layer offers structural support

At 80 µE/($m^2$·s) artificial sunlight and ambient air a doubling time of 6 h was achieved. Since *S. elognatus* UTEX2973 is a rod-shaped bacterium cell area and cell length show a linear correlation. Furthermore, highly synchronous growth behavior was observed.

Conclusion: In the past, the microfluidic technology developed at IBG-1 has proven to be an efficient tool for the evaluation of growth parameters of heterotrophic organisms and co-cultures. An experimental workflow, a dynamic illumination system and a carbon dioxide control have been developed, to meet the needs of photoautotrophic organisms. This is a first step towards the development of a synthetic inclusion vacuole.

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**BT04**

**Controlling macromorphologies of *Aspergillus niger* during high and low shear stress bioreactor cultivation**

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Submersed cultivation of filamentous fungi is widely used in fungal biotechnology. The formation of different macromorphologies, however, range from dispersed mycelia over loose clumps to dense pellets, and thus limits productivity with shear stress as one of the main influencing parameters.

In this study, seed cultures with defined macromorphologies of the cell factory *Aspergillus niger* were exposed to high shear stress in stirred-tank (STR) and low shear stress in rocking-motion bioreactors (RMB). Talcum microparticles at 1 and 10 g $L^{-1}$ were added to the seed cultures to achieve pellet populations with controlled diameter sizes. Physiological and morphological data were comprehensively investigated with high-throughput 2D image analysis and 3D synchrotron radiation based micro-computed tomography. This approach allowed us to determine the distribution of spore agglomerates, pellets and dispersed mycelia as well as hyphal densities and total hyphal lengths.

Our data show that high shear stress in STR leads to breakage of pellets right after the stirrer was switched on. The mechanical stress from stirring also hindered pellets from surpassing a certain diameter during cultivation. In contrast, pellet size increased constantly until glucose was limited during RMB cultivations with largest macromorphological changes during the exponential growth phase.

This work will allow us to estimate hyphal growth rates and pellet breakage as a function of shear stress for the first time and will furthermore pave the way for better understanding of cell-bioreactor interactions, and thus morphology-optimised cultivation processes.

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**BT05**

**Strategies for improved surfactin production by *Bacillus subtilis***

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*Bacillus subtilis*, known as a microbial cell factory, offers great potential for the production of valuable biobased products. In particular, the native biosynthesis of bioactive secondary metabolites offers various potential applications. Here, the class of lipopeptides, including surfactin, fengycin and iturin, shows the potential to replace synthetic surfactants. Particularly the low environmental toxicity and relatively high biodegradability make so-called biosurfactants interesting for industrial applications. However, bottlenecks such as the insufficient productivities of *Bacillus* wild-type strains and the need for adapted bioprocesses due to properties such as strong foaming require further research. Here, we address details of process and genetic engineering approaches for efficient and robust production of surfactin.

A well-known drawback in surfactin bioproduction is excessive foaming during cultivation in aerated bioreactors. For this reason, various strategies have been developed to address this problem. For example, aerobic/anaerobic switch processes have been explored to enable decoupling of aerobic biomass formation and anaerobically inducible surfactin biosynthesis. Another promising approach for surfactin enrichment is in situ product removal by foam fractionation. In addition, the molecular regulatory aspects need to be understood in order to develop *Bacillus* production strains with improved process efficiency and product yield. In this context, the *srfA* operon encoding surfactin synthetase is controlled by multiple transcriptional regulators, including the ComX-mediated quorum sensing network. By means of bioactivity assay, the ComX time course was determined during surfactin production in bioreactor fermentation, providing a model system for molecular process control. Moreover, the impact of several global regulators involved in cell differentiation, such as Spo0A, AbrB, and DegU, on *srfA* operon expression, final surfactin bioproduction, and yield ($Y_{P/X}$ and $Y_{P/S}$) was determined. Thus, studying combinatorial deletion mutants allowed to clarify the regulatory crosstalk on lipopeptide

production. Based on these insights, adapted high cell-density fed-batch fermentations allowed surfactin production up to 26.5 g/L, which is currently the highest surfactin titer known in the scientific literature.

These findings enable the combination of beneficial process and molecular adaptations for economical surfactin production in future studies.

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**BT06**

**Antibiotic effect of high power blue laser**

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Introduction: The association of marine organisms to submerged objects, so called biofouling, leads to serious economic and ecological implications. Biofouling on ship hulls, by added mass and increased water resistance, leads to a higher fuel consumption and can also lead to transfer of foreign species into native ecosystems with detrimental effect. The use of laser light provides an innovative approach to remove the biofouling from ship hulls *in* situ [1] and for disinfecting drinking water [2]. The high power density of the laser allows for shorter irradiation times, making this a feasible approach for large scale application.

Objectives: The mechanism of the lethal damage of antibiotic blue light (448 nm) on different microorganisms is examined using a high power blue laser (1500 W).

Methods: Different bacteria and algae were grown, washed and laser treated. The change in absorption spectra were analysed using different spectroscopic methods. RNA-Microarray experiments were used for transcriptome analyses.

Results: It was hypothesized that blue light irradiation leads to the generation of oxygen radicals via the excitation of pigments like porphyrins and flavins, which in turn lethally damage the cells. To confirm the proposed killing mechanism of antibiotic blue light in our experiments different organisms (*E. coli, P. megaterium, D. shibae, C. fusca* and *P. purpurea*) were irradiated with blue light. The dynamics of the loss of viability could be determined. By comparing the transcriptome of irradiated cells we could show an increase in genes associated with DNA-damage repair, respiration and protein synthesis. Moreover, the role of pigments was elucidated by employing pigment-overproducing bacterial strains compared to the native strains. A loss of pigmentation was observed as well as changes in the absorption spectrum, indicating damage to the pigments.

[1] Zimbelmann et al. (2022). Interaction between laser radiation and biofouling for ship hull cleaning.

[2] Zimbelmann et al. (2022). Disinfection of water by using laser radiation.

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**BT07**

**Valorization of lignin waste by filamentous fungi – Seeking new aromatic compounds**

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More than 50 million tons of lignin waste are generated annually in the pulp and paper industry that, instead of being used as a source for organic compounds, are simply burned. Lignin is one of the most abundant biopolymers. It is one of the main compounds in the vascular plant cell wall, in addition to cellulose and hemicellulose.

By synthesizing these new precursors through a biotechnological process, using industrial waste from the pulp and paper industry, a much better CO2-balance will be achieved.

Therefore, we are looking for suitable filamentous fungi, mainly basidiomycetes, which can modify and de-synthesize the ligninolytic waste products with enzymes like laccases and peroxidases into usable aromatic building blocks, in order to obtain new products with higher value. More than 160 fungi have been cultivated with lignin sulfonate or kraft lignin and the new products are then analyzed by HPLC- and GC-MS.

Four new products have been detected. Two of them are derivatives of dehydroabietic acid, coming from the resin acid abietin and are of particular interest due to their already known biological activities. The structure elucidation and activity testing for cytotoxicity, phytotoxicity and antimicrobial activity of these compounds have been determined.

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**BT08**

**Design of experiment approach for improved fermentative astaxanthin production by *Corynbacterium glutamicum***

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Introduction: Astaxanthin is a commercially important keto-carotenoid. It possesses a red colour and various health benefits, which is why it is used in animal and human nutrition, as well as in cosmetic and pharmaceutical products. Naturally, it is produced by some algae and microorganisms, but it is also found in higher organisms like crabs, shellfish and salmons. Astaxanthin is primarily produced from petrochemicals, but there is a growing market for natural astaxanthin. A potential astaxanthin producer is *C. glutamicum* and while it has been genetically optimized, no optimization of the fermentation has been performed yet.

Objectives: The objective of this study was to establish and optimize a fermentation process for astaxanthin production with *C. glutamicum*.

Materials & methods: All experiments were performed in 2 L BioEngineering KLF bioreactors and CGXII medium with 4% glucose. The strain used was *C. glutamicum* ASTA*. We performed the cube portion of a design-of-experiment with 10 batch fermentations with 4 factors, 2 levels each and 2 center points. The tested factors were aeration rate, dissolved oxygen, pH and initial optical culture density. Further fermentations were performed subsequently, to quantify the effects of pH and aeration rate on astaxanthin

formation. Finally, these results were transferred to a fed-batch process with 60% glucose as feed.

Results: The design-of-experiment showed that almost no astaxanthin is formed, when the pH is lowered to 6. Instead, β-carotene is accumulated in high amounts. Furthermore, lowering the aeration rate shows a beneficial effect on astaxanthin formation at pH 8. Dissolved oxygen and initial OD had no significant effects on astaxanthin production. Further experiments showed that increasing pH beyond 8 and decreasing aeration rate below 0.5 vvm both show negative effects, so true optima were identified. Transferring these results to the fed-batch process made it possible to produce 70 mg/L of astaxanthin within 60 h, which is a significant increase over previously reached titers.

Conclusion: It is possible to significantly increase astaxanthin titers by changing some relatively simple process parameters. In addition, a pH of 6 completely halts the oxygenation from β-carotene to astaxanthin. This is contrary to previous findings e.g. in *P. rhodozyma* where a pH below 6 was beneficial. Furthermore, while aeration rate has a significant influence, the amount of dissolved oxygen does not have any effect.

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**BT09**

**Enzyme engineering to accelerate the Calvin-Benson-Bassham cycle in cyanobacteria**

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Flux control in the Calvin-Benson-Bessham (CBB) cycle is distributed over several reactions operating far from equilbrium.[1] In particular Rubisco and F/SBPase have significant control over carbon fixation for growth or biosynthesis.[2] We have developed an enzyme engineering platform with the ultimate goal of increasing carbon fixation and conversion by the model cyanobacterium *Synechocystis* sp. PCC 6803. Starting with targeted mutagenesis libraries, we used competitive growth coupled to deep sequencing to compare the properties of Rubisco and F/SBPase mutants under different cultivation conditions. As the type I Rubisco of cyanobacteria, green algae and land plants may be phylogenetically constrained regarding mutations and catalytic parameters,[3] we used a type II Rubisco, as these show more sequence diversity and lower folding requirements.[4] We show that the platform identifies enzyme variants of both the type II Rubisco and F/SBPase enzymes that affect cell growth in different conditions. The resulting labeled datasets will be useful for machine-learning assisted design. The establishment of this platform is a first step toward creating optimised enzyme variants to accelerate the CBB cycle in cyanobacteria and possibly chloroplasts.

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**BT10**

**Carbon-efficient production of α-ketoglutarate from C5 and C6 sugars with *Corynebacterium glutamicum***

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Hemicellulose represents a promising second-generation feedstock for industrial biotechnology as it is renewable, non-edible, and highly abundant (Zhou et al., 2011). After hydrolysis, hemicellulosic hydrolysates typically contain various hexoses (d-glucose, d-galactose, d-mannose) and pentoses (d-xylose, l-arabinose). While most microorganisms readily consume available hexoses, many industrially relevant production hosts are not capable of utilizing the pentose fraction. In this study, *Corynebacterium glutamicum*, a workhorse of white biotechnology, was equipped with the Weimberg pathway to convert the pentose d-xylose into high-value TCA intermediate α-ketoglutarate (AKG). This five-step pathway, encoded by a codon-optimized version of the *xylXABCD*-operon originating from *Caulobacter crescentus*, was episomally introduced (Radek et al., 2017). Compared to other d-xylose degrading pathways, the oxidative Weimberg route does not require any ATP and avoids carbon loss in the form of CO2. The uptake of d-xylose by *C. glutamicum* was facilitated through derepression of the chromosomally encoded gene for the glucose/*myo*-inositol permease IolT1 (Brüsseler et al., 2018). For intracellular AKG accumulation, two major routes for l-glutamate synthesis from AKG were abolished through gene deletions. In shake flask cultivations, using the defined medium CGXII with a mixture of d-xylose and d-glucose, the *C. glutamicum* producer strain accumulated AKG in the supernatant at gram-scale. Based on these results, further strain development is envisaged with regard to the formation of common side products and a reduced consumption of AKG within the TCA cycle. These results show that the tailored *C. glutamicum* strain equipped with the Weimberg pathway is a promising host for carbon-efficient AKG production on d-xylose-containing substrates.

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**BT11**

**$N_2$-limitation boosts bio$H_2$ production in *Rhodopseudomonas palustris* dominated mixed species biofilms**

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Introduction: Hydrogen plays an important role in the transition towards carbon neutral energy systems. Here we present a concept for bio-hydrogen (bio$H_2$) production based on mixed species biofilms. These microbial consortia are dominated by *R. palustris*, a purple non-sulfur bacterium that produces bio$H_2$ as byproduct during nitrogen fixation, while obtaining the necessary energy from anoxygenic photosynthesis and the electrons from organic acids like acetate[1]. Cultivation as surface attached biofilms in capillary biofilm reactors (CBR) enables continuous process operation

while providing a high surface-to-volume ratio for optimal light supply[2].

Objectives: The aim of this study is to develop a process concept for bio$H_2$ production suited for continuous operation. Thus, biofilm growth and establishment in the CBR is investigated and process parameters for maximal bio$H_2$ production are determined.

Materials & Methods: *R. palustris* DSM127 and *Pseudomonas taiwanensis* VLB120 eGFP are cultivated in CBRs[3] modified for optimal product capture. Product quantification was achieved using gas chromatography. Biofilm development and population dynamics have been analyzed via confocal laser scanning microscopy and flow cytometry.

Results: Dual-species biofilms comprising *R. palustris* as workhorse and *P. taiwanensis* as supporter strain proved optimal for the establishment of anoxic conditions, stable biofilms and constant bio$H_2$ production. A special glass-casket was designed for the CBR to minimize product loss. Most interestingly, limiting the availability of $N_2$ resulted in a boost in hydrogen production up to 75 mL $L^{-1}$ $h^{-1}$. By adapting the $N_2$ feed it was possible to establish an -in principal- zero growth state in the biofilm, nutrient consumption was heavily reduced while product formation increased significantly.

Conclusion: The necessary anoxic conditions for bio$H_2$ production using *R. palustris* can be established by employing aerobic *P. taiwanensis* as supporter strain for oxygen removal. Furthermore, $N_2$ availability proved crucial for bio$H_2$ production rates, leading to the conclusion, that the hydrogenase activity is utilized as an electron valve, if the organism is grown phototrophic under heavy N-limitation.

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**BT12**

**Hydrogen production with *Parageobacillus thermoglucosidasius* DSM 6285: Impact of hydraulic retention time in a continuous fermentation**


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Introduction: Hydrogen can be produced by hydrogenogenic carboxydotrophs, able to perform the water-gas shift (WGS) reaction ($CO + H_2O \rightarrow H_2 + CO_2$). Some microorganisms have been reported to produce $H_2$ using CO as an energy source. *Parageobacillus thermoglucosidasius* is one such organism found to produce hydrogen through WGS. Furthermore, *P. thermoglucosidasius* has demonstrated an ability to tolerate the toxicity of CO and to direct its metabolism towards the production of organic acids and other products, which has the potential to generate energy through the WGS reaction.

Methods: Previous studies have reported a lag phase between the depletion of $O_2$ and the onset of $H_2$ production in *P. thermoglucosidasius* DSM 6285. However, there is a limitation due to the accumulation of toxic compounds, which makes continuous fermentation an alternative. *P. thermoglucosidasius* DSM 6285 was cultivated in two bioreactors of 1.5 L capacity (Minifors, Infors AG) at 500 rpm, 55 °C and pH of 6.8, performing a two-phase fermentation using a continuous flow rate of 4.46 mmol $min^{-1}$ of air and CO, followed by a gas exchange of 3.57 mmol $min^{-1}$ of a mixture of nitrogen and CO. The reactor medium used was modified ammonium sulfate (mASM). The hydraulic retention time (HRT) was identified as a parameter specific to the bioreactor configuration and substrate that could affect $H_2$ production. Therefore, the effect of HRT on the fermentation process was evaluated by increasing its value.

Results: The increase in HRT resulted in improved overall stability in $H_2$ production. The lowest HRT evaluated was 5 d, with an $H_2$ production rate (HPR) of 0.06 mmol $min^{-1}$ and a slow increase in the production after 4d; a decrease in biomass was also observed, possibly due to cell washout. Increasing the HRT from 8 to 10 d positively impacted HPR, resulting in a rate of 0.29 mmol $min^{-1}$ with the highest HRT. Additionally, the process remained stable for over 5 d of fermentation.

Conclusions: Overall, the increase of HRT has a positive effect up to 10 d on the HPR and the stability of the process. Despite the pH of the experiments being maintained at a constant pH of 6.8, the impact of this parameter on $H_2$ production makes it a compelling area for optimizing fermentation in terms of selecting the optimal HRT. The results emphasize the significance of HRT in ensuring consistent hydrogen production and could serve as a valuable reference for enhancing the biohydrogen platform.

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**BT13**

**Application of the glutathione S-transferase StyI for kinetic racemic resolution towards chiral epoxides**


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Chiral epoxides are of high interest in industry as they have many applications like in drug or agrochemical synthesis. A major issue is to achieve a high yield including regio- and enantioselectivitiy. Enzymatic epoxidation allows for both, but it is limited by the selectivity of respective enzymes. Kinetic resolution emerges as alternative. Few enzymes convert epoxides with a certain selectivity like epoxide hydrolases, glutathione S-transferases (GSTs), CoM-transferases or haloalcohol dehalogenases. But little is known about the selectivity of GSTs which brought these enzymes to our focus. Recently, a bacterial GST (StyI) was found in a styrene degradation route of *G. rubripertincta* CWB2. The unusual GST-presence in styrene degradation appears to broaden the substrate spectrum of the pathway.

We want to uncover the potential of StyI for kinetic racemic resolution to produce enantiopure epoxides.

StyI was heterologously produced in *E. coli*, purified by IMAC, and applied in various formats to convert epoxides. Conversion was followed by product extraction and subsequent GC-FID analysis on chiral columns.

Enzyme production succeeded. Our studies revealed a 3-times higher activity with (*S*)-styrene oxide compared to its (*R*)-enantiomer. This was supported by a 16-times higher affinity and a 1.8-fold higher Vmax for (*S*)-styrene oxide, giving clear evidence for StyI's stereoselectivity. More than 10 substrates comprising different structural motives were tested. For styrene oxide derivatives, like *p*-fluoro- or *p*-chlorostyrene oxide, a high selectivity for the (*S*)-enantiomer was found. The enantiomeric preference of more diverse

structures such as phenylpropylene oxide, indene oxide, and aliphatic epoxides was lower. Since StyI showed selectivity towards (*S*)-styrene oxide indicating its promising application in production of enantiopure (*R*)-epoxide, enzymatic kinetic resolution was performed. The reaction volume was increased to 150 mL scale to proof the feasibility of large-scale kinetic resolution. The approach yielded about 18 mg pure (*R*)-styrene oxide (validated by NMR).

StyI is highly enantioselective for the natural substrate (*S*)-styrene oxide and analogous. A certain selectivity was observed for non-aromatic substrates which highlights the broad application range for this enzyme. The subsequent upscaling of the process allowed to enrich pure (*R*)-styrene oxide. Hence, StyI"s potential is revealed towards large-scale production of enantiopure epoxides.

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**BT14**

**Screening oxygen-dependent promoters for applications of dynamic metabolic engineering**

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Promoters responding to internal or external signals enable the cell to adjust gene expression during the course of a fermentation process and are key for dynamic metabolic engineering concepts. In particular, oxygen-dependent promoters may be of high value for two-stage processes in which an aerobic growth phase is followed by an anaerobic production phase. Several oxygen-dependent promoter candidates have been presented in the past. However, a systematic comparison of these promoters is lacking and only few reported applications made use of oxygen-responsive promoters for metabolic control and process design.

We systematically compared the performance of 15 published oxygen-responsive promoters in *Escherichia coli*. For this purpose, we used a microtiter plate-level screening approach based on an oxygen-independent fluorescence reporter protein and additionally employed flow cytometry analysis. We found varying expression levels and dynamic ranges among the candidates, and six promoters (nar-strong, nar-medium, nar-weak, nirB-m, yfiD-m, and fnrF8) appeared particularly suited for dynamic metabolic engineering. We demonstrate applicability of these candidates for dynamic induction of enforced ATP wasting, a metabolic engineering approach to increase productivity of microbial strains. The selected candidates exhibited sufficient tightness under aerobic conditions while driving the anaerobic expression of the cytosolic F1-subunit of the ATPase from *E. coli* (catalyzing ATP hydrolysis and thus ATP wasting) to levels that resulted in very high specific glucose uptake rates. We finally utilized the nirB-m promoter to demonstrate the optimization of a two-stage lactate production process by dynamically enforcing ATP wasting in the anaerobic (growth-arrested) production phase to boost the volumetric productivity.

We also developed a simple computational approach to identify optimal 2-stage batch processes based on the given characteristics of different single (e.g. aerobic) growth and (anaerobic) production modes.

Our results are valuable for implementing metabolic control and bioprocess design concepts that use oxygen as signal for regulation and induction.

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**BT15**

**Influence of electrode material on the performance, assembly, and composition of the microbial community in microbial electrolysis cells**

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Introduction: Microbial electrochemical technologies, such as microbial electrolysis cells (MECs) can be used for the recovery of resources from the wastewater stream (Modin et al. 2017). In MECs, an applied voltage on the system drives the microbial community on the anode to transfer electrons resulting in the generation of current and production of energy carriers at the cathode (Rozendal et al. 2006). The performance and community composition of these systems are dependent on both deterministic and stochastic factors. The relative importance of the two is poorly understood.

Objective: In this study, we compared the effect of drift to that of anode materials (carbon cloth, graphene, and nickel) on the performance and microbial community structure in single chamber MECs.

Materials & Methods: Replicate hydraulic loops containing MECs with all three materials were operated for 56 days under identical conditions allowing for the study of the ecological processes drift and selection driven by anode material. Metagenomic sequencing was used to analyse the microbial community composition.

Results: The anode material showed a effect on the start-up time for the MECs. The conventional material, carbon cloth, had the least variation and shortest lag time. There were also differences in the peak current density and total current generated between the different materials. MAGs corresponding with taxa from the *Desulfobacterota* phylum, specifically two *Geobacter spp.,* were found to be dominating on the anode, making up between 64-89% of the microbial community. However, drift had an effect of which *Geobacter* species that dominated in the MEC. The MECs within the same hydraulic loop were dominated by the same *Geobacter* species.

Conclusion: Stochastic factors (drift) explained most of the variance observed in microbial community structure while anode material explained most of the variance in bioelectrochemical performance in the MECs.

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electrolysis." International Journal of Hydrogen Energy **31**(12): 1632-1640.

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**BT17**

**An interesting insight into energy efficiency for an oxic microbial electrosynthesis process with *Kyrpidia spormannii***

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The global carbon cycle started to be imbalanced when the humans began to use fossil carbon sources. It"s important to counteract by establishing new production routes which are based on non-fossil carbon, like CO2. One possibility are Microbial Elektrosynthesesis processes (MES), where regenerative electrical energy is used as electron source and CO2 as carbon source. In our oxic Microbial Electrosynthesis Process (O-MES), *Kyrpidia spormannii* grows on a cathode as biofilm. The final product of the process is polyhydroxybutyrate (PHB), which can be converted into biodegradable bioplastic in already established industrial routes.

Energy efficiency data are generally lacking for O-MES processes. With *K. spormannii*, we tackle this challenge. Additionally, we give a suggestion for a continuously operable process.

The biofilms were grown in a flow-cell system. Optical Coherence Tomography (OCT) was used for biofilm quantification. For energy efficiency determination, two main parameters were calculated: The Coulombic Efficiency (CE), which gives the percentage of the supplied electrons that ended up in the final product, and the energy demand which is needed for the formation of 1 kg dry biomass. Furthermore, we developed a method for harvesting the biofilm by discontinuous shearing of biomass from the electrode by hydrogen bubble formation.

We determined the H2/CO2-ratio for *K. spormannii* in a batch system to be 4.23 mol mol-1. With this, we could show a CE of 95.36 % for the O-MES process in its maximum (49.51 % on average). The process showed an overall energy demand of 35.8 kWh kg-1 dry biomass. But when splitting the cultivation in process windows of one day, the minimum energy demand occurred after four days, with only 11.88 kWh kg-1. Here, the process is even more efficient than a reference process with externally produced hydrogen fed to Knallgasbacteria. After harvesting the biomass with hydrogen bubbles, the biofilm was able to regenerate and re-grow to its original height again.

An O-MES system has the advantage of in-situ hydrogen production, with constant hydrogen depletion. In certain process windows, when the growth rate was maximum and the substratum completely covered, the CE reached almost 100 %. Here, the energy demand was even lower than competing production systems. Together with showing a possibility to run an O-MES process continuously, we enable an evidence-based decision for future application in energy conversion as well as CO2 usage and capturing.

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**BT16**

**Biodegradation of phthalic acid esters using immobilized bacteria on a natural carbonaceous porous material**

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Phthalic acid esters (PAEs) are one of the most frequently detected persistent organic pollutants in the environment and are known to be used as plasticizers and non-plasticizers in different industries. It is estimated that the global production of PAEs reached up to 6 million tonnes per year and continues to grow in an alarming speed. Due to their occasionally long aliphatic chains and the possibility to form complex compounds, the biodegradability by bacteria is mostly limited. New environmental-friendly technologies need to be developed in order to reduce the level of PAEs in polluted areas. To tackle this problem, bacterial strain immobilization on a natural carbonaceous porous material has been proposed as a potential path to enhance the biodegradation capacity and rate in polluted environments.

From a biofilm present on a polyurethane tubing, a bacterial strain identified as *Xanthobacter* sp. was isolated and shown to grow with a PAE species as a sole source of carbon and energy. After the metabolization of PAE by *Xanthobacter* sp. was studied in batch cultures, a natural carbonaceous porous material was added to enhance biodegradation.

In order to acquire a tailored bio-based porous material for bacterial immobilization, vine wood (VW) waste was treated through hydrothermal carbonization (HTC). Scanning Electron Microscopy (SEM) analysis showed that the material obtained through HTC (namely hydrochar) had a porous structure with a wide distribution of pore size (~1-20 µm), which may facilitate bacterial colonization and growth of *Xanthobacter* strain. The hydrochar promotes the growth of bacteria, improves the cellular division capacity, reduces the nutrient depletion and enhances the bacterial survivability when facing high levels of PAEs. Moreover, the immobilization efficiency can be optimized by controlling the hydrochar properties, such as surface area, pore volume, and functional groups content.

Therefore, immobilization of a carbonaceous material with PAEs degrading bacteria has the potential to provide an efficient, cost-effective, and sustainable solution for the bioremediation of contaminated soils.

Key words: Phthalic acid esters (PAEs), bacterial immobilization, hydrothermal carbonization (HTC), hydrochar, bioremediation.

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**BT18**

**Engineering *G. oxydans* for the production of functionalized sugar acids and improvement of its slime formation for solid-state fermentations**

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*Gluconobacter oxydans* has a great biotechnological potential due to the ability to incompletely oxidize a large variety of carbohydrates, alcohols, and related compounds and being able to grow in highly concentrated solutions. The microbial oxidations are catalyzed by membrane-bound dehydrogenases (mDHs) with the active site facing towards the periplasm, which avoids transport of a substrate into and the formed product out of the cytoplasm. We developed a platform for functional expression of heterologous mDHs in *G. oxydans* BP9.1, which is devoid of its native mDHs, thereby increasing specific activity of the heterologous mDHs and avoiding unwanted side reactions.

In this study we use this platform to produce cellobionic acid (CBA). CBA is an alternative to the animal-derived lactobionic acid and is used in pharmaceutics, cosmetics and other applications. The membrane-bound glucose dehydrogenase (mGDH) from *Pseudomonas taetrolens* was found to be the most active one in oxidizing cellobiose to CBA. Therefore, we expressed the enzyme in *G. oxydans* BP9.1 to construct a strain that produces CBA with high space-time-yield. The promotors for plasmid based and to avoid the need for antibiotic selection of plasmids, for chromosomal expression were optimized. The production of CBA via oxidative fermentation by *G. oxydans* BP9.1 was evaluated for different fermentation conditions and times.

Common limitations of such processes using acetic acid bacteria are the relatively low space-time-yield due to small cell concentrations and the high energy consumption due to aeration and cooling. Fixed bed reactors, where the cells are immobilized on a suitable carrier material could be an efficient solution for this problem. This immobilization could be achieved by production of sticky exopolysaccharides such as levans. Since our platform strain *G. oxydans* BP9.1 produces only a small amount of levane, we aim at increasing levane production to allow better immobilization. Different strains of acetic acid bacteria (AAB) were screened for their ability to form mucous colonies on sucrose and the best-performing strain was used as a source of a levansucrase gene for functional expression in *G. oxydans* BP9.1. The improvement of the slime formation in the presence of sucrose should help immobilization of *G. oxydans* BP9.1 in solid-state fermentations.

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**BT19**
**A thermostable reporter gene allows for characterization of promoter strength in the thermophilic acetogen *Thermoanaerobacter kivui***

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Introduction: The thermophilic acetogen *Thermoanaerobacter kivui,* which is capable of fixing $CO_2$ utilizing hydrogen and carbon-monoxide as energy sources, shows promise for carbon-neutral chemicals production in a hydrogen-based economy. Genetic manipulation of *T. kivui* is now possible, but sophisticated tools such as inducible promoters are lacking. We have established that gene operons for uptake and metabolism of the sugars mannitol and fructose are up-regulated in response to their respective sugar, and otherwise expressed at very low levels. Therefore, the promoter regions of these operons were identified as candidates for inducible expression of recombinant proteins.

Objectives: The goal of this work was to establish a thermostable reporter-gene system in *T. kivui* that would allow for rapid and accurate assessment of relative promoter strengths at the cells" elevated growth temperature of 66°C. The resulting promoter library will allow for fine-tuned control of protein expression in *T. kivui*. As part of this work the putative sugar-inducible promoters were compared to the constitutive promoter of the S-layer protein ($P_{SLP}$).

Materials and Methods: *T. kivui* lacks a native beta-galactosidase enzyme, so a thermostable version from *Caldicelulosiruptor bescii* ($T_{opt}$ 78°C) was selected as reporter gene and cloned into the genetically tractable Δ*pyrE* strain under the control of various promoters. A combination of qPCR and a pNP-based beta-gal enzyme assay was used to quantify transcription and translation of the reporter gene, and compare relative expression levels between the promoters.

Results: The *C. bescii* reporter gene was functional in *T. kivui*, and changes in transcript level detected by qPCR corresponded to changes in enzyme activity. Beta-gal activity in strains with the sugar promoters increased more than 10-fold when induced by the appropriate sugar, while activity with $P_{SLP}$ was several fold higher than even the induced sugar promoters, regardless of the growth sugar.

Conclusion: The reporter gene established here will allow for rapid characterization of additional natural or synthetic promoters in *T. kivui*. Our results confirm that for very high protein expression the S-layer promoter remains useful, but have also identified moderate strength sugar-inducible promoters with low non-induced activity (low leakiness). The finer control of protein expression these promoters provide will facilitate ongoing metabolic engineering efforts.

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**BT20**
**Heterologous production of c-type cytochromes in *Vibrio natriegens***

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The heterologous production of c-type cytochromes poses a challenge for the host since posttranslational modifications as well as synthesis and incorporation of heme are required. While *Escherichia coli* (*E. coli*) is the most widely used expression host, it has a significant disadvantage in the production of mature c-type cytochromes. The cytochrome c maturation genes *ccmABCDEFGH* are located in an operon together with the periplasmic nitrate reductase genes *napABCDFGH* under the control of an anaerobic regulator. As a consequence, mature c-type cytochromes are not produced under aerobic conditions. Therefore, different expression strategies in *E. coli* were developed over time that require the co-expression of heme c uptake systems or the *ccm* genes. This limits the selection of expression vectors and strains. The Gram-negative non-pathogenic marine bacterium *Vibrio natriegens* (*V. natriegens*) has emerged as a promising heterologous expression host during the last years. In contrast to *E. coli*, the *ccmABCDEFGHI* genes of *V. natriegens* form a separate operon and are expressed during aerobic growth. Therefore, we tested the heterologous production of three different c-type cytochromes from *Acidithiobacillus ferrooxidans* in *E. coli* T7 Express and *V. natriegens* Vmax X2 with and without co-expression of the *ccm* genes under aerobic conditions. Heme-staining revealed bands corresponding to the three c-type cytochromes in *E. coli* and *V. natriegens* cell lysates when *ccmABCDEFGH* where co-expressed. Heme c was

also detected via UV/Vis spectroscopy. Once the *ccm* genes were not co-expressed, no bands were visible after heme-staining of *E. coli* cell lysates and UV/Vis spectroscopy only revealed the presence of heme b, but not heme c. In contrast, heme c and mature c-type cytochromes were detected in aerobically grown *V. natriegens* cells without the co-expression of the *ccm* genes. We propose that *V. natriegens* Vmax X2 may be a superior host for the heterologous production of c-type cytochromes.

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**BT21**

**Methanol-based biotechnology with *Methylorubrum extorquens* – Novel genetic tools and strain optimizations**

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Introduction: *Methylorubrum extorquens* has great potential to become an universal production strain for C1-based bioeconomy. In the last decade, a number of *M. extorquens* strains with heterologously expressed metabolic pathways were described for the methanol-based production of various compounds including 1-butanol, 3-hydroxypropionic acid, mono- and dicarboxylic acids, mevalonate or α-humulene. Although the organism has been an important model organism for methylotrophy, the availability of genetic tools and tailored lab strains is still limited.

Objective: Our work aimed at the development of genetic tools and at strain improvements, which enable straightforward production strain development and provide novel platform chassis.

Material and methods: All developments were enabled after directed evolution approaches, although in one case this was not intended. Strains showing the desired phenotypes were characterized in detail followed by molecular analysis of the underlying mechanisms.

Results: A novel plasmid for *M. extorquens* could be identified. The pBBR1-based vector was found to be suitable for transformation and for co-transformation with the existing pCM vectors after a promoter mutation in the *rep* gene.

An already described promoter system inducible by cumic acid could be strongly improved for applications in *M. extorquens*. A mutated variant of the promoter was found to show suitable background activity levels in contrast to the original version.

Mutant strains with increased methanol tolerance were isolated and characterized. They enable process improvements and simplified lab shake flask experiments. Moreover, underlying mechanisms for methanol resistance were discovered.

To reduce product metabolization after re-uptake, a transporter responsible for uptake of dicarboxylic acids was identified in a straightforward selection approach. Mutant strains were tested with regard to production characteristics for different acids.

Conclusion: Novel genetic tools and strain improvements for *M. extorquens* are described. All developments represent important modules for construction of robust and efficient methanol-based production strains.

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**BT22**

**Expanding the pool of biocatalysts for production of renewable biocommodities through acetogenesis**

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Introduction: Due to their metabolic versatility in substrate utilisation, acetogenic bacteria represent industrially significant production platforms. Their potential biotechnological applications range from the fermentation of industrial waste gases or C1 compounds to microbial electrosynthesis for the production of renewable bulk chemicals and biofuels. The commercialization of these approaches towards a sustainable circular economy is currently limited by the low growth rates and product yields of available acetogenic biocatalysts. To date, over 20 bacterial genera were described to contain acetogenic strains varying in their mode of energy conservation, substrate utilization and fermentation products. Exploring the phylogenetic and metabolic diversity of hitherto uncultured acetogens in nature can be a valuable contribution to the establishment of the genetically and metabolically difficult to access acetogenic bacteria as industrial platform organisms.

Objectives: The project goal is the isolation of novel acetogens from environmental samples followed by an analysis of their metabolic diversity and potential as biocatalysts.

Materials & Methods: Anaerobic enrichment cultures were performed with a variety of environmental samples such as hot spring sediment, bioreactor sludge, compost, river sediment and feces. Acetogens were enriched at mesophilic and thermophilic temperatures by growth on gaseous and C1 substrates. The bacterial composition was monitored by 16S rRNA gene-based community analysis. Enriched acetogens were isolated, genome sequenced and physiologically characterized.

Results: The bacterial community analysis of enrichment cultures showed cultivation of acetogenic and syntrophic bacteria. Within the mesophilic cultures novel bacterial acetogens were enriched belonging to the genera *Terrisporobacter, Oxobacter, Acetoanaerobium, Alkalibaculum* and a novel CO-fermenting genus most closely related to the genus *Sporomusa*. Thermophilic enrichments lead to the growth of a novel *Moorella* species and strains belonging to the species *Moorella thermoacetica* and *Moorella humiferrea*. The isolates were genome-sequenced and the physiological characterizations showed distinct differences between isolates in growth optima and substrate utilization.

Conclusion**:** Novel acetogens have been successfully isolated from environmental samples extending the diversity of available biocatalysts by eight novel strains, two novel species and one novel genus.

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**BT23**

**Towards the development of non-model organisms from the unexplored *Halopseudomonas pertucinogena* lineage**

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The *Halopseudomonas* species form a unique branch within the phylogenetic tree of the *Pseudomonads*[1-5]. Most of them have only been described in the last decade. These strains share a rather small genome indicating a limited metabolic flexibility, nevertheless they can grow in a wide temperature range (4 – 41 °C) and, even more interestingly, within a salinity range of 0 – 15% NaCl. These properties as well as their harsh natural habitats (like oil- or metal-contaminated sites or the Deep sea) suggest a high intrinsic potential of *Halopseudomonas* strains to resist physical and chemical stresses that renders them promising for biotechnological applications[6,7].

However, further exploration of these bacteria is currently hampered by the lack of microbiological and molecular genetic methodology, as protocols for other Pseudomonads can often not readily be transferred. Thus, we selected *H. aestusnigri* VGXO14*, H. bauzanensis* BZ93*, H. litoralis* 2SM5, and H*. oceani* KX20 as four particularly interesting strains and identified first suitable complex and mineral salt cultivation media whereby $C_4$-$C_{10}$ dicarboxylic acids emerged as suitable carbon sources. Supplementation of LB complex medium with succinate increased the growth rates. Furthermore, aiming to harness the strains" potential, we achieved genetic access to *Halopseudomonas* sp. for the first time by developing transfer protocols and testing of vectors with different oriV. Based on this, we established three constitutive and three inducible promotor systems, confirmed by successful heterologous expression of target genes in all selected strains. For specific genomic integration of expression cassettes, two *glmS* genes and therewith *att*Tn7-sites were identified in all selected strains. Using *H. litoralis,* we demonstrated that both sites are accessible for Tn7 transposition. We could show that simultaneous occupation of both sites with cassettes harbouring different target genes leads to functional expression of both the genes.

Consequently, our findings corroborate the potential of these strains and provide a toolbox for the construction of robust chassis strains for biotechnological applications.

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**BYF01**

**Versatile DHN melanin –Spotlight on its function in microcolonial black fungi**

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Dihydroxynaphthalene (DHN) melanin is produced by diverse Ascomycetes via slightly differing biosynthetic routes. The polyketide synthases (PKS) release the heptaketide YWA1, the hexaketide AT4HN or the pentaketide T4HN. The first two products are deacetylated by "yellowish-green" hydrolases to T4HN, and T4HN is further converted by a core set of enzymes to DHN. Final polymerization steps are accomplished by multicopper oxidases. DHN melanogenesis is often regulated in a spatial and temporal fashion resulting e.g., in melanized reproduction and survival structures of the foliar plant pathogen *Botrytis cinerea* (Schumacher 2016, *Mol Microbiol*). In contrast, a polyphyletic group of Ascomycetes (microcolonial fungi/ black yeasts) dwelling in hostile habitats such as bare rock surfaces in hot and cold deserts, exhibits constitutive melanogenesis. Here, DHN melanin builds a protective layer around all vegetative cells thus contributing to the survival of diverse environmental stresses even without specialized reproduction structures. For studying the relevance of constitutive DHN melanogenesis for tolerance of abiotic and biotic stresses, adhesion to substrates and subsequent damage of colonized surfaces, the rock-inhabiting fungus *Knufia petricola* was chosen as gene functions in this fungus can be studied by CRISPR/Cas9-based genome editing. The putative melanogenic genes were identified in the genome of *K. petricola*, deleted to confirm their involvement in DHN melanogenesis and co-expressed in *Saccharomyces cerevisiae* for reconstruction of the synthesis pathway. Phenotypes of DHN-deficient mutants are studied. Here, we will discuss the role of the DHN melanin layer on the outer cell wall in tolerating UV irradiation.

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**BYF02**

**Dark stipe mutants in fruiting body development of *Coprinopsis cinerea***

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Fruiting body development in the dung fungus *Coprinopsis cinerea* is strictly regulated by environmental factors including light, temperature and aeration. At 25° C, it follows a conserved scheme defined by day and night phases, with well predictable distinct stages over the time. Differentiation begins with the formation of primary hyphal knots (Pks) in the dark, which, when exposed to light, transform into compact aggregates, secondary hyphal knots (Sks) in which stipe and cap tissues differentiate controlled by further light signals. Primordium development (stages P1 to P5) takes five days to culminate on day 6 of development in light-induced karyogamy followed by meiosis within the basidia and basidiospore production which parallels fruiting body maturation (stipe elongation, cap expansion). Mature fruiting bodies autolyze on day 7 to release the spores in liquid droplets. Failure in light signaling or also in aeration at steps during Sk to P5 development leads to formation of so-called slender "dark stipes", under proliferation of stipe tissues and blocks in cap tissue development. Scavenger experiments of CO2 with KOH under block of aeration recovered the normal phenotypes in fruiting body development (Sk development, normal continuation of P1 to P4 development, dark spore pigmentation and autolysis) as well as that active mycelial growth was restored with KOH. Accordingly, not a lack of oxygen but increase in concentration of CO2 resulted in abnormal phenotypes. In a larger mutant collection, defects in fruiting do not evenly distribute over the complete developmental pathway which reflects the complexity in the fruiting process. Among our mutants are two (*dst3*, *dst4*) with P3- and P4-type "dark stipe" phenotypes formed under standard fruiting conditions with light and aeration. Genome

sequencing kindly performed by B. Hegedüs and L. Nagy revealed defects linked to the citrate cycle, branched amino acid production, activities of Arf1-like GTPase, and functions of the Cop9 signalosome, suggesting links to CO2 and possibly light regulation. Defects in formerly described genes *dst1* (=*wc1*), *wc2*, and *dst2* caused P1-induced phenotypes by defects in blue-light receptors and a FAD/FMN-binding dehydrogenase of the GlcD superfamily. In the *dst3* mutant, one defect is a loss of start codon in the *arf1-like* gene. Here, we focus on the Arf1-like small GTPase that plays a vital role in vesicular trafficking as activators of phospholipase D in signaling transduction.

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**BYF03**

**Analysis of the *Aspergillus fumigatus* proteomic response to amphotericin B (AmB) reveals involvement of the Rta1-like protein RtaA in resistance**

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Introduction: The filamentous fungus *Aspergillus fumigatus* is an opportunistic human pathogen, which can cause mycoses and allergies in susceptible individuals. Amphotericin B (AmB), which is a member of the long-known polyene family of antifungal drugs, is still frequently used to treat *Aspergillus fumigatus* infections. Increasing resistance to AmB in clinical isolates of *A. fumigatus* is a growing concern. Despite this, the mechanisms of AmB resistance in *A. fumigatus* remain unclear.

Objectives: To get further insights into the drug mechanisms of AmB, we investigated the proteomic profile of *A. fumigatus* in response to AmB and its liposomal formulation by LC-MS/MS analysis

Material and Methods: We compared the proteomes of *A. fumigatus* cultivated in the presence of sublethal concentrations of AmB and liposomal AMB with untreated control cultures using liquid chromatography-tandem mass spectrometry. Selected proteins with significant increase in abundance upon AmB exposure were characterized further by the construction and characterization of single-gene deletion mutants in *A. fumigatus*.

Results: In particular, the level of proteins anchored to the membrane, involved in catabolic processes, aromatic acid degradation, or secondary metabolism increased prominently upon AmB exposure. One of the most upregulated proteins was RtaA, which encodes a fungal Rta1-like family protein. We found that deleting *rtaA* led to polyene sensitivity and overexpression to resistance. Interestingly, *rtaA* expression was only induced by exposure to AmB and by co-cultivation with the secondary metabolite-producing bacterium *Streptomyces rapamycinicus*. Deletion of *rtaA* did not significantly change the ergosterol content of *A. fumigatus*, but decreased fluorescence by the intracellular lipid droplet stain nile red.

Conclusions: The *A. fumigatus* protein RtaA is involved in mediating resistance to the antifungal drug AmB, most likely by maintaining lipid homeostasis and membrane stability. These findings reveal a novel polyene resistance mechanism that warrants further investigation to determine its exact mechanism and importance in clinical resistant isolates of *A. fumigatus*.

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**BYF04**

**Structural and functional analysis of the cerato-platanin-like protein Cpl1 suggests diverging functions in smut fungi**

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Introduction: Plant pathogenic fungi cause significant damage to crops worldwide, leading to severe crop loss. Fungi use a range of strategies to colonize plants, such as avoiding and counteracting the plant immune system and manipulating the host metabolome. Fungi secrete virulence factors that play a crucial role in supporting the infection process. Despite their importance, most of these proteins have not been functionally characterized due the lack of structural and biochemical information. Here, we provide a comprehensive structural and functional characterization of the conserved effector protein Cpl1 from *Ustilago maydis* and its homolog Uvi2 from *Ustilago hordei*.

Objectives: The aim of this study was to gain insights into the molecular function of the cerato-platanin-like protein Cpl1 from *Ustilago maydis* and its homolog Uvi2 from *Ustilago hordei* combining structural, biochemical and cellular approaches.

Methods: X-ray crystallography, precipitation assays, microscale-thermophoresis, HDX-MS, molecular docking, immuno-labeling microscopy, plant infection experiments, co-immunoprecipitation

Results: Our study revealed that both Cpl1 and Uvi2 adopt a double-Ψ β-barrel (DPBB) architecture reminiscent of cerato-platanin proteins, a class not previously described in smut fungi. We furthermore identify a new mode of chitin binding via two extended grooves and unveil a potential function in suppression of chitin-triggered host immunity. Cpl1 localizes to the cell wall of *U. maydis* and may play a role in spatially coordinating other cell wall-degrading and decorating proteins during maize infection. Deletion of cpl1 had only mild effects on *U. maydis* virulence, while deletion of uvi2 strongly impaired *U. hordei* virulence. The two proteins are highly conserved in their central DPBB but vary in the decorating loop regions, which might hint towards diverging functions during plant colonization.

Conclusion: In conclusion, our study provides new insights into the conserved and highly abundant smut virulence proteins Cpl1 and Uvi2, highlighting their potential role in the plant colonization process of *U. maydis* and *U. hordei*. Our findings expand the repertoire of chitin-binding proteins and suggest that Cpl1 may be involved in the spatial coordination of other cell wall-degrading and decorating proteins during infection. Further research is needed to fully understand the mechanisms underlying the function of these proteins in fungal pathogenicity.

**BYF05**

**Spore germination patterns and host-guided chemotropic growth determine maize root infection by *Colletotrichum graminicola***

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The hemibiotrophic fungus *Colletotrichum graminicola* is a relevant maize pathogen able to infect several plant tissues. Recently, we showed that two different types of *C. graminicola* conidia, oval or falcate-shaped spores, differ significantly in their gene expression patterns, secondary metabolite production, and developmental processes. These characteristics cumulate in a specialization for the infection of either roots or aboveground tissues of oval and falcate conidia, respectively. In a current project, we seek to identify relevant processes determining this specialization, focusing on the abilities to germinate in soil and to sense host root-exuded signals.

First, we investigated whether chemotropic growth to maize root exudate (MRE) is relevant for the adaptation for the infection of roots. Testing the response of both *C. graminicola* conidia to MRE, we found that only germlings derived from oval conidia are able to sense MRE-derived signals, tricyclic diterpenoids, and direct their growth towards them. To investigate this response further, we generated mutants in genes encoding for the a-phermone receptor (*Cgste3*) or the scaffolding protein of the cell wall integrity MAPK pathway (*Cgso*), which homologs are responsible for the sensing of root-exuded peroxidases in other fungal root pathogens. Intriguingly, both mutants were unable to sense MRE, indicating a conservation of the perception pathway despite the different chemical nature of the plant-derived signals. However, symptom development of both deletion mutants did not differ from the wildtype, indicating a minor role for this process in the adaptation of oval conidia to maize root infection. Shifting our attention to conidial germination, we investigated soil samples from plant co-incubation experiments after 21d. Intriguingly, we found a massive amount of not-germinated or dead falcate conidia, indicating that those conidia are not adapted to survive or propagate in soil. In the same experiments, no oval conidia were observed. In further analyses assessing germination in soils of different composition, consistently a fast germination pattern of oval conidia was observed, but no germination of falcate conidia.

In summary, we identified germination patterns as the major factor for the root infection adaptation of oval conidia, whereas the ability of those spores to sense MRE signals is of minor relevance.

**BYF06**

**A conserved polyprotein structure escorts the peptide toxin candidalysin through the secretory pathway in *Candida albicans***

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*Candida albicans* damages host cells *via* its peptide toxin, candidalysin. Prior to secretion, candidalysin is embedded in a precursor protein, Ece1, consisting of a signal peptide, the precursor of candidalysin, and seven non-candidalysin Ece1 peptides (NCEPs). Here, we show that the exceptional Ece1 structure does not conform to the canonical definition of a toxin-antitoxin system or resemble the usual precursor structure of peptide toxins. *C. albicans* cells are protected from damage by their own toxin, and single NCEPs adjacent to candidalysin are sufficient to prevent host cell toxicity. Critically, we show that NCEPs play a vital role for intracellular Ece1 folding and candidalysin secretion. Removal of single NCEPs or modifications of peptide sequences cause an unfolded protein response (UPR), which in turn inhibits hypha formation and pathogenicity. Our data indicate that the Ece1 precursor is not required to block premature pore-forming toxicity, but rather to prevent candidalysin auto-aggregation.

**BYF07**

**Histone binding of ASF1 is required for fruiting body development, but not for genome stability in the filamentous fungus *Sordaria macrospora***

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Introduction: Fungal fruiting bodies function in the production and dispersal of sexual spores; however, the molecular mechanisms underlying their formation are insufficiently understood. Previous comparative transcriptomics of fruiting body development in different ascomycetes showed that genes involved in the regulation of transcription and chromatin maintenance are upregulated during fruiting body development in several distantly related species. One of the genes that were identified by this approach is *asf1*, which encodes a conserved eukaryotic histone chaperone for histones H3 and H4. A deletion mutant of *asf1* in the ascomycete *Sordaria macrospora* is viable, but sterile, and shows significant differences in its development-dependent transcriptome as well as reduced overall DNA methylation compared to the wild type.

Objectives and Materials and Methods: In this study, we have performed ChIP-seq and Hi-C analyses of the *S. macrospora* wild type and the *asf1* deletion mutant to determine the molecular influence of ASF1 on chromatin

structure. Furthermore, we performed complementation studies and co-immunoprecipitation analyses with mutated *asf1* constructs to identify regions of ASF1 that are involved in fruiting body formation as well as histone binding. To study the role of ASF1 in genome stability, strains carrying the wild type and mutated versions of *asf1* were also tested for growth on methyl methansulfonate (MMS), which induces DNA double strand breaks.

Results and Conclusions: ChiP-seq and Hi-C analyses showed that the overall chromatin organization in the *asf1* deletion mutant does not differ from the wild type, but that the histone modification H3K27me3 is significantly upregulated in the mutant strain. Analyses of mutated versions of the ASF1 protein showed that amino acid residue V94 is required for both histone binding as well as fruiting body formation, whereas amino acid residue D37 plays a minor role in both processes. Interestingly, histone binding is not required for resistance to MMS, suggesting that ASF1 has a role in chromatin maintenance and stabilization that is independent of its histone binding. Thus, histone binding of ASF1 is required for fruiting body formation, but not for overall genome stability.

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**BYF08**

**Establishment of a CRISPR/Cas9 system for *Neurospora crassa***

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Understanding gene functions and the associated molecular mechanisms relies on the ability to genetically modify the organism of interest. In filamentous fungi, one commonly used approach is homologous recombination (HR) to create knock-outs. However, HR is inefficient and limited in terms of available selection markers [1]. Conversely, the RNA-guided CRISPR/Cas9 system offers high efficiency, is easy to handle and allows a precisely targeted mutagenesis. It has already been successfully applied in some filamentous fungi [2]. For Neurospora, the use of non-replicating plasmids carrying the *cas9* and the gRNA sequence, respectively, was successfull [3]. But so far this method is not implemented as a standard method for Neurospora. Therefore, we developed a different version of the CRISPR/Cas9 system by integrating the *cas9* sequence into the *N. crassa* genome. This system enables the manipulation of more than one gene at a time in a time efficient manner. Allowing the characterization of complex biosynthetic pathways and thereby opening up new possibilities to finding answers to not yet asked questions.

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**CM01**

**Cross-link aided modeling of protein-RNA complexes**

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Ribonucleoproteins (RNPs) are key regulators in many central biological processes. While identification of the specific RNAs binding by a particular protein could be routinely performed, there is a dire need for the precise identification of protein-RNA interactions simultaneously at amino acid and nucleotide resolution. In contrast to mass spectrometric (MS) analysis of chemically crosslinked protein-protein complexes, providing precise identification of the crosslinked residues at the amino acid level, crosslinking of an unlabeled RNA with proteins yields amino acid resolution only on the protein side while exact position on the RNA molecule at the nucleotide level remains inaccessible.

Here, we propose an approach to decipher protein-RNA interactions at single amino acid and nucleotide resolution by combining macromolecular modeling and *in silico* blind docking experiments with crosslink-aided sorting strategy of generated RNP complexes.

Atomic models of *B. subtilis* YlxR and RnpA have been predicted using Alphafold [1], the RNA molecule (RnpB) has been computed using Rosetta RNA modeling protocol FARFAR2 [2]. Macromolecular *in silico* docking experiments yielding 3D models of RNP complexes (*B. subtilis* YlxR-RnpA-RnpB) was performed using ROSETTA [2] and HDOCK [3].

Modeled RNP complexes were individually analyzed in terms of their compatibility with cross-linking data by calculating Euclidean distances between the protein residue (position within the protein is known) and the linked nucleotide/nucleotides (position within the tRNA is not known) that is/are located within specified distances from the protein residue. In that manner both single (the shortest-distance) and multiple crosslink (slacked distances) occurrences have been assessed, counted and stored. The developed sorting strategy provided three populations of distinct RNP complexes that have been verified by mutagenesis experiments.

This approach provides the basis for further functional characterization of the investigated RNP complex.

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**CM02**

**Bacterial nanomotions, combined with supervised machine learning, accurately classify antibiotic susceptibility**

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Background: Antimicrobial resistance (AMR) renders formerly curable infections life-threatening. Many multi-resistant bacteria can no longer be conventionally treated with antibiotics, contributing to the rising number of sepsis-related deaths. Besides finding novel and alternative therapeutics, accelerating antimicrobial diagnostics can lead to faster treatment decisions and more selective usage of antibiotics. In sepsis patients where less critical antibiotics can be administered, quick de-escalation from last-resort antibiotics helps to control the spread of AMR. Most phenotypic antibiotic susceptibility testing (AST) detect bacterial growth, making them reliable but often too slow.

Methods: We used the Resistell Phenotech to develop a fast growth-independent yet accurate AST based on the measurements of bacterial nanomotions. Nanomotions were recorded for four hours for the antibiotics ceftriaxone, cefotaxime, ciprofloxacin, and ceftazidime-avibactam. Each antibiotic was measured at one concentration on a comprehensive set of E. coli and K. pneumoniae clinical isolates. Recordings were subsequently analyzed and classified using assisted machine learning.

Results: Bacterial nanomotions alter depending on their phenotype upon exposure to an antibiotic. For resistant bacteria, we observed an increase in the signal intensity, whereas the signal for susceptible bacteria stagnated or decreased. The antibiotic responses varied among isolates, necessitating machine learning to extract signal parameters and develop accurate classification models. A handful of signal parameters from the nanomotion signal sufficed to classify all strains with an accuracy of 90-100 %. We achieved perfect separation of susceptible and resistant isolates with only one signal parameter in the case of ceftazidime-avibactam.

Conclusions: An accurate AST based on nanomotion technology is measured at a single antibiotic concentration and is completed after four hours, making it significantly faster than gold-standard tests in hospitals. It changes paradigms of assessing antibiotic susceptibility and could supplement current methods in the clinic, offering treatment options faster and containing the spread of AMR for critical drugs.

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**CM03**
**Methylome evolution through lineage-dependent selection in the gastric pathogen *Helicobacter pylori***

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The bacterial pathogen *Helicobacter pylori*, the leading cause of gastric cancer, is genetically highly diverse and harbours a large and variable portfolio of restriction-modification systems. Our understanding of the evolution and functional roles of DNA methylation is limited. Here, we performed a comprehensive analysis of the methylome diversity in *H. pylori***,** using a dataset of 541 genomes that included all known phylogeographic populations. The frequency of 96 methyltransferases and the abundance of their cognate recognition sequences were strongly influenced by phylogeographic structure and were inter-correlated, positively or negatively, for 20% of type II methyltransferases. Low density motifs were more likely to be affected by natural selection, as reflected by higher genomic instability and compositional bias. Importantly, direct correlation showed that methylation patterns can be actively enriched by positive selection and suggests that specific sites have important functions in methylation-dependent phenotypes. Finally, we identified lineage-specific selective pressures modulating the contraction and expansion of a m5c motif, revealing that the genetic load of methylation could be dependent on local ecological factors. Taken together, natural selection shapes both the abundance and distribution of methyltransferases and their specific recognition sequences, likely permitting a fine-tuning of genome-encoded functions not achievable by genetic variation alone.

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**CM04**
**An artificial intelligence tool for lactic acid bacterial species identification**

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Introduction: Rapid identification of cultured microorganisms from environmental or clinical samples has become an indispensable time saver in research and diagnostics. DNA sequencing and matrix-assisted laser desorption ionization (MALDI) with time-of-flight (TOF) analysis (MALDI-TOF) have become standard procedures in most laboratories. The introduction of MALDI-TOF in particular has led to a dramatic time reduction regarding sample preparation. However, these methods are expensive, sophisticated to operate, and require skilled personnel.

Objectives: We address the implementation of an artificial intelligence (AI) tool for fast and reliable identification of bacterial species from the order *Lactobacillales* based on image data from a single bacterial colony.

Material & Methods: Species of Lactic acid bacteria (LAB) were plated onto DeMan-Rogosa-Sharp agar plates supplemented with bromphenol blue (MRS-BPB). Images from LAB colonies were taken in a self-designed photo box. Dataset including imaging capture and digital organization were generated with our self-written LACTOGRAPH software. The neural networks AlexNet, MobileNet2, VGG19, ResNet18 and DenseNet121 were used as deep learning architectures. During data training, stochastic gradient descent (SGD) optimizer and different learning rate methods, such as cyclic learning rate (CLR) and reduced learning rate on plateau (RL-ROP), were applied to all models.

Results: Images from 1893 bacterial colonies were captured in LACTOGRAPH for automatic image organization. Then, a supervised machine learning procedure for image data training was conducted using different neural networks that were selected on our experiences with similar tasks. During training, stochastic gradient descent (SGD) optimizers and different learning rate methods, such as cyclic learning rate (CLR) and reduced learning rate on plateau (RL-ROP), were applied to all models. To further improve the data set, it was augmented during training. Using the DenseNet121 neural network and an RLROP scheduler, we achieved an accuracy of 93.11% on our dataset. We were then able to increase this accuracy by 3.72% to 96.38% by verifying the cell shape of the mislabeled bacteria.

Conclusion: AI tools can accelerate rapid identification of microorganisms at very low cost. The approach may serve as an example to further include machine learning-based methods as powerful tools in bacteriology.

**CM05**

**Application of SERS technique and deep learning algorithms in the rapid differentiation of four Shigella species**

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Accurate discrimination of *Shigella* spp. sits in the core of shigellosis prevention and control. As a label-free method, surface enhanced Raman spectroscopy (SERS) is being intensively investigated for bacterial diagnostics. In this study, we developed a novel method for rapid and accurate discrimination of *Shigella* spp. via label-free SERS coupling with multiscale deep-learning method. In particular, SERS spectral deconvolution was used to generate unique barcodes, revealing subtle differences in molecular composition between *Shigella* spp. Four supervised learning models based on Random Forest (RF), Support Vector Machine (SVM), Convolutional Neural Network (CNN), and One-Dimensional Multi-Scale CNN (1DMSCNN) were constructed and assessed for their predictive capacities of *Shigella* spp. The results showed that 1DMSCNN achieved the best performance, which could quickly distinguish four *Shigella* spp. accurately. Finally, we built a software embedded with 1DMSCNN model to predict raw SERS spectra of *Shigella* spp., which is freely available at https://github.com/4forfull/1DMSCNN_RAMAN_SHIGELLA.

**CM06**

**National research data infrastructure for the research of microbiota (NFDI4Microbiota) – Democratize access to microbiota data and high-end computational analyzes**

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Introduction: Recent technologies like high-throughput molecular sequencing lead to the generation of large amounts of data. However, (re-)use of these data has failed to exploit its full potential. The NFDI (National Research Data Infrastructure) will change this by developing comprehensive research data management. NFDI4Microbiota aims to facilitate digital transformation in the microbiological community, by providing access to data, analysis services, training, and standards.

Objectives: Central for the consortium is the development and provision of the computational infrastructure and analytical workflows required to store, access, process, and interpret various microbiology-related data types. Here, NFDI4Microbiota works on developing and implementing software and standardized workflows for users to analyze their own data (i.e. for quality control, data processing, statistical analysis, and visualizations of different data types and results).

Materials & methods: The German microbial research will be engaged through training, community-building activities, and by creating a cloud-based system that will make the storage, integration, and analysis of microbial data - especially -omics data - consistent, reproducible, and accessible. So, NFDI4Microbiota will promote the FAIR (Findable, Accessible, Interoperable, and Re-usable) principles and Open Science.

Results: NFDI4Microbiota consists of ten well-established partner institutions and is supported by five professional societies (including VAAM) and more than 50 participants. Several workshops and training events were already performed and further will take place frequently. Moreover, the consortium launched an ambassador program to connect with the participants, thereby helping to identify the needs of the community. Technical solutions are developed, tested, and refined in several Use Cases from different fields of microbiology. The recently published Knowledge Base with materials on research data management etc. is accessible via the web portal nfdi4microbiota.de. All information and specific services are and will be made available there as well.

Conclusion: Producers and users of data will benefit from FAIR data more likely to be cited and integrated into a wider microbial inquiry. The current data parasitism would shift to a future data mutualism helping all partners. The NFDI4Microbiota will support the whole community through this process with an elaborate training program and other services.

**CM07**

**Effect of seasonality on microbial community dynamics in a full-scale wastewater treatment plant in Göttingen, Germany**

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Introduction: Wastewater treatment plants (WWTPs) remove pollutants from wastewater and prevent the release of Antimicrobial Resistance Genes (ARGs) and Antimicrobial Resistant Bacteria (ARBs) to the environment. The critical role of microbial communities in WWTPs is affected by seasonality but our understanding of these impacts is limited.

Objective: We investigate the impact of seasonal variation on microbial community structure and function in a full-scale WWTPs in Göttingen, Germany, focusing on microbial metabolic potential and antibiotic-resistant gene abundance.

Methods: FASTQ sequence files from nine stages of the WWTP were retrieved for summer, autumn, winter, and spring, and analysed using SqueezeMeta v1.5.2 in co-assembly mode. Taxonomy and functions were assigned using GenBank and KEGG databases. ARGs and ARBs were identified using the Comprehensive Antibiotic Resistance Database. We constructed Microbial co-

association networks to capture the alterations in interaction patterns among community members across different treatment stages.

Results: The microbial composition shifted as the wastewater was processed from untreated to treated state, with *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Chloroflexi*, *Nitrospirae* being the dominant phyla. Taxonomic analysis showed that samples from before, during, and after treatment formed distinct clusters due to compositional variability. Microbial co-association networks show that phylum level microbial population in Summer was closer to Spring, while Winter was more like Autumn samples. Temporal and spatial changes also occurred in KEGG-based community functional potentials for nitrogen metabolism, carbon metabolism, and xenobiotic degradation. The abundance of AMR genes was higher during summer and spring than winter and autumn, with evidence of AMR gene release in the effluent from pathogens, e.g., *Nocardia farcinica* (rpoB2), *Pseudomonas aeruginosa* (MuxB, MexK, rsmA), *Stenotrophomonas maltophilia* (smeE), *Vibrio fluvialis* (sul1), and *Burkholderia cepacian* (ceoB).

Conclusion: Seasonal variability affects the microbial community structure and WWTP performance.

Keywords: metagenomics, activated sludge, nitrogen metabolism, xenobiotic degradation, antimicrobial resistance

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**CM08**

**Recent updates on Bakta – A rapid & standardized software tool for the comprehensive annotation of bacterial genomes**

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Introduction: In modern microbiology, a thorough genome annotation has become key for many analyses. However, community focus has shifted from manual annotations of single genomes to high-throughput batch annotations. These developments are tremendously accelerated by metagenomics which adds a constant influx of new genomes of unknown species. Addressing these challenges, we recently published Bakta - a new command line software tool for the annotation of bacterial genomes, MAGs and plasmids. Here, we present new developments, added features and improvements.
Objectives: We constantly improve the performance of our software including improved annotation results, technical maintenance, supporting common standards and addressing the various feedback and requests of our active users worldwide. In particular, we addressed requests regarding a lightweight database version and the support of metagenomes.
Results: New features and enhancements have been added improving annotation results and overall software usability. First, we introduced the detection of pseudogenes. A new workflow detects conserved sequences located around de novo-predicted genes and screens elongated alignments for pseudogene determinants or hints for translational exceptions. Second, Bakta now supports the annotation of MAGs adjusting internal gene prediction parameters. Third, we developed a new lightweight database version requiring only 3 Gb of storage, thus helping researchers with limited download bandwidth and storage capacities. By this, wall clock runtimes were significantly reduced. Four, for the annotation of special-interest genes, an import of user-provided protein sequences has been implemented. Five, since de novo gene prediction tools fail to correctly predict translational exceptions, we developed a module to detect and annotate selenocysteine proteins by taking advantage of specific ncRNA motifs. Six, for the streamlined bulk annotation of protein sequences only, we added a new sub command that can be used in addition to the actual whole-genome annotation pipeline. Last but not least, numerous enhancements have been added in terms of annotation quality, long-term maintenance and usability of the software following feedback and requests from the community. Conclusion: We present various improvements of Bakta. This includes important new features and various usability improvements supported by the comprehensive feedback we got from our worldwide and active user community.

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**EME01**

**Electrochemical characterization of recombinant respiratory proteins of the acidophilic iron oxidizer *Ferrovum* sp. PN-J47-F6**

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Moderately acidophilic iron oxidizing bacteria of the genus *Ferrovum* represent a unique model to study adaptations of proteins to the acidic pH of their mine water habitats, because they share common physiological features with both extreme acidophiles and neutrophiles. Earlier studies have shown that homologous periplasmic redox proteins such as high potential iron sulfur proteins (HiPIPs) and c-type cytochromes (CytC) differ with respect to their redox properties in acidophiles and neutrophiles. Because the redox potential of the O2/H2O electron acceptor couple is more positive at acidic pH, acidophiles are assumed to also require respiratory redox proteins with more positive redox potentials than their neutrophilic relatives. However, so far only few redox proteins in acidophilic bacteria have been characterized directly.

Thus, our study aims to characterize the redox properties of recombinant HiPIP-41, CytC-78 and CytC-18 derived from the moderate acidophile *Ferrovum* sp. PN-J47-F6.

Our experimental strategy involves the heterologous production of these three periplasmic redox proteins and their electrochemical characterization.

Our first results strongly suggests that the redox potentials of HiPIP-41 and CytC-78 are indeed more positive than those of homologous proteins in neutrophiles. At the same time they are slightly more negative than those of the extreme acidophiles *Acidithiobacillus* spp.. For example, the redox potential of HiPIP-41 was determined to be 580 mV (vs. SHE) using classic cyclic voltammetry. This is 100 to 400 mV more positive than those of HiPIPs found in neutrophilic phototrophs and about 50 mV more negative than the extreme acidophiles *Acidithiobacillus* spp.. Furthermore, our first measurements of CytC-78 using linear sweep voltammetry at very low scan rates coupled to UV/Vis spectroscopy suggest that the redox potentials of both heme centers differ about 100 mV.

Next, we will fine-tune the experimental set-up to validate our first results. We also plan amending in-silico analyses of structural models predicted by the D-i-Tasser suite to examine the surroundings of the redox centers for potential candidate residues involved in the modulation of the redox properties of HiPIP-41, CytC-78 and CytC-18.

**EME02**

**Between light and shadow – Consequences of exposure to photosynthetically active radiation for *Paramecium bursaria* and its suitability as model random walker in statistical physics**

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*Paramecium bursaria* lives in close mutualistic symbiosis with photosynthetically active endosymbiotic algae, mostly *Chlorella variabilis* or *Micractinium conductrix*. Within this genus, it exhibits a unique behavioral trait, so-called photoaccumulative behavior, i.e., cells accumulating in photosynthesis favoring areas. Implementing a refined protocol to not only qualitatively but also quantitatively analyse photobehavioral responses as a consequence of exposure to photosynthetically active radiation we assessed differences in behavioral responses of *P. bursaria* (i) infected with different intracellular algae, (ii) naturally symbiont-free cells, and (iii) cells experimentally deprived of their algal endosymbionts. Previous studies reported contradictory observations for the latter, i.e., aposymbiotic cells either still exhibiting such behavior or not, and different hypotheses on how photoaccumulation is mediated were proposed. By considering the aposymbiotic cultivation duration, the time elapsed between the cell"s confirmed aposymbiotic status and the respective experimental assessment, our data provide a unifying explanation. Moreover, under laboratory conditions aposymbiotic *P. bursaria* successfully re-establish symbiosis with their original or with another photobiont, respectively, and subsequently exhibit overall positive response to light accompanied by accumulation.

Furthermore, we will demonstrate *Paramecium* as a random walker, an excellent model organism assessing distribution and motion of Brownian particles. By assessing its swimming behavior in strictly defined environments, we test potentially influencing parameters, e.g., bacterial gradients and different illumination scenarios. Such knowledge allows to regulate particle motion in order to develop functional materials possibly obstructing biofilm growth or, consequential to specific characters, to even separate particles. Based on our data we interpret photoaccumulative behavior as a complex, adaptive strategy of *P. bursaria* to its symbiotic lifestyle. Our assessment of *Paramecium*"s swimming behavior is highly relevant in predicting e.g. its feeding strategy, response to disturbances, or distribution. We furthermore confirm *Paramecium* as a simple model suitable to verify predictions in statistical physics, active soft matter, and ecosystem modeling.

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**EME03**

**Bacterial and viral response to treated wastewater in an urban stream**

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The microbial community of freshwater streams plays a crucial role in ecosystem services being a cornerstone in central biogeochemical cycles. In densely populated areas, streams are under permanent stress by effluents of wastewater treatment plants, which can make up more than half of the total water during dry conditions. Yet, the effects of wastewater microbiome and resistome on the community of a previously anthropogenic stressed river remain largely unknown. Here, we show that the introduction of treated wastewater results in a temporal, but drastic change of the bacterial and viral community returning to the reference state within ten days by coupling a mesocosm approach (AquaFlow) to full-length 16S rRNA gene Nanopore sequencing and strain-resolved metagenomics. In this indoor mesocosm setup, two metal flow channels filled with stream sediment are connected to three water tanks. For ten days, restored river water with and without added treated wastewater (30%) was run in circle in triplicates and sampled for DNA-based analyses. As revealed by strain-resolved metagenomics, treated wastewater introduced a high bacterial and viral diversity that diminished over time. Analyses of the encoded resistome showed that low abundant microbes introduced additional antibiotic resistance genes (ARG) in treatment systems, yet resistant microbes from the reference stream were responsible for an increase of total ARG counts after ten days. We conclude that a substantial introduction of wastewater only temporarily alters the microbial community of a previously stressed river which seems to be resilient to a repeated stressor impact.

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**EME04**

**Resistance of freshwater sediment bacterial communities to salinity disturbance and implication for industrial salt discharge and climate change based salinization**

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The impact of salinization on freshwater ecosystems became apparent during the industrial salt discharge in the Oder River (2022) that caused an ecological disaster. How bacterial communities respond to salinization caused by industrial salt discharge, or climate change driven events like storms or sea level rise, depends on the sensitivity of these complex bacterial communities. To investigate the sensitivity to pulse salinization on bacterial communities we performed experiments in the salinity range 0.1-6. In addition, we sampled similar salinities in the littoral zone of the Baltic Sea where the bacterial communities are permanently exposed to the respective salinities. To simulate a major disturbance, we also included antibiotics (ampicillin/streptomycin) manipulation in the experiment. While the addition of antibiotics and increase in salinity had a significant impact on the water bacterial community richness and community composition, only antibiotics had an effect on the sediment bacterial community in the experiment. In contrast, sediment bacterial communities from the Baltic Sea littoral zone clustered according to salinity. Hence, sediment bacterial communities are more resistant to pulse changes in salinity than water bacterial communities, but are able to adapt to a permanent change without loss in species richness. For industrial salt discharge and climate change scenarios our results indicate that moderate pulse salinization events of freshwater bodies will cause changes in the water bacterial community with unknown consequences for ecosystem functioning. Sediment bacterial communities however will be probably unaffected in their ecosystem services. Long term disturbance like sea level rise or constant salt discharge will cause permanent changes in the sediment bacteria

community composition and established communities can maintain ecosystem functioning.

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**EME05**

**Function-based screening for novel CODH enzymes from hydrothermal vent environments and marine sediment**

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Carbon monoxide dehydrogenases catalyze the reversible reaction of carbon monoxide (CO) and water to carbon dioxide (CO2), protons, and electrons. These enzymes are used by a variety of phylogenetically diverse aerobic and especially anaerobic microbes for autotrophic carbon fixation and energy conservation such as in the reductive acetyl-CoA pathway. Therefore, the enzymatic inventory involved in this CO metabolism is a promising resource for industrial as well as biotechnological applications as CODHs drive the conversion of the greenhouse gas CO2 into valuable commodities. However, given that the vast majority of microbes on earth are currently not cultured, identification of truly novel environmental CODH enzymes is limited and thus the exploration of a large biochemical potential remains inaccessible using present culture-dependent and sequence-based approaches. Facing this problem, we use a culture-independent method and apply an activity-based colorimetric screen that enables us to detect (novel) CODH enzymes from the environment. To investigate which CODHs from different microbial phyla can be targeted by this method, the screen was successfully applied to fosmid clones prepared with genomic material from *Rhodospirillum rubrum*, *Desulfovibrio vulgaris*, *Moorella thermoacetica* and *Methanosarcina mazei.* Our screen was then applied to two different metagenomic fosmid libraries constructed with both anoxic marine sediments from Eckernförde Bight (Baltic Sea, Germany) and material from hydrothermal vents (Sisters Peak, Mid-Atlantic-Ridge). Screening latter metagenomic fosmid library recently resulted in the detection of two putative CODH active fosmid clones, which will be further investigated, characterized, and optimized for application in electrochemical cells to potentially reduce anthropogenically emitted CO2.

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**EME06**

**Proteomic insight into the degradation of arabinogalactan by marine, particle-associated bacteria *Maribacter***

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Planktonic microalgae contribute nearly 50% to the global net photosynthesis. In temperate regions, an annual succession of microalgae is observed, starting with diatoms which are followed by *Phaeocystis*. The breakdown of these microalgae fuels heterotrophic bacterioplankton. Abundant carbohydrates in *Phaeocystis* biomass are arabinose and galactose, which are the main components of arabinogalactan (Alderkamp *et al.*, 2007). Arabinogalactan was recently identified as a main carbohydrate of diatoms in the North Sea (Vidal-Melgosa *et al.*, 2021). One realized arabinogalactan function is the decoration of lipoproteins and these large molecules anchor the cell wall in the cytoplasmic membrane (Veenhof and Popper, 2020). Currently the degradation of arabinogalactan by marine microorganisms has not been explored. Therefore, we studied the proteomes of *Maribacter* strains grown on polysaccharides to gain insight in the degradation of arabinogalactan and other cell wall polysaccharides. *Maribacter* is a genus of marine *Flavobacteriia* that live associated to particles, which are the nutrient rich hot spots in the oligotrophic sea. The role of the particle-associated bacteria in the remineralisation of photosynthesis-derived organic matter into carbon dioxide is likely underestimated, but definitely underexplored.

We grew *Maribacter* strains in liquid medium on several different mono- and polysaccharides, including arabinogalactan. Extracted proteins were digested and resulting tryptic peptides were analyzed by LC-MS/MS. We used MaxQuant with a strain-specific protein sequence database and Perseus software for protein identification, quantification and statistical data evaluation (Tyanova *et al.*, 2016; Tyanova and Cox, 2018).

The expressed proteins revealed candidate carbohydrate-active enzymes (CAZymes) for polymer degradation and arabinose and galactose pathway. *Maribacter* strains were able to use either the glycolysis or the pentose phosphate pathway to feed their citrate cycle. The induced enzymes, which possibly play a role in the utilization of arabinogalactan, were not located in a polysaccharide utilization locus (PUL), an operon like genetic region encoding the proteins for hydrolysis and transport of polysaccharides on the chromosome (Grondin *et al.*, 2017).

These observations provided a first insight in the arabinogalactan utilization pathway by particle–associated *Maribacter*.

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**EME07**

**Antarctic krill gut communities are a reservoir of biopolymer hydrolysing enzymes**

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Introduction: The Antarctic krill (*Euphausia superba* Dana) is a key species in the Southern oceans and its gut most likely represents a distinct nutrient rich environment when compared to surrounding habitats. The diverse food spectrum of Antarctic krill, which is composed of algae, diatoms, copepods up to lithogenic particles, provides the present gut microorganisms with a broad range of biomacromolecules like lipids, proteins and diverse polysaccharides. Due to the sheer number of Antarctic krill, its combined gut volume represents the enormous reaction volume of up to $8x10^7$ $m^3$ for the turnover of biomacromolecules. Despite its uniqueness as a habitat, the associated microbial assemblages of the Antarctic krill gut received limited attention in the study of Arctic microbial communities. Hence, it represents a promising and largely untapped reservoir for the exploration of biopolymer hydrolysing microbial communities and enzymes.

Aim: Aim of this study was to gain insights in the structure of the microbial communities present in the Antarctic krill, as well as their theoretical and verifiable capacity to degrade biomacromolecules.

Material & methods: To answer these questions, classical cultivation-based approaches were applied to generate a biobank of cultivatable krill gut bacteria and test them with a biodegradation assay. Results were substantiated with molecular analysis using state-of-the-art amplicon sequencing via NanoPore as well as the generation of metagenomic datasets for the cultivatable and uncultivatable microbial communities.

Results: The results indicate that the microbial krill gut community, which is composed mainly of members of *Proteobacteria* and *Bacteriodetes*, are characterized by their detectable capability to hydrolyse a broad range of biomacromolecules like proteins, lipids and diverse polysaccharides, which include among others chitin, alginate, xylan and cellulose. This is furthermore reflected by the high number of genes present in the metagenomic datasets for the respective depolymerisation pathways.

Conclusion: These microbial assemblages not only bear the potential to play an important role in the turnover of ingested biomacromolecules in the Antarctic krill gut and thereby in the Antarctic carbon-cycle, but are also an interesting starting point to find hydrolytic enzymes or microbes, which could be applied in biotechnological processes.

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**EME08**

**Effect of multiple stressors and their release on microbial biomass recycling in sediment of the river boye**

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Microorganisms play a key role in the functioning of healthy river ecosystems because they recycle nutrients and carbon from dead biomass (necromass), but little is known about how these fundamental processes are affected by multiple stressors such as increased temperature and salinity. In this study, we investigated the recycling of necromass by microorganisms in the river Boye before, during, and after a period of increased temperature and salinity using an outdoor flow-through system (ExStream). Rates of necromass recycling were measured as an increase in $^{13}CO2$- concentration over time resulting from the microbial degradation of $^{13}C$ -labelled necromass, which was offered either in the form of intact (but dead) or lysed *E. coli* cells. Preliminary results indicate that necromass recycling was highest in the first days of incubation, suggesting that it is an easily biodegradable carbon source. Interestingly, increased temperature strongly increased the rate of whole cell-necromass recycling compared to the unstressed control, while it slightly decreased the rate of lysed cell recycling. Increased salinity did not seem to affect necromass recycling alone or in combination with increased temperature. After stressor removal, necromass recycling rates were not distinguishable from the unstressed control. Overall, microbial necromass recycling is strongly affected by temperature but resistant to salinity stress, and the effect of temperature stress depends on the necromass type. The fast recovery after stressor removal indicates high resilience of these microbial processes.

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**EME09**

**CO2 degassing drives metabolic processes in unique Eger rift subsurface microbiome**

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Frequent seismic activity and consistently high $CO_2$ fluxes make the Eger Rift in Western Bohemia (CZ) a rare subsurface ecosystem and scientifically relevant location to study microbial behavior and assess how geologically derived compounds are utilized at depth. Explorations into microbial life in this unique ecosystem provide the opportunity to investigate how high $CO_2$ levels and the associated mineralogy influence microbial community composition and metabolic activity. Furthermore, seismic activity in this region can cause abiogenic production of $H_2$, potentially providing the basis for primary production through methanogenic archaea.

To gain insight into microbial processes associated with the high $CO_2$ Eger Rift subsurface we investigated temporal diversity and compositions of bacterial and archaeal communities in sediment samples from drill cores and 100 m deep groundwaters. Using metagenomic approaches we were able to identify metabolic attributes and functional processes shaping microbial life in this unique high $CO_2$ ecosystem. Metagenomic binning and annotation resulted in the recovery of nearly complete genomes and reconstruction of metabolic networks. In addition, culturing efforts allowed us to enrich and further characterize active members of the Eger subsurface community.

Our work revealed microbial communities driven by high $CO_2$ conditions and frequent groundwater movement as well as an unexpected archaeal diversity. Metagenomic investigations resulted in the recovery of more than 20 high quality metagenome assembled draft genomes (MAGs), featuring acetogenic, potentially $CO_2$ fixating *Acetobacterium* and sulfate reducers including *Desulfomicrobium* and *Desulfovibrio*. Characterization of *Methanobacterium* and *Methanosphaerula* MAGs was highlighted by the metabolic capability to utilitze tectonically released $H_2$ through methanogenesis. In vitro experiments using Eger enrichment cultures provided further details on the active biological utilization of $CO_2$ and $H_2$, and the role of key metabolic pathways in natural $CO_2$ driven subsurface environments.

Going forward our data will be used to develop microfluidic culturing experiments. This approach, together with metatranscriptomics will be used to further investigate cellular processes under high $CO_2$ conditions, with the aim to identify biomolecules of industrial and biotechnological relevance which may be used as substrates for microbially synthesized products.

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**EME10**

**Mycorrhizosphere community structures: Plant *versus* soil impact at a former uranium mining site**

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Post-mining disturbed ecosystems can be used with reforestation, where trees must establish beneficial interactions with soil microorganisms to adapt to the harsh abiotic conditions.

We wanted to test whether patterns of microbial community structure are depending on soil chemical parameters or tree species.

The mycorrhizospheres of oak, pine and birch stands at a former uranium mining site near Ronneburg (Germany) were analyzed for bacterial and fungal communities and ectomycorrhizal root tips. Statistically relevant correlations to bioavailable elements from soil were extracted from the data.

Among the most abundant bacterial classes were *Acidobacteriia*, *Alphaproteobacteria*, *Ktedonobacteria*, *Bacteroidia* with about 50-60 % of all bacterial sequences, while the fungal community was dominated by *Thelephoraceae*, *Inocybaceae*, and *Russulaceae* contributing 50-80% of abundances. Whereas bacterial communities correlated with soil chemical parameters, in particular the content of toxic metals, total nitrogen and C/N ratios, fungal communities and their distribution patterns revealed close associations with the plant species and primary versus secondary succession. Ectomycorrhizal communities showed a low diversity of morphotypes with a preference for contact and short-distance exploration strategies; only the abundant ascomycete *Meliniomyces bicolor* was found with all trees. The contact morphotypes correlated with high Al, Cu, Fe, and Sr contents, and the medium fringe type with rhizomorphs on oaks correlated with total nitrogen.

Metal contaminated, afforested substrates in post-mining landscapes showed selection of ectomycorrhizal fungi in a tree species-dependent manner, with exploration types likely improving tolerance of the trees to specific abiotic conditions of the environment. Although soil pH value had a minor direct effect on the structure of bacterial communities, abiotic soil parameters led to ectomycorrhizophere community structuring. This implies an indirect effect through the change of mobility of toxic metals and their bioavailability.

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**EME11**

**Sodium chloride affects biosynthesis and emission of the sesquiterpene sodorifen in *Serratia plymuthica* 4Rx13**

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Introduction: *Serratia plymuthica* 4Rx13 is a rhizobacterium isolated from the rhizosphere of *Brassica napus.* It emits more than 100 organic and inorganic volatile organic compounds (VOCs), with the sequiterpene sodorifen (C16H26, unique structure, highly polymethylated and bicyclic)1 being the most dominant2. A four-gene cluster with a new type of methyltransferase (FPPMT) and a non-canonical terpene synthase (SODS) are indispensable for its biosynthesis3, 4.

Objective: As the rhizosphere is a dynamic habitat where salts from fertilizer deposits, geological weathering of rocks, and industrial effluents may function as environmental stress on rhizobacteria and their interactions, we hypothesized that salts may influence gene expression and emission of sodorifen of *S. plymuthica* 4Rx13.

Methods: Different concentrations of sodium chloride and other salts were supplemented to Davis Minimal Medium, and growth, FPPMT and SODS gene expression, enzyme levels were followed for 72h in *S. plymuthica* 4Rx13. Furthermore, the EnvZ/ompR two-component NaCl uptake system was investigated.

Results: The expression of EnvZ/ompR two-component system was significantly upregulated under salt treatment, indicating an increased NaCl uptake at 200mM NaCl. Under these conditions the VOC emission profile showed a significant reduction of the amount of sodorifen emitted per cell. This reduction was further supported by the observation that the FPPMT and SODS genes were less expressed in the exponential and late growth phases in the NaCl-treated culture. In contrast, the expression of both genes was much higher in the salt treated cultures during the transition from exponential to the stationary phase (at 15 and 24 hours), while sodorifen emission was still lower in the NaCl-treated culture. To unravel this discrepancy, we determined the SODS protein levels by Western blots. The amounts of SODS protein were not different in treated and untreated cultures. These controversial results might point towards a yet unknown sodorifen emission regulation under ion-induced osmotic stress. We are currently investigating whether NaCl stress reduces SODS activity and/or whether VOC transporters are inhibited under NaCl-induced osmotic stress conditions.

Conclusions: Here we showed that the regulation of the emission of the unusual VOC sodorifen is altered during salt treatment in different growth phases of *S. plymuthica* 4Rx13.

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**EME12**

**More than defense metabolites? – Revisiting the effect of diverse glucosinolates on microbial leaf colonization in *Arabidopsis thaliana***

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Introduction: Commensal leaf bacteria are critical for plant health, but in contrast to root bacteria little is known about whether and how they are actively recruited. Glucosinolates (GLS) are a major group of secondary metabolites in Brassicaceae plants like the model plant *Arabidopsis thaliana* (*At*). GLS speciation is controlled by plant genotype: well-studied *At* Col-0 mainly produces 4MSOB-GLS whereas a local genotype isolated in Jena (NG2) biosynthesizes allyl-GLS. GLS and their breakdown products isothiocyanates (ITCs) are well known for their defensive effect against herbivorous insects and plant pathogenic microbes.

Objectives: In this study we compare the effects of aliphatic GLS on leaf commensal bacterial colonization in NG2 and Col-0 *At*.

Methods: We characterized the sensitivity of ~150 bacterial isolates from wild *At* leaves to GLS breakdown products, including allyl-ITC and 4MSOB-ITC. We then studied how different GLS affect natural leaf colonization in healthy

plants. We compared bacterial load and 16S rRNA diversity using the wildtypes Col-0 and NG2 and their respective mutants, *myb28/myb29* and NG*myb28*, neither of which produce aliphatic GLS. We also performed *in-vitro* enrichments from wild NG2 leaf washes with GLS as sole carbon source.

Results: Most isolates were sensitive to GLS breakdown products, only *Pseudomonas* and *Xanthomonas* strains were resistant to both tested ITCs. Genome sequencing confirmed that these isolates carry ITC resistance genes. Although the ITCs were toxic, we observed no significant GLS effect on commensal leaf colonization in Col-0, confirming other studies. In NG2, however, there were significant effects on total but not endophytic bacterial communities. Differentially abundant taxa were surprisingly always enriched in NG2 leaves compared to NG*myb28* without aliphatic GLS. Similar taxa were also more abundant in wild *At* populations compared to other plants. Ongoing experiments evaluate the role of GLS for the leaf surface colonization of 7 diverse bacterial strains enriched from wild NG2 leaf washes.

Conclusion: GLS are important defense metabolites, but their presence on leaves can also play attractive roles, for example in insect oviposition. Our data supports their role in recruitment of specific leaf bacteria, probably as a carbon source. Recruitment differs among GLS species, linking plant genotype to leaf bacterial recruitment. Thus, similar to roots, leaf chemodiversity can shape the leaf microbiome.

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**EME13**

**Acidobacteriota under the spotlight – A metabolomic and genomic endeavour**

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Introduction: Global challenges call for novel natural products (NP), but the discovery rate has declined for decades. Existing challenges include unknown cultivation conditions for promising microorganisms, such as the phylum Acidobacteriota. While metagenomic data indicate the presence of novel biosynthetic gene clusters (BGCs), their NP repertoire and ecological role are scarcely described.

Objective: We planned to combine cultivation-dependent and independent approaches to gain insights into the biosynthetic potential of the Acidobacteriota by employing our in-house metabolomics and bioinformatics platform.

Material & Methods: A set of Acidobacteriota was cultivated in an OSMAC approach. Crude methanolic extracts were screened for bioactivity and the presence of unknown masses of interest. Based on these findings, plant growth-promoting characteristics were investigated both microbiologically and *in planta*. 618 acidobacterial genomes, including three novel isolates, were quality-checked and bioinformatically analysed.

Results: Extracts of *Acidicapsa borealis* and FhG110214 showed activity specifically against the fungal phytopathogen *Septoria tritici*. MS analysis of the active fractions indicated the presence of **Pityriacitrins** A and B (later confirmed by NMR) and putative Malassezindoles A and B. In addition, unknown derivatives of the Malassezindoles, as well as an unknown peptide, were detected. Extracts also contained indole-3-acetic acid (IAA) and N$^6$-isopentenyladenine (N6). Production of IAA increased in the presence of Tryptophan (TRP) and was highest in *A. borealis* (100 µM). FhG110214 produced the most N6 (1.4 µM). Applying crude extracts of a TRP-induced culture of *A. borealis* increased barley seedlings' biomass compared to the culture without TRP but not compared to the medium control. Bioinformatic analyses indicate an evident accumulation of biosynthetic potential in certain phylogenetic groups.

Conclusion: This work highlights the vast opportunity for further studies of this phylum regarding its NP repertoire and related ecological functions. Bioactivity-guided and novelty-guided metabolomic analysis revealed the production of compounds previously only known to be produced by fungi but also showed the presence of unknown compounds of interest. Genomic analyses indicated hotspots of biosynthetic potential within this phylum that can be used for more targeted ecological studies.

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**EME14**

**Traversing the canopy – Throughfall-mediated transport of microorganisms interconnects microbial habitats within forest tree crowns**

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Phyllosphere microbiomes are subject to microbial import from various sources and undergo substantial changes during different developmental stages of plants. While these dynamics are well understood for herbaceous plants, much less is known about how forest canopy microbiomes are shaped by plant-driven controls versus random colonization and transport of microbes and nutrients. Here, we propose that throughfall, rainwater percolating through the tree canopy, plays a central role in the distribution of microorganisms within the canopy. We leveraged the infrastructure of the Leipzig Canopy Crane Facility (Germany), located in a floodplain hardwood forest, to (i) follow the transport of microorganisms by throughfall across top, mid and bottom position of the tree crown for three species - *Quercus robur* L., *Fraxinus excelsior* L., and *Tilia cordata* Mill., and (ii) characterize microbial exchange between throughfall, leaves and bark. Throughfall samplers were deployed within the canopy for a two-weeks-period in March, May, and October 2021, and May 2022. Throughfall communities were clearly distinct and more variable in March compared to the months after the emergence of foliage, while the abundance of transported microorganisms increased by up to two orders of magnitude from March to May. In May, transported bacteria were dominated by *Oxalobacteraceae* and *Pseudomonadaceae*, while *Beijerinckiaceae*, *Sphingomonadaceae*, *Acetobacteraceae* and *Erwiniaceae* contributed a large fraction to the bark and phyllosphere communities. Between 14 and 32% of the phyllosphere community members, and 16 – 27% of the bark community members were shared with throughfall, pointing to considerable microbial exchange between percolating rainwater and leaf and bark surfaces. Sampling month explained about 15% of the community variation of transported microorganisms, while tree species identity accounted for 8-12% of variation. Our results suggest that rainwater-driven microbial transport plays a crucial role for the import of bacterial taxa to the phyllosphere, and for their

establishment and redistribution, modulated by phenology-driven changes of the host trees.

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**EME15**

**Denitrifiers vs. nitrate ammonifiers: How land use intensity and environmental parameters influence the competition in grasslands**

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Introduction: Denitrification is the sequential reduction of nitrate via nitrite and nitrous oxide to dinitrogen, and nitrate ammonification reduces nitrate via nitrite to ammonium. Both pathways compete for nitrate and nitrite, and play a decisive role in channeling nitrate either to gaseous N-products leading to N-loss in soils or to ammonium remaining available for plant nutrition.

Objectives: Although parameters like pH or C/N ratio are well known to impact the competition between denitrifiers and nitrate ammonifiers, in-depth studies from grassland soils relative to impact of management practices are lacking. Thus, we investigated the influence of land use intensity and other environmental parameters on the competition between denitrifiers and nitrate ammonifiers.

Material and Methods: We quantified bacterial 16S rRNA genes and denitrification/nitrate ammonification marker genes (*narG*, *napA*, *nirK*, *nirS*, *nrfA*, *nosZ*I and *nosZ*II) in 150 grassland soils in Germany via qPCR. Land use intensity and other important environmental factors, e.g. pH, C/N ratio, water content or temperature were determined and correlations between environmental factors and qPCR results were calculated. Furthermore, 16S rRNA derived cDNA was Illumina NextSeq sequenced, classified, and analyzed in regard to significant correlations between amplicon sequence variants and marker gene abundance.

Results: Land use intensity was negatively correlated with 16S rRNA gene normalized *nirK* and *nrfA* abundances. Furthermore, denitrifier to nitrate ammonifier ratio as indicated by *nirK* and *nirS* over *nrfA* ratios was positively correlated with C/N and fungi/bacteria phospholipid fatty acid (PLFA) ratios, and negatively correlated with total PLFA, soil water content and pH. Denitrifier and nitrate ammonifier related marker gene abundance data correlated with certain 16S rRNA derived ASVs belonging to *Acidobacteria*, *Actinobacteria*, *Chlamydiae*, *Chloroflexi*, *Dependentiae*, *Entotheonellaeota* and *Proteobacteria*. ASVs of *Firmicutes, Planctomycetes, Rokubacteria* and *Latescibacteria* correlated well with denitrifier-related marker gene abundance.

Conclusion: We identified land use intensity, C/N ratio and pH as key parameters impacting denitrifier and nitrate ammonifier competition in soil, and identified potential taxa hosting or positively interacting with denitrifiers and nitrate ammonifiers.

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**EME16**

**Bacterial drivers of nitrogen cycling in grasslands under different land use intensities**

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Soil bacteria are important drivers of nutrient cycles. They influence total and available soil nutrient pools by nutrient uptake, storage and immobilization in biomass, nutrient transformation and release by exudation or cell-death. This is particularly true for the nitrogen (N) cycle, where soil bacteria mediate the transformation of inorganic and organic N forms by N fixation or mineralization. *Vice versa*, pH, nutrient pools and land use shape bacterial communities and their activities.
We want to understand how species composition and functional gene abundance of the N cycle differ in varying land use intensities and parent soil material. Therefore, we used a metagenomics approach to reconstruct the bacterial nitrogen cycle on a functional and taxonomic level. Soil samples were taken from temperate grassland soils of the Biodiversity Exploratories (www.biodiversity-exploratories.de). The effect of land use intensity, soil variables and the correlation of relative gene abundances to each other were analyzed on paired-end metagenomes (Illumina® NextSeq500). Quality filtering, trimming as well as taxonomic and functional annotation was performed on the MGrast server using the NCBI RefSeq and KEGG database, respectively.
The top 5 abundant bacterial families involved in the transformation of N belonged to the Actino- and Proteobacteria and were Mycobacteriaceae, Bradyrhizobiaceae, Burkholderiaceae, Nocardioidaceae, and Brucellaceae. On functional gene level, highest relative abundances were detected for genes in the organic nitrogen cycle related to glutamate transformation (*gudB*, *gltB*, *glnA*), peaking in highest land use intensity in the soils with the highest C, N and P stocks. Within the inorganic N cycle, genes related to the dissimilatory nitrate reduction (*nirBD*) were highly abundant and peaked in soils with medium land use intensity in the region with medium N and P stocks. *narGH*/*nxrAB*, coding for the transformation of nitrate to nitrite and *vice versa*, decrease with land use intensity in the region with low N and P stocks, but increases in the region with medium and high N and P stocks with land use intensity. The processes within the bacterial nitrogen cycle highly depended on land use intensity. The correlation analysis revealed that the turnover of organic and inorganic N substances were closely interlinked while these effects were region specific.

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**EME-MBE01**

**Active soil microbial composition and proliferation are directly affected by the presence of biocides from building materials**

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Combinations of biocides are commonly added to building materials to prevent microbial growth, which can cause degradation of the façades. These biocides reach the environment by leaching from façades and thereby pose an environmental risk. Although ecotoxicity to the aquatic habitat is well established, there is hardly any data on the ecotoxicological effects of biocides on the soil habitat. Therefore, this study aimed to characterize the effect of the biocides terbutryn, isoproturon, octhilinone, and combinations thereof on the total and active soil microbial community composition and functions. Total soil microbial community was retrieved directly from the nucleic acid extracts, while the DNA of the active soil microbial community was separated by an immunocapture approach after bromodeoxyuridine labelling. Bacterial 16S rRNA gene and fungal transcribed spacer region gene-based amplicon sequencing was carried out for both active and total, while gene copy numbers were quantified for the total soil microbial community. Additionally, soil respiration and physico-chemical parameters were analyzed to investigate overall soil microbial activity. The bacterial and fungal gene copy numbers were significantly affected by single biocides and combined biocide soil treatment but not soil respiration and physico-chemical parameters. Moreover, results showed that single and combined biocide treatment only had minor effects on the total soil microbiome. While the total soil microbiome experienced only minor effects from single and combined biocide treatment, the active soil microbiome was significantly impacted in its diversity, richness, composition, and functional patterns. The active bacterial richness was more sensitive than fungi. However, the negative effects of the biocide combination treatments on soil bacterial richness were highly dependent on the identities of the biocide combination. Our results demonstrate that the presence of biocides frequently used in building materials affects the active soil microbiome. Thereby, it can be used as an ecotoxicological measure for effect on complex soil environments in future studies.

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**EME-MBE02**

**Direct-geneFISH to link antibiotic resistance gene presence and phylogeny in model strains and microbial populations**

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Linking antibiotic resistance genes (ARGs) with cell phylogeny can provide direct proof of the antibiotic resistance potential of a specific bacterial group and can be used to trace the abundance and spread of such genes in microbial populations. Here we applied a modified direct-geneFISH approach, which combines rRNA-targeted catalyzed amplification reporter deposition - fluorescence *in situ* hybridization (CARD-FISH) and *in situ* gene detection, for visualization, identification, and quantification of ARG-containing cells. Three *sul1*-targeting polynucleotide probes and a non-sense probe (*Nonsul*) were designed to specifically target *sul1.*The proof of principle experiment was conducted on model organisms: *Escherichia coli K12 J53* and *Acinetobacter defluvii* strains that contain the *sul1* gene, and *Pseudomonas alloputida* that do not contain this gene. The method was then applied to a mixture of naturally formed river biofilms and *sul1*-containing *Acinetobacter defluvii*. According to the results, the *sul1* probe had a detection efficiency of over 96% for *Escherichia coli K12 J53* and 90% for *Acinetobacter defluvii*. False positive signals in *Pseudomonas alloputida* were found to range from 5.8% to 22.2%. The number of *sul1* gene copies ranged from 1 to 12 in *Escherichia coli K12 J53*, and from 1 to 7 copies in *Acinetobacter defluvii*. Direct-geneFISH on the artificially constructed mixture showed that the *sul1*-containing *Acinetobacter defluvii* could be traced back within the mixture. In summary, our preliminary results demonstrate that the direct-geneFISH method can be employed to detect and track ARG-containing bacteria in model systems, and has the potential to identify and quantify ARG-carrying cells in environmental samples.

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**EME-MBE03**

**Adaptation of *Listeria monocytogenes* to quaternary ammonium compounds**

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The human pathogen *Listeria monocytogenes* can thrive under various environmental conditions and is ubiquitously found in nature. Upon ingestion of contaminated food products, *L. monocytogenes* can switch to a pathogenic lifestyle and cause mild or severe infections depending on the immune status of the patient. *L. monocytogenes* can easily enter food-processing facilities due to contamination of raw materials. For the elimination of bacterial contaminations on surfaces, disinfectants that usually contain quaternary ammonium compounds such as benzalkonium chloride (BAC) and cetyltrimethylammonium bromide (CTAB) are used. Several BAC-tolerant isolates of *L. monocytogenes* have been identified over the past years. These strains often possessed efflux pumps, which are either located on the chromosome or on mobile genetic elements. Here, we aimed to elucidate the potential of the commonly used *L. monocytogenes* wildtype strain EGD-e to adapt to BAC and CTAB. All isolated BAC-tolerant *L. monocytogenes* strains acquired mutations in *fepR* or its promoter region resulting in the overproduction of the efflux pump FepA, which has already been previously described (1). In contrast, adaptation to CTAB resulted in overproduction of the efflux pumps SugE1 and SugE2, whose expression is regulated by SugR. *L. monocytogenes* strains lacking FepA or SugE1/E2 could still be adapted to BAC and CTAB and further analysis revealed that the remaining efflux pump(s) could compensate for the deleted one(s). Interestingly, we identified mutations in the diacylglycerol kinase encoding gene *lmo1753* (*dgkB*), when a *fepA sugE1E2* double mutant was adapted to BAC and CTAB. DgkB converts diacylglycerol to phosphatidic acid, which is subsequently reused for the synthesis of phospholipids, one of which is required for lipoteichoic acid biosynthesis (2). This suggests that other efflux systems of *L. monocytogenes* are less effective against both compounds than alterations in the membrane composition or modification.

(1) Douarre et al. (2022) Front Microbiol. 13:864576.

(2) Jerga et al. (2007) J Biol Chem. 282:21738-45.

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**EME-MBE04**

**The disinfectant glutaraldehyde induces antibiotic tolerance underpinned by phenotypic heterogeneity and transcriptome remodeling**

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Introduction: Glutaraldehyde (GTA) is commonly used to disinfect medical equipment, in animal husbandry and in hydraulic fracturing. Its wide use bears the risk that microorganisms in different environments are exposed to potentially non-lethal doses of glutaraldehyde. To date, little is known about the effects of glutaraldehyde on the susceptibility of bacteria to antibiotics and its role in the selection of tolerant phenotypes.

Objectives:

- To determine the effect of glutaraldehyde exposure on the survival of *E. coli, S. aureus* and *P. aeruginosa* to antibiotics
- To find the mechanistic basis for increased antibiotic tolerance upon glutaraldehyde exposure

Materials & Methods: Four bacterial isolates were exposed to sub-inhibitory levels of glutaraldehyde. Antibiotic tolerance was determined by time-kill assays. Regrowth dynamics (lag times) were determined with ScanLag. *E. coli* was further investigated, using RNAseq to identify genes and processes involved in antibiotic tolerance. Mutants of candidate genes were screened for their antibiotic tolerance and heterogeneous target gene expression under stressed and unstressed conditions.

Results: Short-term exposure to sub-inhibitory levels of glutaraldehyde induced tolerance to high doses of bactericidal antibiotics. Tolerance to antibiotics was associated with highly heterogeneous regrowth dynamics and global transcriptome remodeling. Differentially expressed genes represented diverse biological functions and cellular components, including antibiotic efflux, metabolic processes, and the cell envelope. The heterogeneous regrowth dynamics and the diversity of the differentially expressed genes are likely related to the unspecific mode-of-action of glutaraldehyde. Among the many differentially expressed genes, several genes were identified that were not previously associated with antibiotic tolerance or persistence, which, when overexpressed alone, increased antibiotic tolerance.

Conclusion: Our results highlight how the big advantage of a disinfectant, its unspecific mode-of-action, can induce transient tolerance to antibiotics in bacteria. These findings have implications for 1.) settings where disinfectants and antibiotics are used in proximity, such as hospitals and animal husbandry, and 2.) for the selection dynamics of tolerant bacteria in fluctuating environments because of the trade-off that arises from overcoming the lag phase as fast as possible and maintaining antibiotic tolerance.

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**EME-MBE05**

**Trade-offs between translational speed and polyproline-induced ribosomal stalling in bacteria**

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Bacterial growth rate depends on the speed of translation—genome characteristics correlated with growth rate (rRNA gene copies, tRNA gene copies, and codon bias) all influence the rate of translation. The rate of translation is also affected by the specific amino acid being incorporated into the growing polypeptide chain. In particular, proline is a poor peptidyl acceptor and donor, and successive stretches of prolines cause ribosomes to stall. In bacteria, polyproline (PP) induced stalls are mitigated by translation elongation factor P (EFP). While EFP increases the speed of proline transfer by optimally positioning the pro-tRNA, ribosome profiling studies have shown that EFP does not completely eradicate these stalls. Accordingly, there is selection against PP motifs in fast-growing species like *E. coli*, especially in highly expressed proteins. Slow-growing bacteria are under reduced selection for translational speed—they have reduced codon usage bias in growth-related genes and fewer tRNA and rRNA gene copies than their fast-growing counterparts. Therefore, we wondered if PP motifs would be more widespread in slow-growing bacteria, and if reduced selectional pressure on translation speed would allow these motifs, and potentially new phenotypes, to occur in ways that were inaccessible to fast-growing bacteria. We measured the occurrence of PP motifs across a set of >3000 bacterial genomes spread across 35 phyla and found that species predicted to be slow growing have more PP motifs in general, and in proteins expected to be highly expressed. Throughout our study, we found that PP motifs were common in protein domains known to interact with nucleotides. Interestingly, we found several cases where ribosomal proteins that interact directly with RNA elements encode conserved PP motifs in slow-growing bacteria but not in fast-growing bacteria. High pressure to maintain translational efficiency in fast-growing species may limit their ability to encode PP motifs in highly expressed proteins where they may otherwise be beneficial.

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**EME-MBE06**

**Reductive genome evolution in Ca. Ancillulaceae (Bifidobacteriales), a novel lineage of endosymbionts in termite gut flagellates**

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The cellulolytic gut flagellates of lower termites are essential for the symbiotic digestion of lignocellulose. They are associated with a diverse but host-specific consortia of ecto- and endosymbiotic bacteria, whose functional roles in the multilayered symbiotic system are largely unknown. Here, we investigated a termite-specific clade of *Bifidobacteriales* (Actinobacteria) using 16S rRNA gene libraries of capillary-picked flagellates, fluorescence *in situ* hybridization (FISH), and comparative genome analysis of metagenome-assembled genomes (MAGs). We found that *Ca.* Ancillulaceae comprises two phylogenetically and functionally distinct groups that experienced substantial genome reduction. *Ca*. Ancillula (average genome size 1.48 Mbp) are endosymbionts of *Trichonympha* spp. and retained the capacity to synthesize amino acids, vitamins, and co-factors. *Ca*. Opitulatrix (average genome size 1.2 Mbp), a hitherto unknown lineage of putative flagellate symbionts, has lost most biosynthetic capacities and depends on the uptake of numerous metabolites from its flagellate hosts. Both groups retained an amino acid export system and acquired novel gene functions in response to their intracellular lifestyle. This includes novel phosphate sugar transporters that enable the endosymbionts to tap the ample supply of sugar phosphates in the cytoplasm of their flagellate hosts. Homologs of these transport systems are absent from their closest free-living relatives among *Bifidobacteriales*, but present in other, unrelated termite gut bacteria. We hypothesize that the convergent evolution of flagellate endosymbionts is based on frequent events of lateral gene transfer among the bacterial gut microbiota and driven by the need to maintain the stability of the symbiosis

by compensating for gene losses that inevitably occur during an ongoing genome reduction.

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**EME-MBE07**
**Evolution of a novel lipoate assembly system in bacteria and archaea**
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Introduction: Lipoic acid is an essential biomolecule found in all domains of life and is involved in central carbon metabolism and dissimilatory sulfur oxidation. Until now, de novo lipoate synthesis in bacteria was thought to be achieved by a single lipoate synthase LipA and either the octanoyltransferase LipB in Proteobacteria or the distantly related octanoyltransferase LipM in Firmicutes. Alternatively, free lipoate can be scavenged by a lipoate:protein ligase. In the sulfur-oxidizing Alphaproteobacterium Hyphomicrobium denitrificans, a novel lipoate assembly pathway involving a specific sLpl(AB) lipoate:protein ligase, and two lipoate synthases, LipS1 and LipS2, is required for lipoate assembly on LbpA, an essential component of the sulfur oxidizing sHdr system [1]. For other lipoate-dependent enzymes, lipoate is provided by the simultaneously active canonical LipA/LipB de novo synthesis pathway.

Objectives: Here, the evolutionary history of lipoate synthesis was reconstructed to answer the question of why two distinct and non-redundant lipoate synthesis systems operate in the same organism.

Materials & methods: Extensive homology searches combined with genomic context analyses allowed us to precisely distinguish between the new and established pathways and map them on the tree of life. The rooted phylogenies of the octanoyltransferases and lipoate:protein ligases were calculated to reconstruct the evolutionary origin of either system.

Results: Our results show that dedicated machineries for both de novo lipoate biogenesis and scavenging from the environment were implemented early in evolution and that their distribution in the two prokaryotic domains was shaped by a complex network of horizontal gene transfers, acquisition of additional genes, fusions and losses. This revealed a much wider distribution of lipoate biogenesis systems than expected. Furthermore, the evolutionary origin of the novel lipoate assembly system could be located in the archaeal domain, with the bipartite archaeal LplAB ligase being the ancestor of the bacterial sLpl(AB) proteins. LipS1/S2 have a more complex evolutionary history with multiple of such events but probably also originate from the archaeal domain.

Conclusion: The novel slpl(AB)-LipS1/S2 lipoate assembly pathway most likely emerged in the archaeal domain and was later transferred into the bacterial domain.
[1]Koch, Dahl 2018 ISME J 12, 2479

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**EME-MBE08**
**Diversity of far-red light photoacclimation responses in cyanobacteria**
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The discovery of cyanobacteria capable of harvesting far-red light has changed the paradigm that oxygenic photosynthesis is only driven by visible light and exclusively by chlorophyll *a*. There are two known types of far-red photosynthesis. Firstly, a constitutive adaptation that uses a majority of chlorophyll *d,* which is restricted to a single genus (*Acaryochloris*). Moreover, an acclimation response, known as Far-Red Light Photoacclimation (FaRLiP), which uses chlorophyll *f* and is present in phylogenetically diverse cyanobacteria. FaRLiP involves the extensive remodelling of the photosynthetic machinery, including Photosystem I, Photosystem II and phycobilisomes. Here, I will highlight the similarities and differences of FaRLiP among cyanobacteria on a cell, membrane and protein level by using biochemical and biophysical methods. The study focusses on cyanobacteria of the genus "*Chroococcidiopsis*", as well as the phylogenetically early-branching group of "*Halomicronema*/*Synechococcales*". A strain was discovered that only contains a partial FaRLiP cluster, without genes for a far-red PSI variant, but with a normal growth behaviour under far-red light. This questions the minimal requirements for FaRLiP but also opens up new possibilities for engineering cyanobacteria of biotechnological interest to tune their absorption properties.

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**IB01**
**How to survive in a sepsis patient: Using bioinformatics to elucidate strategies of potentially pathogenic bacteria**
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Introduction: The gut microbiome is a balanced group of microorganisms with a close relationship to the host in health. However, in the case of sepsis, an excessive immune response by the host, high doses of antibiotics are often administered as a treatment. Unfortunately, this can disturb the balance of the gut microbiome. In sepsis. The origin of these bacteria might derive from the remaining microbial community within the patient's gut.

Objectives: To isolate surviving *Enterobacteriaceae* from fecal samples of a sepsis patient were characterize using sequencing and bioinformatic analysis. To assess the antibiotic resistance profiles *in silico* and *in vivo* analyses and to form biofilms. To investigate the presence of core biofilm genes in gut microbiomes of critically ill adult patients undergoing systemic antimicrobial therapy

Materials and Methods: Fecal samples were obtained from a critically ill patient. Initial isolates of *Enterobacter* or *Escherichia coli* were sequenced and analyzed in vivo and with bioinformatics.

Results: Whole genome sequencing revealed a diverse range of species, such as *Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Kosakonia sp.,* or *Shigella sonnei.* Antibiotic resistance profiles were assessed through in silico and in vivo analyses, and did not explain all survival. All isolates were able to form biofilms. That was evaluated using techniques like confocal laser scanning microscopy, live/dead staining, and computer image analyses. Genomic

analyses identified biofilm-related genes in Enterobacteriaceae, including crl, bscABZC, ehaB, csgBA, csgDEFG operons, and csgB-to-csg-D interaction regions, which were crucial for survival in the antibiotic-treated gut microbiome. The presence and enrichment of core biofilm genes were compared between antibiotic treated and untreated intensive care unit patients. Notably, there was a significant enrichment of core biofilm genes.

Conclusion: We isolated and characterized surviving and potentially pathogenic Enterobacteriaceae from fecal samples and performed bioinformatic analysis. We analyzed the antibiotic resistance profiles *in silico* and *in vivo* analyses and the ability to form biofilms. We show that the presence of core biofilm genes is enriched in gut microbiomes of critically ill adult patients undergoing systemic antimicrobial therapy. These findings suggest that targeting these genes could serve as potential therapeutic interventions.

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**IB02**

**Outer membrane-selective antimicrobial peptides derived from innate bacterial proteins as new antibiotic potentiators**

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The outer membrane (OM) of Gram-negative bacteria is a crucial barrier that prevents antimicrobials from reaching targets that are located in the inner membrane (IM) or cytosol. Permeabilization of the OM is an attractive strategy to sensitize Gram-negative bacteria to antibiotics. However, the only OM-targeting drugs currently in use are colistin and polymyxin B, which are not selective for the OM but also attack the IM. Unfortunately, this dual activity is accompanied by high cytotoxicity, limiting their use to last resort cases. This prompted us to explore OM-selective peptides as specific and non-cytotoxic antibiotic potentiators.

We have observed that Gram-negative proteomes contain peptide sequences that can interact with their own OM but not IM. Based on this observation, we screened the proteome of *Escherichia coli* for such peptides. Bioinformatic tools were applied to select the most promising sequences based on their hydrophobicity, hydrophobic moment, amphipathicity, length, and discriminatory factor. A set of 14 peptides was synthesized and investigated with respect to their OM activity and selectivity, their potential as antibiotic potentiators, and their hemolytic and cytotoxic activities.

We observed OM permeabilization for 12 of these peptides using the OM-impermeable fluorescent dye NPN. We then assayed IM activity using both the membrane potential-sensitive fluorescence dye DiSC(3)5 and a GFP fusion to the cell division regulation protein MinD, which is sensitive to depolarization as well as changes in membrane fluidity and architecture. None of our peptides showed strong activity in these assays, demonstrating their selectivity for the OM. Using synergy assays, we showed that all 12 OM-permeabilizing peptides potentiated the activity of antibiotics that are not normally active against Gram-negative bacteria by several orders of magnitude. Using antibiotics of different size, we were able to discern the extent of OM disruption. Finally, we found that only one peptide was hemolytic and only 2 showed toxicity against liver and kidney cells, while the other peptides 13 showed no detectable effects on mammalian cells.

Taken together, our results show that we can efficiently identify OM-active and selective peptides from bacterial proteomes and that these peptides show promising potential as potentiators. In a second iteration, we have synthesized another 16 peptides from *Mycobacterium tuberculosis*, which are currently undergoing analysis.

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**IB03**

**Expression and activation control of the T6SS4 machinery in *Yersinia pseudotuberculosis***

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The type VI secretion system (T6SS) is a contact dependent protein delivery system of Gram-negative bacteria to intoxicate prokaryotic or eukaryotic cells. The enteropathogen *Yersinia pseudotuberculosis* (*Ypstb*) encodes four complete clusters (T6SS1-4) with potentially different functions controlled by intricate, independent regulatory pathways. Under laboratory conditions, only T6SS4 is mildly expressed which makes thorough analysis of each system difficult. The recent identification of the T6SS4 activator RovC allows a more detailed investigation of the regulation of this cluster.

Flow cytometry was used to analyse T6SS4 expression on a single cell level. We observed heterogenous expression of T6SS4 (T6SS4+ and T6SS4- subpopulations) within a *Ypstb* population, which strongly depends on temperature and growth phase. Heterogeneous expression is not only limited to T6SS4, as also *rovC* is heterogeneously expressed, indicating an unknown additional transcriptional regulator upstream of *rovC*. Once activated, daughter cells of a T6SS4+ population continue to express T6SS4, even independently of RovC. This suggests that the signal for T6SS4 expression is passed on to the next generation and moreover, additional transcriptional pathways are involved besides RovC. However, overexpression of T6SS4 compounds alone does not result in a firing event. Analysis with fluorescence microscopy could show that several additional triggers such as high cell density and osmotic pressure are required to activate the T6SS4.

Our preliminary data show a tight regulation on transcriptional and posttranslational levels, that differ from already known pathways in other organisms. However, as little is known about the T6SSs of *Ypstb* so far, further research is required to understand their function and the purpose of this heterogeneous expression.

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**IB04**

**Dual mechanism antimicrobial agents – Trans-translation inhibition synergizes with copper stress**

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Ribosome rescue mechanisms are essential in bacteria. The most efficient rescue mechanism, *trans*-translation, is a compelling target for new antimicrobials as it is critical for bacterial survival. KKL-40 and KKL-55 are small molecules that inhibit *trans*-translation [1,2]. However, previous studies have found that KKL-35, an inhibitor of the same family of KKL-40, can inhibit bacterial growth independently from

*trans*-translation [3]. Moreover, a comparison of proteomic responses (CoPR) in *Bacillus subtilis* showed no similarity to RNA or protein synthesis inhibitors. Instead, the KKLs elicit a response similar to that of ionophores, which engage in metal homeostasis of divalent ions [4]. Based on the proteomic response elicited by KKL-40 and KKL-55 in *B. subtilis* 168, we analyzed their capacity to act as ionophores. KKL treatment resulted in an accumulation of copper in *B. subtilis* in minimal medium to which no copper was supplemented. We assessed the ability of KKL-40 and KKL-55 to transport copper and to form complexes with copper. We demonstrated that KKLs transport copper into lipid vesicles, and both the concentrations of Cu(II) and KKLs increase in *B. subtilis* when $CuCl_2$ is added during KKL treatment. KKL-40 forms a complex with Cu(II) with a stoichiometry of 3:1 KKL-40:Cu(II) and a KD of ~$4 \times 10^{-19}$ M. The KKL-55-Cu(II) complex has a 2:1 stoichiometry and a KD of ~ $3 \times 10^{-15}$ M. The lipophilicity of both complexes is higher than that of the KKLs, explaining their ability to cross lipid membranes. However, removing available copper traces from the media, cause a reduction in the antimicrobial activity of KKL-55. Cotreatment with non-inhibitory concentrations of $CuCl_2$ and either KKL caused growth stalling and protein synthesis inhibition. We used gel-free-based quantitative proteomics to evaluate the physiology of the cotreatments and the results indicated a synergy between copper stress and *trans*-translation inhibition. The study revealed an additional antibiotic mechanism of KKLs (copper toxicity). It shows the importance of understanding antibiotic action not only on an isolated target but also on a system level.

[1] doi:10.1128/AAC.02362-18

[2] doi:10.1128/AAC.01199-17

[3] doi:10.1128/AAC.01459-17

[4] doi:10.1128/AAC.01373-20

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**IB05**

**The VBNC state as global stress response in different *Acinetobacter baumannii* strains**

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Introduction: *Acinetobacter baumannii* has become a global threat to healthcare institutions worldwide. Besides increasing multidrug resistances the viable but non-culturable (VBNC) state is an additional challenge for the treatment of pathogens. This state is a stress-induced persistence mechanism found in several Gram-negative bacteria. In the VBNC state, bacteria become unculturable in medium which would normally support their growth. Those cells are defined as metabolically active and more resistant to antibiotics. Addressing the molecular function of the VBNC state may support treatment options for nosocomial pathogens. Here, we describe that *A. baumannii* can enter a VBNC state, a previously unrecognized feature of this pathogen.

Objectives: The aim of the study was to ask whether *A. baumannii* enters a VBNC state and, if so, to determine parameters that induce transition to the VBNC state.

Materials & methods: Long-term culturability studies, resuscitation analysis, flow cytometry measurements, electron microscopy, transcriptomics.

Results: Long-term culturability studies revealed that several strains of *A. baumannii* lost culturability in extended stationary growth phases. Loss of culturability was enhanced in the presence of stressors such as high salt, anoxic conditions, cold stress, heat stress and desiccation stress. However, when cells that had lost culturability were diluted in sterile phosphate buffered saline to remove the stressor and were incubated for two days, culturability could be regained, i.e. cells were resuscitated. To prove that cells were viable in the state of non-culturability, flow cytometry measurements with the fluorescent dyes Syto 9 (stains viable cells) and propidium iodide (stains dead cells) were performed. This live-dead-staining indeed identified these cells to be viable proving the entry into a VBNC state. Morphology of VBNC cells was further characterized by electron microscopy. Transcriptome analysis of VBNC state cells revealed a profound alteration of gene expression in comparison to culturable cells.

Conclusion: These data demonstrate that various strains of *A. baumannii* are able to survive unfavorable environmental conditions by entering the VBNC state.

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**IB06**

**Community dynamics of milk microbiota in healthy and mastitis cows**

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Introduction: Mastitis is an inflammatory disease of the mammary gland and one of the largest sources of economic loss and reduced animal welfare in dairy industry. While diverse pathogenic bacteria are known to cause mastitis, in many mastitis cases no causative agent can be identified, and it remains unclear if these inflammations are associated with pathogenic microorganisms at all.

Objectives: By monitoring temporal changes in the milk microbiome during mastitis in comparison to the microbiome of healthy cows we aimed to identify potentially overlooked bacteria causing the mastitis. Also by correlating those microbiome changes to cow health parameters like milk yield and rumen boli data, we aim to determine parameters suitable for early mastitis detection.

Materials & methods: State-of-the art bacteriological analysis of quarter milk samples in combination with somatic cell counts were used to identify causative organisms for every cow with a positive California mastitis test. Further, we performed 16S-rDNA amplicon sequencing on time series of whole milk samples collected from 29 mastitis (according to California Mastitis Test) and 29 healthy cows between January and April 2023. Cow health parameters like milk yield, body temperature, drinking intervals and rumination activity were monitored simultaneously for all cows investigated.

Results: Mastitis episodes were associated with drastic changes of the milk microbiome that were distinguishable from temporal fluctuations in milk from healthy cows. Microbiome alterations accompanied or preceded the deterioration of cow health parameters. We observed distinct increases in relative abundances of specific bacterial populations within milk samples prior to mastitis diagnosis.

While in many cases the increasing populations were in accordance with bacterial species identified by bacteriological diagnosis, our results suggested that additional bacteria were associated with the development of mastitis.

Conclusion: Our investigations of the temporal dynamics of milk microbiome composition provided novel insights into the microbiology and etiology of bovine mastitis. In combination with monitoring of cow health parameters, our data are used for the development of early mastitis detection methods.

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**IB07**

**Extremely short-lived peptide-polyene antimicrobial enables nasal commensal to eliminate *Staphylococcus aureus***

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Introduction: The microbiomes of human skin and upper airways play crucial roles in human health and predisposition to various diseases. Microbiome compositions govern susceptibility to and severity of chronic diseases such as atopic dermatitis and acne, and microbiomes can include facultative bacterial pathogens such as *Staphylococcus aureus*, which colonizes the anterior nares of ca. 30% of the human population. Microbiome dynamics are shaped by both, antagonistic and mutualistic interactions between microbiome members.

Objectives: The study was designed to identify antagonistic bacterial interactions between members of the nasal microbiome. Hereby, we focused on a *Staphylococcus epidermidis* strain that exhibited broad antimicrobial activity against a multitude of Gram-positive bacteria.

Materials & methods: Bioactivity-guided enrichment methods and subsequent preparative reversed-phase high-performance liquid chromatography (RP-HPLC) enabled us to purify a highly active but surprisingly unstable novel compound. A combination of Nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectrometry (HR-MS) enabled us to elucidate the structure of the peptide-polyene-tetramic acid.

Results: We report a new type of antimicrobial, named epifadin, produced by nasal *Staphylococcus epidermidis*. It has an unprecedented architecture with a non-ribosomally synthesized peptide, a polyketide, and a modified terminal amino acid moiety. Epifadin combines a wide antimicrobial target spectrum with an extraordinarily short life span. It is highly unstable under *in vivo*-like conditions, presumably to limit collateral damage of bacterial mutualists. However, *Staphylococcus aureus* is effectively eliminated by epifadin-producing *epidermidis* during co-cultivation *in vitro* and *in vivo.* We describe a new microbiome-derived antimicrobial class and suggest that limiting the half-life of an antimicrobial may help to balance its beneficial and detrimental activities.

Conclusion: In addition to the previously described non-ribosomally synthesized lugdunin from *Staphylococcus lugdunensis* we present another novel molecule produced by a member of the human nasal microbiome. This new finding underscores the importance of secondary metabolites for bacterial competition, also indicating that epifadin-producing commensals could help prevent nasal *S. aureus* carriage.

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**IB08**

**Evolutionary trajectories of gut bacteria determine resistance against pathogen colonization**

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The human gut is a complex and highly dynamic microbial ecosystem with major impact on host physiology. Key microbiome functions, such as colonization resistance against enteric pathogens, are mediated by the metabolic potential of the intestinal microbiota, which is influenced by compositional, transcriptional and evolutionary changes. While microbiome composition and transcriptional adaptation have been extensively studied, the functional impact of within-host evolution remains largely unknown. Here, we employ experimental evolution as a tool to study the adaptation of a bacterial synthetic community (Oligo-Mouse-Microbiota; OMM$^{12}$) to the mouse gut and explore its role in mediating colonization resistance against *Salmonella enterica* serovar Typhimurium (*S.* Tm.). We show that mice are protected from *S.* Tm. infection only after long-term colonization with the OMM$^{12}$, suggesting a role for bacterial evolution in mediating colonization resistance. By analyzing the genomes of 122 bacterial re-isolates from OMM$^{12}$ mouse lines housed in two different facilities, we identify numerous mutations which drive intra-species diversification and influence the metabolic potential of bacteria. By transplantation of a specific set of evolved strains we identify a minimal set of mutants which are required for the observed colonization resistance phenotype. In summary, our work establishes bacterial within-host evolution as a mediator of colonization resistance against enteric pathogens. A mechanistic understanding of the forces driving the evolution of bacterial communities will aid the improvement of targeted microbiome manipulations towards a healthy state of the host.

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**IM-PB01**

**The abortive infection system CBASS controls mode of action and resistance to antifolate antibiotics in *Vibrio cholerae***

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Introduction: Most bacteria carry toxic modules, such as toxin-antitoxin (TA) or abortive infection (Abi) systems, which are involved in a wide range of cellular processes, e.g. stabilization of genetic elements, stress response, persister formation, or phage defence. Little is known about the contribution of these systems to the treatment outcome of antibiotics and other antimicrobial substances, which is often species specific and not only influenced by the primary target of an antibiotic, but also by proteins and pathways which are apparently not related to it.

Objective: Here, we study if and how the presence/absence of toxic systems changes the antimicrobial effect.

Methods: We selected the cyclic-oligonucleotide-based anti-phage signalling system (CBASS) antiphage Abi system from *Vibrio cholerae* El Tor N16961 as a representative toxic module. Using a phenotypic screening approach, which is blind to whether a compound directly or indirectly targets the toxic module, we identified candidate drugs that interact with CBASS. The interaction of the system/drug pairs was further characterized by genetic modifications, microscopy, and bioinformatic analysis.

Results: We show that CBASS increases sensitivity to the well-established antifolate antibiotics sulfamethoxazole and trimethoprim. In addition, it interferes with the synergy of the two drugs, and ultimately enables bacterial lysis by antifolates - classic bacteriostatic antibiotics, in *V. cholerae*. As CBASS operons are widespread in bacteria, we tested systems from other bacteria and found that the CBASS-antifolate interaction is specific to closely CBASS systems found for example in *E. coli*.

Conclusion: Altogether, our findings illustrate that toxic modules, such as the anti-phage defence CBASS system, can dramatically impact antibiotic activity, and open the possibility that endogenous metabolites could act as trigger/silencers of toxic modules under stress beyond antibiotic treatment, such as phage infection, biofilm formation or disease environments.

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**IM-PB02**

**Isolation, characterization, and comparative genomic analysis of bacteriophage Ec_MI-02 from pigeon feces infecting *Escherichia coli* O157:H7**

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*Escherichia coli* O157:H7 is the most important serotype of Shiga-toxigenic *E. coli* (STEC) for its role in causing foodborne illnesses. *E. coli* O157:H7 has a low infectious dose of 50–100 colony-forming units (cfu) g-1 of solid materials or 50–100 cfu mL-1 of liquid materials due to its stress resistance mechanisms and surviving in low pH environments such as acidic food. *E. coli* O157:H7 could cause various gastroenteritis symptoms such as diarrhea, hemolytic uremic syndrome, hemorrhagic colitis and thrombotic thrombocytopenic purpura and may even cause death. The main sources of *E. coli* O157:H7 infection are livestock, poultry and their products, and polluted water. Elimination of *E. coli* O157:H7 during food processing and storage is a possible solution. Bacteriophages have a significant impact on bacterial populations in nature due to their ability to lyse their bacterial host. In the current study, a virulent bacteriophage Ec_MI-02 was isolated from the feces of a wild pigeon for potential future use as a biopreservative or in phage therapy. Based on morphology and genome analysis, Ec_MI-02 belongs to the genus Tequatrovirus under the order Caudovirales. The adsorption rate constant (K) of Ec_MI-02 was found to be 1.55 × 10−8 mL min−1. The latent period was almost 50 min though no distinct burst size was observed in the one step growth curve when the phage Ec_MI-02 was propagated in the host cell *E. coli* O157:H7. Ec_MI-02 was found to be stable at a wide range of pH, temperature and commonly used laboratory disinfectants. Its genome is 165,454 bp long with GC content of 35.5 % and encodes 266 protein coding genes. Ec_MI-02 has genes encoding for rI, rII and rIII lysis inhibition proteins that support the observation of delayed lysis in the one step growth curve. The current study provides additional evidence that wild birds could also be a good natural reservoir for bacteriophages that do not carry antibiotic resistance genes and could be good candidates for phage therapy.

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**IM-PB03**

**Structural characterization flavin specific phosphatases**

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Introduction: Roseoflavin is a structural analog of Vitamin B2 produced by *S.davaonensis* and *S.cinnabarinus*. RoFMN and RoFAD, which are derivatives of Roseoflavin, bind competitively with the flavoproteins in target cells and impair their function, as observed in many Gram-positive bacteria. The roseoflavin biosynthetic pathway has been extensively studied and it was found that enzymes RosB, RosC and RosA catalyze the first, second and third steps respectively. RosC was recently identified as a unique phosphatase belonging to the histidine phosphatase superfamily that dephosphorylates flavins. RosC primarily converts AFP (8-demethyl-8-amino-riboflavin-5′-phosphate) to AF (8-demethyl-8-amino-riboflavin) which further acts a substrate for the next enzyme, RosA. It was also observed that the enzyme acts on FMN as well as RoFMN which are structurally like the natural substrate of the enzyme, AFP. Objectives: 1. Elucidation of the crystal structure of RosC to highlight the important residues for a better understanding of the enzyme.
2. Comparative analysis of RosC with the naturally occurring FMN hydrolases belonging to a completely different superfamily that are found in all organisms and play an important role in flavin metabolism. Materials and Methods: Primarily, structural similarity to well characterized histidine phosphatases gave a very good idea about the important residues. Kinetic studies of RosC mutants were used to validate findings from bioinformatics and structural data obtained from X-ray crystallography. Results: In line with the mechanism of action of the members of the superfamily, H34 accepts the phosphate group from the substrate (AFP or FMN).The transfer of phosphate group is facilitated by conserved residues- Arg33, Arg83 and H165 which interact electrostatically with the phosphate group. E107 is an important residue that acts as proton donor in the phosphate binding pocket. Residues F128 and W181 position and stabilize the isoalloxazine ring of the flavins. An aspartate residue at position 166 in RosC is a key residue and is present in the position occupied typically by aliphatic residue in the superfamily. This residue involved in imparting substrate specificity to the enzyme. Conclusion: RosC is an interesting candidate to study with respect to its unique role in dephosphorylating AFP and FMN. In this study, we have established the mechanism of action for this enzyme as well as identified all the key residues involved in catalysis.

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**IM-PB04**

**Predicting phage infection in *Staphylococcus aureus* with receptor-binding proteins**

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Introduction: *Staphylococcus aureus* can cause life-threatening infections that are often resistant to multiple antibiotics due to horizontal gene transfer of antibiotic resistance genes from other staphylococci. One way to

combat these antibiotic resistant infections are bacteriophages. The host range of *S. aureus* phages is determined by the species-specific structure of wall teichoic acids (WTA), which is currently the only known *S. aureus* phage receptor. While other Staphylococci such as *S. epidermidis* carry a glycerol-phosphate WTA, nearly all *S. aureus* strains possess WTA consisting of ribitol-phosphate repeats.

Objectives: This study aims to investigate the binding capabilities of *S. aureus* ribitol-phosphate binding phages to their host cells through the identification of receptor-binding proteins (RBPs). Through this, we want to understand the underlying mechanism leading to adsorption and thus infection of the phage, which might ultimately enable us to predict the host range of *S. aureus* phages.

Materials & Methods: We used bioinformatic analysis to identify putative RBPs necessary for phage adsorption. Over 350 *S. aureus* phage genomes were analyzed to identify *S. aureus* ribitol-phosphate binding RBPs, which were then classified based on amino acid homology. Protein fusion constructs were created by addition of a fluorescent N-terminus to the phage RBPs, and the specific binding of these proteins to different *S. aureus* WTA-mutants was investigated via flow cytometry and microscopy.

Results: We found various RBPs necessary for phage adsorption and identified several different groups of *S. aureus* ribitol-phosphate binding phages based on their predicted RBPs. The created phage clusters allow for the prediction of phage adsorption to different WTA glycosylation types during the initial stage of phage infection.

Conclusion: This study provides insights into the host range of both known and novel phages and may be useful in developing phage-based therapeutics against *S. aureus* infections. The findings suggest that *S. aureus* ribitol-phosphate binding phages can be classified into different groups based on their RBPs, which can be used to predict their binding capabilities and success in phage adsorption to different WTA glycosylation types.

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**IM-PB05**

**Metabolic burden and resource allocation in *Pseudomonas putida* during the expression of a synthetic pathway for geranic acid production**

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The implementation of a heterologous metabolic pathway into a bacterial host leads to an increased demand for energy and cellular resources such as amino acids, ribosomes, and polymerases. The result is a redistribution of normal cellular functions towards the additionally implemented task. Restriction of any of these resources usually leads to a reduction in growth rate and cessation of heterologous production, a condition defined as metabolic burden.

In this work, we investigate the metabolic burden in *Pseudomonas putida* derived from the introduction of a synthetic pathway for geranic acid (GA). GA is a monoterpenoid that shows great potential for various industrially relevant applications, e.g., as a fragrance or antifungal agent. *P. putida* is an attractive biotechnological host for the production of monoterpenoids, because it exhibits several mechanisms that counteract the toxicity of these organic solvents. To be able to track cellular capacity during expression of heterologous proteins *P. putida* CAP was employed, which carries the gene encoding the fluorescent mCherry protein under the control of a constitutive promoter in the chromosome. The mCherry signal serves as a proxy for the cellular capacity, as it reflects the share of cellular resources distributed to non-regulated gene expression. The capacity monitor together with the growth rate contribute to the characterization of the metabolic burden. In order to study the effect of the introduction of a synthetic pathway for GA production, the first step was to introduce this pathway into *P. putida* CAP. Therefore, six genes of the mevalonate (MVA) pathway of *Myxococcus xanthus* and the geraniol synthase gene of *Ocimum basilicum* were introduced into the organism. Therefore, we chose a plasmid based approach, which allowed us to study the impact of the two modules independently from each other. We could show in a first screening that the expression of the MVA pathway genes had a higher effect on the growth of the host strain, than the expression of *ges* alone. These effects will be studied in more detail by measuring product formation rates, yield, growth rate, and capacity of mCherry production under different conditions. At the end of the day, this collected quantitative data will enter a mathematical model providing a detailed description of resource allocation and metabolic burden in *P. putida*, which may lay the basis for tuning the performance of heterologous pathways in future applications.

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**IM-PB06**

**Fermentation with a twist: Combining fermentative metabolism with respiratory modules**

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(1) In anaerobic conditions, if no external electron acceptor is available, cells must produce fermentation products to maintain redox balance. This process can be exploited through metabolic rewiring to stoichiometrically convert fed carbon sources to industrially relevant products. However, since redox balance between the fed carbon source and the desired product must be maintained, the range of possible carbon feedstocks is limited to a few sugars.

(2) Previous engineering attempts to allow "unbalanced" or "impossible" fermentations include the supply of an external electron acceptor or electro-chemical wiring with an anode as a sink for excess reducing power. These strategies increase the complexity and/or cost of industrial fermentations. Therefore, we aim to establish a novel type of aerobic fermentation that permits the addition of individual oxidation reactions instead of requiring external electron sinks. These respiratory modules allow the selective use of O2 as an electron acceptor for individual reactions.

(3) Through model-guided genetic engineering, we eliminate all NADH dehydrogenases and quinone-reducing reactions in *Escherichia coli.* The resulting strain has an intact electron transport chain but cannot transfer electrons to its ubiquinone pool. It can therefore only generate ATP through substrate level phosphorylation and O2 may not be used as an electron acceptor unless quinone-reducing reactions are reintroduced.

(4) To characterize the resulting strain, we first investigate its fermentative growth phenotype and demonstrate stoichiometric conversion of glucose to the fermentation product lactate under aerobic conditions. Then, we reintroduce glycerol-3-phosphate:ubiquinone oxidoreductase (GlpD) and demonstrate the same stoichiometric conversion from glycerol to lactate, which is considered and "impossible fermentation". Finally, to highlight the industrial potential of our novel fermentation concept, we replace lactate dehydrogenase with heterologous production routes for different industrially relevant products and demonstrate their stoichiometric production from glucose and glycerol under aerobic conditions.

(5) Ultimately, this study yields insights into the plasticity of central metabolism by demonstrating a new concept for obligatory fermentation. This approach increases the range of carbon sources that fulfil the requirement of redox balancing, allowing novel bioproduction routes while simplifying bioprocessing considerations.

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**IM-PB07**

**Developing a standardized cloning toolbox to metabolically engineer *Lactiplantibacillus plantarum* as an aroma-adding microorganism in beverage fermentation**

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*Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) is a Gram-positive lactic acid bacterium (LAB) that produces lactic acid as the main product of fermentation. Due to their generally recognized as safe (GRAS) status, LAB are widely used in food industry and production of pharmaceuticals. In beverage production, *L. plantarum* is used for sour beer fermentation. Previous studies found that the major compounds that add to the flavor of beer are two monoterpenoids linalool and geraniol present in hops. These compounds can also be found in lavender, rose and other aromatic plants, but both the raw material and the extraction process are costly. In this study, we aim to genetically engineer *L. plantarum* WCFS1 as an efficient aroma producer.

First, we developed a modular and standardized Golden Gate Assembly-based toolbox for the *de novo* assembly of shuttle vectors from *Escherichia coli* to LAB. A collection of the most relevant genetic parts, e.g. different origins of replication, inducible and constitutive promoters, was incorporated into our toolbox and characterized by flow cytometry and microplate reader. Each genetic part can be combined freely into a plasmid in one step, due to their standardized fusion sites, which effectively shortens the cloning process. Next, we screened four plant-derived linalool and geraniol synthase genes. The production of these two volatiles was quantified by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS). *L. plantarum* endogenous farnesyl diphosphate synthase was also engineered to supply more precursor of monoterpenoid, which was verified both *in vivo* and *in vitro*. The best performer was co-expressed with two bottleneck enzymes of the synthesis pathway using our toolbox. Detectable amounts of linalool and geraniol were produced, with the linalool titer well above the odor threshold.

Overall, we have developed a highly efficient and flexible cloning toolbox for engineering LAB as promising probiotics and biofactories. Two flavorful monoterpenoids were produced in *L. plantarum* with potential applications in food processing.

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**IM-PB08**

**A toolbox for the design and construction of synthetic small regulatory RNAs in bacteria**

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Introduction: Bacteria use small RNAs (sRNAs) for post-transcriptional regulation of gene expression. By complementary binding, they are able to destabilize mRNAs and/or repress their translation. Prototype sRNAs typically consist of (i) a seed region that binds to target mRNAs and (ii) a scaffold region that contains structural features and binding sites for RNA chaperones, such as Hfq. These two regions can be seen as versatile building blocks in synthetic biology approaches to create custom-made sRNA regulators, which can be applied to control expression of any gene of interest.

Objectives: Computational tools and convenient cloning systems are mandatory for efficient design and construction of synthetic sRNAs in bacteria. Rules for synthetic sRNA design can be deduced from testing of multiple sRNA variants.

Material and Methods: Here, we apply our recently developed DIGGER-Bac toolbox (SEEDling and G-Garden tools) for design of synthetic sRNAs in *Escherichia coli*. The SEEDling tool considers IntaRNA binding energies and structural features to predict seed regions for efficient target regulation. The *acrA* gene, encoding an efflux pump component, was chosen as proof-of-concept target. Repression of *acrA* results in an increased susceptibility to the β-lactam antibiotic oxacillin, which can be tested in phenotypic screens. Synthetic sRNA expression plasmids are constructed by Golden Gate assembly, which is assisted by the primer design tool G-GArden.

Results: We identified the minimal seed region length for efficient *acrA* repression to be ~12 nucleotides for two different sRNA scaffolds. Furthermore, we validated that targeting the translation initiation region of *acrA* is favorable to achieve strong repression. When increasing the seed region length, binding energies to the target are increased as well. However, we observed that in this case the scaffold has a considerable impact on the regulatory outcome.

Conclusions: The presented toolbox speeds up design and construction of synthetic sRNAs. Experimental testing of multiple sRNA variants helps to establish reliable design rules for future applications.

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**MC01**

**Genetic manipulation of a synthetic co-culture to expand the product spectrum**

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As climate crises becomes more apparent, it is essential to reduce greenhouse gas emissions, especially of global economy. This can be achieved by adopting sustainable production methods that produce less greenhouse gases or even consume them. A photoheterotrophic co-culture established in our laboratory is based on this approach: Under salt stress, the cyanobacterium *Synechococcus elongatus* (*S. elongatus*) PCC 7942 *cscB* accumulates sucrose from light and carbon dioxide. The disaccharide is then released into the surrounding medium and used by the soil bacterium *Pseudomonas putida* (*P. putida*) EM178 *att*Tn7::*cscRABY* to synthesize the potential plastic alternative polyhydroxyalkanoates (PHA). Due to its design, the co-culture represents a key process for sustainable production. In order to apply it in a wider range, it is necessary to expand its product range.

This goal was pursued by following a plasmid-based approach. Requiring little effort and time, *P. putida* can be easily equipped with the required genetic elements for the synthesis of the desired product. To avoid the employ of antibiotics, an inducible transfer plasmid was generated and selected *via* the sucrose operon *cscRABY*. Subsequently, the initial plasmid was reduced in two steps. First, the operon's negative regulator *cscR* was eliminated, and finally a minimal plasmid was designed. The latter carries exclusively the gene for the membrane porin CscY, which is essential for sucrose uptake. In a next step, different plasmid variants were designed. To assess the effect of plasmid carriage on growth, the first variant contained only the selection marker. The other two carried a cargo, but just one version was expressible. This served to distinguish the impact of cargo and protein expression. The first "product" was eGFP, allowing an easy determination of protein expression due to its autofluorescence.

After successful generation of all plasmids and their transformation into *P. putida*, growth behavior was studied in axenic growth experiments, both in microtiter plates and shake flasks. The strains generated grew on sucrose and expressed eGFP. Compared to *P. putida* EM178 *att*Tn7::*cscRABY* with 0.37 ± 0.02 $h^{-1}$, they achieved a specific growth rate of about 0.14 ± 0.02 $h^{-1}$. Insights into the behavior of a plasmid-based co-culture were obtained: A one-week co-culture with *S. elongatus* confirmed that the new strains are suitable for the co-culture and are not disadvantageous to the phototrophic partner.

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**MC02**

**Isolation & characterization of Trace Amines (TAs) producing human skin commensals**

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Introduction**:** Biogenic amines are produced by microorganisms, plants and mammals mainly by decarboxylation of aromatic amino acids. One group of monoamines represent the so-called 'trace amines' which refers to the low abundance in brain tissue. The major TAs in mammals include tryptamine (TRY), tyramine (TYM), phenylethylamine (PEA) and octopamine (OCT), which act as neurotransmitters or neuromodulators. TAs are not only produced by the host but also by a number of bacterial species belonging to the human microbiota. It has been shown that the bacterial production of such TAs interact with adrenergic receptors thus controlling invasion of bacteria into host cells or wound healing.

Objective**:** Certain staphylococcal species possess a staphylococcal aromatic amino acid decarboxylase (SadA) which is unusually unspecific. Here we analyzed the spectrum of TA-producing species on the human skin.

Methods and Results: From 30 subjects, we isolated about 2200 skin bacteria under aerobic conditions. The number of CFU in the subjects varied considerably from 2x102 to 1.3x106 CFU/100cm2. The culture supernatants of all skin isolates were analyzed for their TA production by RP-HPLC. We found TAs in the culture supernatant in 450 isolates (19.8%). We could divide the TA producers into those that produced 1, 2, or all 3 TAs. By 16S rDNA analysis we could group the isolates into three major bacterial families: *Staphylococcacae* (81.04%), *Bacillacae* (17.16%), and *Micrococcacae* (1.81%). Remarkably, isolates producing all 3 TAs were almost exclusively staphylococcal species; whereas bacteria from other genera showed greater diversity in TA production.

Conclusion: In our study, we not only revealed the spectrum of neuromodulator-producing skin bacteria, but also uncovered the responsible enzymes. This work is an important contribution to better understand the role of the skin microbiota in interacting with neuronal receptors.

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**MC03**

**Macroporous silicone chips for decoding microbial dark matter inenvironmental microbiomes**

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Introduction: Microorganisms appear in an almost infinite variety on earth, colonizing a huge range of diverse habitats. The majority of these microorganisms is still unknown and are therefore called "microbial dark matter". Since most of these microorganisms do not grow in isolated and planktonic form, cultivation in the laboratory is difficult and their biotechnological potential can therefore hardly be exploited.

Thus, there is a great demand for matrices that allow the cultivation of microbial communities.

Objective**:** Development of a macroporous elastomeric silicon foam (MESIF) as a matrix integrated in a chip with a media reservoir to enable growth of complex microbial communities from arbitrary habitats. Integration of the matrix into an analytical platform for time-resolved documentation of bacterial growth, also depending on the surface properties of the MESIF materials.

Method**:** MESIF materials were prepared with differing pore sizes and surface modifications. The MESIF was integrated into a chip and characterized regarding its colonization by the model organism *Escherichia coli* and environmental microbiomes. In this context an analysis platform was developed, which enables the automated, time-resolved documentation of the colonization. The effect of the MESIF on the enrichment of environmental microbiomes was studied via sequencing of genomic DNA.

Results: To validate the characteristics of the biocompatible and modifiable chip containing MESIF, initial growth studies with the model organism *Escherichia coli* were performed. The chip containing the MESIF was then incubated in environmental habitats, and the microbial communities enriched from it were subsequently subjected to bioinformatics analysis. A broad cross section of organisms typically found in the respective habitat were identified. For example organisms belonging to the *Candidate Phyla Radiation* were found in chips placed in a moving bed biofilter of a fish tank.

Conclusion: The MESIF materials serving as enrichment matrix, can be easily prepared with reproducible properties. The chip design offers the possibility of enriching microorganisms that have not yet been cultured, allowing the systematic study of microbial dark matter.

Literature**:**

Zoheir AE, Meisch L, Velaz Martín M, Bickmann C, Kiselev A, Lenk F, Kaster A-K, Rabe KS and Niemeyer CM.: Macroporous Silicone Chips for Decoding Microbial Dark Matter in Environmental Microbiomes. *ACS Applied Materials & Interfaces* (2022) *14* (44):49592

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**MC04**

**Born with a silver spoon in your mouth: Establishing an oral biofilm microbiome model**

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Introduction: The oral cavity theoretically represents a garden Eden for microorganisms. A constant flow of delicious substrates, a moist environment, and inviting locations to settle down are reasonable motives to stay. However, the hosts defensive strategies like the high salivary flow rate and several specialized peptides and proteins (eg lysozyme, lactoferrins, agglutinins) raise high hurdles preventing microorganisms from reaching this goal. Forming microbial biofilms known as plaque lowers this hurdle, enabling the establishment of (uninvited) ecosystems. This conflict zone between shifting bacterial equilibria, pHs, and the occasional mechanical cleaning by toothbrush is the home of oral care ingredients. These products are designed to prevent tooth decay, a typical result of microbial metabolism when bacteria turn consumed sugars to acids.

Objectives: We developed and validated an *ex vivo* biofilm model for the oral cavity. Using this, we monitor what happens when free floating saliva microbiota decide to settle down.

Methods: Using saliva from 13 to 20 participants, we were able to generate stable microbial biofilms forming on hydroxyapatite. Through 16S rRNA gene sequencing, we were able to retrace the composition shift in the microbial communities. Additionally, we quantified the microbial activity photometrically through fluorescein diacetate (FDA) hydrolysis to establish several benchmarks as positive controls.

Results: Using the *ex vivo* biofilm model, we were able to simulate the conversion of the free-floating saliva microbiome towards a geographically fixed plaque microbiome. Using information derived from this model, we succeeded in describing different mechanisms of action for both benchmarks and newly developed test substances. Additional to traditional benchmarks, which usually reduce the bacterial activity, we found substances modulating the oral care microbiota in desired directions.

Conclusion: The developed model represents both free floating and settled down parts of the human oral cavity microbiome. In both cases it can be used to study the microbial ecology of this region. Through eliminating random external factors, this model may facilitate the determination of specific cases. Our results highlight the relevance of pre-testing new cosmetic ingredients for potential microbiome effects.

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**MC05**

**Airway microbial metagenomics in health and bronchiectasis**

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Introduction: People with the chronic lung disease bronchiectasis present with abnormal dilatation of the bronchi and clinical symptoms such as cough, sputum production and shortness of breath. Disease progression arises from a cycle of persistent infections, inflammation and airway damage. Our study examines the airway metagenome of people with non-CF bronchiectasis.

Objectives: The global objective of our ongoing studies is to define microbial dysbiosis in chronic lung disease. The extraction of microbial signatures in health and lung disease allows a characterization of microbial communities distinguishing dysbiosis and balanced states.

Materials & Methods: We performed whole genome shotgun sequencing of sputum samples from healthy people (n=156) and people with bronchiectasis (n=107). Fragment libraries were sequenced on an Illumina NextSeq system. Quality filtering and alignment of the short reads to a reference database consisting of bacterial, fungal and viral genomes was performed by our in-house developed pipeline Wochenende (1), which additionally normalized the read counts.

Results: The individual metagenomes of people with bronchiectasis clustered by the absence or presence of *Haemophilus influenzae* and *Pseudomonas aeruginosa*. The individual patterns of commensals differed from that of the average healthy control, even in the absence of any pathogen. Common inhabitants of the respiratory tract of healthy subjects including *Neisseria subflava* and *Fusobacterium periodonticum* were rarely detectable in bronchiectasis.

Conclusion: The suppression of indicator commensal species is diagnostic for microbial dysbiosis in people with bronchiectasis.

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**MC06**

**Mapping interactions of staphylococcus aureus with the human nasal microbiome using high-throughput network analysis**

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Introduction: *Staphylococcus aureus* is a major human pathogen, which colonizes the human nares. Nasal carriage of *S. aureus* is a significant risk factor for severe infections. Successful colonization by *S. aureus* relies on complex interactions with the human microbiome, which is shaped by competition for nutrients and metals, phage predation, as wells as the presence of antimicrobial substances such as bacteriocins.

Objectives: Identifying molecular interventions to interfere with *S. aureus* colonization by mapping interaction networks of the nasal microbiome.

Material & Methods: Liquid and agar-based high-throughput methods supported by robotic systems.

Results & Conclusion: Here we present our plans of describing diverse aspects of interrelationships between *S. aureus* and the nasal microbiota by adopting techniques established for high-throughput bacterial network analysis. For this purpose, we collected nasal microbiomes of 11 volunteers and analysed the respective metagenomes. This collection will serve as the basis for our interaction network analysis with the aim of identifying molecular interventions to interfere with S. aureus colonization.

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**MC07**

**Revealing taxonomy, activity, and substrate assimilation in mixed bacterial communities by GroEL-based stable isotope probing**

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Bacterial communities are crucial in ecosystem dynamics, human health, and biotechnological applications. To investigate the metabolic activity and substrate assimilation of these communities, protein-based stable isotope probing (protein-SIP) has become increasingly popular. However, the complexity of samples often hinders the detection of low-abundant proteins and requires a sample-specific database for precise protein identification. In this study, we present a protein-SIP approach, referred to as GroEL-SIP, which directly links taxa to metabolic activity and substrate assimilation. GroEL (also known as Cpn60 and Hsp60) suits as a taxonomic marker protein since it is highly conserved and abundantly present in nearly all bacteria. The GroEL-SIP method established by us involves the following steps: the incubation of bacterial communities with an isotopically labeled substrate, extraction of proteins followed by high-resolution mass spectrometry, identification of both isotopically labeled and unlabeled GroEL-derived peptides using a Galaxy workflow, and subsequent protein/taxonomy inference by our Python-based analysis script. To reduce sample complexity and enhance GroEL identification while decreasing the instrument time, we propose pre-separating proteins from crude extracts by SDS-PAGE. Subsequently, gel bands corresponding to the molecular weight of GroEL (approximately 60 kDa) are excised and subjected to mass spectrometry, followed by the established GroEL-SIP workflow. To validate the effectiveness of GroEL-SIP, we applied this approach to raw metaproteome data obtained from synthetic microbial communities cultured with 13C, 18O, or 2H-labeled substrates. Our results show that GroEL-SIP reliably identified isotopically-labeled peptides. In low complex bicultures, taxonomic characterization was accurate at the genus level. In more complex communities, taxonomic characterization was robust at the family level. The significant advantage of GroEL-SIP is its compatibility with a pre-computed and expandable sample-independent database saving time and costs for generating a sample-specific one. In brief, our experimental data and our computational analyses show that GroEL-SIP enables rapid and efficient protein-SIP analyses of bacterial communities, providing robust taxonomic resolution. Consequently, GroEL-SIP facilitates protein-SIP experiments on a larger scale with enhanced replication capabilities.

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**MC08**

**Compositional and functional analyses of microbial enrichments to unravel their potential of lignocellulose degradation**

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Engineered microbial communities provide environmentally friendly solutions to industrial processes, such as green chemical production. Valuable chemical compounds from complex biomass can replace fossil feedstock-derived chemicals. To that, optimal composition and function of microbial communities must be considered for feasible green chemical production.

In ERA CoBioTech project Cell4Chem, we use microbial consortia to convert lignocellulosic biomass into medium-chain carboxylates (MCC), for which there are several agro-industrial applications. The most challenging step of this process is lignocellulose hydrolysis. Previously, we enriched microbial communities sampled from different environments to perform this function. Here, we describe the compositional and functional profile of these communities and discuss their potential application.

Three different inocula (marshland soil, digestate and compost, and cow manure) were enriched in synthetic media with different carbon sources (cellulose or xylan). The anaerobic enrichments were transferred into fresh medium periodically. The microbial composition was accessed by 16S rRNA gene amplicon sequencing. Shotgun metagenome sequencing was used to access the functional potential of the microbial communities, and metagenome-assembled genomes (MAGs) were reconstructed.

Samples from the original inocula exhibited higher diversity than samples from enrichment cultures. Different inoculum sources yielded different communities under various carbon sources, but communities enriched on cellulose sources showed greater similarity. Within each enrichment process, the communities changed but remained relatively stable in later transfers. Regarding taxonomic composition, notable families for cellulose enrichments included Bacteroidaceae, Desulfovibrionaceae and Fibrobacteraceae, while Lachnospiraceae and Clostridiaceae were prominent in xylan enrichments. Analysis of MAGs from later transfers revealed representatives of these families, such as *Bacteroides*, *Desulfovibrio* and *Lacrimispora*. In total, over 100 high-quality MAGs were recovered. Currently, we are investigating the genetic potential of these MAGs and have identified genes associated with cellulose and hemicellulose hydrolysis, including β-glucosidase and β-xylosidase.

We successfully enriched stable and potentially lignocellulose-degrading microbial communities. Now, consortia will be constructed with other microorganisms in order to efficiently produce MCC.

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**MT02**

**Puzzle pieces to a bigger picture: Identification and characterization of novel amino acid transporters in *Bacillus subtilis***

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To steadily sustain protein biosynthesis, every living organism is dependent on a constantly available pool of amino acids. Apart from biosynthesis, *B. subtilis* can take up amino acids via various importers. However, a large fraction of amino acid importers has not been characterized yet and for several amino acids, like asparagine, no transporter has been identified (1). We used different approaches to elucidate asparagine homeostasis. First, we isolated suppressor mutants that can resist D-asparagine stress. This demonstrates that MleN, the malate/lactate antiporter, is also involved in D-asparagine uptake. We also obtained suppressors under L-asparagine stress in the Δ*ansA* Δ*ansZ* double deletion strain, which lacks the two genes coding for asparaginases in *B. subtilis* and is therefore unable to degrade the amino acid. The corresponding mutations revealed a mechanism for asparagine export, which is made possible by AzlCD, an exporter for azaleucine and histidine (2). We also elucidated new functions of the major amino acid importer AimA, as it is involved in the uptake of more amino acids, in addition to the so far known substrates glutamate and serine (3).

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(2) Meißner, J et al. (2022) J. Bacteriol. 204: e0035322.

(3) Krüger, L et al. (2021) PLoS Genet 17: e1009092.

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**MT03**

**Structure, cofactor composition and electron transfer pathway of the Rnf complex from *Acetobacterium woodii***

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Introduction: Acetogenic bacteria are a group of strictly anaerobic bacteria that produce acetate as the main product using the Wood-Ljungdahl pathway (WLP). The WLP is hooked up to a respiratory enzyme, which is the ferredoxin:NAD oxidoreductase (Rnf) in the model acetogen *Acetobacterium woodii*. Exergonic electron transfer is coupled to $Na^+$ export from the cells[1,2] and the $Na^+$ gradient drives ATP synthesis *via* a $Na^+$ coupled $F_1F_o$-ATP synthase[3].

Objectives: We aimed to elucidate the structure, the cofactor composition and the electron transfer pathway of the Rnf complex of *A. woodii*.

Materials & methods: Generation of Rnf variants, growth experiments, protein purification, enzymatic assays.

Results: The structure of the Rnf complex of *A. woodii* was resolved and the cofactor composition was identified by Cryo-EM. To probe the electron transfer pathway variants were generated. Therefore, the binding-sites for different iron-sulfur cluster and flavins were deleted by site-directed mutagenesis. The plasmids were transformed into a Δ*rnf*-deletion mutant of *A. woodii*. The deletion of the iron-sulfur center B1 and AE1 as well as of the FMN in RnfG and the riboflavin in RnfD abolished growth on $H_2 + CO_2$ and NAD:ferredoxin oxidoreductase activity demonstrating their essentiality in electron transfer from ferredoxin to $NAD^+$.

Conclusion: Structural analysis together with site directed mutagenesis revealed the electron transfer pathway in the Rnf complex of *A. woodii*.

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[2] Wiechmann, A., Trifunovic, D., Klein, S., Müller, V. (2020). Biotechnol Biofuels 13, 208

[3] Fritz, M., Müller, V. (2007). FEBS J. 274:3421-3428

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**MT04**

**Protein dynamics control toxin injection through the bacterial type III secretion system**

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Bacteria manipulate eukaryotic target cells by injecting proteins through type III secretion systems (T3SS), large molecular machines. In contrast to the well-defined, engine-like structures that come to mind, many biological molecular machines are dynamic and adaptive. Using live cell microscopy, single particle tracking, proximity labelling, proteomics and molecular dynamics modelling in

combination with functional and infection assays, we found that large parts of the T3SS, including the membrane-spanning core apparatus, exchange subunits, and that bacteria modulate these dynamics in order to optimize the assembly and function of the T3SS, and ultimately the outcome of the interaction with eukaryotic cells. In addition to these new findings, I will show how we can exploit protein dynamics by using optogenetics to control the activity of the T3SS, a new approach for the biotechnological and medical application of bacterial molecular machines.

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**MT05**

**Investigating transfer protein functions and key amino acid residues in pIP501 conjugative resistance transfer**

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Antibiotic resistance is a growing global health concern that poses significant challenges in treating bacterial infections. Conjugative plasmids are major contributors to the problem. These plasmids not only carry antibiotic resistance genes but also encode all the necessary proteins for their own transfer.

The enterococcal broad-host range plasmid pIP501 encodes for a specialized multiprotein complex, called the type IV secretion system (T4SS), which mediates the pIP501 transfer from donor to recipient cells. The T4SS of pIP501 is composed of 15 transfer genes (*traA*-*traO*), which are organized in a single operon. pIP501 has become a valuable model for studying conjugative transfer in gram-positive bacteria.

To investigate the specific roles of the transfer proteins, single and double pIP501 knockout mutants of *traA* and *traN* were created. The relaxase TraA functions as an initiator of conjugation prior to the actual transfer. The repressor protein TraN has a proposed role as a T4SS transcriptional regulator. Furthermore, putative key residues enabling TraN to interact with pIP501 DNA were identified. To exclude polar effects on downstream genes, wild type *tra* genes were used to complement deletion mutations, leading to the complete restoration of conjugative transfer. Biparental mating assays were performed to evaluate the effects of the knockout mutants and complemented strains on pIP501 transfer. The results demonstrated that TraA was essential for pIP501 transfer, whereas deletion of *traN* resulted in significantly enhanced conjugative transfer. Additional complementation studies of the *traN* knockout were performed with TraN variants containing alanine substitutions of selected key residues. Purified TraN variants were applied to microscale thermophoresis to determine binding affinities of mutant proteins to their cognate pIP501 target DNA. Three out of four variants showed decreased binding affinity to their cognate pIP501 target DNA compared to wildtype TraN. These results are consistent with those obtained from biparental mating for these TraN variants, where an increased transfer rate was detected.

We identified the important role of several key residues in specific TraN DNA binding, highlighting their role in TraN repressor activity and demonstrated that TraA is essential for pIP501 transfer. Further *in vitro* studies are in progress to elucidate possible protein-protein interactions between the two regulatory T4SS proteins, TraA and TraN.

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**MT06**

***Pseudomonas aeruginosa* responds to altered membrane phospholipid composition by adjusting the production of two-component systems, proteases and iron uptake proteins**

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Membrane protein and phospholipid (PL) composition changes in response to environmental cues and during infections. To achieve these, bacteria use adaptation mechanisms involving covalent modification and remodelling of the acyl chain length of PLs. However, little is known about bacterial pathways regulated by PLs. Here, we investigated proteomic changes in the biofilm of *P. aeruginosa* phospholipase mutant (Δ*plaF*) with altered membrane PL composition.[1] The results revealed profound alterations in the abundance of many biofilm-related two-component systems (TCSs), including accumulation of PprAB, a key regulator of the transition to biofilm.[2] Furthermore, a unique phosphorylation pattern of transcriptional regulators, transporters and metabolic enzymes, as well as differential production of several proteases, in Δ*plaF*, indicate that PlaF-mediated virulence adaptation involves complex transcriptional and posttranscriptional response. Moreover, proteomics and biochemical assays revealed the depletion of pyoverdine-mediated iron uptake pathway proteins in Δ*plaF,* while proteins from alternative iron-uptake systems were accumulated. These suggest that PlaF may function as a switch between different iron-acquisition pathways. The observation that PL-acyl chain modifying and PL synthesis enzymes were overproduced in Δ*plaF* reveals the interconnection of degradation, synthesis and modification of PLs for proper membrane homeostasis. Although the precise mechanism by which PlaF simultaneously affects multiple pathways remains to be elucidated, we suggest that alteration of PL composition in Δ*plaF* plays a role for the global adaptive response in *P. aeruginosa* mediated by TCSs and proteases. Our study revealed the global regulation of virulence and biofilm by PlaF and suggests that targeting this enzyme may have therapeutic potential.

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**MT07**

**Understanding the transport of homodimeric Tat substrates**

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Research on molecular mechanisms of protein transport across membranes contributes fundamental knowledge for

prospective applications in various fields. In particular, transport of folded proteins by the twin-arginine translocation (Tat) pathway is interesting for biotechnology, as this system can ensure transport of folded proteins, which can help to reliably produce recombinant pharmaceutical proteins of highest specific activity. Recognition and transport of Tat substrates depend on N-terminal signal peptides that contain two eponymous arginines in a conserved motif. While transport of monomeric or heterooligomeric proteins requires only one signal peptide, many Tat substrates are homodimers or even homotrimers, and it is unknown whether more than one signal peptide in one protein can be functionally important for Tat transport. By using multifaceted molecular biological and biochemical approaches including in-frame gene deletion, site-directed mutagenesis, subcellular fractionation, and cross-linking analysis, we investigated thoroughly the Tat transport of homodimers, using PvdN of *P. fluorescens* A506 as model Tat substrate. Our cross-linking data indicated that cofactor-binding is a prerequisite for dimerization, and transport analyses with wild type and differently mutated PvdN variants demonstrated that both signal peptides of the dimer are involved in mediating transport. Mechanistic models for this process are presented.

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**MT08**

**Resurrecting ancestral membranes with minimal bacterial cells**

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Looking across the domains of life, we find a staggering diversity in the lipid composition of biological membranes. Since lipids determine the physical properties of membranes, which in turn influence their function, the evolution of membrane property and function is, at least indirectly, encoded in the diversification of lipid complexity. However, no theory has yet been able to account for the range of lipid chemistries across the three domains of life. We hypothesize all living membranes share conserved properties encoded in their redundant yet diverse lipidomes, and that the specific biophysical properties of membranes required to occupy different functional niches were achieved through the evolution of diverse lipid chemistries. We are working to elucidate this relationship between lipid chemistry and biophysical properties across the evolution of membranes. To this end, we have developed minimal living membrane systems to understand how life employs the collective properties of lipids to build responsive organizational interfaces between cells and their environment. For this presentation, I will report on our insights from recent advances that allow us to tune lipidome composition in the mycoplasmas and the Minimal Cell (JCVI-Syn3.0) from fewer than 10 to more than 100 lipid species. By resurrecting ancestral lipidomes, we aim to reveal the conserved features of modern membranes and introduce a new paradigm for understanding why life needs so many lipids.

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**PCB01**

**Principles of circadian organisation exemplified in *B. subtilis***

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Introduction: Circadian clocks are intracellular biochemical oscillators that temporally structure physiological and behavioural processes over 24h. Circadian clocks are pervasive throughout nature, yet we know very little about circadian clocks in non-photosynthetic bacteria. Our group recently reported the existence of a circadian system in a lab strain of *Bacillus subtilis*.

Objectives: We challenge the circadian system of *B. subtilis* with respect to a catalogue of chronobiology protocols to investigate to which extent the clock in *B. subtilis* is similar to characterised clock systems.

Material and Methods: Using the promoter region of promoter genes fused to the bacterial luciferase cassette, we examine rhythms in bioluminescence in constant darkness and during entrainment to various zeitgeber structures.

Results: We report that circadian rhythms occur in wild isolates of this prokaryote. Furthermore, *B. subtilis* can entrain to variously structured cycles of blue light or red light and darkness. We find surprising hallmarks of other circadian systems, namely masking, after-effects and systematic dependence of the free-running period on light intensity (Aschoff"s Rule). Population-level rhythms in *B. subtilis* measured using gene expression are dependent on biofilm formation. We furthermore characterise alternative colony morphologies which manifest the circadian clock. Interestingly, we find that circadian organisation and differentiated state of this bacterium are entangled processes.

Conclusion: *B. subtilis* circadian clock shows complexity in its entrainment properties, similarly to what has been described in higher, multicellular organisms. We propose that investigation of circadian organisation in *B. subtilis* might reveal principles of communication and local coordination in clock systems, which are still poorly understood in higher species.

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**PCB02**

**ATPase activity of *B. subtilis* RecA affects the dynamic formation of RecA filaments at DNA double strand breaks**

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Introduction: RecA is likely one of the most studied proteins with regards to nucleic acid interaction, together with its ortholog Rad51 in eukaryotic cells it is the central player in homologous recombination, touching vital processes such as DNA replication and recombination. In spite of a large body of literature, a key element in the activity of RecA is still unclear: how do ssDNA filament arising at sites of recombination or of double strand breaks (DSBs) in the DNA reach to the homologous DNA duplex of the sister chromosome. For Rad51, sister chromosomes are physically paired during S-phase through to mid-M phase, so there is spatial proximity. However, in bacteria, duplicated chromosome regions are rapidly moved away 1 to several micrometres, yet RecA/ssDNA filaments can set up

crossovers between a break site or a recombination site and a faraway homologous region.

Objectives: We addressed the question if loss of ATP binding or ATPase activity affect properties of RecA *in vitro*, or RecA/ssDNA filaments ("threads") dynamics *in vivo*. For that propose we have generated Walker A mutant forms of RecA, to study the biological effects of lowered ATP binding or loss of ATPase activity, to better understand the structure–function relationship of RecA and the effect of mutations on RecA dynamics in *B. subtilis*.

Materials & methods: By combining biochemistry and using Single Molecule Microscopy and tracking of RecA protein of *B. subtilis*, at 20 ms stream acquisition speed.

Results: Single molecule tracking of RecA revealed incorporation of freely diffusing and non specifically DNA-bound molecules into filaments upon induction of a single DSB. This change of dynamics was highly perturbed in the absence of ATPase activity, revealing that filamentous forms of RecA as well as their dynamics depend on ATPase activity. Our data suggest that RecA/ssDNA filaments change in subcellular localization and length involving ATP-driven homology search.

Conclusion: Our study provides key information on RecA from the Gram-positive model bacterium *Bacillus subtilis*, suggesting that ATPase driven homology search along the length of RecA/ssDNA filaments provides the driving elements of crossover formation in time and space.

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**PCB03**

**Different mobilities of cell wall hydrolases within the cell wall of *Bacillus subtilis***

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Introduction: Bacillus subtilis has multiple cell wall hydrolases, which are involved in different tasks. They are located in different areas of the cell wall (CW) and are expressed during various time points of the growth cycle. Some proteins are involved in stochastic CW lysis, allowing for the incorporation of new CW strands, such as LytC and LytD, considered as the major hydrolases in Bacillus. They are thought to be located within the outer layer of the CW. On the other hand, LytF and its homolog CwlS are specific for cell separation and are thus only functional in the exponential growth phase.

Objectives: How proteins move through the CW to find their target sites is still unclear. Is protein mobility in the CW similar to that of DNA-binding proteins, or is there free diffusion? In our studies we wished to visualize the motion and the dynamics of specific hydrolases in B. subtilis.

Materials & methods: Because of the location of the proteins outside of the cytoplasm, modified fluorescent proteins (FPs) are necessary. We achieved to combine LytC and LytF with an FP to investigate the dynamics of the proteins within living cells, using single molecule tracking. The fusion was placed at the original locus.

Results: We find evidence for three distinct mobilities for LytC and LytF: a rather static fraction, likely for CW binding and cleavage, a medium-mobile fraction (constrained motion via non-specific CW-binding), and a fraction that shows diffusion constants similar to freely diffusive cytosolic proteins. This fraction strongly supports the existence of a periplasmic space in B. subtilis. In Comparison of the mobilities, LytF is rather static and almost entirely found at the cell poles and the septum. LytC is mostly free diffusive and only a small fraction is static. If a deletion of lytD is combined with the LytC-fusion, more molecules become static. Likewise, the LytF-fusion combined with ΔcwlS results in a change in mobility.

Conclusion: Our study shows that a) FP fusions of LytC and LytF can be analyzed at single molecule level, b) free diffusion exists outside the cytosol, and c) the mobility of the two CW-binding proteins is highly distinct, conforming to the assumed functions of the proteins, stochastic cuts at the lateral sides by LytC, activity at new division sites by LytF. The finding that LytC and LytF become more static in the absence of their homologs supports the idea of finding more substrate sites in the absence of a redundant hydrolase.

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**PCB04**

**Mechanisms of contact-dependent prey cell killing by the predatory soil bacterium *Myxococcus xanthus***

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Predatory bacteria are found in various habitats and have adapted different mechanisms to kill and consume other microorganism. We investigate the predation mechanisms of *Myxococcus xanthus*, a soil bacterium, which preys on a broad range of bacteria and fungi, and employs a multilayered predation strategy. Secreted bacteriolytic proteins can lyse Gram positive prey at a distance, but predation of Gram negative bacteria requires direct cell-cell contact with M. xanthus. Aiming to reveal the molecular basis of contact-dependent prey cell killing, we hypothesized that dedicated protein secretion systems might mediate the targeted translocation of killing factors. Therefore, we analyzed mutants of three gene clusters that encode envelope-spanning protein secretion systems in co-culture experiments that follow predation on different time scales.

We find that two different protein secretion systems fulfill distinct functions during contact-dependent prey killing: one is necessary to induce prey cell death upon cell contact with *M. xanthus*, while another system is required for prey cell lysis. Fluorescence microscopy of individual components revealed that both secretion systems localize to the predator-prey contact site prior to killing, and that their respective accumulation depends on each other. Swarm expansion assays showed that both secretion systems are required to utilize live prey bacteria and that *M. xanthus* can extract sufficient nutrients from prey bacteria to power cell motility. In conclusion, our observations hint at a functional interaction of two atypical secretion systems, which mediate killing and lysis of bacterial cells by *M. xanthus* upon direct cell-cell contact.

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**PCB05**

**Bacterial type 4 pili dynamics shape *Neisseria gonorrhoeae* interactions from single cells to biofilms**

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Bacterial type 4 pili (T4P) are filamentous dynamic cell appendages that are used as adhesins as well as motility systems. It consists of a type 2 secretion system like basel body, that elongates and retracts the dynamic pilus fiber in and out of the cell. In *Neisseria gonorrhoeae*, an exclusively human pathogen and one of the most common sexually transmitted disease, those molecular nano machines are positioned around the cell envelope and randomly move the cell around biotic and abiotic surfaces. At higher cell densities, cells start interacting via their T4P which leads to the formation of spherical microcolonies within minutes. Those microcolonies contain hundreds of individual cells and develop into fullgorwn biofilms over time.

Since the T4P are crucial for the *N. gonorrhoeae* lifestyle, we aimed to investigate their dynamics and interactions in single cells, micro-colonies, under environmental and antibiotic stressors. To do so we combine live cell fluorescence microscopy with quantitative image analysis.

By establishing an Alexa Flour488C5 maleimide based visualization protocol, we were able to follow T4P dynamics in live cells and could show a single *N. gonorrhoeae* produce around ~200 T4P min-1. Treatment with azithromycin or ceftriaxone reduces T4P production rate, while low pH results in stationary elongated pilus fibers with extended lengths (Kraus-Römer et al., 2022). By focusing on events that lead up to colony formation, we could show that in neutral conditions, interaction between pili happens randomly when two fibers touch and retract which pulls single bacteria into multi-cellluar aggregates. The T4P interaction interface during those events seems to be limited to a small part in the tip arrea. On mono-cell layers, pili dynamics can still be observed. In later-stage microcolonies, new arriving cells, attach from the outside and penetrate into the microcolony with their T4P in a matter of minutes, anchoring them to the pili matrix of those colonies.

Overall, our maleimide based microscopy protocol allows us to investigate T4P dynamics in situ in living cells in different stages and under many stressors. It further enables us to document how interactions on a single cell level lead up to establish and shape a whole biofilm.

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**PCB06**

**The role of glutathione in periplasmic redox homeostasis and oxidative protein folding in *Escherichia coli***

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Many extracytoplasmic proteins depend on the introduction of disulfide bonds for correct folding, a process termed "oxidative folding". In bacteria the oxidative protein folding machinery is located in the periplasm, a compartment more oxidizing than the cytosol. In *E. coli*, it consists of the oxidoreductase pair DsbA/B for oxidative power and the DsbC/D system as its complement for isomerization of non-native disulfides. While the standard redox potentials of those systems are known, the *in vivo* redox potential imposed onto protein thiol disulfide pairs in the periplasm remains unknown.

Here, we used the genetically encoded redox-active probes roGFP2 and roGFP-iL, targeted to the periplasm, to directly analyze the thiol redox homeostasis in this compartment. These GFP-derivatives contain two cysteine residues, that upon oxidation form a disulfide bond. The resulting changes in the excitation spectra of the probes allow a ratiometric determination of the probe´s oxidation state using fluorescence spectroscopy.

We showed that the cysteines of roGFP2 are virtually completely reduced in the cytoplasm of *E. coli*, but once exported into the periplasm, form a disulfide bond. Even in the absence of DsbA periplasmic roGFP2 was fully oxidized, suggesting the presence of an alternative system for the introduction of disulfide bonds. However, the absence of DsbA shifted the steady state periplasmic thiol-redox potential from -228 mV to a more reducing -243 mV. Additionally, the capacity to re-oxidize periplasmic roGFP2 after a reductive pulse was significantly decreased in this mutant. Interestingly, exogenous oxidized glutathione (GSSG) restored re-oxidation in a DsbA-deficient strain, while reduced glutathione (GSH) accelerated re-oxidation of roGFP2 in the WT. In line, a strain devoid of endogenous glutathione showed a more reducing periplasm, and performed significantly worse in oxidative folding of PhoA, a native substrate of the DsbA/B pair. Addition of exogenous GSSG enhanced PhoA oxidative folding in the WT and fully restored PhoA activity in a Δ*dsbA* mutant. Taken together this suggests the presence of an auxiliary, glutathione-dependent thiol-oxidation system in the bacterial periplasm.

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**PCB07**

**Assembly of non-photosynthetic organelles by Tic20-like proteins in bacteria**

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Organelle-specific protein translocation systems are essential for organelle biogenesis in eukaryotes but thought to be absent from prokaryotic organelles.

Although magnetosomes represent one of the best characterized bacterial organelles, the molecular mechanisms of magnetosome assembly and protein targeting remained poorly understood.

Here, in combining bioinformatic in-depth analyses with systematic molecular, cell biological, quantitative proteomic as well as biochemical studies and careful evaluation of magnetism-related phenotypes, we show that magnetosomal MamF-like proteins (MFPs) involved in bacterial magnetosome biogenesis share an ancient origin with Tic20 protein translocases found in plastidal organelles.

Deletion of mamF-like genes in *Magnetospirillum gryphiswaldense* results in severe defects in magnetic biomineralization, organelle positioning, and cellular navigation. Consistent with translocase-like functions, these defects are caused by the loss of magnetosome targeting of a subset of organellar proteins containing C-terminal glycine-rich transmembrane domains. MamJ, one of the most affected proteins, is carbonate resistant and largely dissociated from MamF-like proteins but soluble in their absence.

Our findings suggest that organelle-specific protein translocation systems, may indeed play a role in bacterial organelle formation.

**PCB08**

**Some noteworthy updates on the planctomycetal cell biology**

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Since their discovery, members of the phylum *Planctomycetota* have surprised scientists with their unique features. Although the molecular mechanism is yet to be elucidated, bacteria from the class *Planctomycetia* are well known for their budding cell division. Recently, several environmental isolates belonging to the phylum *Planctomycetota* were discovered, which challenge commonly accepted principles of bacterial cell biology yet again. Three isolates were retrieved from the Baltic Sea during a large sampling campaign in 2014 and 2015. Based on phylogenetic analyses, we propose them as members of the novel class *Saltatorellae* class nov. with the name referring to their unusual locomotion that makes them appear to be "dancing" under the microscope. Another isolate was reported by Shiratori et al. (2019), which seems even more conspicuous than the *Saltatorellae* class nov.: In addition to its eukaryotic-like locomotion and division, it is capable of engulfing prey bacteria in an endocytosis-like mechanism. Thus, this presentation will be about some of the most striking and noteworthy news on planctomycetal cell biology.

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Shiratori, T., Suzuki, S., Kakizawa, Y., and Ishida, K. I. 2019. 'Phagocytosis-like cell engulfment by a planctomycete bacterium', Nature Communications, 10: 5529.

**PPM01**

**Rates of iron(III) reduction coupled to elemental sulfur or tetrathionate oxidation by acidophilic microorganisms and detection of sulfur intermediates**

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Bioleaching processes and acid mine drainage (AMD) generation are mainly driven by aerobic microbial iron(II) and inorganic sulfur/compound oxidation. Dissimilatory iron(III) reduction coupled to sulfur/compound oxidation (DIRSO) by acidophilic microorganisms has been described for anaerobic cultures, but iron reduction was observed under aerobic conditions as well. Aim of this study was to explore reaction rates and mechanisms of this process. Cell-specific iron(III) reduction rates for different *Acidithiobacillus* (*At.*) strains during batch culture growth or stationary phase with iron(III) (~40 mM) as electron acceptor and elemental sulfur or tetrathionate as electron donor (1% or 5 mM, respectively) were determined. The rates were highest under anaerobic conditions for the *At. ferrooxidans* type strain with 6.8 x 106 and 1.1 x 107 reduced iron(III) ions per second per cell for growth on elemental sulfur and tetrathionate, respectively. The iron(III) reduction rates were somehow lower for the anaerobically sulfur grown archaeon *Ferroplasma acidiphilum*, and lowest for the sulfur grown *At. caldus* type strain under aerobic conditions (1.7 x 106 and 7.3 x 104 reduced iron(III) ions per second per cell, respectively). The rates for five strains of *At. thiooxidans* (aerob) were in between those for *At. ferrooxidans* (anaerob) and *At. caldus* (aerob). There was no pronounced pH dependence of iron(III) reduction rates in the range of pH 1.0-1.9 for the type strains of all species but rates increased with increasing pH for four other *At. thiooxidans* strains. Thiosulfate as sulfur intermediate was found for *At. ferrooxidans* during anaerobic growths on tetrathionate and iron(III) but not during anaerobic growths on elemental sulfur and iron(III), and a small concentration was measured during aerobic growths on tetrathionate without iron(III). For the *At. thiooxidans* type strain thiosulfate was found with tetrathionate grown cells under aerobic conditions in presence and absence of iron(III), but not with sulfur grown cells. Evidence for hydrogen sulfide production at low pH was found for the *At. ferrooxidans* as well as the *At. thiooxidans* type strains during microaerophilic growth on elemental sulfur and for *At. ferrooxidans* during anaerobic growths on tetrathionate and iron(III). The occurrence of sulfur compound intermediates supports the hypothesis that chemical reduction of iron(III) ions takes place by sulfur compounds released by the microbial cells.

**PPM02**

**Two homologous metabolic enzyme complexes as novel targets for bispecific protein-protein interaction inhibitor antibiotics**

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Introduction: Multi-resistant bacteria are a rapidly emerging thread to modern medicine. It is thus essential to develop novel antibiotics directed towards hitherto unexploited targets. Such potential targets include the homologous and heterodimeric enzymes aminodeoxychorismate synthase (PabA-PabB complex) and anthranilate synthase (TrpG-TrpE complex), that are involved in folate and tryptophan biosynthesis, respectively. The activity of these enzymes depends on side chain interactions between the respective subunits that are conserved throughout most bacteria, including pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. We therefore hypothesized that molecules blocking subunit association will simultaneously inhibit both enzymes, thereby suppressing two essential metabolic pathways in bacteria.

Objectives: To explore the potential of PabA-PabB and TrpG-TrpE as novel drug targets for protein-protein interaction inhibitors, we assessed the growth of *Escherichia coli* strains bearing mutations that disrupt subunit association.

Methods: We assessed the role of selected interface residues for heterodimerization by gel filtration and enzymatic assays. Mutations in PabA and TrpG that prevented the protomers to interact with their partners PabB and TrpE were then introduced to genomic DNA of *E. coli*. In growth experiments, we analyzed the viability of the constructed strains.

Results: Our modified strains displayed a phenotype of severely retarded growth, which was compensated by adding tryptophan and para-amino benzoate, a folate precursor. The

individual disruption of either the PabA-PabB or TrpG-TrpE complex led to moderately weaker phenotypes, supporting the additional benefit of targeting both complexes at the same time. Notably, we observed the different phenotypes both on minimal medium and in the presence of heat-deactivated human plasma, indicating that accessible metabolite concentrations in the human body are too low to compensate for the lack of folate and tryptophan within the tested bacterial cells.

Conclusion: We validated PabA-PabB and TrpG-TrpE as novel targets for bispecific protein-protein interaction inhibitors. The bacteriostatic effect triggered by impairing subunit interactions is observable even under near-physiological conditions. A potential inhibitor targeting both enzymes will feature a broad spectrum of target pathogens to which resistance is less likely to evolve.

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**PPM03**

**Conformational variability of cyanobacterial ChlI, the AAA+ motor of magnesium chelatase involved in chlorophyll biosynthesis**

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Magnesium chelatase is a conserved enzyme complex responsible for the first committed step of chlorophyll biosynthesis in photosynthetic organisms, which is the addition of magnesium to the chlorophyll precursor, protoporphyrin IX. The complex is composed of the catalytic subunit ChlH, the bridging subunit ChlD, and the subunit ChlI, which serves as the motor that drives the entire complex. Although the enzyme is well-characterized functionally, high-resolution structures are available only for individual subunits. Hence, the full assembly and the molecular mechanism of the enzyme complex remains unknown. Here, we used cryo-EM, supported by biochemical analysis and mass photometry, to determine structures of the ChlI motor subunit of magnesium chelatase under turnover conditions in the presence of ATP. Our data reveal the molecular details of ChlI oligomerization and conformational dynamics upon ATP binding and hydrolysis. These findings provide new insights into the mechanistic function of ChlI and its implications for the entire magnesium chelatase complex machinery.

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**PPM04**

**An enzymatic bottleneck defines the product spectrum of the alkyl quinolone biosynthetic pathway of *Burkholderia thailandensis***

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Introduction: Bacterial 2-alkylquinoline-4(1*H*)-ones (AQs) play important roles in cell-cell communication, interspecies competition, and bacteria-host interactions. Environmental and pathogenic *Burkholderia* spp. predominantly produce a distinct series of *trans*-Δ2-unsaturated and 3-methylated AQs (MAQs), which exhibit antimicrobial activity and are supposed to be involved in intraspecies signaling, whereas their *N*-hydroxylated congeners are potent inhibitors of the bacterial respiratory chain. The biosynthetic pathway of MAQs in *Burkholderia* spp. has been proposed based on homologies to the well-described AQ biosynthesis of *Pseudomonas aeruginosa*, supported by a series of mutagenesis studies (1). However, biochemical studies on MAQ biosynthetic enzymes are rare, and evidence for several hypothetical pathway intermediates is missing.

Objectives: Our aim is to give a detailed description of the MAQ biosynthetic pathway of *B. thailandensis* on an enzymatic level. To this end, we investigate the individual reactions catalyzed by HmqBC, HmqG, HmqF and HmqL. *In vitro* and *in vivo* reconstitution of defined parts of the pathway will moreover give insights into the interplay between the enzymes and allow identification of short-lived intermediates.

Material and Methods: Individual and multi-enzyme assays were either continuously monitored spectrophotometrically, or discontinuously analyzed by UPLC-MS. *In vivo* reconstitution of defined branches of the biosynthetic pathway by heterologous expression of *hmq* genes in *P. putida* complemented the *in vitro* experiments.

Results: We identified HmqG as a SAM- and cation-dependent methyl transferase which competes with the flavoprotein *N*-hydroxylase HmqL for the substrate 2-aminobenzoylacetate, displaying a central branching point of the pathway. Multi-enzyme assays moreover allowed the identification of two elusive pathway intermediates, 2-aminobenzoylpropionate and 2-hydroxylaminobenzoylpropionate. By analyzing substrate preferences and enzyme kinetics, we moreover found that the downstream condensing enzyme HmqBC acts as a bottleneck, channeling the biosynthetic flux towards production of MAQs and hampering synthesis of non-methylated AQs.

Conclusion: Our data provide a biochemical rationale for the high abundance of MAQs and their *N*-oxides over non-methylated AQs produced by *B. thailandensis*.

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**PPM05**

**Investigating and engineering novel carbon metabolism in *Rhodobacterales***

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Introduction: The *Rhodobacterales* are a large and diverse clade of gram-negative bacteria that are abundant across marine and terrestrial ecosystems. While some *Rhodobacterales* species have served as model organisms to investigate specific metabolic processes – such as carbon dioxide fixation in *Rhodobacter sphaeroides* and denitrification in *Paracoccus denitrificans* – the metabolic diversity of this clade is still poorly understood. The recent discovery of the β-hydroxyaspartate cycle (BHAC) in *Rhodobacterales* and its successful application in metabolic engineering approaches of bacteria as well as plants demonstrate the enormous untapped potential that can be found in the carbon metabolism of these microbes.

Objectives: Here, we aim to investigate and engineer novel carbon metabolism in diverse *Rhodobacterales* species, with the dual goal of 1) improving our understanding of the physiology of these abundant bacteria and 2) discovering

previously unknown enzymes and pathways that can be applied in metabolic engineering approaches.

Materials & methods: We used a combination of enzyme biochemistry, bacterial genetics, and bioinformatics to identify and characterize novel enzymes of biotechnological relevance in *P. denitrificans*. Furthermore, we applied targeted mutant generation and proteomics to study the regulation of central carbon metabolism in this metabolically versatile bacterium. Finally, we generated novel genetic tools to extend the possibilities for investigating and engineering the metabolism of marine *Rhodobacterales*, including *Ruegeria*, *Phaeobacter*, and *Dinoroseobacter*.

Results: We characterized previously unknown enzymes for the assimilation of ethylene glycol (monomer of PET) and 6-aminohexanoate (monomer of nylon), which will find future applications in the biotechnological valorization of these plastics. Furthermore, we characterized the regulation of central carbon metabolism in *P. denitrificans* in order to better understand the hierarchical utilization of different carbon sources. Finally, we created and validated versatile vectors for constitutive and inducible gene expression that are applicable in a variety of *Rhodobacterales* species.

Conclusion: The discovery of highly efficient enzymes and metabolic pathways and the establishment of novel genetic tools confirm that *Rhodobacterales* continue to be an exciting field of study, both for fundamental microbiology and for applied metabolic engineering.

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**PPM06**

**Culturing-dependent polymorphism and specialized metabolite production in the entomopathogenic bacterium *Xenorhabdus doucetiae***

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Entomopathogenic bacteria, as of the genera *Xenorhabdus* or *Photorhabdus*, are promising sources for novel bioactive compounds. In the recent years, research focus was hence put on their biotechnological and biopharmaceutical role [1,2]. As an example, selective activation of biosynthetic gene clusters via promoter exchange enabled the production, purification and characterization of specific specialized metabolite derivatives [3]. The cell biology and microbial ecology of these bacteria, however, was mainly neglected, although the ecological function of many specialized metabolites is still unknown. In this work, we investigate the physiology of the entomopathogenic bacterium *Xenorhabdus doucetiae*. We find a surprising polymorphism that only occurs under growth conditions close to the cells' native environment. Proteomic analysis and fluorescence microscopy revealed that these conditions induce biofilm formation and activate the Type-6 secretion system in a phenotype-dependent manner. Promoter exchange in front of the operon required for the production of the exopolysaccharide poly-*N*-acetylglucosamin, a widely distributed component of biofilms, further showed that biofilm formation significantly effects the expression of most biosynthetic gene clusters. This demonstrates that (i) studying the biology of entomopathogenic bacteria creates insights that are relevant for their biotechnological use and (ii) that the bacterial lifestyle has a large effect on specialized metabolite production.

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**PPM07**

**DNA methylation, a regulator of keystone enzyme of chlorophyll biosynthesis in *Synechocystis* sp. PCC 6803**

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Epigenetics acts as important regulator of gene expression in eukaryotes as well as prokaryotes. In bacteria, it regulates DNA repair, cell replication and gene expression by methylation of specific nucleotides in the DNA. The modifications where provided by DNA methyltransferases (MTases). The model organism *Synechocystis* sp. PCC 6803 harbors at least five functional MTases.

The aim of this project is to reveal the purpose of the genomic DNA methylation in cyanobacteria and analyze the impact of the different MTases.

Mutants lacking the MTase M.Ssp6803II (*sll0729*) possesses an altered phenotype. Cells of this strains are decreased in size, contained less chlorophyll a and are sensitive for UV exposure. However, this phenotype is unstable, because after long-term cultivation of the Δ*sll0729* strain single clones appeared that are displaying wild-type-like phenotype (suppressor clones). Whole genome sequencing of suppressor clones revealed a single nucleotide exchange in the promoter of *slr1790*. This gene encodes the protoporphyrinogen IX oxidase (HemJ), a keystone enzyme of the chlorophyll biosynthesis. Partial knockout of HemJ displays a reduced chlorophyll content and is complemented by overexpression of homolog enzyme.

Transcriptome data of the original Δ*sll0729* clones showed significantly reduced amounts of *slr1790* transcripts. HPLC measurements revealed accumulation of phototoxic chlorophyll precursors in Δ*sll0729* cells. To verify the hypothesis that the mutated *slr1790* promoter is responsible for the wild-type like phenotype of the Δ*sll0729* suppressor clones, *slr1790* promoter::Δ*sll0729* double mutants with and without a specific *slr1790* promoter mutation in the methylation side were analyzed. Phenotype, long-term cultivation behavior and chlorophyll precursor accumulation

of these strains support the *slr1790* promoter hypothesis. The native promoter double mutant shows similarly high levels of chlorophyll precursors like the ∆*sll0729* single mutant. The Chlorophyll precursors in the mutated promoter double mutant are on a wild-type-like level.

To analyze differences in HemJ levels in all these strains, a specific antibody will be used for immunoblotting. An induced expression system shall enable expression of *slr1790* temporally to complement the ∆*sll0729* phenotype. In summary, we propose altered expression of *slr1790* as reason for ∆*sll0729* phenotype and suppressor clones, suspect indirect regulation by promoter methylation due to M.Ssp6803II.

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**PPM08**

**Microbial $CO_2$ recovery – Understanding resource allocation in methanogen using a quantitative biology approach**

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To effectively mitigate global warming and climate change, society must develop new technologies for transitioning from a fossil carbon-based to a CO2-based economy. CO2 captured from point sources or directly from air can be utilized as a feedstock in CO2 reduction to make carbon-based products using renewable electricity. Significant amounts of renewable electricity is becoming available at costs cheaper than fossil-based electricity, allowing for fossil-fuel independent, economically viable-electrification of fuel production and chemical syntheses. Metabolism of methanogenic archaea provides a unique opportunity to convert CO2 into CH4 with high selectivity at ambient conditions from green H2 from renewable electricity combined with recycled CO2, providing a solution for energy storage.

To promote the application of methanogen, I will describe how substrate flux (i.e., feed inflow) affects the physiology - metabolic activity, biomass synthesis and composition - of methanogens using a quantitative and systemic biology approach. Our results shows that methanogen does not follow some canonical microbial physiological rules established based on model microbes such as E. coli. In addition, I will present preliminary results of how starvation affects the activity of methanogens.

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**PPM09**

**The principle of microbial infallibility revisited: How wastewater bacteria learned to feed on a previously persistent xenobiotic**

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The long-standing paradigm of microbial infallibility - the expectation that any organic substance in nature that can be oxidized will be oxidized by microbes - has repeatedly been challenged by the persistence of anthropogenic pollutants. One example is the artificial sweetener acesulfame K (ACE), which has been in use since the 1990s and is generally considered recalcitrant to biological wastewater treatment. Therefore, it is used as a marker substance for detecting wastewater discharges into groundwater and surface water. However, there is growing evidence of ACE biodegradation in wastewater treatment plants during the last decade, and recently we isolated bacteria that can grow on ACE as sole carbon and energy source. To elucidate the genetic and enzymatic background of ACE degradation, we used comparative genomics and heterologous expression of candidate genes. By studying the ACE metabolism in *Bosea* and *Chelatococcus* species, we experimentally confirmed the previously postulated route of two subsequent hydrolysis steps via acetoacetamide-N-sulfonate (ANSA) to acetoacetate and sulfamate. Genome comparison of wildtype *Bosea* sp. 100-5 and a spontaneous degradation-defective mutant revealed the involvement of two plasmid-borne gene clusters. The ACE-hydrolyzing sulfatase is strictly manganese-dependent and belongs to the metallo beta-lactamase family. In all degraders analyzed, it is encoded on a highly conserved gene cluster embedded in a composite transposon. The ANSA hydrolase is an amidase signature domain enzyme encoded in another gene cluster of variable length. Transposition of the sulfatase gene cluster between chromosome and plasmid explains how the two catabolic gene clusters recently combined for ACE degradation. Searching published genomes and metagenomes for the respective genes indicated that the ACE plasmid evolved and spread worldwide in short time. The ACE sulfatase gene was rarely found in metagenome datasets and appears to be unprecedented and unique for ACE degraders, whereas the ANSA amidase genes occurs in different genetic environments and likely evolved for the degradation of other substrates. Our study revealed a strikingly high similarity of ACE degradation gene clusters retrieved from sites on different continents. Evolution of the ACE degradation pathway might have been supported by the presence of structurally related natural and anthropogenic compounds, such as aminoacyl sulfamate ribonucleotide or sulfonamide antibiotics.

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**PPM10**

**Discovery of a new non-canonical sesquiterpene synthase from actinobacteria**

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Introduction: Terpenes represent the largest class of secondary metabolites. It is all the more surprising that of the >80,000 known terpenoids, only about 1.3 % are produced by bacteria [1]. Considering the huge number of bacteria estimated to exist on Earth (5x1030), the lack of knowledge on bacterial terpene biosynthesis becomes apparent. Recently a new facet of bacterial terpene diversity was discovered, which includes the methylation of prenyl pyrophosphates (IPP, GPP or FPP) followed by the terpene synthase reaction, e.g., during the elucidation of the biosynthesis of the extraordinary compound sodorifen (C16H26) of the gamma-proteobacteria *Serratia plymuthica* 4Rx13 a cyclic C16 compound became the substrate of the following terpene synthase (SODS, sodorifen synthase) [2].

Objective: The aim of our project is to find new non-canonical terpene synthases (TPS) in bacteria with special focus on actinobacteria and to identify their substrate and product spectra. By phylogenetic analysis, a potential sesquiterpene methyltransferase (MT) and terpene synthase (TPS) of the

actinobacterium *Streptomyces acidiscabies_a10 (S.a)* was identified.

Method: Since the strain could not be acquired commercially, the MT and TPS genes were synthesized by Genewiz and were cloned into overexpression vectors. The heterologously produced protein was purified via Ni-NTA affinity chromatography. The enzymes were tested *in vitro* for their substrate and product spectra, products were analysed by GC/MS and NMR.

Results: MT/TPS in double enzyme assay with FPP as substrate yielded two new non-canonical sesquiterpenes, α- and β-acidiphene. In addition, unmethylated FPP and GPP were also substrates of the TPS in single enzyme assays, and canonical terpenes (e.g. nerolidol and linalool) were detectable. The TPS is a multisubstrate and a multiproduct enzyme.

Conclusion: Using a genome mining approach, we identified the first actinobacterium that produces non-canonical sesquiterpenes and also canonical terpenes. The new terpene synthase is a promiscuous enzyme, as it accepts methylated and unmethylated FPP and GPP as substrate.

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**PPM11**

**Investigating microbial carbon use efficiency using soil-free microbial cell extracts: A new approach to understanding microbial carbon utilization in soils**


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The fate of soil organic matter depends largely on microbial carbon (C) use efficiency (CUE); the proportion of C assimilated by microorganisms and retained within microbial biomass versus being released as CO2 during decomposition. To better understand and elicit the factors (i.e., nutrient chemistry, availability) influencing CUE and determine metabolic activity and C utilization patterns, soil-free microbial cell extract (SFCE) may enable a more precise per-cell perspective compared to studying microorganisms within the complex soil matrix. SFCE provides a means to directly measure and quantify microbial biomass and activity for a complex, near-to-natural soil microbiome, which is essential for calculating CUE. By isolating microbial cells from the soil matrix, we aim to accurately determine the amount of C taken up by microorganisms and assess the efficiency with which that C is utilized.

For this purpose, we extracted microbial cell suspensions from agricultural soil with a Nycodenz density gradient and maintained them in pre-extracted dissolved organic matter (0.22um filtered and autoclaved). The cells were stained with Syto9 and enumerated with flow cytometry. The extracted cell suspension received glucose and other substrates and cell metabolic activity (heat release) was determined by a microcalorimeter (TAM Air) in comparison to the intact soil. Our preliminary results suggest that SFCE biomass can indeed be used to study the relative CUE of different microbial groups and identify key factors that drive variations in CUE without the confounding factors present in the soil. The data generated from studies using SFCE can be used to develop and refine models that simulate microbial processes and CUE dynamics in soil ecosystems. These models will help to predict how changes in environmental conditions, or climate scenarios may impact microbial CUE and C cycling in soils.

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**PPM12**

**Maintenance of cellular metal-dependent biochemistry is mediated by evolutionary genome plasticity via acquired and fine-tuned homeostasis systems of the resistome in *Cupriavidus metallidurans* in the face of multiple metals stressor**


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Introduction: Life depends on biometals, such as trace elements like Fe, Zn, Co, Ni, Mo and Cu, in catalytically active enzymes, structurally in cofactors or when they are involved in signaling and regulation processes. An adapted and controlled interplay of efflux, uptake and storage systems are required to maintenance the cellular metal homeostasis. The essential metal requirement of a cell is counterbalanced by the danger of poisoning in case of excess, the two faces of biometals. As a result, the cellular metal quotas of the essential metals are strictly controlled in face of the changing environmental conditions. *Cupriavidus metallidurans* possess of a large number of metal efflux systems and an entire repertoire of systems for uptake in combination with a cellular buffer capacity of transition metal cations.

Objectives: A deeper insight into the interaction of these different systems under varying environmental conditions is imperative for understanding the cellular responses at system level.

Materials & methods: Metallomic - cellular metal content determination by ICP-MS (metallome), transcriptomic via RNA sequencing (transcriptome), proteomic via bottom-up approach by LC-MS/MS (proteome), reporter gene assays, DNA binding assays and physiologically characterization under exposure of multi metal stress.

Results: In combination with a two-stage efflux, metal controlled, consisting of P-type ATPases, CDF (cation diffusion facilitator) proteins and RND systems, it is able to tolerate highly toxic metal environments. With a fine-tuned regulation of efflux and uptake system as well as a cellular metal buffer *C. metallidurans* restores metal homeostasis. Furthermore, a high cellular metal binding capacity could be determined under these multitox conditions. This indicates possible applications for bioremediation and biorecovery, even under multiple metal contaminations.

Conclusion: The plasticity of genome composition and fine tuning at the regulatory level of these evolutionarily acquired homeostasis systems provide insight into a deeply interwoven cellular network. This regulation and a complex

interplay of the involved systems makes the organism to a survivalist in metal contaminated environments, as well as in times of scarcity.

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**PPM13**

**Genetics, physiology, and evolutionary origin of a novel bacterial degradation pathway for the anthropogenic buffer compound TRIS**

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TRIS (2-amino-2-hydroxymethyl-propane-1,3-diol) is a synthetic chemical that was first synthesized in the early 20th century and is now widely used as a buffer, emulsifier, and pharmaceutical. TRIS was long considered biologically inert and no degradation pathway for TRIS was known. However, based on its abundant use and its high water solubility significant amounts of TRIS are presumably released into our waterways and wastewater treatment facilities, facilitating the emergence of a new degradation pathway due to repeated exposure of the microbial community to TRIS.

We isolated eight *Pseudomonas* strains from different wastewater treatment plants that can grow with TRIS as only carbon and nitrogen source. Genome sequencing and transcriptomic analyses revealed that TRIS degradation is encoded by two adjacent gene clusters encoding a transporter protein of the amino acid-polyamine-organocation (*APC*) superfamily, a choline dehydrogenase-like protein and an aldehyde dehydrogenase (cluster I), and a serine hydroxymethyltransferase and a serine dehydratase (cluster II). TRIS transformation assays with heterologously expressed proteins in *E. coli* confirmed that the APC transporter is required for TRIS uptake, before TRIS is oxidized to 2-hydroxymethyl serine in two steps by the choline dehydrogenase-like protein and the aldehyde dehydrogenase. Subsequently, the serine hydroxymethyltransferase presumably catalyzes the removal of formate to produce serine, which is degraded into pyruvate and ammonium by the serine dehydratase.

The TRIS degradation gene clusters are part of a composite transposon, which was found either in the chromosome or on large circular plasmids in the sequenced strains. Conjugational transfer of these plasmids conveyed TRIS degradation capability to a recipient *Ps. putida* strain that was originally not able to metabolize TRIS, confirming that TRIS degradation can be disseminated among *Pseudomonas* strains by horizontal gene transfer.

Our data suggest that TRIS degradation evolved by a recombination of genes involved in the metabolism of polyamines, choline, and serine, enabling Pseudomonads to use TRIS as a novel carbon and nitrogen source. The high sequence similarity of the mobile genetic element encoding TRIS degradation suggests that the TRIS degradation pathway evolved only recently representing an unprecedented model system for pathway evolution studies.

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**PPM14**

**The regulatory axis of PII-PirC-PGAM in cyanobacterial carbon metabolism**

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The balancing of C/N metabolism is crucial for the maintenance of cyanobacterial homeostasis. During vegetative growth, fixed carbon is mainly used for the formation of essential building blocks. When the non-diazotrophic cyanobacterium *Synechocystis* sp. PCC 6803 is deprived of combined nitrogen fixed carbon is initially stored as glycogen. Carbon partitioning for the C/N balance occurs at the cofactor-independent phosphoglycerate mutase (PGAM) reaction controlled by the regulatory axis of PII-PirC-PGAM. PII is a sensor of the energy- and nitrogen status using ADP, ATP and 2-oxoglutarate (2-OG) as metabolic status reporter. N-limitation leads to 2-OG accumulation causing dissociation of the PII-PirC complex. Free PirC now inhibits the PGAM to redirect carbon flow from lower glycolytic routes towards glycogen. In a PirC-deficient mutant (ΔpirC), carbon flow towards Acetyl-CoA is not turned down, leading to increased polyhydroxybutyrate (PHB) levels during nitrogen starvation. To reveal the mode of regulation of PGAM by PirC, bioinformatic analysis of available PGAM sequences was performed, highlighting two exclusive sub-domains in cyanobacterial PGAM sequences with a yet unknown function. An AlphaFold prediction of the *Synechocystis* PGAM revealed proximity of these two domains. The exclusivity of PirC and these sub-domains of cyanobacterial PGAM suggested functional relations. To confirm this hypothesis, we carried out *in vitro* experiments with mutated PGAMs, which were deleted in either of these domains or in both (Δloop, ΔCT or ΔloopΔCT) and constructed strains expressing the variants for *in vivo* analysis. PGAM activity either decreases in the Δloop variant or increases in ΔCT while the activity of ΔloopΔCT remains constant. The activity of all these variants no more responds to PirC. During N-depletion, the mutant strains carrying these PGAM variants produce lower amounts of glycogen and higher amounts of PHB similar to the ΔpirC mutant. To further elucidate the interaction between PII and PirC, we used Biolayer interferometry with PirC and the various PII variants, confirming that the T-loop of PII is critically involved in binding PirC. Moreover, an in silico prediction shows similar PII's T-loop binding properties towards PirC than PipX. The elucidation of the structure of PGAM- and PII-PirC is a prospective goal for a better understanding of the regulatory axis of PII, PirC, and PGAM, required for further engineering of cyanobacterial metabolism.

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**PPM15**

**Bacterial cell wall recycling: Still much more to be discovered**

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Introduction: It was first recognised in the 1980s that *Escherichia coli* recycles about half of the peptidoglycan of its cell wall during one cell doubling. Since then the so-called peptidoglycan recycling metabolism has been extensively studied and it is now considered to be mostly understood. Still, many mysteries remain to be solved and, in addition, it became evident that what holds for *E. coli* may not hold for other organisms (1). The ability to recover components of their own cell wall is a common and pivotal feature of bacteria, with every species having its peculiarities.

Objectives: We are studying the cell wall recycling metabolism in different bacteria and aim at providing a comprehensive view of how bacterial species recycle their own cell wall and how this affects survival in (co-)culture and the natural environment.

Materials & methods**:** We use genetic and biochemical screenings, bioinformatic tools and mass spectrometry analytics to discover unknown genes, proteins, or pathways involved in the cell wall recycling process as well as study their regulation and importance for viability under stress conditions (e.g. antibiotic treatment).

Results: We recently discovered two exo-lytic N-acetylmuramidase, *B. subtilis* NamZ and *S. aureus* MupG, involved in two distinguished routes of sequential turnover of peptidoglycan in these organism (2,3). They cleave MurNAc- and MurNAc-6P-entities, respectively, from the non-reducing end and, thus, act differently from the well-known "lysozyme-like" N-acetylmuramidases that have endo-lytic action. The access to suitable artificial and natural substrates allowed their identification and kinetic and mechanistic assessment. MupG and NamZ are now classified as founding member of the new glycosidase families 170 and 171 (GH170; www.cazy.org/GH171.html and GH171; www.cazy.org/GH171.html), respectively.

Conclusion: We identified and characterized new enzymes and pathways involved in the cell wall turnover and recycling metabolism. Given the effort bacteria make with the cell wall recycling, it is not surprising that it provides much more benefit than previously assumed. Thus, a better understanding of the importance of this metabolic processes will be crucial for improvements in biotechnological processes and antibiotic treatments.

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**PPM16**

**Same same but different –Ggene expression changes in response to lanthanum concentration and lanthanide elements in a lanthanide-storing methylotroph**

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Lanthanides are central to methanol oxidation in diverse taxa, including many previously not associated with methylotrophy. We recently characterized a novel, lanthanide-dependent, and lanthanide-accumulating methylotroph, *Beijerinckiaceae* bacterium RH AL1, that naturally depends on lighter lanthanides (La, Ce, Nd) for methanol oxidation. We show that lanthanum concentration and lanthanide (Ln) elements significantly affect gene expression and Ln uptake under methylotrophic growth conditions. Using incubations with either La (50 nM or 1 µM), Nd (1 µM), or a lanthanide cocktail ([Ce, Nd, Dy, Ho, Er, Yb], 1 µM), differential gene expression analysis revealed that up to 41% of the encoded genes were differentially expressed. The effects of lanthanum concentration and Ln elements were not limited to genes linked to lanthanide-dependent methanol oxidation but reached into many parts of metabolism. We observed that the flagellar and chemotactic machinery is controlled by lanthanides, which was additionally confirmed by a motility assay. Gene expression associated with carbohydrate metabolism, the uptake and processing of alkane sulfonates, various metal uptake and efflux systems, and polyhydroxyalkanoate (PHA) metabolism was highly responsive to Ln elements. We postulate that lanthanides may function as calcium analogues or antagonists and similarly affect bacterial physiology. As described for calcium intake, lanthanide uptake and storage might be linked to channels of short-chain PHA and polyphosphate. Using advanced electron microscopy, we could previously identify periplasmic lanthanum deposits closely located to polar PHA granules. We now expanded this insight into intracellular lanthanide deposition and detected deposits consisting of mixtures of lanthanides near PHA granules when strain RH AL1 was grown with a lanthanide cocktail. The different lanthanide elements were not evenly represented in the deposits and complexed differently compared to previously found lanthanum deposits. Our findings suggest that lanthanides are taken up and stored selectively by *Beijerinckiaceae* bacterium RH AL1. Periplasmic lanthanide deposition could be a key mechanism for lanthanide homeostasis. The ability to discriminate lanthanides and links between lanthanides and the biosynthesis of valuable secondary metabolites such as PHA make *Beijerinckiaceae* bacterium RH AL1 an attractive target for developing bioeconomic applications.

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**RNA01**

**Functional characterization of the RNA interference machinery of *Aspergillus fumigatus***

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RNA interference (RNAi) is a conserved eukaryotic gene regulatory pathway that generates and uses small RNAs to control gene expression. In the fungal kingdom, the influence of RNAi has been implicated in processes such as defense against invading nucleic acids like viruses and transposons, as well as regulation of endogenous genes that are important for adaptation to stress, drug resistance, and pathogenesis. In the WHO fungal priority pathogen, *Aspergillus fumigatus*, the RNAi system is only known to be intact and functional. In this study we dive deeper into the mechanism and function of the *A. fumigatus* RNAi pathway using techniques like comparative genomics, inverted-repeat silencing, proteomics, and RNA sequencing.

*A. fumigatus* has orthologs each of the canonical RNAi components, which includes the dicer-like protein, the argonaute protein, and the RNA-dependent RNA polymerase protein. First, we investigated the evolutionary conservation of these RNAi genes in a large collection of environmental and clinical genomes and we found that these genes show only minor genetic variation across all the sequenced genomes, suggesting that they may play a role in fungal biology. In order to gain more insight into the molecular mechanism of this pathway, we generated single and double deletion knockouts of each canonical RNAi gene ortholog

mentioned earlier and endogenously expressed an inverted-repeat transgene complementary to *pksP* gene, which is important for the biosynthesis of melanin in the fungus. This approach allows for a phenotypic readout based on the production of melanized spore, and indeed we showed that a subset of the RNAi componentry is active in inverted-repeat transgene silencing. To further investigate the biological processes that might be influenced by RNAi, we did mRNA and small RNA sequencing of the wild-type and RNAi knockouts at 24 and 48 hours of growth, as well as in conidia. Our analysis suggests that RNAi has a major influence on conidial transcriptome and linked the *A. fumigatus* dicer-like enzymes and RNA-dependent RNA polymerases to regulation of conidial ribosome biogenesis genes.

In conclusion, *A. fumigatus* RNAi seems to play an active role in regulation of conidial ribosome biogenesis genes and our inverted-repeat transgene experiment result suggests that with efficient delivery of effective small interfering RNAs, the pathway might be exploited to target and silence genes in the fungus.

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**RNA02**

**Early response to antibiotic exposure is characterized by induction of small genes in *Sinorhizobium meliloti***

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Introduction: Bacteria respond to antibiotic exposure at all levels of gene regulation including the RNA level. However, information about very early steps in response to antibiotics exposure is scarce, although these steps could be decisive for bacterial adaptation. Soil and plant-interacting bacteria often have high intrinsic resistance or tolerance to antibiotics. Therefore, bacteria such as *Sinorhizobium meliloti* could be useful for discovery of novel adaptation mechanisms.

Objectives: In this study, we aimed to identify new genes involved in adaptation of *S. meliloti* to tetracycline (Tc) exposure.

Materials & methods: RNA-seq was used to identify genes with changed RNA leves 10 min after addition of subinhibitory Tc amount to exponential cultures. To validate Tc-induced genes and to study induction mechanisms, RT-qPCR, Northern blot, reporter fusions (analyzed by fluorescence measurements and Western blot) and suitable mutants were used.

Results: The RNA-seq revealed 273 genes with changed mRNA levels (at least two-fold change; max. p-value of 0.01). Among the 24 genes with highest induction, 13 encode proteins < 100 aa, 11 of them with unknown functions. Induction was observed already 3 min after Tc addition and mRNA levels increased in time. Increasing Tc concentrations also resulted in gradually increased mRNA levels. The highest induction was detected for the conserved gene of unknown function SM2011_RS13250 (83 aa). Its Tc-induction was detected at the level of mRNA and protein, and depends on the translation of a small upstream ORF (uORF, 29 aa), which was identified recently by ribosome profiling [1].

Conclusion: The early transcriptome response of *S. meliloti* to Tc exposure is characterized by induction of small genes with unknown function. Posttranscriptional mechanisms are part of this response. Their importance for bacterial adaptation to antibiotic exposure is under investigation.

Reference:

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**RNA03**

**Characterization of Kti12, a tRNA binder and partner protein of the Elongator complex**

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Introduction: In order to accurately transmit the information of the genetic code during translation and protein biosynthesis, transfer ribonucleic acids (tRNA) are decorated with a plethora of chemical modifications in all living organisms. Especially the anticodon, the contact point between messenger RNA (mRNA) and tRNA, is heavily modified in all organisms in order to ensure functional decoding of mRNA (McCown et al., 2020). In eukaryotes, the Elongator complex is responsible for carboxymethylation (cm5) of 11 different substrate tRNA carrying a uridine at position 34 . In mammals, either perturbations or mis-regulation of the complex were found to be associated with distinct disease such as amyotrophic lateral sclerosis or cancer (Hawer et al., 2019) and the modifier was found to be essential during embryogenesis.

Objectives: The protein Kti12 was found to bind tRNA, interact with Elongator and enable its tRNA modification activity. To better understand the function of the Elongator pathway protein, we characterized the ATP and tRNA binding properties of Kti12 by mutagenesis.

Material & methods: We investigated functional regions in Kti12, by placing genomic mutations according to sequence similarities with O-phosphoseryl-tRNA kinase (PSTK). The activity of Elongator in the created mutants was assayed using a toxin, that relies on the cm5 decoration by Elongator to cut anticodons and kill cells. Further, uridine modifications were quantified by liquid chromatography coupled to mass-spectrometry.

Results: The disruption of ATP or tRNA binding by Kti12 inactivates the wobble uridine modification by Elongator, thereby establishing a link between Kti12 and Elongator activity. We additionally confirm the functional conservation between Kti12 and PSTK, albeit both proteins do not participate in the same tRNA modification pathway.

Conclusion: Kti12 can be viewed as a valid regulator of Elongator function *in vivo,* which should help address future characterization of Elongator roles in associated human diseases.

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**RNA04**

**Improved RNA stability estimation indicates that transcriptional interference is frequent in diverse bacteria**

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Gene expression in bacteria is regulated by a variety of mechanisms. This includes transcriptional interference that has not been globally studied, thus far. Using stochastic simulations and experimental data from four proteobacterial and cyanobacterial model organisms (*E. coli*, *K. aerogenes*, *Synechococcus* PCC7002, *Synechocystis* PCC6803), we provide evidence that rifampicin time-series data allow to globally monitor and quantify termination by the collision mechanism of transcriptional interference. We show that transcription termination caused by the collision of sense and antisense transcription-complexes is likely a common and underappreciated mechanism of bacterial gene regulation. Furthermore, differential RNA decay, partial termination and internal transcriptional start sites often deviate from the given gene annotations. As a result transcript segments with different half-lives and other properties exist within one and the same annotated gene. We introduce "rifi", a platform-independent workflow for analyzing transcriptomic data from rifampicin time series that takes such differences into account. "rifi" uses a dynamic programming based segmentation that identifies individual transcripts. This allows much more accurate estimates of RNA stability and detection of a wide range of transcriptional events.

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**RNA05**

**Functional and structural characterization of type IV-A CRISPR-Cas-mediated DNA interference**

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Introduction: Bacterial Type IV CRISPR-Cas systems produce RNA-guided effector complexes with CRISPR RNAs (crRNAs) that most often display plasmid-derived spacers. Our laboratory investigates Type IV-A1 CRISPR-Cas systems of *Pseudomonas oleovorans* strain DSM 1045 and *Methylorubrum extorquens*. Both organisms' Type IV CRISPR arrays are located on megaplasmids and reveal spacer sequences that match plasmid, transposon, and viral targets with a consensus 5'-AAG-3' PAM motif.

Objectives: In this study, we aimed to define the mechanism of Type IV-A CRISPR-Cas-mediated DNA interference.

Methods & Results: RNA_seq analysis revealed processing of CRISPR RNAs for highly degenerated repeat sequences. *P. oleovorans* was found to produce crRNAs that target the host *pilN* gene (1). The deletion of this crRNA resulted in upregulated *pilN* expression, which impacts Type IV pilus generation. Synthetic crRNAs with engineered spacers were introduced into *P. oleovorans* as guide molecules for native effector complexes. Downregulation of their target gene expression was confirmed via tryptophan auxotrophy or pigment accumulation phenotypes. In addition, a reporter gene assay was established to analyze the (i) stringency and (ii) spatial coordination of CRISPRi effects on *gfp* expression.

Recombinant Type IV-A CRISPR-Cas effectors were produced in *Escherichia coli* and showed activity against plasmid, virus, and host gene targets. Purified recombinant CRISPR ribonucleoproteins (crRNPs) consist of single copies of the Cas proteins Csf1 (Cas8-like), Csf3 (Cas5-family), the crRNA endonuclease Csf5 (Cas6-family), and five copies of Csf2 (Cas7-family) backbone proteins bound to the crRNA spacer. The helicase Csf4 (DinG) was found to be recruited to the effector crRNP upon target recognition, and a structure of DinG and crRNP interactions was obtained.

Conclusions: Our results highlight that Type IV-A CRISPR-Cas systems regulate a wide range of DNA targets in the absence of conventional DNase activities.

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**RNA06**

**The fate of RNA in a cyanobacterial cell: Translation-independent RNA localisation in *Synechocystis***

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RNAs are known to localize heterogeneously throughout eukaryotic cells. Here, different mechanisms involved in RNA-trafficking were discovered over the past few years. Special cis-acting sequential motifs determine the final RNA localization and further provide binding sites for RNA-binding proteins, necessary for RNA-transport. Although those processes were thought to solely occur in eukaryotes, recent evidence suggest that translation-independent, directed RNA-trafficking could be important for local regulation of gene expression in prokaryotes as well[1]. We aim to get a better understanding of translation-independent RNA organization using two independent experimental approaches. The RNA-sequencing technique "Rloc-sequencing" combining cell fractionation and RNA-sequencing and the visualization technique fluorescence in situ hybridization (FISH) combined with high-resolution microscopy[2,3]. Using Rloc-sequencing we further intent to identify different sequential motifs and corresponding RNA-binding proteins responsible for directed RNA-transport. Current results show, that different transcripts encoding proteins involved in the photosynthesis accumulate at the thylakoid membrane in a translation-independent manner. However, not only the process of RNA-transportation plays an important role determining the fate of RNAs. Other players like RNA polymerase, RNA degradasomes and ribosomes could be involved in spatio-temporal RNA organization. Therefore, we try to unravel the fate of mRNAs in *Synechocystis* sp. PCC 6803 by investigating not only the

subcellular RNA organization, but also RNA-binding proteins involved in local mRNA enrichment[4].

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**RNA07**

**RNA interaction and localization of NudC, the NAD-RNA decapping enzyme in *E. coli***


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Introduction: 5´-NAD-RNA capping was described as the first transcriptional modification in bacteria, in which the NAD-cap provides transcript protection against endonuclease degradation, like RNAse E.

In *E. coli*, NudC was originally described as a Nudix hydrolase, hydrolyzing NAD into AMP and NMN. However, NudC is the main NAD-decapping enzyme, since it has a stronger affinity towards 5´-NAD-RNA, hydrolyzing it into 5´-P-RNA and NMN.

Although the NudC structure has been resolved, no RNA-binding motif has been identified, and the mechanisms for the NudC-RNA interaction remain elusive.

Intrigued by these observations we hypothesized that the NudC-RNA interaction promotes NudC catalytic activity, such interaction might further result in the association with the RNA degradosome complex to facilitate RNA turnover in the degradosome.

Objectives:

- Identify the key residues of NudC required for RNA interaction.
- Elucidate NudC localization and analyze changes upon RNA depletion.
- Define the NudC interactome and demonstrate its association with the RNA degradosome.

Material and Methods:

- To identify residues required for NudC-RNA interaction, Hydrogen-Deuterium exchange (HDX) assays were performed. Site-directed mutagenesis was performed on the identified residues, recombinant proteins were purified and used for *in vitro* NAD-decapping assays.
- To analyze subcellular localization, NudC was fused to the fluorescent protein mNeongreen, and single molecule tracking (SMT) assays were performed.
- To identify the NudC interactome, a proximity labeling strategy (BioID) was followed.

Results**:** HDX assays led to identifying two main residues of NudC, which showed a significatively reduced activity towards NAD-RNA when these residues were substituted.

Furthermore, it was observed that NudC displays precise confinement towards the membrane and a static behavior, which was severely modified upon RNA depletion. Both phenotypes were lost when NudC no longer had the ability to interact with RNA, displaying cytosol localization and fast diffusive behavior.

Conclusion: Altogether, the presented data demonstrate the mechanisms by which NudC interacts with RNA. Such interaction promotes its decapping activity and results in NudC confinement to the membrane and association with the RNA degradosome.

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**RNA08**

**Role of *edited in fruiting body development* (*efd*) genes in *Sordaria macrospora* sexual development**


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RNA editing is the selective insertion, deletion, or substitution of nucleotides and is conserved in all domains of life. RNA editing of protein-coding transcripts leads to sequence changes in the transcript as well as the protein that could alternatively be directly encoded in the DNA. In filamentous ascomycetes, adenosine (A) to inosine (I) RNA editing was recently detected to occur in protein-coding transcripts during sexual reproduction. Interestingly, in fungi, amino acid codons, but also stop codons tend to be affected by editing, the latter leading to a change of *TAG* or *TGA* codons to *TGG* tryptophan codons. Why editing occurs during sexual development, how it is mediated and why the induced protein sequence changes are not directly DNA-encoded, is still under investigation. However, it has been hypothesized that editing is required for ascospore formation and / or ascospore germination and that the diversified proteome provides an advantage for the progeny.

To gain insight into the biological role of editing, we analyzed genes whose transcripts are affected by editing in the ascomycete *Sordaria macrospora*. Deletion of several *efd* genes indeed revealed a function of these genes during ascospore formation and/or discharge. Complementation studies with mutations of the native stop codon to a *TGG* (always long protein) or a *TAA* (always short protein) revealed possible functions for the editing sites. Further studies on the function of editing during ascospore germination in different physiological conditions are underway, with a focus on EFD7, a sorting nexin.

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**STGR01**

**Interkingdom communication between insect pathogenic *Photorhabdus luminescens* and plants via the LuxR solo SdiA**

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The Gram-negative entomopathogenic bacterium *Photorhabdus luminescens* lives in mutualistic symbiosis with *Heterorhabditis* nematodes. During insect infection, a part of the population undergoes phenotypic switching from the nematode-symbiotic primary cells (1°) to the secondary cell variant (2°). The 2° cells lack 1°-specific phenotypes like pigmentation, antibiotic production, bioluminescence, and are not able to undergo symbiosis with the nematodes anymore. After a successful insect infection cycle, the 2° cells remain in the soil, exhibit high chemotaxis activity toward plant root exudates, to finally colonize the plant as novel host. Here we show that, to sense and colonize the plants, *P. luminescens* 2° use the LuxR solo SdiA mediating interkingdom communication between the bacteria and the plants sensing a plant-derived signaling molecule. However, the chemical nature of this signaling molecule is still unknown. We could show that SdiA acts as bidirectional transcriptional regulator controlling expression of its own gene and neighboring *aidA* gene. This activity is negatively influenced by plant root exudates. Indeed, in a *P. luminescens* Δ*sdiA* mutant expression of *aidA* is highly upregulated indicating an involvement of AidA in plant colonization. Furthermore, the Δ*sdiA* deletion mutant exhibits differences in biofilm formation and motility compared to the wild type. Lastly, after sensing and colonizing plants, *P. luminescens* 2° protect them from infection from phytopathogenic fungi via chitinolytic activity. In summary, our data underline a novel mechanism in *P. luminescens* 2° involving SdiA in *Photorhabdus*-plant interkingdom signaling. Since the 2° cells display biocontrol activity protecting plants from fungus infection, *P. luminescens* might find biotechnological application in agriculture in the future.

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**STGR02**

**How the LysR-type transcription factor LsrB navigates the plant-pathogen *Agrobacterium tumefaciens* through stress**

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*Agrobacterium tumefaciens* is a soil-borne plant pathogen, which is broadly known for its ability of interkingdom gene transfer. In the rhizosphere *A. tumefaciens* needs to effectively navigate itself through abiotic and biotic stress and can ultimately escape the harsh soil environment through host colonization. However, successful colonization heavily depends on the ability of *A. tumefaciens* to withstand the host-defense mechanisms. Therefore, the transition and adaptation from one environment to another requires tightly regulated response systems. In our present study, we investigated the role of the LysR-type transcription factor LsrB in the stress response of *A. tumefaciens*.

We found, that the LysR-type transcription factor LsrB acts as superior regulator of various stress response systems required for both lifestyles of *A. tumefaciens*. Transcriptome profiling via RNA-Seq revealed that *lsrB*-deletion leads to differential regulation of more than 1000 genes, including genes corresponding to ROS-defense, beta-lactam resistance or small RNAs. Via *in vitro* binding assays we confirmed that the thioredoxin-, catalase- and beta-lactam resistance system, as well as the small RNA AbcR1 are under direct control by LsrB. Phenotypic analysis confirmed that deletion of *lsrB* leads to beta-lactam- and ROS-sensitivity, as well as reduced virulence of *A. tumefaciens*. By combining *in silico* modeling and bio-physical/chemical analysis, i.e. microscale thermophoresis we identified two environmental signals which are putatively perceived by the regulator LsrB: a plant derived secondary metabolite and a ligand-independent mechanism, solely relying on redox-sensing by LsrB. The given results illuminate the global regulatory role of LsrB and its importance in the stress response of *A. tumefaciens.*

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**STGR03**

**The putative membrane protein RcrB provides exquisite protection to uropathogenic *Escherichia coli* during phagocytosis and exposure to neutrophilic oxidants**

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Background & Question: Activated neutrophils generate reactive oxygen and chlorine species (RO/CS) to eliminate invading pathogens in a process named phagocytosis. Hypochlorous acid (HOCl) is the most potent neutrophilic RO/CS generated in the neutrophil phagosome and kills pathogens mainly through widespread oxidative damage of the cellular macromolecules. However, bacteria have likewise evolved mechanisms to counter the detrimental effects of RO/CS-stress, *although we know very little about how inflammation-associated bacteria sense and defend RO/CS-stress.*

Methods & Results: Using growth curve-based assays, we discovered that uropathogenic *Escherichia coli* (UPEC), the common etiological agent of urinary tract infections, are substantially more resistant to HOCl exposure and neutrophil-mediated killing compared to intestinal *E. coli* pathotypes. We identified the molecular mechanism behind UPEC"s increased HOCl resistance using transcriptional, biochemical, and phenotypic approaches: upon exposure to HOCl, UPEC cells upregulate the expression of an operon consisting of three uncharacterized genes *rcrA*, *rcrR*, and *rcrB*. We identified RcrR as a HOCl-sensing transcriptional repressor that represses the operon during non-stress conditions. During HOCl-stress and in the phagosome of neutrophils, however, RcrR becomes inactivated resulting in the expression of all three genes, which contribute to UPEC"s increased survival in these stressful environments. Expression of *rcrB* appears to be particularly crucial as *rcrB*-deficient cells are as sensitive to HOCl and phagocytosis as intestinal *E. coli* pathotypes. Alternatively, recombinant expression of RcrB in HOCl-sensitive intestinal *E. coli* strains renders them highly resistant to HOCl. Using flowcytometry and fluorescence microscopy, we compared differences in intracellular macromolecular damage in *rcrB*-deficient and wildtype UPEC as a proxy to quantify the intracellular HOCl level and found indeed substantially increased protein, lipid, and DNA damage in UPEC cells that lack RcrB.

Conclusions & Outlook: We propose that UPEC responds to HOCl stress, which it experiences in the phagosome of neutrophils, with the production of the membrane protein RcrB, which may limit the HOCl uptake into the cell. Our goal is now to decipher the precise mechanism of how RcrB controls the cellular HOCl influx, which will help us to better understand how UPEC survives in HOCl-rich environments such as the urinary tract.

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**STGR04**

**Role of zur-regulated transporters in *Mycobacterium avium* spp. *paratuberculosis* during zinc starvation**

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Zinc homeostasis in bacteria is essential for many biological processes and survival. Maintenance is achieved by expression of specific systems, e.g. alternative ribosomal proteins (ARPs) and transporters, which are mainly regulated by the zinc-sensitive global **z**inc **u**ptake **r**egulator Zur. *Mycobacterium avium* ssp. *paratuberculosis* (MAP) is the causative agent of Johne's disease in ruminants and suggested to be involved in Crohn"s disease in humans. While many bacteria possess only one zinc importer, MAP and the environmental, nonpathogenic *M. smegmatis* (MSMEG) possess three zinc uptake systems, which are induced upon zinc-starvation and/or Zur deletion. These systems comprise three importers in MAP (*znu*ABC$^{MAP}$, *znu*ABC-like, *mpt*ABC) and two zinc transporters and a porin in MSMEG (*znu*ABC1, *znu*ABC2, *msp*D).

Our study was performed to show whether and how the expression of the zinc uptake systems is orchestrated *in vitro* and *in vivo* and to elucidate the role of Zur in regulation of these systems.

*In vitro* time- and concentration-kinetics were performed with MAP and MSMEG upon zinc starvation with TPEN. Gene expression of transporters, porins and ARPs was analysed by qRT-PCR. *In vivo* expression of zinc marker genes was tested after infection of macrophages with MAP. β-galactosidase assays were performed to analyse the impact of Zur on *znuABC-like* and *znu*ABC$^{MAP}$ expression. Promoters were fused to *lac*Z and activity was tested in MSMEGwt and MSMEG∆*zur*.

*In vitro* MAP ARP *rpm*E2 was induced first, followed by *mpt*ABC, *znu*ABC-*like* and *znu*ABC$^{MAP}$. *rpm*E2 and *mpt*ABC were also induced after infection. In MSMEG *znu*ABC1 was induced before *znu*ABC2, *rpm*G and *msp*D. β-galactosidase assay revealed a zinc- and Zur-dependent regulation of *znu*ABC$^{MAP}$ but a zinc-dependent/Zur-independent regulation of *znu*ABC-like.

Our data clearly indicate a time and concentration dependent induction of zinc uptake systems. A different order of induction in MAP (*rpm*E2 → *mpt*ABC → znuABC-like → *znu*ABC$^{MAP}$) and MSMEG (*znu*ABC1 → *znu*ABC2 → *rpm*G → *msp*D) suggests diverging zinc response strategies. In macrophages MAP is subjected to zinc deficiency. Promoter analyses of *znu*ABC-like indicate additional regulatory mechanisms for zinc uptake systems in MAP.

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**STGR05**

**Ribosome profiling reveals novel small proteins and adaptations to mild and severe acid stress in *Escherichia coli***

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Ribosome profiling (Ribo-Seq) has significantly advanced the current understanding of translational regulation in bacteria and allows deep-sequencing of ribosome-protected mRNA fragments (RPFs), which are obtained by nuclease digestion of non-ribosome covered RNA. The applications of Ribo-Seq are manifold, and include monitoring protein synthesis rates across the proteome, identifying novel small open reading frames (sORFs), and determining protein copy numbers per cell during steady-state growth. In contrast to mass spectrometry-based approaches, Ribo-Seq is independent of protein biochemistry and allows detection of sORFs encoding fewer than 50 amino acids.

The evolution of molecular systems conferring acid tolerance and/or acid resistance is an important survival strategy for neutralophilic bacteria, including the human enteropathogens, which must survive the bactericidal barrier of the stomach before they infect the human host. Known mechanisms to counteract acid stress include inducible amino acid decarboxylase-antiporter systems, proton pumps, acid-dependent chemotaxis, chaperones, membrane remodeling and induction of acid shock proteins. In this study, we used Ribo-Seq for the first time for exponentially growing *E. coli* cells under culture conditions with different pH levels (pH 7.6, pH 5.8, and pH 4.4) in. In parallel, we performed RNA-Seq to investigate transcriptional differences as well as pH-dependent alterations in translational efficiency.

Our systemic analysis using the high-throughput HRIBO pipeline revealed hundreds of genes with different mRNA and RPF levels under mild and severe acid stress, as well as examples of pH-dependent changes in translation efficiency. In particular, we identified new adaptation processes, including increased iron uptake, glycerol catabolism, copper efflux, and nucleotide biosynthesis, as well as decreased membrane transport and metabolic processes for amino acids and sugars. Moreover, we also identified additional transcription factors that play key roles under acid stress, and 18 new candidate sORFs which were detectable only in cells exposed to severe acid stress. All acid-specific adaptations were elaborated using an autoencoder-based comparison with other stressors.

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**STGR06**

**Solute/sodium symporter-dependent signal transduction: Significance and molecular mechanism of function of the two-component system MxtR/ErdR of *Pseudomonas putida* KT2440**

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Transporters of the solute/sodium symporter (SSS) family transport sugars, amino acids, vitamins, and ions into the cells of all kingdoms of life. However, SSS proteins also form domains in prokaryotic signal transduction systems. For example, SSS domain-containing sensor kinases of bacterial two-component systems are widely distributed among proteobacteria. Here, we investigate the physiological role and molecular mechanism of function of the two-component system MxtR/ErdR of the soil bacterium *Pseudomonas putida* KT2440.

Genes of the two-component system and potential target genes were deleted, and the growth of the resulting mutants on different carbon sources was studied. The binding of ErdR to the promoters of target genes was tested with

electromobility shift assays. Target gene products with unknown function were purified and characterized. The influence of individual domains of the sensor kinase MxtR on signal transduction was tested.

We found that individual deletion of *mxtR* significantly reduced the ability of *P. putida* KT2440 to colonize wheat and maize seedlings. In addition, we observed that MxtR/ErdR and individual target genes are essential for the growth of *P. putida* KT2440 on acetate and other organic acids. Direct interactions of the response regulator ErdR with the promoter regions of genes involved in the metabolism of these organic acids were demonstrated. Physiological and biochemical analyses showed that the target gene *scpC* encodes a protein involved in acetate detoxification and replaces succinyl-CoA synthetase in the citric acid cycle. The SSS domain of MxtR was found to be essential for signal transduction.

The results indicate that the MxtR/ErdR system is an important regulator of *P. putida* KT2440 metabolism, with the SSS transporter domain interfering in the sensing and signaling process. The observations underscore the importance of transporters for signal transduction.

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**STGR07**
**Structure and function of the high-torque bacterial flagellar motor from *Helicobacter pylori***
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The bacterial flagellar motor is a membrane-embedded rotary macromolecular machine that converts the electrochemical energy of the proton gradient into the mechanical energy of rotation. The knowledge about the bacterial motor is a source of inspiration for nanotechnology and one of the first steps towards making artificial motors on the same scale. Recent breakthrough electron cryotomography studies have revealed proteinaceous periplasmic structures adjacent to the stator (the powerhouse) of polar flagellar motors, which are essential for the stator assembly and function. These structures are either disk-shaped, as is the case with *Vibrio* spp., or form a round cage, as is the case with *Helicobacter pylori*. It is now recognized that such additional periplasmic components are a common feature of polar flagellar motors, which sustain higher torque and greater swimming speeds compared to peritrichous bacteria such as *Escherichia coli* and *Salmonella enterica*. The presentation at the meeting will showcase the cutting-edge research on the structure, composition, and function of the periplasmic scaffold in the polar bacterial flagellar motor of *Helicobacter pylori*. This microorganism displays high motility in the very viscous mucous layer of the stomach, which enables us to use it as a model system to study the polar motor specialised for locomotion in highly viscous fluids. The presented work will illustrate the advantages of an interdisciplinary approach combining biology and physics. The presentation will conclude with the discussion of the new paradigm for how the previously unseen accessory components control the function of the flagellar motor.

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**STGR08**
**Regulation of flagellar motility by the global anaerobic regulator FNR in *Salmonella* Typhimurium**
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Introduction: *Salmonella* Typhimurium is an enteropathogenic bacterium encountering different oxygen level during the infection process, i.e. low oxygen in the small intestine. Here, flagellar motility plays a crucial role for *Salmonella*pathogenicity by enabling directed movement towards host cells. The global transcriptional regulator FNR enables the switch from aerobic to anaerobic metabolism and has been shown to be important for flagellar motility. However, the detailed mechanism of FNR-dependent regulation of motility is not yet understood.

Objectives: Here, we investigated how FNR maintains flagellar motility under anaerobic conditions.

Material & methods: We analyzed *Salmonella* Typhimurium wildtype, *fnr* deletion and complementation strains under aerobic and anaerobic conditions in MOPS-buffered medium supplemented with D-xylose to avoid potential effects of catabolite repression or pH.

Results: We confirmed that FNR is essential for flagellar motility under anaerobic conditions using soft agar plates. However, in contrast to previous reports, flagellin immunostaining demonstrated that cells were still flagellated in absence of FNR, although flagellar gene expression was downregulated. We found that this effect was independent of the flagellar master regulator *flhDC*. Rather, live-cell microscopy on single cell level revealed that, in absence of FNR, the run-and-tumble swimming pattern was drastically affected indicating loss of chemotaxis or impaired PMF.

Conclusion**:** We found that FNR is important for proper flagellar function under anaerobic conditions and hypothesize that the FNR-dependent switch between aerobic and anaerobic metabolism is important to provide enough energy required for efficient flagellar motility. This might be advantageous for *Salmonella* during the infection process, in which bacteria first encounter the anaerobic lumen enabling FNR-dependent flagellar motility, followed by increased oxygen level at the intestinal epithelium, which might inactivate FNR and flagellar motility enabling efficient host cell invasion.

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**AMZ-FG04**
**Important roles of Zinc Finger µ-Proteins in *Haloferax volcanii***
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Zinc finger proteins are known to fulfil many different roles in eukaryotes, e.g. as transcription factors, ribosomal proteins, regulators of membrane proteins, or molecular scaffolds. In archaea, they are severely understudied. In addition, most well-studied eukaryotic zinc finger proteins are large proteins containing several small zinc finger domains. In contrast, in archaea the majority of putative zinc finger proteins are very small and await experimental characterization.

*Haloferax volcanii* is a halophilic archaeon that can easily be cultivated, making it a frequently used model organism. Its genome encodes 282 small proteins (less than 70 amino acids), most of them having no annotated function yet. 43 of those proteins contain at least two CPXCG-like motifs, making them putative one-domain zinc finger proteins of unknown function.

For the analysis of these zinc finger µ-proteins more than 30 single gene *in frame* deletion mutants have been generated until now. They were compared to the wildtype concerning e.g. growth behaviour in different media, swarming, and biofilm formation. 5 genes turned out to be essential. Most of the mutants showed a strong phenotype under at least one of the conditions tested, revealing that the zinc finger proteins fulfil various important functions in haloarchaea[1]. For further analyses, co-affinity purifications of His-tagged versions have been initiated to screen for interaction partners (proteins, DNA, RNA). A biochemical assay was performed to investigate which of the putative zinc fingers actually bind zinc ions.

The proteins HVO_0758 and HVO_2753 have been studied in detail[2]. RNA-Seq analyses (coll. with C. Sharma, Würzburg) revealed that movement/chemotaxis genes are downregulated in the deletion mutants, in excellent agreement with their inability to swarm. Determination of the NMR solution structures (coll. with H. Schwalbe, Frankfurt) revealed distinct structural differences between the two proteins. HVO_0758 has a N-terminal alpha-helix with highly-conserved positively charged amino acids that could facilitate DNA-binding. The N-terminal region of HVO_2753 is flexible and could be intrinsically disordered.

Taken together, we could show that haloarchaeal zinc finger µ-proteins are important for various biological functions, e.g. glycerol metabolism, biofilm formation and swarming. More in-depth studies of selected proteins are underway.

[1]Nagel et al. (2019) Genes 10:361
[2]Zahn et al. (2020) FEBS J. 288:2042

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**AMZ-FG05**
**Linking cell architecture and genomics features in the genus Achromatium**
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*Achromatium* is large, hyper-polyploid, and the only known heterozygous bacterium. Single cells contain approximately 300 different chromosomes with allelic diversity far exceeding that typically harbored by single bacterial genera. Surveying all publicly available sediment sequence archives, we show that *Achromatium* is common worldwide, spanning temperature, salinity, pH, and depth ranges normally resulting in bacterial speciation. Saline and freshwater *Achromatium* spp. are not phylogenetically separated, and the genus *Achromatium* contains a globally identical, complete functional inventory regardless of habitat. *Achromatium* spp. cells from differing ecosystems (e.g., from freshwater to saline) are, unexpectedly, equally functionally equipped but differ in gene expression patterns by transcribing only relevant genes. We suggest that environmental adaptation occurs by increasing the copy number of relevant genes across the cell"s hundreds of chromosomes, without losing irrelevant ones, thus maintaining the ability to survive in any ecosystem type. This, so far unique, mechanism is possible due to the complex cellular architecture, which we show using high-resolution fluorescence and electron microscopy. The complex membranal structure separates chromosomes into clusters allowing them to evolve independently one from another, without the need to equally replicate all chromosomes at all times. We demonstrate this by sequencing cell parts and cells during division, revealing the intracellular genomic diversity which results in non-clonal daughter cells. As such, this may be a bacterial paralogue to sexual reproduction. Using fluorescent labeling, we further show that processes like DNA replication, and RNA and Protein synthesis are not temporally synchronized across the large cell. The functional versatility of *Achromatium* and its genomic features reveal alternative genetic and evolutionary mechanisms, expanding our understanding of the role and evolution of polyploidy in bacteria while challenging the bacterial species concept and drivers of bacterial speciation.

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**BT-FG01**
**Recent Advances in Enzymatic Plastic Recycling and Upcycling**
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The escalating amount of plastic waste and its detrimental effects on the environment have sparked a growing interest in sustainable plastic recycling methods. Enzymatic degradation of plastic has emerged as a promising circular economy solution, providing a sustainable and efficient alternative to conventional mechanical and chemical recycling methods.

Having conducted extensive 18-year research on polyester hydrolase discovery and engineering, researchers have significantly advanced our understanding of enzymatic hydrolysis for polyethylene terephthalate (PET), a widely used plastic in packaging and textiles. This deeper insight has led to the design of highly efficient biocatalysts suitable for large-scale PET recycling. Despite global scientific efforts, significant progress in effectively breaking down other highly resilient petrochemical plastics remains nascent.

Recent reports highlight newly discovered enzymes that facilitate the industrial recycling of particular polyurethanes (PUR) and identify enzyme cascades with the potential to degrade vinyl polymers. These breakthroughs hold significant promise for accelerating biocatalysis-driven plastic recycling and upcycling, as well as expanding substrate processability, including those found in solid waste streams.

This lecture aims to shed light on recent research advances, addressing both the challenges and opportunities associated with the implementation of large-scale enzymatic plastic degradation. By examining how enzymatic methods can promote sustainability, a deeper understanding of strategies to combat plastic waste and reduce ecological damage can be attained.

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**BT-FG02**
**Directed evolution of a nonribosomal peptide synthetase**
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The synthesis of analogues of antibiotics through the engineering of biosynthetic enzymes is an enticing strategy. Non-ribosomal peptide synthetases (NRPSs) play a critical

role in the production of important antimicrobial peptides. The adenylation domains of NRPSs select amino acid substrates for incorporation into peptide. The objective of this study [1] was to demonstrate that directed evolution of an adenylation domain of a Pro-specific NRPS module can switch substrate specificity to a non-standard amino acid, piperazic acid (Piz), bearing a labile N-N bond. To this end, we employed LC-MS/MS-based screening of small libraries of the gramicidin S NRPS to detect peptide formation. The mutagenesis strategy combined the analysis of natural sequences and site-directed saturation mutagenesis of functional hot-spots. By only screening 1200 mutants in total, we completely switched substrate specificity of the adenylation domain to Piz, which enabled production of a Piz-derived gramicidin S analogue. The applied technique can presumably be replicated with a larger number of substrates and NRPS modules. Thus, we give new impetus to the too-early dismissed idea that widely accessible low-throughput methods can switch the specificity of NRPSs in a biosynthetically useful fashion.

[1] Stephan, P.; Langley, C.; Winkler, D.; Basquin, J.; Caputi, L.; O"Connor, S. E.; Kries, H. Directed Evolution of Piperazic Acid Incorporation by a Nonribosomal Peptide Synthetase. BioRxiv 2023. https://doi.org/10.1101/2023.04.03.535426.

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**BT-FG03**

**Discovery and engineering of coenzyme $F_{420}$-dependent glucose-6-phosphate dehydrogenase from *Thermomicrobium roseum***

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Intorduction and Objective: The deazaflavin redox coenzyme $F_{420}$ is involved in various metabolic processes such as methanogenesis, antibiotic biosynthesis, and xenobiotic degradation. Due to the low redox potential of the coenzyme, $F_{420}$-dependent enzymes are promising candidates for challenging reductions in biocatalysis. However, such processes require the reduced coenzyme $F_{420}H_2$. $F_{420}$-dependent glucose-6-phosphate dehydrogenase (Fgd) catalyzes the conversion of glucose-6-phosphate (G6P) into phosphogluconolactone, with the concomitant reduction of coenzyme $F_{420}$. However, G6P is a relatively costly molecule, so that the discovery of enzymes accepting cheaper substrates is highly appreciated for *in vitro* applications.

Methods and Results: The thermophilic bacterium *Thermomicrobium roseum* is known for the production of the rare coenzyme $F_{420}$ and bioinformatic analyses suggested the presence of several potential $F_{420}$-dependent enzymes. Bioactivity-guided screening and heterologous expression of candidate enzymes followed by biochemical characterization revealed a thermostable Fgd homolog with side activity towards glucose (besides G6P). Crystallographic structural elucidation and computational modeling assisted in the identification of potential substrate-binding residues. Saturation mutagenesis-based engineering and screening were undertaken to direct the substrate specificity of the enzyme toward glucose.

Conclusion: These results helped to establish a more economic regeneration system for coenzyme $F_{420}$. Moreover, our work inspires future efforts to discover further $F_{420}$-dependent enzymes, to enhance their native biochemical functions, and thereby foster biotechnological applications of the coenzyme in the future.

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**BT-FG04**

**Driving bacterial cytochrome P450s: Investigation of a novel specialized, yet broadly applicable electron transport system from *Acinetobacter* Sp. EB104**

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Cytochrome P450 monooxygenases (CYPs) are versatile enzymes that catalyze various oxidation reactions. They require electron transfer (ET) partners to transfer electrons and activate the oxygen species. Bacterial P450s have diverse ET partners, including NAD(P)H-specific flavin-containing reductase and ferredoxins. Identifying native redox partners is challenging due to spatial differences in the gene locus, but crucial for efficient coupling. Their specific interactions are often unmatched by non-natural redox partners, yet a certain promiscuity makes it possible to use "universal" ET systems to efficiently drive these reactions.

Fourty years ago, a CYP153A and its corresponding ET system were found on one operon in *Acinetobacter* sp. EB104 [doi: 10.1002/abio.370030404]. We thus aimed to characterize the two ET enzymes ferredoxin (Fd) and ferredoxin reductase (FdR) with respect to activity, thermal-stability, and options to couple this ET pair to native and non-native CYP partners. For this, we combined the three enzymes, ferredoxin reductase, ferredoxin and CYPs, in free and linked forms to evaluate their potential for general application.

Genes coding for Fd, FdR, as well as for a panel of CYPs were cloned into suitable vector plasmids, individually as well as in form of fusion constructs. The individual enzymes were heterologously produced in *E. coli,* isolated and purified by nickel-affinity chromatography. They were then characterized for thermostability (thermo shift assay) and for activity via suitable coupled assays (NADH reduction, cytochrome c reduction, CO-reduction). The individual and linked forms were used to perform hydroxylation reactions with a panel of substrates and CYPs *in vivo*.

Fd and FdR proved to be appreciably active in both, individually produced and linked, forms. Their coupling to a panel of CYPs was successful, providing access to a series of hydroxylation products.

With the long-known, yet until now disregarded, proteins Fd and FdR from *Acinetobacter* sp. EB104, an ET protein pair with great potential in biotechnological setting has been elucidated. It is applicable in non-fused systems, enabling the fast co-expression with other proteins e.g. in a screening setting, and also valid as a fusion protein system, where the wisely chosen linker enables an efficient ET from NADH to the catalytic center of the desired CYP. We propose this ET system to be a powerful tool for future couplings to CYP monooxygenase enzymes.

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**BT-FG05**

**Metabolic engineering of *Corynebacterium glutamicum* for the production of the low-caloric natural sweetener D-allulose**

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Introduction: High-calorie sugars have a bad record in the field of nutrition, since their consumption has been linked with an increased risk of obesity, type 2 diabetes and cancer. Therefore, low-caloric natural sweeteners that provide a sweet taste without adding much calories gained public and economic interest, since they represent an attractive alternative for sweetening processed foods. D-Allulose is a promising candidate, which is currently produced in industry from fructose in fixed-bed reactors at high temperatures with immobilized allulose or tagatose 3-epimerases. This process requires purification and immobilization of large quantities of protein and an elaborate separation of the product from the substrate.

Objectives: Microbial processes for allulose production should be established, which could offer advantages compared to the current process with immobilized enzymes.

Materials and methods: A *C. glutamicum* strain unable to grow with fructose as sole carbon source was constructed by gene deletion via homologous recombination. The genes required for the production of allulose were cloned into the pPREx2 plasmid, in which target gene expression is regulated by the tac promotor via IPTG induction. Transformed strains were cultivated in minimal medium with 2 % (w/v) glucose and 2 % (w/v) fructose in baffled shake flasks. Fructose consumption and allulose formation were detected via HPLC.

Results: For microbial production of allulose from fructose, the industrially established cell factory *C*. *glutamicum* was used as host. To inhibit metabolization of fructose in central metabolism, a fructose-negative strain was constructed. Two pathways were tested for allulose production: one based on the conversion of fructose to allulose by the allulose 3-epimerase, and a second one based on the conversion of fructose 6-phosphate to allulose 6-phosphate followed by dephosphorylation to allulose. Both pathways led to the production of allulose from fructose. The strain with allulose 3-epimerase enabled 21.5 % conversion within the first 48 h of cultivation. For the strain which used allulose 6-phosphate dephosphorylation, a conversion of 13.2 % was obtained.

Conclusion: Two microbial production pathways for allulose were established in *C. glutamicum*, of which the direct epimerization of fructose to allulose is currently more efficient. Future studies aim at further improvement of the strain using the dephosphorylation method for the production of D-allulose.

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**BT-FG07**
**Antibiotic-free plasmid maintenance – A long-awaited tool for biotechnology**
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Antibiotic-based plasmid selection and maintenance is a core tool in molecular biology; however, while convenient, this strategy has numerous drawbacks when it comes to biotechnological production. Overuse of antibiotics and antibiotic resistance genes (ARG) contributes to the development of antimicrobial resistance (AMR), which is a growing threat to modern medicine. Antibiotics themselves are costly and therefore often omitted in large-scale fermentations, leading to a decrease in yield due to plasmid loss. Furthermore, constitutive expression of a plasmid-encoded antibiotic resistance gene imposes an unnecessary metabolic burden on the cells. Ultimately, the use of antibiotic resistance genes for many fermentation products, e.g. in the area of nutrition, is subject to strict official regulation and should therefore be avoided. We present a simple method for plasmid selection and maintenance with stringent selection pressure that is independent of antibiotics and ARG, and can be used without any restrictions regarding culture medium or temperature. The developed method involves modification of a bacterial strain such that an essential gene is expressed genomically under the control of an inducible promotor. As a result, the strain is only viable when supplemented with the inducer. A copy of the same essential gene with its endogenous promotor is supplied on a plasmid for selection. In the absence of the inducer, cells rely on expression of the plasmid-encoded gene copy, leading to tight selection for plasmid maintenance. We demonstrate long-term, tight selection for plasmid maintenance with a variety of different plasmids and *E. coli* strains. This method facilitates plasmid-based fermentations by eliminating the need for antibiotic selection and improving plasmid stability. Importantly, the engineered strain can be transformed with any required plasmid containing the essential gene as a selection marker without requiring helper plasmids or repetitive genome engineering.

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**FB-FG02**
**Unveiling the impact of UPR modulation on pathogenic development of *Ustilago maydis***
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The Unfolded Protein Response (UPR) is a signaling pathway that is conserved in all eukaryotes and ensures endoplasmic reticulum (ER) homeostasis under stress conditions. The UPR serves to increase the ER folding capacity and to reduce the amount of un- or misfolded proteins. If protein homeostasis cannot be achieved, apoptosis is induced. In order to avoid cell death, the UPR needs to be tightly regulated. Biotrophic growth of the corn smut fungus *Ustilago maydis* requires modulation of the UPR by physical interaction between the developmental regulator Clp1 (Clampless1) and the central UPR regulator Cib1 (Clp1 interacting bZIP). Formation of the Cib1/Clp1 complex leads to stabilization of both proteins and promotes enhanced ER stress tolerance. Moreover, modulation of Cib1 transcriptional activity is connected to altered Cib1 phosphorylation, which often precedes ubiquitination and subsequent proteasomal degradation. We are currently investigating the effects of altered Cib1 phosphorylation on the transcriptome during the fungal plant interaction and aim to identify the involved kinases and phosphatases.

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**FB-FG03**
**Elucidating the ability of *Trichoderma* to distinguish between plant-beneficial and plant-pathogenic fungi**
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The application of plant-beneficial microorganisms as bio-fertilizer has gained traction in recent years, as both agriculture and forestry are facing the challenges of decreasing soil quality and climate change. This includes the ascomycete genus *Trichoderma*, which members can promote plant growth by translocation of nutrients, production of plant hormones, induction of plant systemic resistance and direct antagonism towards plant-pathogenic

fungi. However, the rhizosphere also contains plant-beneficial fungi, such as mycorrhizal fungi. The mycotrophic nature of *Trichoderma* could, therefore, impair the symbiotic interactions between plants and native mycorrhiza. The outcome of these multi-species interactions of root-associated fungi and the plant can be either mutualistic or detrimental and is likely mediated by the exchange of signaling molecules such as soluble exudates or volatiles. In this regard, it was shown previously that the ectomycorrhizal fungus *Laccaria bicolor* can alter the volatile emission profiles of *Trichoderma* strains during *in vitro* co-cultivations and partially inhibit colony growth even without direct mycelia contact. To further predict the risk of *Trichoderma* applications on native mycorrhiza, we aimed to answer the question whether *Trichoderma* strains can distinguish between plant-beneficial and plant-pathogenic fungi. We therefore investigated the ability of two different *Trichoderma* species to differentiate between the ectomycorrhizal *L. bicolor* and the plant-pathogenic *Fusarium graminearum* as potential preys by using a novel bio-choice assay. The developed "olfactometer-like" race tube system allows to investigate how another fungus influences the physiological response of *Trichoderma* in terms of hyphal growth direction over time. While the results confirmed that without any physical contact *L. bicolor* negatively influences hyphal growth of *Trichoderma,* the opposite was observed during confrontation with the plant-pathogen *F. graminearum*. Intriguingly, an expression analysis of mycoparasitism-related genes revealed differences in the response of both *Trichoderma* strains to the plant mutualist and the plant pathogen. The expression of several fungal cell wall-degrading enzymes in *Trichoderma* were significantly lowered in the presence of *L. bicolor,* while these genes were activated during confrontation with *F. graminearum.* In a next step we will include a common woody host, poplar (*Populus* x *canescens*), to our experimental set-up to further explore the mechanisms of the tritrophic interaction between poplar and the two potentially plant beneficial fungal species, ectomycorrhizal *L. bicolor* and plant growth promoting *Trichoderma* spp..

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**FB-FG04**

**The isoprenyl chain length of coenzyme Q mediates the nutritional resistance of fungi to a predatory amoeba**

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Prey-predator interactions among microorganisms are one of the main drivers in microbial evolution and can influence the diversity of entire microbial communities as well as the evolutionary adaptations of single microbial species. *Protostelium aurantium* is a fungivorous amoeba that co-exists with a variety of fungal species in nature and feeds on a wide range of fungal species including filamentous and yeast-like ascomycetes and basidiomycetes. Food source screenings identified the members of the *Saccharomyces* clade as one of the very few taxonomic groups that showed resistance to the amoebae, despite rapid uptake by the phagocyte (1, 2).

One of the very few fundamental molecular differences of this clade in comparison to other fungi and bacteria is the different lengths of the side chain of the major mitochondrial electron carrier, ubiquinone, or coenzyme Q. While most fungi and bacteria use coenzyme Q with chain lengths of 8-10 isoprenyl units (Q8 to Q10), *Saccharomyces sp*. generally use only Q6. The biological reason for this evolutionary switch to shorter isoprenyl chain lengths is currently unknown. Genome analyses of *P. aurantium* suggested the absence of a functional biosynthetic pathway for the coenzyme and indicated that this vital cofactor is supplied only via predatory feeding on its fungal prey. External feeding of Q9 or the introduction of a functional pathway for Q9 biosynthesis restored amoeba predation on *S. cerevisiae.* Hence, the inability of *P. aurantium* to feed on members of *Saccharomyces* clade indicates that a nutritional resistance (switch to Q6) can function as an efficient strategy to escape environmental predation and may be easily achieved by a fermentative microorganism that could potentially tolerate a less efficient version of coenzyme Q.

1. Radosa et al., 2019; *Environ Microbiol,* 21,1809-1820.
2. Radosa et al., 2021; *Cell Microbiol,* 23,e13389.

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**FB-FG05**

**Exploring the methylotrophic yeast *Ogataea polymorpha* as a host organism for producing platform chemicals from methanol**

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Introduction: C1-molecules, such as methanol, have the potential to become sustainable substrates for microbial fermentations as they can be derived from $CO_2$ and green $H_2$, without requiring arable land. The methylotrophic yeast *Ogataea polymorpha* is an established organism for the production of recombinant proteins due to its many beneficial traits, including the ability for high protein secretion rates and high thermotolerance.

Objective: In the project METAFOR our goal is to use the yeast's native ability for methanol assimilation to extend its product spectrum by metabolic engineering to produce platform chemicals. We further expanded the genetic toolbox of *O. polymorpha* by assessing the suitability of genetic parts for using them in methanol-based cultivations.

Material & Methods: Several promoters and terminators were characterized during growth on methanol. For this characterization, a short half-life GFP variant was chosen, which allows a precise temporal resolution of gene expression. We then applied the characterized genetic parts to metabolically engineer *O. polymorpha* for the production of different industrially relevant chemicals with methanol as the sole carbon source. Production was boosted through further strain engineering, adaptive laboratory evolution, and optimization of the cultivation conditions.

Results: By varying the terminators alone, a 6-fold difference in gene expression was achieved with the homologous MOX terminator boosting gene expression by around 50 % compared to the second strongest terminator [1]. The characterized genetic elements were used to produce malate from methanol in metabolically engineered *O. polymorpha* strains. We could achieve titers of up to 11.5 g/L malate, which is the highest malate titer reported to date in yeast with methanol as the sole carbon source. Further, we demonstrated the production of lactate, acetone, and isoprene as additional heterologous products in *O. polymorpha.*

Conclusion: These results stress the necessity to carefully choose suitable genetic elements for strain engineering and highlight how *O. polymorpha* can be applied as a versatile cell factory for the production of platform chemicals. Thus, we showcase how methylotrophic yeasts could contribute to sustainable biomanufacturing based on C1-compounds.

[1]Wefelmeier, et al. "Mix and match: promoters and terminators for tuning gene expression in the methylotrophic yeast Ogataea polymorpha." *Front. Bioeng. Biotechnol.* (2022): 759.

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**FB-FG06**

**Biologically and economically sustainable: Development of a fungal heterologous host for enhanced production of astin C**

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Astin C is a non-ribosomal cyclic chlorinated pentapeptide (CCPP) produced by the endophytic *Lecanoromycete* fungus *Cyanodermella asteris*. Whole-genome sequencing allowed the identification and prediction-based functional characterization of all relevant key enzymes of the astin C biosynthetic pathway in the natural producer fungus. The biosynthesis of astin C represents a highly complex intercompartmental process requiring a fungal-specific endomembrane system, the transport of intermediates and substrates across membranes as well as signal peptide-guided sorting of biosynthesis enzymes. As astin C shows promising anthelmintic properties, it represents a potential anti-parasitic agent.

Due to the presence of an unique, chemically particularly unstable amino acid building block, known as (3S,4R)-dichloroproline, the chemical synthesis of which is also extremely environmentally harmful and uneconomical, classical peptide synthesis represents no reasonable approach. Consequently, microbial fermentation remains as the only alternative for large-scale production of astin C. However, the time-, labor-, and cost-intensive processing of axenic cultures of *C. asteris*, combined with very low yields and, at the same time, unreliable production due to the epigenetic adaptation of secondary metabolism, prevent the provision of the required amount of astin C.

Consequently, bio-sustainable and reliable large-scale production of astin C can only be provided in a genetically manipulable heterologous host that allows successful reconstitution of the highly complex astin biosynthetic pathway. Therefore, the goal of this project is the development of an astin C-producing *Aspergillus nidulans* strain which overcome the drawbacks of the natural producer enabling an enhanced production of astin C.

In order to achieve this objective, all relevant biosynthetic genes are cloned under strong constitutively active promoters and transferred into *Aspergillus nidulans* protoplasts by PEG-mediated transformation. The generated mutant strains are validated at small production scales by HPLC-MS to demonstrate the potential of the modified *Aspergillus* strains for more sustainable and reliable astin C production.

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**FB-FG07**

***Aspergillus niger* as a cell factory for the production of pyomelanin, a molecule with UV-C radiation shielding activity**

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Melanins are complex pigments with numerous biological functions that have potential applications in space exploration and biomedicine due to their radioprotective properties. *Aspergillus niger* is, a fungus with naturally high radiation resistance, is well established as a biotechnologically harnessed cell factory, and is an eligible candidate for melanin production.

Within this study, we demonstrate that *A. niger* can produce a radioprotective fungal pyomelanin ($Pyo_{Fun}$) via L-tyrosine inducible overproduction in a recombinant *ΔhmgA* mutant strain (OS4.3). $Pyo_{Fun}$ as well as a synthetic pyomelanin ($Pyo_{Syn}$) control were extracted and purified for further characterisation and radiation shielding capacities.

The production sequence for pyomelanin from OS4.3 has not yet been optimized for a large upscaled production but the average $Pyo_{Fun}$ yield extracted from a 200 mL culture of OS4.3 has shown an excellent bioconversion rate from L-tyrosine as a precursor. The characterization was succesfully performed using three spectroscopic methods (UV-Vis, FTIR and RAMAN spectroscopy) as well as physiochemical tests. Furthermore, its strong antioxidant properties were assessed with DPPH-assays. Additionally, the study evaluated the protective effect of $Pyo_{Fun}$ and $Pyo_{Syn}$ against non-ionizing radiation (monochromatic UV-C) and displayed a significant protective effect against the radiation induced stress for UV-C dosages in the range of space surroundings (1038 J/m2). Here, $Pyo_{Fun}$ also demonstrated superior protection compared to the synthetically produced control pyomelanin ($Pyo_{Syn}$).

These findings suggest that $Pyo_{Fun}$ has significant potential as a biological shield against harmful radiation, particularly for the research field of in - situ resource utilisation (ISRU) for future space exploration settings, since the generated pyomelanin is synthesized extracellularly and there is no need for cell lysis for pigment purification. Although the pigments have only been assessed in dissolved form so far, further research is necessary to test them in a dried form, similar to how melanin pigments are found in nature. However, the findings of this study show that pyomelanin could be a valuable biological derived agent for shielding human tissue and coat materials against harmful radiation, highlighting the significance of further exploration in this area, since in all human space travel endeavours harmful radiation protection is an ongoing and crucial element of research.

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**FGB-FG01**

**A comprehensive comparison of feature selection methods for machine learning on microbial datasets**

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(1) The analysis of microbial datasets is challenging due to their high dimensionality, sparsity and compositionality as well as the complex interactions within microbial communities. Feature selection addresses these challenges by identify subsets of relevant microbial taxa while removing irrelevant or redundant features. These methods, therefore, enable the identification of microbial taxa that contribute to ecosystem dynamics, biogeochemical processes, and ecological interactions and help us understand the roles of microbes in environmental systems.

(2) This study provides a comprehensive comparison of feature selection methods for environmental microbial amplicon datasets evaluated in the context of machine learning workflow.

(3) We compared an exhaustive set of feature selection methods, including filter, wrapper and embedded techniques by applying them on 13 different environmental amplicon datasets in a meta-analysis. Feature selection methods were evaluated in a supervised machine learning setting based on their ability to improve model accuracy in predicting environmental metadata. Furthermore, interpretability, simplicity, power consumption and runtime of the resulting models were considered.

(4) Overall, our comprehensive comparison of feature selection techniques demonstrated the effectiveness of these methods in improving machine learning model performance. Our results reveal dataset specific and general trends concerning the different feature selection techniques. We also provide an insight into the ecological interpretation of the results, accomplished through the respective methods.

(5) In conclusion, this study provides a comprehensive comparison of feature selection methods for the analysis of microbial amplicon datasets and their interpretation in an ecological context. The results contribute to advancing the field of microbial ecology by identifying the most effective techniques for selecting informative features and improving model accuracy.

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**FGB-FG02**

**Using artificial intelligence and a novel media database to predict cultivation conditions for bacteria**

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Growing new types of bacteria in the laboratory is a basis for their biochemical and physiological characterization but to date the design of appropriate cultivation conditions remains a challenge.

As a basis for the prediction of suitable cultivation media from genome information, we developed Media*Dive* (https://mediadive.dsmz.de), a comprehensive and expert-curated cultivation media database, which comprises recipes, instructions and molecular compositions of more than 3,200 standardized cultivation media for more than 45,000 microbial strains from all domains of life, including media and strains from the collections of DSMZ, JCM and CCAP. Media*Dive* is designed to enable broad range applications ranging from every-day-use in research and diagnostic laboratories to knowledge-driven support of new media design and artificial intelligence-driven data mining. It offers a number of intuitive search and comparison tools, as for example the identification of media from related taxonomic groups and the integration of strain-specific requirements. Besides classical PDF archiving and printing, the state-of-the-art website allows paperless use of media recipes on mobile devices. External user engagement is enabled by a dedicated media builder tool.

The standardized and programmatically accessible data of Media*Dive* open new avenues for the rational design of cultivation media, especially when targeting the vast majority of uncultured microorganisms. Based on the genome information of more than 16,000 microbial strains, we developed an AI-guided approach for the prediction of cultivation media. Users will be able to upload a (meta-)genome sequence and retrieve suggestions on medium compositions based on a *k*-Nearest-Neighbor (*k*NN)-based algorithm. Additionally, predictions of relevant phenotypes such as optimal temperature, pH and the ability to utilize various carbon and nitrogen sources are available. Thereby, Media*Dive* in combination with AI will be able to guide the future design of cultivation media for microbial dark matter.

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**FGB-FG03**

**Functional characterization of hypothetical proteins in *Escherichia coli* using artificial intelligence and big data**

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Introduction: The task of fully characterizing microbial diversity is almost incomprehensibly vast. Strikingly, even todays" best-studied microorganisms have not been fully elucidated yet. In the "favorite pet" of microbiologists for over 100 years - the model organism *Escherichia coli* - the function of more than 30% of proteins has not been determined experimentally and more than 2% of protein-encoding genes have no characterization at all. These so-called hypothetical proteins (HPs) represent a huge knowledge gap and hidden potential for biotechnological applications – particularly in uncultured microbes. HPs can so far not be characterized by classical *in vivo*, *in vitro*, and/or *in silico* methods - a challenge rapidly growing alongside the advent of NGS technologies and its enormous extension of "omics" data in public databases. Opportunities for leveraging this "Big Data" have proliferated with the use of artificial intelligence (AI)[1].

Objective: To employ AI for predicting functions of HPs from *E. coli* K-12.

Materials & methods: In this study, we developed specific functional hypotheses for uncharacterized HPs from *E. coli* K-12 by adapting machine learning models that use gene expression data collected from public repositories along with information on metadata. A decomposition algorithm was applied to identify co-expressed genes, followed by using a clustering algorithm. The resulting gene clusters were then assigned to experimentally derived "regulons". To find the best candidates for experimental testing, data was further analysed regarding operon arrangement, evolutionary conservation, and distant homologs calculated based on Alphafold structures. To validate the functions *in vivo,* gene knockouts and phenotypic assays were performed.

Results: Clustering for 72 of the 108 HPs of *E. coli* K-12 into regulons was achieved, giving clues to likely functions. Further analysis of the operon arrangement, sequence and structure conservation added additional detail to the functional hypotheses, particularly for genes yhdN and yeaC. Phenotypic assays of gene knockouts confirmed aspects of our predictions using this novel workflow.

Conclusion: Our approach provides new insights into the functions of previously uncharacterised HPs that can be extended to other microbial species in the future.

1. Ardern et al. (2023). *FEMS microbiology reviews,* 47(1), fuad003. https://doi.org/10.1093/femsre/fuad003

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**FGB-FG04**

**Exploring the theoretical limits: Kinetic modelling of cyanobacterial metabolism for optimization of terpenoid production in *Synechocystis***

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All photosynthetic organisms on earth utilize the light absorbing properties of photosynthetic pigments to collect energy that drives photosynthesis. These pigments are derived in part from terpenoid products. In cyanobacteria, the progenitors of oxygenic photosynthesis: chlorophyll, carotenoids and plastoquinone are all at least partly derived from terpenoid compounds, produced by the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway. By introducing additional terpenoid synthase genes into *Synechocystis,* other industrially relevant terpenoids can be produced sustainably from CO2. However, these heterologous terpenoids compete with photosynthetic pigments for metabolites, so their synthesis may have an impact on cell growth. Thus, the objective of metabolic engineering is to both maximize the synthesis of the terpenoid of interest and ensure a minimum of pigmentation for functional photosynthesis.

To explore the theoretical boundaries of terpenoid production in *Synechocystis*, a kinetic model of the MEP pathway and terpenoid synthesis was constructed. Previously published models of the MEP pathway in cyanobacteria or plant chloroplasts yielded promising results, but did not quantify the impact of the competition between terpenoid production and pigment synthesis on growth.

Our kinetic model addresses the biochemical regulation of the MEP pathway by its intermediates, the cost of amino acid synthesis for the expression of each involved enzyme and the quantitative dependence of photosynthetic growth on pigments derived from the MEP pathway. The model has been calibrated using literature data and validated against the experimental data from pigment mutants.

We have performed Metabolic Control Analysis (MCA) to identify key reaction steps that hold the control over the production pathway. Results of MCA have been then used to optimize the model to yield the theoretical maximum in the light-driven production of terpenoids by *Synechocystis*. This theoretical study will guide strain engineering for the enhanced production of valuable terpenoids such as squalene and valencene.

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**FGB-FG05**

**Synthetic intra- and inter-species symbiosis between E. coli and V. natriegens helps understanding the fundamental regulations of abundance ratios in microbial communities**

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Synthetic intra- and inter-species symbiosis between E. coli and V. natriegens help understanding the fundamental regulations of abundance ratios in microbial communities

In nature microbes live almost exclusively in communities. The advent of omic technologies opened a new route to analyse microbial communities such as the human gut microbiome. However, a lot of technical and bioinformatic expertise is required to gain and properly understand omics data. Additionally, the underlying mechanics of how microbial communities are regulated are still barely understood: how do factors like cooperativity, enzyme occupation rates and individual growth rates affect the relative abundance in the community? How does the metabolism of an individual partners adapt to the nutrient exchange? To complement meta-omic-based top-down approaches, it is necessary to study extremely simplified microbial model communities. Optimally, these communities should not be naturally occurring to reduce the amount of cross talk between partners in order to further simplify the system.

In this study, we designed simple, stable microbial communities based on amino acid auxotrophies that force microbial partners to exchange nutrients in between cells in order to grow. Knockout mutants tagged with fluorophores of *E. coli* MG1655 and *Vibrio natriegens* ATC14048 were created and we showed that they manage to thrive in stable intra- and inter-species communities. Using flow cytometry data, we were able to measure relative abundances of the fluorescent partners and the use of individual metabolomics and proteomics data allowed us to look deeper into the intracellular adaptations to this novel interaction.

We discovered that these communities autoregulate themselves to an inherent abundance ratio independent of their starting ratio. By perturbing the growth conditions of communities in various ways we tried to manipulate this ratio in order to elucidate the factors and mechanics that regulate a stable community. These fundamental principles help to better understand naturally occurring communities based on nutrient exchange and offer a starting point to create synthetic communities that share metabolic burden to produce high value compounds in biotechnological setups.

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**FGB-FG06**

**Identification of modulators of microbial abundances based on genome-scale metabolic modelling**

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Introduction: The modulation of the abundance of microbes is of paramount importance, as health and the environment are influenced by them. Therefore, there is a need for the

development of precision approaches that target specific species of interest.

Objectives: Our objective was to develop a methodology for the identification of compounds that modulate the abundance of microbes in a targeted way.

Materials & methods: To address this question, we used the worm *Caenorhabditis elegans* as a model organism. Also, we utilised the *C. elegans*-related bacterium *Pseudomonas lurida* MYb11 which is immune-protective and the bacterium *Ochrobactrum vermis* MYb71 which is also part of the community and a persistent coloniser of the worm. Therefore, in this project, we focused on this host-microbial system to explore the development of precision prebiotics.

We employed genome-scale metabolic modelling to simulate a bipartite bacterial community that is comprised of MYb11 and MYb71. Based on flux balance analysis, we simulated the supplementation of dietary compounds in this community and we investigated whether there were compounds that specifically boost the abundance of the host-beneficial MYb11 inside the community. To this end, we cross-checked the predicted candidates with published *Biolog* datasets, as they provide information on sources utilisation for each species. Following this informed selection of candidates, we validated their effects *in-vitro* and *in-vivo.*

Results: Metabolic modelling proposed 17 candidates that could support the growth of MYb11. L-serine, L-threonine, D-mannitol, and γ-aminobutyric acid were further experimentally validated *in-vitro*. L-serine showed the biggest effect and therefore it was further assessed *in-vivo*. Our results demonstrated that L-serine can cause an increase in MYb11 abundance in the worm host and in a concentration-dependent way.

Conclusion: Our project demonstrates a proof of concept, as metabolic modelling is a powerful tool for the development of approaches to manipulate microbial abundances. This can be regarded as a first step for microbiome-targeted therapies and further in environmental settings, where adjustment of microbial abundances is needed.

Marinos, Hamerich *et al.*, **Metabolic model predictions enable targeted microbiome manipulation through precision prebiotics**, 2023, DOI: 10.1101/2023.02.17.528811

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**FGB-FG07**

**Technological advances and synthetic yeast for the next level of genome investigation**

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Introduction: Synthetic biology has enabled molecular biology to the next level of genome engineering. Standardization, novel technologies and the integration of laboratory automation are key driving forces for the field. In recent years, efforts were made to synthesize and subsequently characterize microbial chromosomes and genomes. This particular field is termed synthetic genomics. Currently, only fully synthetic bacterial genomes have been described but the international synthetic yeast consortium (Sc2.0) is on the finishing line. Multiple chromosomes have been synthesized and characterized in individual cell lines and the process of consolidating multiple synthetic chromosomes for the final Sc2.0 strain resulted in a strain with 7.5 synthetic chromosomes. In contrast to other synthetic genome projects, the Sc2.0 project made ambitious alterations towards the genetic content. One of the most relevant ones may be the *Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution* system, short SCRaMbLE, allowing to create on demand a population of genotypes from a single genotype. However, creating synthetic genomes is merely a rewriting of existing sequences and "creative" genome writing, e.g. writing many genome variants to better understand chromosome biology, is still challenging.

Objectives: Combining synthetic biology and laboratory automation can be used to understand biological functions on a global scale. We establish and use various methodologies to study genome organization and biological processes by generating large-scale genome variation in stochastic and rational manners.

Materials & Methods: Synthetic biology DNA assembly strategies are used for the construction and use of part libraries to reconstruct chromosomal segments and the construction of neochromosomes. Synthetic yeast populations are created by SCRaMbLE and genotyped with a high-throughput genotyping strategy prior to solving the genomes of selected candidates with long-read sequencing. Laboratory automation is used throughout the experimental procedures.

Results & Conclusion: Here we present our initial steps towards the next level of synthetic microbial genomes. We have established a set of tools and methods to efficiently and quickly construct, validate, diversify, and analyze engineered synthetic yeast strains. We utilize laboratory automation and synthetic biology principles.

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**FGB-FG08**

**Application of high-throughput sequencing technologies for comprehensive analysis of bacterial genome methylation and epigenetics in the model bacterium *Helicobacter pylori***

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Background and Questions. Bacterial epigenetics is a recently expanded field of study, which has shown that numerous bacterial species express several methyltransferases (Mtases) which methylate genomic DNA at specific nucleotide motifs. It is well established that DNA methylation is contributing to genomic DNA integrity and replication, however many recent studies expanded Mtase functions also to global transcript regulation. *Helicobacter pylori* is currently one of those bacterial species which possess the highest number and the most variably expressed set of DNA Mtases [1,2]. Although we have a basic understanding of *H. pylori* epigenetics, we need to better characterize the activity and basic biology of the set of methyltransferases under different environmental conditions. More quantitative measurements of global genome methylation are also wanted.

Methods and Results. We have used various methods, including long-read sequencing, enzymatically aided detection, biochemical methods, and mutant analyses, to quantitate specific methylation patterns of the *H. pylori* genome under various conditions. We have thereby collected information on hemi-methylating and fully methylating Mtase enzymes, local methylation, and identified conditions that modulate global genome methylation in *H. pylori*.

Conclusions. We have obtained quantitative information on diverse genome methylation patterns in *H. pylori*. This not only helps our fundamental understanding but also makes bacterial epigenetic modulation more accessible for possible therapeutic approaches.


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**IUS-FG01**

**What is in a name? Not much without a description!**


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Cultivation-dependent and -independent methods to study microbiomes have led to the discovery of thousands of novel species. While the number of names validly published under the International Code of Nomenclature of Prokaryotes (ICNP) increases each year, the hundreds being named, pales in comparison to those identified via sequencing. This has led to the development of the SeqCode, a genome-based alternative to the ICNP, which requires isolation, and deposition, of the bacteria.

The development of the SeqCode has led to the proposal of human-understandable names to all sequenced taxa that currently lack any name other than a genome ID. The placement of these taxa is based solely on the output of the Genome Taxonomy Database (GTDB), which is also the only descriptive data provided within their taxonomic description i.e. protologue.

While naming these unknown taxa is important, without critical assessment of their taxonomic placement, or description of their functional and ecological features, the naming process lacks any content. These key details help prevent the creation of erroneous taxa, and provide a starting point for future researchers interested in the described taxon.

We developed Protologger to provide such key information and facilitate the detailed description of cultured bacteria, and have now applied it to the uncultured fraction. This involves generating taxonomic, functional, and ecological datasets for all species within GTDB, with the goal that this information will be used to describe the taxa. To facilitate this, all generated information will be hosted on a dedicated Wiki, allowing the community to access the information, and modify the description of the taxa into a curated protologue. Once curated, aim to submit the descriptions to the SeqCode for validation, along with the proposed names, with the goal that once isolated, the names transition to the ICNP validation list as well. This workflow aims to assure that each taxon being described has both a name and a description, helping to maintain the high-quality of species descriptions that currently exist for cultured species.

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**IUS-FG02**

**Selectively targeting microbes associated with childhood stunting**


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Stunted growth, characterized as low height for age, is the result of chronic or recurring malnutrition that affects millions of children worldwide. It is associated with environmental enteric dysfunction (EED), increased morbidity and mortality, lost physical growth potential, reduced neurodevelopmental and cognitive function, and increased risk of chronic disease in adulthood.

Recent evidence suggests a microbial contribution to stunting through an imbalance in the microbiome. Both duodenal fluid and fecal samples from stunted children have a distinct microbial signature dominated by oropharyngeal bacteria, with butyrate-producing Clostridia underrepresented.

Using a systematic in vitro high-throughput screening method for bacterial growth, we have identified a number of compounds that selectively inhibit the stunting-associated taxa while sparing the commensals. Subsequently, clinical isolates from stunted children were isolated and identified using a MALDI-TOF MS library method, which allows for the rapid and cost-effective identification of human commensal gut bacteria. The most promising drug candidates were then tested for selective inhibition in clinical isolates from stunted children as well as in synthetic and patient stool-derived bacterial communities.

The restoration of a healthy balance in the microbiome is expected to have anti-inflammatory effects, which could consequently alleviate the symptoms of stunting and have a positive impact on the health and development of children.

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**IUS-FG03**

**Host and microbiome jointly contribute to adaptation to a complex environment**


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Background: Most animals and plants are host to a community of microbes, their microbiomes, which often provide essential life-history functions. Given these functions, microbiomes have the potential to contribute to adaptation of the host-microbiome assemblage, especially as they can change more rapidly than their hosts, either through changes in microbial community composition or individual microbial lineages. To date, however, it is not well understood to what extent and how precisely host and microbiome jointly mediate adaptation to a novel environment.

Objectives: The objectives of our study are to disentangle the contributions of hosts and microbiomes to evolutionary adaptation using a novel experimental study system.

Methods: We established a new mesocosm approach, with which we adapted the nematode *Caenorhabditis elegans* as host with its diverse microbiome to a novel complex environment (laboratory compost). After approximately 30 nematode generations (100 days), we harvested worm populations and associated microbiomes and subjected them to a common garden experiment, in order to unravel the impacts of microbiome composition and host genetics on adaptation.

Results: We found that adaptation took different trajectories in different mesocosm replicates, where some increased while others decreased their fitness in the novel environment. Interactions between host and microbiome played a critical role in the observed evolutionary paths. By focusing on two exemplary mesocosms, we reconstructed the specific changes in microbiome composition (for both bacteria and fungi) and also host genetics that accounted for the observed change in fitness.

Conclusion: Our study provides experimental evidence that adaptation to a novel environment is jointly influenced by host and microbiome, highlighting that both need to be considered and analyzed for a full appreciation of the process of evolution.

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**IUS-FG04**

**Using natural experiments to reconstruct symbiotic adaptation in lucinid clams and their bacterial partners**

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Studying (co-)evolution in animal-microbial symbioses is challenging due to the differing generation times of animals and bacteria and the inability of typical research projects to replicate the necessary scales of time and space involved in host and microbe adaptation. To overcome these challenges, we are using natural experiments in the sea to reconstruct how symbiotic bacterial partners helped lucinid clams (Lucinidae) adapt to a changing environment. One such natural experiment is the closure of the Isthmus of Panamá, which separated the Caribbean Sea from the Tropical Eastern Pacific about 3 million years ago, resulting in lucinid clams now living in very different habitats on either side of the Isthmus. Using metagenomic sequencing, we characterized the biodiversity and metabolic potential of lucinid symbionts on both sides and found that their genomic functions were very similar, although the potential to fix nitrogen was only found in the Caribbean Sea. Combining our dataset with other symbionts from more than 20 different host species in diverse environments, we showed that bacterial symbionts can easily drop and regain nitrogen fixation genes in nitrogen-limited habitats, regardless of phylogeny and host identity. We are now investigating whether nitrogen fixation varies with seasonality and ultimately benefits the host clams.

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**MB-FG01**

**Resistance to host antimicrobial peptides mediates resilience of gut commensals during infection in *Drosophila melanogaster***

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The microbiota is exposed to host immune effectors triggered by the pathogens during intestinal infection. Yet, unlike pathogens, healthy gut microbial communities remain stable through the infection[1,2]. How commensals stably persist in the gut during infection-induced immune response remains mostly unexplored. Here, we used *Drosophila melanogaster* and its natural pathogen *Pectobacterium carotovorum as a model* to investigate how infection shapes the commensal communities in the host gut. First, using 16s rRNA sequencing, we found no significant effect of infection on microbiota species richness and diversity. Next, focusing on a major *Drosophila* commensal *Lactiplantibacillus plantarum*[3], we showed that it remains stable throughout the time-lapse of infection and is resistant to *Drosophila* antimicrobial peptides (AMPs), unlike pathogens. By a transposon screening, we identified *L. plantarum* mutants sensitive to AMPs. These mutants were impaired in peptidoglycan or teichoic acid modifications, resulting in increased negative cell surface charge and higher affinity to cationic AMPs. AMP-sensitive *L. plantarum* mutants were cleared from the gut after infection and aging-induced gut inflammation in wild-type, but not in AMP-deficient flies, suggesting that the resistance to host AMPs is essential for commensals to persist in an inflamed gut environment. These results together with our previous findings[4] illustrate the importance of microbial resistance to AMPs in host-pathogen and host-commensal interactions.

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**MB-FG02**

**Antibiotic resistances spread through wastewater treatment plants – and can be restrained**

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The alarming spread of antibiotic resistances is one of the severest threats to human health and development of our time (https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance). Wastewater treatment plants (WWTPs) are a potential hot spot for antibiotic resistance spread and dissemination, especially for those against non-naturally occurring antibiotics. In order to assess the actual role of WWTPs as source of antibiotic resistances, we analyzed the microbial communities of two WWTPs over two years on the taxonomic and functional level. We show

that WWTPs release proteins conferring antibiotic resistance and bacteria resistant even against last-resort antibiotics. While the taxonomic and antibiotic resistance profiles of microbial communities change substantially over the course of the wastewater treatment, several antibiotic resistance protein classes are significantly higher abundant in the WWTP effluent than in the receiving environment. In addition, many antibiotics are not completely removed during treatment. Moreover, a *Citrobacter braakii* strain isolated from wastewater exhibits resistance to last-resort carbapenems encoded on plasmids that are presumably shared between several members of the *Enterobacteriaceae*.

We investigated whether physical cold plasma treatment can be a potential remedy for the spread of antibiotic resistances through WWTPs. Indeed, plasma treatment leads to substantial decrease in cultivable bacterial counts, while not selectively enriching resistances or microbial groups of special concern.

Taken together, the effluent of WWTPs might lead to shifts in the antibiotic resistance profile in natural microbial communities, with as yet unclear ramifications for ecosystems and human health. Physical cold plasma treatment can potentially combat this contingent threat.

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**MB-FG03**

**Predatory bacteria influence host-associated microbial communities and *C. elegans* fitness**

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According to ecological theory, the presence of higher trophic level organisms such as predators may influence alpha-diversity by reducing dominant members of a community and creating niches for less common species to thrive. Ecosystem stability has been linked to an elevated alpha diversity, hence the presence of predatory bacteria may have a positive effect on various ecosystems including hosts.

Indeed, a meta-analysis corroborated the hypothesized relationship between predator presence and diversity, with BALO presence being associated with an increase in alpha-diversity of microbiomes from distinct host groups(1). We then supported this correlation by *in vitro* experiments with 12- and 50-member microbial communities in the presence of the bacterial predator *Bdellovibrio bacteriovorous* MYbb2 in liquid medium. Over time, the richness of amplicon sequence variants (ASVs) decreased significantly less in the presence of the predator.

We sought to extend these findings to a host system and chose the hermaphroditic nematode *Caenorhabditis elegans* as a model host due to the ease of manipulating its microbiota. We performed controlled laboratory experiments and found that *Bdellovibrio bacteriovorous* MYbb2 presence impacted the 12- or 43-member worm microbiota, with certain community members displaying significant differential abundances when compared to BALO-free controls. Additionally, worms exposed to *Bdellovibrio* produced a significantly greater number of offspring and lived significantly longer than those from control treatments.

Taken together, these findings underscore the importance of predators in host-associated communities and their impact on host fitness.

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**MB-FG04**

**Presence of microplastic-antifouling paint particles alter microbial communities in surrounding marine sediment**

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Introduction: While microplastic (MP) pollution is now a well-establish sub-field of aquatic toxicology, some types of MPs are better studied than others. One type of MP still very much understudied are paint particles. Paint particles can end up in marine sediment due to degradation of painted layers on maritime objects, namely ships, and can be highly numerous in certain areas, sometimes being the most prominent MP type, especially in harbour sediment samples. Antifouling paints contain biocides, often heavy metals, particularly zinc and copper, which reduce the formation of biofilms. Therefore, there is the theoretical propensity for such MP-antifouling paint particles (APPs) to impact sediment microbiology.

Objectives:

1. Determine whether APPs affect microbial communities in surrounding marine sediment
2. Determine which paint types or additives have the strongest effect on microbial communities
3. Estimate whether changes in microbial communities have implications for microbially-mediated sediment biogeochemical cycles

Materials & methods: APPs were produced from 7 different antifouling paint types were produced according to Tagg (20213- in Review). Baltic Sea sediment was collected by Van Veen grab from a pier in Heiligendamm in North-Eastern Germany. Sediment was homogenised and added to individual 50 ml microcosm tubes along with a given type of APPs. Starting community (T0) samples were also analysed, as well as non-antifouling PP and glass beads were included as controls. Tubes were incubated at 10$^{o}$C for 30 to 60 days before undergoing DNA extraction and 16S amplicon sequencing (Illumina).

Results: Results show a clear shift in the incubations where APPs were added. In general, the shift is similar across all APPs. The non-antifouling paints, as well as glass beads saw very little comparative change throughout incubation. The effect was strongest after 30 days, with a continued but diminished effect after 60 days. Based on estimations using FAPROTAX, several sediment biogeochemical processes appear to be affected by APPs, notably sulphate respiration. Additional results will be presented.

Conclusion: APPs cause a clear change in the microbial community in surrounding sediment following contamination, while non-antifouling PP appear to have no strong effect on community composition.

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**MB-FG05**

**Susceptibility of human oral bacteria to roseoflavin, a vitamin $B_2$ analogue**

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A recent study [1] showed, that *Streptococcus mutans* from the human oral cavity is auxotrophic for riboflavin (RF, vitamin $B_2$). This finding might open up new therapeutic options with regard to caries. In soil, *Streptomyces davaonensis* produces the antivitamin roseoflavin (RoF), a RF analogue, to reduce growth of microbial competitors [2]. Hence, the oral application of RoF might be a novel approach to reduce the cariogenic potential of the human oral microbiota. However, little is known about the antimicrobial efficacy of RoF against human oral bacteria.

Here, we obtained bacterial pure cultures from saliva samples of 25 healthy human volunteers and analysed them by using Matrix Assisted Laser Desorption Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS). So far, 26 different species were identified, all representing well-known human oral bacteria. These isolates were tested regarding their susceptibility to RoF using the disk diffusion method. While all Gram-negative species (12) were found to be RoF resistant, 13 out of 14 Gram-positive species were clearly inhibited by RoF. Additionally, the isolated bacteria were tested with regard to RF-auxotrophy using a RF-free culture medium. After 24 h of incubation in RF-free medium with and without added RF, bacterial growth was measured using a photometer at 600 nm. Based on two independent replications, all Gram-negative bacteria did not show RF-auxotrophy, which nicely corroborated previous studies [3]. In addition, a few Gram-positive bacteria were strictly RF-auxotrophic, while several other Gram-positive species showed at least partial dependency on exogenic RF. These data suggest that RoF-treatment might indeed be suitable to modulate the human oral microbiota.

Ongoing research aims at increasing the number of tested bacterial strains and at characterizing the inhibited species and the biochemistry of the underlying inhibition processes in more detail.

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**MP-FG01**

**A novel T10SS contributes to the virulence of *Yersinia enterocolitica* towards invertebrates**

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Introduction: The human pathogen *Yersinia enterocolitica* strain W22703 exhibits insecticidal and nematocidal activity. This phenotype is caused by proteins of the toxin complex (Tc) encoded on the pathogenicity island Tc-PAI*Ye*. The toxin subunits, as well as the activator TcaR2, are expressed at environmental, but silenced at body temperature. The three subunits form a tripartite ABC-type toxin complex, with subunit C causing cell death through its ADP-ribosyltransferase activity.

Objectives: Molecular and pathophysiological details of insect larvae infection and killing by strain W22703 have not been dissected. In particular, the mechanism by which the Tc proteins are translocated across the bacterial membranes still lacked disclosure.

Methods: Using larvae of the Greater wax moth, *Galleria mellonella*, we established an oral model of infection that allowed us to follow the natural route of infection. For this purpose, larvae were infected with strain W22703 and its *tc*-gene mutants, and the survival rate of the insect were determined in correlation with *Y. enterocolitica* cell numbers. In addition, we isolated W22703 cells via immunomagnetic separation at two time points after infection to delineate its transcriptional response to the host, and applied electron microscopy to visualize the infection process.

Results: Time course experiments allowed the dissection of distinct *Y. enterocolitica* infection stages starting with gut colonisation followed by invasion of the hemolymph, where the pathogen strongly proliferated (Sänger *et al*. 2022 PLoS Pathogens 18:e1010991). The *in vivo* transcriptome of strain W22703 is mainly characterized by a drastic reprogramming of the energy, amino acid and carbohydrate metabolism, by an increase of motility and signaling molecules, and by cell membrane rearrangements. Several phenotypes including penetration of the gut epithelium depend on the presence of the Tc proteins. Strikingly, a mutant lacking a holin/endolysin cassette, which is located within Tc-PAI*Ye*, resembled the phenotypes of W22703 D*tcaA*.2

Conclusion: Our data demonstrate the pivotal role of the Tc proteins in the infection and killing of insect larvae to which *Y. enterocolitica* adapt by a specific transcriptional program. A holin/endolysin pair is essential for insecticidal activity and constitutes an example of the novel type ten secretion system (T10SS) for toxin release.

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**MP-FG02**

**Identification of translocation inhibitors targeting the type III secretion system of enteropathogenic *Escherichia coli***

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Infections with enteropathogenic *Escherichia coli* (EPEC) cause severe diarrhea in young children. The non-invasive bacteria adhere to enterocytes of the small intestine and use a type III secretion system (T3SS) to inject effector proteins into host cells to modify and exploit cellular processes in favour of bacterial survival and replication. Several studies have shown that the T3SSs of bacterial pathogens are essential for their virulence. Furthermore, the loss of T3SS-mediated effector translocation results in increased immune recognition and clearance of the bacteria. The T3SS is, therefore, considered a promising target for novel antivirulence strategies.

Here, we report the results of a high-throughput screening assay based on the translocation of the EPEC effector protein Tir fused to β-lactamase reporter. Using this assay, we screened more than 13,000 small molecular compounds of six different compound libraries and identified three substances which showed a significant dose-dependent effect on translocation without adverse effects on bacterial or eukaryotic cell viability. In addition, these substances reduced bacterial binding to host cells, effector-dependent cell detachment, and abolished attaching and effacing lesion formation without affecting the expression of components of the T3SS or associated effector proteins. Moreover, no

effects of the inhibitors on bacterial motility or Shiga-toxin expression were observed.

In summary, we have identified three new compounds that strongly inhibit T3SS-mediated translocation of effectors into mammalian cells, which could be valuable as lead substances for treating EPEC and enterohaemorrhagic *E. coli* infections.

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**MP-FG03**

**The zoonotic pathogen *Chlamydia psittaci* exits the host cell via formation of *Chlamydia*-containing spheres, a novel non-lytic egress pathway**

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The Gram-negative bacterium *Chlamydia psittaci* acts as zoonotic pathogen, causing the respiratory disease psittacosis which can lead to severe pneumonia. *Chlamydiae* are characterized by an obligate intracellular development that includes host cell invasion followed by intracellular growth and replication and a final egress from the host cell. Here, we characterize and mechanistically dissect a novel non-lytic egress pathway of *C. psittaci* - the formation of *Chlamydia*-containing spheres (CCS). We found that in late *C. psittaci* inclusions, a caspase-3-independent proteolytic cleavage of the peptide DEVD occurs, that continues during CCS formation. CCS formation is preceded by an increase in intracellular calcium concentration of infected cells, followed by blebbing of the plasma membrane and rupture of the inclusion membrane. Finally, blebbing cells detach, thereby forming CCS. CCS are spherical, low phase contrast structures surrounded by a membrane, and contain infectious progeny and cellular DNA. The surrounding membrane exposes phosphatidylserine, but still maintains its barrier function. Thus, the novel non-lytic egress mechanism of CCS formation is fundamentally different to extrusion formation, the previously described non-lytic egress mechanism of *C. trachomatis*. Similar to the egress of other intracellular pathogens, CCS formation shares characteristics of apoptotic cell death.

Host cell egress is essential for intracellular pathogens to spread inside human bodies and for pathogen transmission. Here we describe CCS formation as a novel egress pathway for the intracellular, zoonotic bacterial pathogen *C. psittaci*. This non-lytic egress pathway is fundamentally different to previously described *Chlamydia* egress pathways which suggests that *C. psittaci* exits epithelial cells by a currently unknown mechanism. In addition, CCS formation shares characteristics of apoptotic cell death. However, the sequence of proteolytic activity, followed by plasma membrane blebbing and the final detachment of a whole phosphatidylserine exposing former host cell is unique for *C. psittaci*, which underlines that CCS formation represents a fundamental new egress pathway for intracellular pathogens.

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**MP-FG04**

**Protein sociology of the secreted zinc metalloprotease ProA from *Legionella pneumophila***

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*Legionella pneumophila* is the causative agent of Legionnaires' disease, a life-threatening, atypical pneumonia. Due to demographic and climatic changes, the number of cases continues to rise and the environmental bacterium became the most significant waterborne pathogen in terms of its spread and the severity of disease. The zinc metalloprotease ProA represents one of the most important virulence factors during human infections promoting pulmonary tissue destruction and immune evasion via its versatile interaction partners. By experiments on different organizational levels, we aimed to characterize the role and interplay of ProA with different bacterial or host substrates using proteolytic degradation assays, cell line reporter studies, structural modeling of protein-protein interactions and immunohistochemistry in human lung tissue explants (HLTEs). In this *ex vivo* model, thickening of alveolar septa was shown as a direct consequence of ProA-mediated derangement of collagen IV at the basal lamina. Degradation of structural proteins was also verified *in vitro* explaining how the protease is able to facilitate transmigration and proliferation of *L. pneumophila* in lung tissue. Furthermore, increased susceptibility of a *proA*-deficient mutant to human serum revealed a central role of the protease in antagonizing humoral immune defense. HEK-Blue reporter cell studies, moreover, indicate that ProA is able to reduce TLR5-mediated activation of the pro-inflammatory NF-κB signaling pathway due to the efficient cleavage of bacterial flagellin monomers. However, flagellation itself as an important pathogenicity factor remains unaffected. AlphaFold v2.2 multimer modeling revealed that ProA specifically binds the FlaA subunit in its polymerization domain, which is no longer accessible in the assembled filament. Overall, we demonstrated that the zinc metalloprotease contributes to pathogenesis of Legionnaires' disease by destroying human lung tissue, promoting replication and dissemination of *L. pneumophila*, and antagonizing host defense mechanisms via a diverse spectrum of bacterial or host specific targets.

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**MP-FG05**

**Structure-function relationships underpin disulfide loop cleavage-dependent activation of *Legionella pneumophila* lysophosholipase A PlaA**

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Introduction: *Legionella pneumophila,* the causative agent of a life-threatening pneumonia, intracellularly replicates in a specialized compartment in lung macrophages, the *Legionella*-containing vacuole (LCV). Secreted proteins of the pathogen govern important steps in the intracellular life cycle including bacterial egress. Among these is the type II secreted PlaA which, together with PlaC and PlaD, belongs to the GDSL phospholipase family found in *L. pneumophila*. Additionally, PlaA was previously described as a factor promoting bacterial exit in the absence of the type IVB-secreted effector SdhA.

Objectives: We here focus on the characterization of the phospholipase PlaA, investigate the mode of activation mechanism, and 3D structure of PlaA.

Materials and methods: For detection of enzymatic activity, recombinant PlaA was purified and subjected to lipid hydrolysis assay. Additionally, the effect of the zinc metalloproteinase ProA on PlaA integrity and activity was

determined. Moreover, the 3D structure of PlaA was analyzed via crystallization.

Results: PlaA shows lysophospholipase A (LPLA) activity which increases after secretion and subsequent processing by the zinc metalloproteinase ProA at residue E266/L267 located within a disulfide loop. The 3D structure of PlaA shows a typical α/β hydrolase fold and reveals that the uncleaved disulfide loop forms a lid structure covering the catalytic triad S30/D278/H282. This leads to blockage of both substrate access and membrane interaction before activation; however, the catalytic and membrane interaction site gets accessible when the disulfide loop is processed. After structural modelling, a similar activation process is suggested for the GDSL hydrolase PlaC, but not for PlaD. Furthermore, the size of the PlaA substrate binding site indicated preference towards phospholipids comprising ~16 carbon fatty acid residues which was indeed verified by lipid hydrolysis, suggesting a molecular ruler mechanism.

Conclusion: Our analysis revealed the structural basis for the regulated activation and substrate preference of PlaA and suggests that protein activation of a potentially lytic enzyme may be important to maintain bacterial integrity.

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**MP-FG06**

**The expanding toolbox to investigate intracellular *Staphylococcus aureus***

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*Staphylococcus aureus* is an opportunistic pathogen of humans. Persistence of *S. aureus* carriage, chronicity of infection, recurrence of disease, and dissemination from the site of infection is attributed to intracellular reservoirs of *S. aureus* in the host. *S. aureus* is readily internalized by non-professional phagocytes (NPPC) including epithelial cell lines.

We can modulate bacterial uptake by interfering with either host factors, or by deletion of pathogen adhesion such as fibronectin-binding proteins. Within NPPC, we can induce phagosomal escape of *S. aureus* by expression of phenol-soluble modulins (PSM). The development of an escape reporter not only illustrated that *S. aureus* replicates within the host cytoplasm in NPPC, but also identified a non-ribosomal peptide synthase to contribute to escape in a mutant screen for escape phenotypes. Further, we recently used induction of escape for targeted delivery of a pathogen protease by an otherwise non-cytotoxic S. aureus cloning strain. This cysteine protease, staphopain A, is involved in detachment of host cells from the substratum. Inactivation of the corresponding gene scpA however, does only delay host cell death and leads to extended replication of the mutant suggesting other virulence contribute to host cell death once bacteria reach the host cytoplasm.

Out genetic toolbox thus expands by another tool, allowing for the inducible control of virulence processes in intracellular *S. aureus* that enable investigation of *S. aureus*-host interactions in the future.

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**MV-FG02**

**Prophage maintenance is determined by environment-dependent selective sweeps rather than mutational availability**

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Prophages, viral sequences integrated into bacterial genomes, can be beneficial and costly. Despite the risk of prophage activation and subsequent bacterial death, active prophages are present in most bacterial genomes. However, our understanding of the selective forces that maintain prophages in bacterial populations is limited. Combining experimental evolution with stochastic modelling, we show that prophage maintenance and loss are primarily determined by environmental conditions that alter the net fitness effect of the prophage. When prophages are too costly, they are rapidly lost through environment-specific sequences of selective sweeps. Conflicting selection pressures that select against the prophage but for a prophage-encoded accessory gene can maintain prophages. The dynamics of prophage maintenance additionally depends on the sociality of this accessory gene. Non-cooperative genes maintain prophages at higher frequencies than cooperative genes, which can protect phage-free "cheaters" that may emerge if prophage costs outweigh their benefits. Our simulations suggest that environmental variation plays a larger role than mutation rates in determining prophage maintenance. Our findings highlight the complexity of selection pressures that act on mobile genetic elements and challenges our understanding of the role of environmental factors relative to random chance events in shaping the evolutionary trajectory of bacterial populations.

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**MV-FG03**

**Structural insight into the exceptional binding mechanism of the halovirus HFTV1**

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Archaea are single-celled microorganisms that share evolutionary similarities with eukaryotes and bacteria. They are ubiquitous and play an important role in extreme hypersaline environments where they dominate the cellular biomass. Like all domains of life, they are subject to viral infections, which drive archaeal evolution and regulate their global biodiversity and biogeochemistry. Since the archaeal cell surface and its appendages are fundamentally different from those of bacteria, their viral invaders must overcome different obstacles to successfully infect their host. But, compared to their bacteriophage counterparts, archaeal viruses are still largely unexplored and are among the most enigmatic representatives of the viral sphere.

Here, we demonstrate the comprehensive characterization of a model system to study the infection strategies of halophilic archaeal viruses. We used the recently isolated Haloferax

tailed virus 1 (HFTV1) together with its euryarchaeal host *Haloferax gibbonsii* LR2-5 to determine the mechanism of interaction for archaeal tailed viruses (arTVs). A detailed analysis of host features and in-depth characterization of the virulent life cycle enabled us to identify the factors governing attachment to the host cell. We found that HFTV1 exhibits an unusually rapid adsorption mechanism. Electron microscopy of infected cells showed that virus particles often make initial contact with their heads to the cell surface. As a binding site for infection, HFTV1 uses the surface (S)-layer protein of the protective cell wall.

We used electron cryo-microscopy to elucidate the architecture of the virus particles and determined the composition of the major structural proteins of HFTV1. Furthermore, we identified structural elements that contribute to the rapid adsorption and may be related to the unusual binding mechanism. Our results reveal parallels between the entry mechanisms of archaeal viruses and those of bacterial jumbo phages and bacterial gene transfer agents. With this work, we intend to advance our understanding of archaeal virus-host interactions and provide a more comprehensive insight into the function and evolution of microbial viruses.

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**MV-FG04**

**_Streptomyces_ development during phage infection and the role of the phage-encoded transcriptional regulator WhiB**

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Bacteria of the genus *Streptomyces* undergo a complex life cycle starting from single spores developing into a branched mycelium that is followed by formation of aerial hyphae and maturation of spores. Sporulation and related cell division in *Streptomyces* is controlled by a regulatory network involving WhiA and WhiB transcriptional regulators, which co-regulate genes critical to development. In this study, we focused on investigating the impact of phage infection on cellular development using the model species *Streptomyces venezuelae*. We observed that phage infection on solid media triggered the development of aerial hyphae and sporulation at the infection interface of the plaque. Analysis of mutant strains defective at different stages of *Streptomyces* development further confirmed the importance of cellular development for the establishment of transient phage resistance promoting the containment of viral infections. Genome analysis of phages infecting actinobacteriophages showed that WhiB-like proteins represent the most abundant transcriptional regulator in these viruses. Specifically, 31 % of phages infecting *Streptomyces* harbor *whiB* including many containing further developmentally relevant genes. Global transcriptome analysis suggest a suppression of cellular development during the early stages of phage infection. Expression of phylogenetically diverse phage-encoded *whiB* genes in *S. venezuelae* imaged on microfluidic chips revealed drastic changes in cellular development. These findings indicate that development is playing an important role during phage infection, both as host-specific phage defense and as target for phage-driven host manipulation. Additionally, genetic engineering of *whiB*-encoding phages infecting Streptomyces and ChIP-sequencing will expand our understanding of phages involvement in the development of their host.

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**MV-FG05**

**Interplay of lytic phages and the bacterial stringent response**

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Bacteria and their viruses (bacteriophages) are in constant evolution. To avoid infection, bacteria rely on diverse defense mechanisms. Meanwhile, phages have developed multiple counter-defense systems. During this fight, bacteria and phages can directly affect diverse cellular processes including replication, transcription, and translation[1]. These bacterial mechanisms are also regulated by bacterial alarmones (p)ppGpp, which are signaling molecules during the stringent response (SR). They influence multiple cellular processes dynamically reshaping the bacterial metabolism and physiology during different stress situations[2]. Since the replication of phages largely relies on the functionality of the host machineries and processes that are targets of the (p)ppGpp alarmones, we are investigating if and how the stringent response and phage infections are interconnected.

To tackle this, we are focusing on host- and phage-related factors. To study the host factors, we employ *B. subtilis* and its lytic phage SPP1. We found that cells lacking the bifunctional (p)ppGpp synthetase and hydrolase enzyme Rel are hypersensitive towards phage infection. Systematic analyses of different Rel variants carrying point mutations highlight that *B. subtilis* becomes hypersensitive towards lytic phages when the synthetase domain of Rel is inactivated, suggesting that the production of (p)ppGpp is protective against phage infection. On the other hand, and focusing on phage-encoded factors, bioinformatical analysis led to the identification of small (p)ppGpp hydrolyzing enzymes (SAH) in several lytic phages[3]. Our results demonstrate that the giant phage phiKZ infecting *P. aeruginosa* encodes a small alarmone hydrolase (phiKZSAH) that is able to degrade (p)ppGpp, (p)ppApp and NADPH in a $Mn^{2+}$-dependent manner. The tridimensional structure is still unknown, yet the Alphafold model is highly similar to other crystalized SAHs. We are now investigating the biological role of phiKZSAH during the host infection, and hypothesize that it might serve as an effector lowering the concentration of alarmones during phage replication.

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**MV-FG06**

**To lyse, or not to lyse: Identification of YosL, a key player controlling the lytic cycle of the _Bacillus_ phage SPβ**

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SPβ-like phages employ a sophisticated lysis/lysogeny management system that controls the entry into the lysogenic or lytic cycle. The virus of the SPβ archetype, which resides as a prophage in the genome of the strain 168 of the model bacterium *Bacillus subtilis*, is currently being intensively studied. Recently, several novel components of the lysis/lysogeny management system have been identified (1,2). Moreover, the SPβ c2 prophage mutant is of particular interest to study the lysis/lysogeny management system because it enters the lytic cycle upon heat exposure due to a single amino acid exchange in the master repressor MrpR (YopR) (3,4). Recently, we observed that a heat-treated culture of *B. subtilis* cells carrying SPβ c2 does not completely lyse. The surviving lysogens are still heat-sensitive, proving heat-based induction incomplete and the remaining SPβ c2 prophage intact. This finding prompted us to isolate SPβ c2 suppressor mutants that had lost the heat-sensitivity. Genome sequencing uncovered that two independently isolated SPβ c2 suppressor mutants had acquired loss-of-function mutations in the prophage gene *yosL*. Surprisingly, neither heat nor mitomycin C treatment activate the lytic cycle of a SPβ c2 mutant lacking YosL. Thus, YosL seems to be essential for prophage induction at a very early stage of the lytic cycle. However, the observation that the artificial expression of *yosL* during logarithmic growth of a SPβ c2 lysogen did not induce lysis indicates that additional components are involved in the activation of the lytic cycle. Indeed, *yosL* expression in combination with heat treatment re-established the temperature-dependent induction of SPβ c2. *In silico* analyses revealed that YosL might be a DNA-binding protein involved in transcription regulation. Since the *yosL* gene is not expressed by the dormant prophage (5), we hypothesise *yosL* itself is induced during lysogeny resolvement. Very likely, the SPβ master repressor MrpR and associated proteins employ YosL as an activator and signal multiplicator in a downstream signaling cascade. Taken together, YosL is a novel component of the lysis/lysogeny management system in SPβ-like phages.


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**MV-FG07**
**Identification of huge phages from wastewater metagenomes**

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Wastewater contains complex microbial communities, including bacteriophages, most of which are derived from the human gut. Large phage genomes (>200 kb) have recently been described and knowledge about this category of viruses is still neglected, although they are widespread in Earth"s ecosystems. We hypothesized their genetic reservoir is relevant to transfer genes involved in pathogen control and antimicrobial resistance.

In this study, we described the taxonomic classification and functional potential of huge phage genomes and indicate their potential impact in human, animal and environmental health.

We selected 165 wastewater metagenomes publicly available in the TerrestrialMetagenomeDB. After, we used the Multi-Domain Genome Recovery pipeline to identify viral contigs. Viral contigs were dereplicated and quality checked using CheckV. Complete and high quality viral genomes with more than 200 kb were used for further analysis. We manually checked phage genomes by identifying sequences containing phage hallmark genes (i.e., terminase, portal, capsid, tail). Misassemblies, long repeats and concatenated genome sequences, were removed from the data set. All candidate genomes were checked for circularisation and manually curated to remove N's and local misassemblies. We annotated coding sequences searching proteins in PFAMs, TIGRFAMs, VOGs and pVOGs and performed phylogenetic analysis PhyML based on concatenated amino acid sequences of the terminase, major capsid and polymerase genes.

We identified approximately 2.5 million putative vOTUs, of which 629 were >200 kb in length. Based on their quality and the predicted high number of viral genes, 51 vOTUs were subjected to full annotation and curation. In total, four phage sequences were circularised and three genomes remained linear (genome sizes: 204 kb to 304 kb). Functional annotation identified phage-specific structural and nucleotide metabolism genes, while the function of approximately 87% of the proteins remained unknown. We identified between six and 50 tRNAs per genome and metabolic genes involved, for example in the biosynthesis of vitamins and antibiotics. Phylogenetic analysis grouped the genomes into new clades among Caudoviricetes.

We reconstructed seven novel huge phage genomes from wastewater metagenomes, predicted their functional potential and placed them in new taxonomic clades. Their genetic reservoir may contribute to modulation of host metabolism and virus-mediated host evolution.

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**REG-FG02**
**Interplay between GTP, (p)ppGpp and c-di-GMP in controlling biofilm formation by *Escherichia coli* via the diguanylate cyclase DgcE and its GTPase partner system**

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The second messenger c-di-GMP promotes bacterial biofilm formation by playing diverse roles in complex signaling networks. It gets synthesized and degraded by multiple diguanylate cyclases (DGC) and phosphodiesterases (PDE) with DgcE serving as the top-level trigger to produce the extracellular matrix in a *E. coli* macrocolony biofilm [1,2,3,4].

While the c-di-GMP signaling cascade for matrix production downstream of DgcE has been studied in detail, the input into DgcE is less well understood. We could previously show that a complex of the proteins RdcA and RdcB directly interacts and activates DgcE to trigger the c-di-GMP signaling cascade that finally results in biofilm matrix production [5].

To further investigate the mechanism of RdcAB-DgcE signaling, we used a combination of *in vitro* protein and *in vivo* interaction assays as well as macrocolony morphology as an output for DgcE activity. The characterization of RdcA, containing conserved motifs for GTP hydrolysis at its N-

terminus, confirmed that RdcA is a GTPase and revealed that DgcE activation and interaction depend on GTP hydrolysis, but are still possible with a variant not binding GTP or only the C-terminal part of RdcA. This suggested that GTP acts as an inhibitor and that the decrease of GTP during entry into stationary phase could be an input signal sensed by RdcA. Additionally, we found that the binding of the arlamone (p)ppGpp by RdcA inhibits its enzymatic activity, probably by competitive binding. Thus (p)ppGpp may promote RdcA's interaction with DgcE as it prevents binding of the inhibitor GTP.

Taken together, we were able to characterize the RdcAB-DgcE signal transduction with RdcA being a sensor of the cellular GTP/(p)ppGpp pools to activate DgcE's enzymatic activity when cells enter into stationary phase where GTP levels decrease and (p)ppGpp accumulates. Our data show that a complex interplay of three guanosin-based nucleotides, i.e., GTP, (p)ppGpp and c-di-GMP, which are sensed and/or controlled by the RdcAB/DgcE complex, decide on the production of the extracellular biofilm matrix in *E. coli*.

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**REG-FG04**

**A bacterial-type phosphorelay in the methanogenic archaeon *Methanosarcina acetivorans***

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For organisms to survive, it is essential to rapidly react to environmental changes by perceiving, processing, and reacting to signals. Bacteria often use two component systems, which consist of a sensor kinase and a response regulator. In addition to the classic two-component systems, phosphorelay systems have been described consisting of a hybrid kinase with a bound receiver domain, an HPt domain and a response regulator. Within this system, a phosphorylation cascade proceeds via a four-step phosphorelay (His1-Asp1-His2-Asp2) enabling cross regulation. While these systems are well known for bacteria, the knowledge of archaeal signal transduction is still limited. It is believed that archaea acquired two component systems via horizontal gene transfer from bacteria [1]. Here, the major question arises how these systems have adapted to work together with the archaeal transcription machinery, which is more like the eukaryotic transcription machinery than that of bacteria. In the genome of *M. acetivorans,* 51 putative sensor kinases have been found [2]. One of them, MA4377, is a classical bacterial-type hybrid histidine kinase consisting of input domains (CHASE, HAMP, PAS), a kinase catalytic core and two receiver domains. In order to understand the function of MA4377, the protein and truncated variants were recombinantly produced in *Escherichia coli* and purified using affinity chromatography. Kinase Assays employing radioactively labelled γ-ATP confirmed autokinase activity of MA4377 at the conserved histidine residue 497. In addition, intramolecular transphosphorylation to both bound receiver domains and to the downstream encoded stand-alone receiver MA4376 were observed. While we were able to identify the first steps of this archaeal phosphorelay, further steps of this signal transduction system are currently unknown. Comparison to a classical bacterial phosphorelay would ask for an intermediate HPt protein/domain and then likely a final regulatory protein with an output domain. In this context it should be mentioned that only two potential HPt domains could be identified bioinformatically. The organism furthermore contains a large number of stand-alone receiver proteins that likely require interaction with other proteins for signal transduction. Further studies will be directed towards understanding this missing link in this archaeal signal transduction.

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**REG-FG05**

**Genetic code expansion with backbone modified amino acids**

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Introduction: Nature employs a limited and conservative set of amino acids to synthesize proteins. The ability to genetically encode an extended set of building blocks can be used in diverse applications, including approaches to study and control protein function as well as to design novel therapeutics. Non-natural amino acids (NAA) are co-translationally incorporated into proteins by orthogonal pairs consisting of aminoacyl-tRNA synthetase (aaRS) and cognate tRNA. However, the current repertoire can neither display the full natural diversity of NAAs and is especially limited for backbone modifications.

Results: We have suceeded in engineering a protein ligase into an aaRS capable of charging an amber supressor tRNA ($tRNA^{Am}$) with β-amino acids (βaa) both in vitro and in vivo.

Conclusion: Our new-to nature-nature aaRS/$tRNA^{Am}$-pair allows for the co-translational incorporation of βaa into the nascent chain and will ultimately allow the cost-effective production of antimicrobial peptides with high protease stability and increased activity against multidrug-resistant pathogens.

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**UM-FG01**

**Effect of elevated atmospheric CO2 and warming on the soil microbiome of a grassland ecosystem**

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Elevated $CO_2$ concentrations in the atmosphere account for the largest share of anthropogenic greenhouse gases and are a crucial driver of climate change. Although several experiments have investigated the effects of elevated $CO_2$ levels on the soil microbiome, few studies have assessed the interplay between increased $CO_2$ and warming levels. The responses of soil microbial communities to elevated $CO_2$ and warming are highly variable, and the presence of plants and plant roots plays a significant role in the magnitude of the impact on microbial communities. Studies of the effects of warming have shown reduced microbial biomass, reduced fungal abundance, and shifts in microbial communities.

Warming effects depend upon water availability as they strongly influence bacterial population size and diversity.

This research aims to study the combined effects of elevated atmospheric $CO_2$ and temperature on the soil microbiome of a grassland ecosystem by applying a metabarcoding approach alongside assessing soil chemical parameters, soil gas fluxes, and soil microbial activity.

Soil samples were taken in the summer of 2019 and 2021 at the Giessen Thermo FACE experiment on a permanent grassland site near Giessen, Germany, which combines elevated $CO_2$ and elevated temperature. RNA extraction and 16S rRNA (cDNA) metabarcoding sequencing were performed from bulk and rhizosphere soils, and the obtained data were processed with QIIME2 and R. Also, 16S rRNA qPCR was performed to determine bacterial abundances. Physiochemical soil parameters, soil respiration, and gas fluxes were also determined.

The results of this work showed that the site factor and soil physiochemical parameters mainly influenced the structure and composition of the soil microbiome and species richness. Nonetheless, gas fluxes, soil respiration, and bacterial 16S rRNA copy numbers were particularly increased by the elevated $CO_2$ and warming. Also, gas fluxes significantly increased under elevated $CO_2$ conditions. However, $N_2O$ fluxes did not increase with the combination of elevated temperature and $CO_2$ but did so with the increment of atmospheric $CO_2$. Our results indicated that elevated $CO_2$ and temperature affect the soil microbiome even with a short exposure time.

In conclusion the soil chemical parameters strongly drove the soil microbiome composition. Therefore, further research will be necessary to assess the long term effects of these two parameters on this ecosystem in a climate change scenario.

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**UM-FG02**

**Unraveling the impact of pioneer bacterial communities on the transformation of arid and semiarid soils under simulated humid climate conditions**

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Pioneer bacterial communities are essential for the initial soil formation in arid and semiarid environments. However, the impact of climate change on these communities and their influence on early soil transformation is poorly understood. In this study, we evaluated the effects of decreased temperature and increased moisture on soil-forming bacterial communities of the arid site of Pan de Azúcar and the semiarid site of Santa Gracia and their combined effect with biocrust and plants on soil-transforming processes. Samples from both sites were collected from soil horizons A and B and incubated under simulated conditions for sixteen weeks, with different treatments organized as a successional gradient: sterile soil, ubiquitous microorganisms alone, microorganisms combined with biocrusts, and microorganisms combined with pioneer plants. Our results show that plant-microorganism treatment increasingly influenced the physicochemical properties of the soil, mainly in aggregate stabilization. However, the difference between plant-microorganisms treatment and sterile soil was not significant. Under simulated conditions, arid soils exhibited a decreased bacterial diversity, while structural changes in bacterial community compositions were observed in arid and semiarid soils. Bacteria of arid soils reassembled into a new community dominated by *Proteobacteria*, *Actinobacteriota*, and *Planctomycetota*, while semiarid soils were dominated steadily by *Acidobacteriota* and *Proteobacteria*. Co-occurrence network analysis revealed a less developed arid soil with a specialized and sensitive bacterial community, while the semiarid soil exhibited a more complex, versatile, and stable community that could use nutrients more efficiently. Our findings indicate that the abiotic environment is the stronger driver in soil formation under the simulation conditions evaluated and that the legacy of past climate conditions influenced the response of the bacterial community to changing climate conditions.

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**UM-FG03**

**Hunting for pigments in plastic biofilms from the Great Pacific Garbage Patch**

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Each year, an immense amount of plastic debris enters marine ecosystems, much of which ends up in the Great Pacific Garbage Patch (GPGP). The plastic fragments are home to a diverse community of microorganisms, and while researchers have gained a better understanding of these marine plastic biofilms, we lack insights into the physiology and genomic potential of the bacteria that colonize them. Examining the lifestyle of plastic colonizers from the GPGP is particularly intriguing, as the high concentration of plastics in this accumulation zone might allow for a microbial adaptation to this unique man-made habitat. A range of pigmentation was observed in 67 isolated strains obtained directly from plastic pieces sampled from the GPGP surface water. Sequence comparison between the cultivated bacterial strains and the 16S rRNA gene amplicon dataset confirmed that most of the cultivates could also be captured through DNA-only methods. Whole genome analysis of four taxonomically diverse representatives revealed multiple carotenoid pathways, including those to produce less common glycosylated carotenoids, like sarcinaxanthin glucoside. Further, we identified a potentially new *Rhodobacteraceae* species containing a photosynthetic gene cluster (PGC). Absorption analysis confirmed the actual production of the carotenoids and bacteriochlorophyll a. Floating plastics represent a habitat with strong UV-light exposure, making the protection with antioxidant carotenoids as well as the ability to use light as an energy source highly beneficial traits for plastic colonizers. Our findings indicate, that the production of pigments is a common adaption mechanism for plastic-associated bacteria, and that plastic

biofilms present a so far overlooked source of rare carotenoids and light-harvesting mechanisms.

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**UM-FG04**

**Heterotrophic marine bacteria employ a laminarin-fueled intra-population α-glycan recycling loop**

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Glucan storage polysaccharides are the dominating glycans during seasonal blooms of microalgae (phytoplankton) with a global annual production of the β-(1,3)-glucan laminarin estimated at 18 gigatons [1]. Together with β-glucanases, α-glucanases and their respective transport systems belong to the most prominent markers of bacterial activities during these blooms [2, 3]. Yet, the source of α-glucans warranting such a response remained obscure. Our analyses indicated that not phyto- and zooplankton, but the bacteria themselves are major producers of α-glucan during microalgal blooms. 18S rRNA gene sequencing and metatranscriptome analysis of bloom samples revealed that bacteria expressing α-glucanases increase their numbers and activities within few days of phytoplankton peaks. Metaproteome analyses indicated that these bacteria synthesize glycogen for intracellular storage. Interestingly, they also express an uptake machinery for extracellular glycogen. We detected α-glucan produced by these bacteria in bloom samples and *in vitro* and isolated marine bacterial strains grew on this polysaccharide. Through proteogenomic and biochemical analyses we uncovered the protein machinery of bloom-associated bacteria for marine α-glucan degradation, characterizing three main glycoside hydrolases. Moreover, proteome analyses allowed detection of a specific induction of the α-glucan-targeting protein expression.

Polysaccharide-specialized bloom-associated bacteria grow to high cell densities and are therefore susceptible to mortality factors such as viral lysis or predation [4]. We demonstrate that such marine bacteria not only produce large amounts of α-glucans from the available dissolved excess carbon of algal origin, but also seem to have evolved to recycle their own storage polysaccharide to utilize resources becoming available through lysis.

1. Becker S et al. (2020) Laminarin is a major molecule in the marine carbon cycle. Proc. Natl. Acad. Sci. USA, 117, 6599–6607.
2. Sidhu C et al. (2023) Dissolved storage glycans shaped the community composition of abundant bacterioplankton clades during a North Sea spring phytoplankton bloom. Microbiome, 11.
3. Francis TB et al. (2021) Changing expression patterns of TonB-dependent transporters suggest shifts in polysaccharide consumption over the course of a spring phytoplankton bloom. ISME J, 15, 2336–2350.
4. Bartlau N et al. (2022) Highly diverse flavobacterial phages isolated from North Sea spring blooms. ISME J, 16, 555–568.

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**UM-FG05**

***Candidatus* thiovulum stygium, a new veil-forming giant bacterium from caves and subterrenean aquifers**

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*Thiovulum* spp. (*Campylobacterota*) are large sulfur bacteria that form veil-like structures in aquatic environments. The sulfidic Movile Cave (Romania), sealed from the atmosphere for ~5 million years, has several aqueous chambers, some with low atmospheric O2 (~7%). The cave"s surface-water microbial community is dominated by bacteria we identified as Thiovulum. We show that this strain, and others from subsurface environments, are phylogenetically distinct from marine *Thiovulum*. We assembled a closed genome of the Movile strain and confirmed its metabolism using RNAseq. We compared the genome of this strain to one we assembled from public data from the sulfidic Frasassi and despite the great spatial and temporal separation, the genomes of the Movile and Frasassi *Thiovulum* were highly similar, differing greatly from the marine strains. We concluded that cave *Thiovulum* represent a new species: *Candidatus* Thiovulum stygium. Based on their genomes, cave *Thiovulum* can switch between aerobic and anaerobic sulfide oxidation using $O_2$ and $NO_3^-$ as electron acceptors, the latter likely via dissimilatory nitrate reduction to ammonia. Thus, Thiovulum is likely important to both S and N cycles in sulfidic caves. Electron microscopy analysis suggests that at least some of the short peritrichous structures typical of *Thiovulum* are type IV pili, for which genes were found in all strains. These pili may play a role in veil formation, by connecting adjacent cells, and in the motility of these exceptionally fast swimmers.

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**UM-FG06**

**Bromoxynil changes virus-prokaryote host interactions in soils, a genome-centric analyses of metagenomes recovered from a microcosm succession study**

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Introduction: Bromoxynil is a selective herbicide, and its widespread and continued use may affect ecosystem processes controlled by microorganisms.

Objectives: In this study, we tested the addition of Bromoxynil (50 mg/Kg, in accordance with OECD 307) in the dynamics of virus-host interactions in soils.

Materials & methods: We sequenced 54 metagenomes (36 Bromoxynil and 18 Control) from soils at 0, 4, 8, 16, 29, and 64 days and used MuDoGeR to recover metagenome-assembled genomes (MAGs). The quality of MAGs was determined by CheckM, and their taxonomy by GTDB-tk.

The MAGs were clustered into prokaryotic operational taxonomic units (pOTUs) at an average nucleotide identity of 0.95. We used VirSorter2, VirFinder, and VIBRANT to recover putative viral contigs. Their quality was determined by CheckV. We consider complete and high-quality contigs as uncultivated virus genomes (UViGs). PhaGCN2.0 was used to assign UViG taxonomy. Further, we performed host predictions using WiSH, and lifestyles using DeePhage.

Results: We recovered 307 MAGs that were clustered into 193 pOTUs (Bacteria 175 and Archaea 18). The most recovered pOTUs belonged to *Actinobacteriota* (n=97) and *Proteobacteria* (n=36). All Archaea were affiliated with *Thaumarchaeota* (n=18). We recovered 88 UViGs (temperate phages, n=77, lytic phages, n=11), and the most abundant viral families were *Vilmaviridae* (n=17) and *Mesyanzhinovviridae* (n=17). We could predict hosts for ≈61% of the temperate and ≈45% of the lytic phages. We also identified tRNAs (42.9%), head and packaging (17.9%), and DNA, RNA and nucleotide metabolism (13.5%) from 724 coding sequences (CDS) with predicted function while 3,153 of CDS were assigned as hypothetical proteins. We identified four phages carrying auxiliary metabolic genes (AMGs) involved in carbohydrate cofactors metabolism. The virus-host interaction dynamics in our succession were affected as follows: (i) viruses induced only in the controls (3); (ii) viruses induced in the contaminated soils but not in the controls (15); and (iii) viruses induced in the contaminated soils that did not correlate with a lower relative abundance of their hosts (12).

Conclusions: Over one-third of the recovered viruses were affected by the addition of Bromoxynil to the soil. As most Prokaryotes in soils are infected by viruses that, when induced, may hijack their host metabolism, our data suggest that many of the changes in ecosystem processes in contaminated soils may be caused by viruses.

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**WA-FG04**

**Establishment of a SARS-CoV-2 wastewater surveillance system in Berlin**

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Introduction: As the causative agent of the Coronavirus disease (COVID-19), the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a pandemic. During a SARS-CoV-2 infection, virus and RNA particles are shed in feces, urine and saliva, which will be collected in communal sewers. Thus, SARS-CoV-2 RNA can be detected in wastewater. The monitoring of infection events within the human population by medical testing systems, which often tested each person individually, is time-delayed and strongly dependent on the test willingness within the population. Since infected people already release SARS-CoV-2 RNA particles before the disease onset, wastewater-based surveillance (WBS) facilitates a rapid monitoring of infection events of the whole population

Objectives: We aimed to establish a WBS system to extend the monitoring of SARS-CoV-2 infection events in Berlin.

Material & Methods: We collect communal wastewater of three wastewater treatment plants and the airport BER by automatic samplers. In our laboratory, we extracted nucleic acids from wastewater and quantified SARS-CoV-2 RNA amounts using quantitative polymerase chain reaction (qPCR) and digital PCR (dPCR). In addition, RNA sequencing was performed to analyze the predominant virus variants. Finally, we developed the digital platform "Hygiene Monitor" to map the infection events and circulating virus variants in Berlin.

Results: Our WBS system could precisely display the trends of infection events and the predominant virus variants in Berlin. Interestingly, we were able to detect increased SARS-CoV-2 RNA amounts in wastewater several days before the seven-days-incidents arise. This prediction of the rising seven-days-incidents disappeared due to the appearance of the Omicron virus variants. However, our WBS system could show the trend of rising infection events after abrogation of the mandatory testing. Moreover, we register event-related increases of infection events, like Christmas or the abrogation of mandatory mask-wearing.

Conclusion: Our WBS system is a powerful tool to get an overview of the occurrence of infections within the human population at a distinct gathering ground. Thereby, it works faster and more cost-effective than a medical testing system. In the future, we aim to analyze the RNA/DNA amounts of several human pathogens, e.g. bacteria, viruses and parasites, and antimicrobial resistance genes to monitor the infection events of various pathogens within the Berlin population.

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**AMP001**

**Characterizing the bacteria-like O-demethylase system of the methanogenic archaeon *Methermicoccus shengliensis***

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Lignin is one of the most abundant biomolecules on earth. This complex macromolecule is mainly composed of aromatic monomers, some of which exhibit methoxy moieties. As a crucial step of lignin biodegradation, these methoxy groups can be *O*-demethylated by microbial enzyme systems. While this type of metabolism has extensively been studied for bacteria, only recently the first archaeal *O*-demethylation system (MtoABCD) was discovered in the methanogen *Methermicoccus shengliensis* [1]. Remarkably, this system resembles that of acetogenic bacteria, rather than already known archaeal methyltransferase systems [1]. Initial results gained by biochemical assays with native and heterologously produced proteins showed that the first *O*-demethylase (MtoB) transfers the methyl group derived from methoxylated aromatics to a second methyltransferase (MtoA) via a corrinoid CH3-carrier (MtoC) that is activated by a reductive activase (MtoD). In contrast to other methanogenic methyltransferases, tetrahydromethanopterin (H4MPT) acts as the terminal CH3-acceptor rather than coenzyme-M (CoM), very likely resulting in an altered energy metabolism and redox (im)balance [1]. Currently, we aim for establishing an efficient procedure for heterologous production of MtoB in *Escherichia coli* and its purification. UV-Vis spectroscopy and activity assays will be performed with purified MtoB1 and MtoB2 isoforms to characterize and compare substrate utilization and reaction kinetics. An analogous approach will be applied for MtoA to study the kinetics of methyl transfer from MtoC to H4MPT. With help of the developed procedure for heterologous production of the Mto components, we are then able to produce and test Mto systems of not yet cultivated strains such as Bathyarchaea strains.

[1] Kurth, J. M., et al. (2021). Methanogenic archaea use a bacteria-like methyltransferase system to demethoxylate aromatic compounds. *The ISME Journal*, *15*(12), 3549-3565.

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**AMP002**

**From pyrolysis by-products to short-chain carboxylic acids: The metabolic versatility of anaerobic mixed cultures enables carbon and energy recovery during the co-fermentation of syngas and different pyrolysis wastewaters**

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Introduction: The pyrolysis of municipal and agricultural waste represents an important technology for biofuel production and waste management. However, the process generates two by-products, namely, pyrolysis syngas (PS) and aqueous condensate (PAC), which together can contain a significant portion of the carbon and energy of the waste material. Their composition depends on type of waste and process conditions. PS primarily consists of a mixture of CO, $CO_2$, $CH_4$, and $H_2$. The PAC is toxic to microorganisms even at low concentrations and can contain organic acids, organic and N-aromatics, ammonium and nitrate. Although PS and PAC have been used separately as substrates in anaerobic fermentations for methane or carboxylates production, limited information is available about their co-fermentation.

Objectives: This study investigates the co-fermentation of PS and PACs obtained from biomass, sewage sludge, or polyethylene with mesophilic and thermophilic anaerobic mixed cultures to improve the carbon and energy recovery from the pyrolysis process.

Materials & methods. Kinetic studies, run at 37 and 55°C in 250 mL bottles at 20 kPa CO, 25 kPa $CO_2$, 6 kPa $H_2$ and increasing PACs loading, were performed to assess the impact of the different PACs on microbial metabolism and PAC components degradation. Then, PS and the biomass-PAC were continuously fed for over 200 days into two 1.5 L STR (37 and 55°C, pH of 5.5, HRT of 20 days) to enrich two polytrophic mixed cultures and study their microbial composition. The carboxylates produced during the bioreactor enrichments were fed to a secondary fermentative process. At regular intervals, 10 mL of the medium from each bioreactor were centrifuged and inoculated with *A. oryzae* to produce l-malate.

Results: The mesophilic and thermophilic mixed cultures could perform multiple, simultaneous ecological functions. During the kinetic experiments, the mixed cultures degraded aromatic and N-heterocyclic compounds and denitrified, while simultaneously fixing C1 compounds. The mesophilic bioreactor co-fermentation enriched for *Clostridium* sensu stricto 12. The thermophilic co-fermentation led to the enrichment of *M. thermoacetica*, though regular re-inoculations were necessary to maintain stable process operation. *A. oryzae* converted all carboxylates into L-malate up to a yield of 25 mol%.

Conclusions: Integrating pyrolysis and biological processes can improve the recovery of carbon and energy, leading to the production of high-value chemicals.

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**AMP003**

**Lactate formation from fructose or C1 compounds in the acetogen *Acetobacterium woodii* by metabolic engineering**

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Introduction: Anaerobic, acetogenic bacteria have gained interest for a sustainable biotechnology since they capture and convert the greenhouse gas carbon dioxide to acetate. Furthermore, they can perform homoacetogenesis by converting one mol hexose to three mol acetate. Recently, we described that *A. woodii* performed mixed acid fermentation of fructose after genetic inactivation of the $CO_2$ reduction pathway[1]. This offered the possibility to engineer *A. woodii* strains to convert fructose or even C1 compounds to reduced end products, such as ethanol or lactate.

Objectives: Here, we investigated lactate production from fructose or C1 compounds in the metabolically engineered strain Δ*hydBA*/*hdcr*[2]. In order to verify that lactate production is catalyzed by the electron-bifurcating lactate dehydrogenase, the complex should be genetically inactivated.

Materials & methods: A Δ*hydBA*/*hdcr*/*lctBCD* mutant was generated. Lactate production from fructose or glycine betaine + CO was analyzed in resting cells of Δ*hydBA*/*hdcr* and Δ*hydBA*/*hdcr*/*lctBCD*. The transcript levels of the *lctBCD* genes in the Δ*hydBA*/*hdcr* mutant were analysed *via* quantitative real-time PCR.

Results: Resting cells of the Δ*hydBA*/*hdcr* mutant performed heterolactate fermentation from fructose, with a fructose:lactate ratio of 1:1.2 and an acetate:lactate ratio of 1:2.8. Glycine betaine + CO were also converted to lactate and acetate, with a acetate:lactate ratio of 1:1.1, and as a minor product ethanol was detected. Lactate formation was $CO_2$ and $Na^+$ dependent. When the *lctBCD* genes were deleted, lactate formation from glycine betaine + CO was completely abolished. This is consistent with the fact that the *lctBCD* genes were highly upregulated in the Δ*hydBA*/*hdcr* mutant during glycine betaine + CO fermentation.

Conclusion: Metabolic engineering of the homoacetogen *A. woodii* leads to lactate formation not only from fructose but also from C1 compounds, which gives a potential for sustainable industrial applications.

[1] Moon, J., Schubert, A., Poehlein, A., Daniel, R., Müller, V. (2023). Environ. Microbiol. Rep., in press

[2] Moon, J., Schubert, A., Waschinger, L. M., Müller, V. (2023). ISME J., in press

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**AMP004**

**A higher number of DNA phages integrate in microbial genomes after a starvation event in a biomethanation reactor showed by a genome-centric analyses of 54 metagenomes**

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We hypothesized that viruses influence the resilience of hydrogenotrophic communities during intermittent starvation. To test our hypothesis, we evaluated how H2 and CO2 starvation affect the dynamics of prokaryotes and viruses in biomethanation reactors. We operated three controls and three treatment bioreactors in parallel for approximately 100 days. Samples for metagenomics were collected in parallel at nine-time points in three experimental phases: stabilization, starvation, and recovery. Using MuDoGeR, we recovered metagenome-assembled genomes (MAGs) and uncultivated viral genomes (UViGs) from the 54 metagenomes. All UViGs were dereplicated and reassessed using VIBRANT. Recovered MAGs and UViGs were dereplicated at an average nucleotide identity (ANI) of 0.95 (a proxy for species). Subsequently, the MAGs in each cluster were manually curated and defined as operational taxonomic units (pOTUs, proxy for strain level), and dereplicated complete and high-quality UViGs were defined as viral operational taxonomic units (vOTUs). We also assigned the virus-host pairs using WiSH. From the 54 metagenomes, we recovered 877 MAGs dereplicated into 49 pOTUs. Although Firmicutes A encompassed ~45% of the observed pOTUs, Methanobacterium spp. (Archaea) showed the highest relative abundance in all libraries (81-95%). We identified 12 vOTUs (8 Caudoviricetes and 4 unclassified viruses). Lifestyle analyses indicated six Caudoviricetes vOTUs and one unclassified vOTU as a temperate virus. Host prediction analysis using WISH indicated three temperate vOTUs that may infect Bacteria and Archaea. WiSH predicted PTOGUVIG_KN11 (Caudoviricetes) and PTOGUVIG_KN12 (Unclassified virus) may infect Methanobacterium spp. (Archaea, p=0.041), Aminobacterium colombiense (Bacteria, p=0.032) and eight species from Clostridia, including Clostridium V ultunense (Bacteria, p=0.023). Statistical analysis (t-test) indicated that the number of MAGs with prophages was significantly higher in treated bioreactors than in the parallel controls during the starvation and recovery phase ($p < 0.05$). Our data suggest viruses can switch from the lytic to the lysogenic cycle under starvation in bioreactor systems. This switch may be a survival strategy when Prokaryotes are depleted of nutrients and energy limits virus replication. Understanding how to control virus survival strategies will open new doors for bioreactor engineers by the targeted introduction of novel functions into Prokaryotes using viruses.

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**AMP005**

**Metabolic pathways involved in anaerobic degradation of C1 or C2 compounds during axenic versus syntrophic growth of *Thermacetogenium phaeum***

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*Thermacetogenium phaeum*, a thermophilic acetogen, is known for its capacity to degrade C1 and C2 compounds under axenic or syntrophic conditions. Although the degradation of acetate is thermodynamically unfavorable, *T. phaeum* can degrade it in collaboration with a methanogenic partner in syntrophic culture. However, the mechanisms underlying energy conservation in *T. phaeum* are still not fully understood. Unlike the well-studied model acetogen *Acetobacterium woodii*, *T. phaeum* lacks the Rnf-complex, which is considered essential for energy conservation in anaerobic acetogenic bacteria. In this study, we compared the proteome data obtained from the growth of *T. phaeum* with ethanol, methanol, ethanolamine, and acetate as substrates under axenic or syntrophic conditions. Proteomics analysis revealed that the Wood-Ljungdahl pathway (WLP) enzymes, which form the central metabolic pathway in degradation of C1 and C2 compounds in *T. phaeum*, showed high abundance under all growth conditions. Regardless of the growth substrate, aldehyde:ferredoxin oxidoreductase (AOR) was found to be constitutively abundant, indicating that acetaldehyde is the central metabolite during the degradation of C1 and C2 compounds since AOR catalyzes the oxidation of acetaldehyde to acetate. Moreover, one ATP could be "saved" by using AOR instead of acetate kinase in acetate degrading direction. Additionally, membrane-bound formate dehydrogenase and hydrogenase were regulated differently under syntrophic and axenic conditions. Most likely, these enzymes are responsible for transferring reducing equivalents to the syntrophic partner, thus promoting the generation of a transmembrane ion gradient. We suggest that AOR, membrane-bound formate dehydrogenase and hydrogenase are the key energy-conserving enzymes for syntrophic acetate oxidation in *T. phaeum*.

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**AMP006**

**Evaluation of biotechnological propionate production in *Bacteroidetes***

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Background: *Bacteroidetes* play an important role in the gastrointestinal tract of animals and humans, as they degrade a variety of polymers to short chain fatty acids (acetate, succinate, propionate) [1]. Propionate serves as a precursor in many industries including food- and feedstock preservation and is needed in large quantities. However, the majority is still produced via petrochemical processes and current advances for microbial production have not yet met industrial standards due to low yield and/or productivity [2].

Objectives: Here, we investigate the propionate pathway and biotechnological application of *Bacteroidetes*, since they degrade industrially relevant feedstocks.

Methods: To evaluate common propionate producers, 10 strains from the genera *Bacteroides*, *Parabacteroides* and *Phocaeicola* were screened in defined minimal medium with 15 mM of glucose (DMMG) and 0.5 g/l yeast extract (DMMG-Y). Furthermore, $CO_2$ dependence of the two best propionate producing strains was tested in growth experiments with $NaHCO_3$ concentrations from 0 to 50 mM.

Results**:** Propionate formation varied among the strains. The highest yield of 0.39 $g_{pro}/g_{gluc}$ was achieved by *B. propionicifaciens,* followed by *B. graminisolvens* (0.25 $g_{pro}/g_{gluc}$). While the growth rate of *B. propionicifaciens* was enhanced by yeast extract, *B. graminisolvens* was not affected. Low concentrations of $NaHCO_3$ did not affect the propionate yield of *B. propionicifaciens,* but the growth rate decreased from 0.2 $h^{-1}$ (50 mM) to 0.12 $h^{-1}$ (0 mM). The propionate yield of *B. graminisolvens* decreased with lower concentrations due to the formation of lactate and the strain did not grow without $NaHCO_3$, showing that it is more dependent on $CO_2$ than *B. propionicifaciens.* When *B. propionicifaciens* was grown in a pH-controlled fed-batch with 135 mM glucose, it produced 119 mM propionate and reached an $OD_{600}$ of 8.12. Succinate was produced in elevated amounts, and the maximum yield was 0.38 $g_{pro}/g_{gluc}$.

Conclusion: We developed a standardized protocol to identify the two best propionate producers among the 10 strains tested and enhanced the propionate titre of *B. propionicifaciens* to 8,8 g/l in a pH-controlled batch fermentation. Currently, we are aiming to improve fermentation conditions as well as genetically engineering

the strains towards industrial platform organisms for the conversion of polymers to propionate.

Thomas *et al*. 2011, Front Microbiol 2: 93.

Gonzalez-Garcia, *et al*. 2017, Fermentation 3 (2): 21.

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**AMP007**

**Characterization of ferredoxins from the thermophilic, acetogenic bacterium *Thermoanaerobacter kivui***

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Introduction: The iron-sulfur-containing and electron-transferring protein ferredoxin is a major electron carrier in the energy and carbon metabolism of the acetogenic model organism *Thermoanaerobacter kivui*. The genome encodes four potential, different ferredoxins whose function remained elusive.

Objectives: To shed light on the biochemistry and functional role of the four encoded putative ferredoxin-like proteins of *T. kivui* (TKV_c09620, TKV_c16450, TKV_c10420, TKV_c19530).

Materials & methods: Individual deletion mutants *T. kivui* Δ*TKV_c09620*, Δ*TKV_c16450*, Δ*TKV_c10420* and Δ*TKV_c19530* were created. Growth experiments and transcriptional analysis were done. All four genes were cloned, with an added His-tag encoding sequence, into a vector for protein production in *T. kivui* and purified. Their interaction partners were revealed by enzyme assays.

Results: The deletion of the individual ferredoxin-like genes led to a slight reduction in growth of all mutants on pyruvate or $H_2$ + $CO_2$. The determination of relative transcript level revealed an upregulation of *TKV_c09620* transcripts in the Δ*TKV_c16450* mutant and conversely *TKV_c16450* in the Δ*TKV_c09620* mutant. Enzyme assays showed that TKV_c09620 and TKV_c16450 were used as electron acceptors for the pyruvate:Fd oxidoreductase, electron-bifurcating hydrogenase, monomeric CODH, bifunctional CODH and methylene-THF reductase. The iron content determined matched the prediction that TKV_c09620 and TKV_c19530 contain two [4Fe4S]- and TKV_c16450 and TKV_c10420 one [4Fe4S]-cluster, respectively.

Conclusion: In sum, our data revealed that TKV_c09620 and TKV_c16450 are indeed ferredoxins. Biochemical and genetic analysis showed that TKV_c09620 and TKV_c16450 are involved in autotrophic and heterotrophic metabolism of *T. kivui*.

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**AMP008**

**New insights into the interaction between the individual subunits of the benzylsuccinate synthase (Bss) and their influence on its activation**

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In recent years, anaerobic toluene degradation has been shown to proceed by a common pathway in various unrelated groups of bacteria which couple toluene degradation to anaerobic respiration or fermentation in syntrophic cocultures [1]. The pathway is initiated by (*R*)-Benzylsuccinate Synthase (BSS), which belongs to the family of glycyl radical enzymes and contains a conserved glycine residue close to the C-terminus, which is converted to a glycyl radical moiety by an activating enzyme [1]. The enzyme catalyzes the addition of the methyl group of toluene to the double bond of a fumarate cosubstrate and contains two small [FeS]-cluster containing subunits in addition to the large catalytic subunit[1] [2]. The structure and reactivity of BSS provides a prototype for many other fumarate-adding enzymes (FAE) initialising anaerobic degradation of other hydrocarbons, such as xylenes, cresols, methylnaphthalene, or alkanes.

In our ongoing study, we are creating and characterizing variants of BSS via targeted mutagenesis to understand more of the reaction mechanism of BSS, especially the contributions of conserved amino acids of the active center and the small subunits for catalysis. In addition to expand or alter the substrate range of BSS, we are also trying to characterize other fumarate-adding enzymes and test them for potential applications.

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**AMP009**

**Metagenomic and metatranscriptomic analyses reveal a new limonene degradation pathway in a methanogenic enrichment**

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Introduction. Limonene is a biogenic hydrocarbon, classified as a cyclic monoterpene, and naturally occurs in the peel of citrus fruits and is responsible for the lemon-like scent. It is often used as a flavoring or fragrance compound in cosmetics, food and cleaning products. Annual industrial extraction of limonene from natural sources of approximately 60 000 tons is an indication for its abundance in nature. The degradation of limonene in methanogenic enrichments has been observed, however, anoxic degradation pathways of limonene under methanogenic conditions has so far not been described.

Objectives: A limonene-degrading methanogenic enrichment culture had been maintained for over twenty years. This study analyzed metagenomes and metatranscriptomes of the limonene-degrading enrichment culture to identify the degradation pathway for the biogenic hydrocarbon limonene.

Materials & methods: The DNA was sequenced using PacBio HiFi sequencing technology. The metagenomic reads were assembled using Flye and corrected using Inspector. The contigs were binned using Vamb, MaxBin2 and MetaBAT2 and a non-redundant set of bins was selected using DAS Tool. Annotation of contigs and bins was performed by Bakta. The RNA was sequenced by Illumina and after quality trimming, the reads were mapped onto the open reading frame sequences of the contigs and the transcripts per million (TPM) were calculated.

Results: 32 metagenomic assembled genomes were retrieved from a PacBio metagenome including 5 genes coding for proteins of the benzylsuccinate synthase superfamily known to be involved in the degradation of

aromatic and aliphatic hydrocarbons. Metatranscriptomic analysis revealed that one of these genes, a potential limonenylsuccinate synthase in a *Syntrophobacteraceae*, is among the 300 most highly expressed genes. A 6-oxocyclohex-1-ene-1-carbonyl-CoA hydrolase was also highly expressed. This enzyme may catalyze the ring-opening of a limonenylsuccinate compound.

Conclusion: Metagenomic and metatranscriptomic analysis revealed the presence of a new limonene degradation pathway under methanogenic conditions. The proposed name for the enzyme catalyzing the initial step of the limonene degradation is limonenylsuccinate synthase. It enlarges the substrate range of hydrocarbonsuccinate synthases to biogenic monoterpenes.

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**AMP010**

**Ethanologenesis from glycerol in the gut acetogen *Blautia schinkii***

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Introduction: Acetogens play an important role in the human gut microbiome. In 2008 different bacteria of the human gut microbiome were reclassified as "*Blautia*"[1], which play a crucial role in the human well-being[2]. However, only little is known about the physiology of different *Blautia* species. Here, we characterized for the first time the glycerol metabolism of the human gut acetogen *Blautia schinkii*.

Objectives: We aimed to unravel glycerol metabolism of *B. schinkii*. The pathway for glycerol fermentation was determined by bioinformatic, physiological and biochemical methods. In addition, we studied ways to increase ethanol production from glycerol by *B. schinkii*.

Materials & methods: Growth experiments, fermentation analyses in resting cells, enzyme assays, genome analysis and analysis of gene expression were used to identify the glycerol degradation pathway and to determine fermentation products.

Results: *B. schinkii* is able to grow on glycerol as substrate. Glycerol is first oxidized by a glycerol dehydrogenase to dihydroxyacetone (DHA), activated by a DHA kinase and metabolized *via* glyceraldehyde-3-phosphate to pyruvate. Fermentation products are acetate, ethanol and trace amounts of lactate and $H_2$. At low glycerol concentrations ethanol and acetate are produced in almost equal amounts but at higher glycerol concentrations ethanol is the almost only product. *B. schinkii* was able to tolerate glycerol concentrations up to 1.5 M. Furthermore, *B. schinkii* is able to grow on DHA, producing acetate and ethanol as major products.

Conclusion: The human gut acetogen *B. schinkii* can perform homoethanolgenesis at high glycerol concentrations, presenting a novel feature of acetogenic gut bacteria that might be of relevance not only for the human well-being but also for bioindustrial applications.

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**AMP011**

**The formate-hydrogenlyase complex of *Trabulsiella guamensis***

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Introduction: Many *Enterobacteriaceae* produce hydrogen ($H_2$) through the activity of the formate hydrogenlyase (FHL) complex during their mixed-acid fermentation. The detoxification of formate and removal of protons ($H^+$) from the cytoplasm are the main functions of the FHL complex. The soluble, cytoplasmically-oriented arm of the complex catalyses two reactions: 1. Formate oxidation to $CO_2$ and a $H^+$ is catalysed by a formate dehydrogenase (FdhH); 2. Both electrons from this oxidation are transferred via iron-sulfur clusters to the hydrogenase, where they reduce two $H^+$ to $H_2$. The soluble arm is furthermore attached to a membrane domain, which, depending on the FHL complex, comprises a variable number of membrane-integral subunits. The biochemically well-characterized FHL-1 complex in *E. coli* has two membrane subunits (HycCD), while the less well-characterized FHL-2 has five membrane subunits (HyfB-F). *Trabulsiella guamensis* is an enterobacterium which only encodes an FHL-2 complex.

Objectives: Despite the similarity of FHL complexes to complex I of the respiratory chain, the possible involvement of the membrane subunits in proton pumping and their contribution to the catalytic reactions of the soluble arm remain to be established. Purification of an active FHL-$2_{Tg}$ complex is the first step in studying this potential contribution.

Methods: We generated plasmids carrying the *T. guamensis hyf* genes and introduced them into a hydrogenase-deficient *E. coli* strain. FHL-$2_{Tg}$ was anaerobically purified from this strain via Ni-NTA IMAC. Analyses were performed via activity-assays, gas chromatography, SDS- & Blue-Native PAGE, as well as western blots.

Results: We report the purification of the active FHL-$2_{Tg}$ complex. Aerobically purified complexes do not catalyse formate-dependent $H^+$ reduction, despite detectable formate dehydrogenase and hydrogenase activities measured using viologen dyes. Anaerobic purification of the complex does, however, result in an active complex that catalyses $H_2$ production from formate.

Conclusion: We were able to purify active FHL-$2_{Tg}$ complex. This is the first required step in investigating the potential proton-pumping activity of this complex.

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**AMP012**

**Single-particle CryoEM structure of a tungsten-containing aldehyde oxidoreductase that forms nanowires**

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Introduction: Bacterial aldehyde oxidoreductases (AOR) are proteins that belong to the tungstoenzymes family. The AOR from *Aromatoleum aromaticum* (AOR*Aa*) is not only able to oxidize a wide spectrum of aldehydes to their acid form, but can also reduce organic acids by using low-potential electron donors or even hydrogen [1]. Previously, the only structural information of these enzymes relied on AORs from the hyperthermophilic archaeon *Pyrococcus furiosus*. In contrast, AOR*Aa* differs from its archaeal homologs given its activity at room temperature, a higher oxygen tolerance and multi-subunit architecture [2]. The AOR*Aa* complex is composed by the ferredoxin-like subunit AorA, a FAD-containing subunit AorC and the catalytically active AorB which carries the tungsten-*bis*-MPT cofactor (W-cofactor). However, the lack of a protein structure limits the understanding of its mechanistic insights.

Objective: We aimed to determine a high-resolution structure of a bacterial multimeric AOR, with special focus in the binding coordination of the W-cofactor.

Materials and methods: We combined single-particle cryo-electron microscopy (CryoEM), mass photometry (MP) and site-directed mutagenesis with QM:MM methods to establish a model of AOR*Aa*.

Results: The tridimensional structure of the filament-forming AOR*Aa* was determined by CryoEM at 3.22 Å resolution, showing an asymmetrical Aor(AB)2C complex where the repeating AorAB protomers are capped by the NAD-binding subunit AorC. In the catalytic subunit AorB, QM:MM modelling was used to fit the tungsten-center from the W-cofactor within the electron density at the active site. Interestingly, the subunit AorA containing four [4Fe-4S] clusters oligomerizes in a polyferredoxin-like fashion to resemble an electron-conducting nanowire. To test the role of AorA in the filament formation, we constructed a variant lacking the C-terminal helix proposed to be the binding region that leads to higher-order AOR*Aa*. *In vitro* enzymatic assays with Δhelix-AOR*Aa* showed a 2-fold decrease in the specific activity and, supported by MP experiments, we validated that this variant was unable to form filaments.

Conclusion:

Altogether, the structural information provided by single-particle CryoEM and the experimental evidence confirmed the formation of nanowire-like filaments by AOR*Aa*, a unique redox-enzyme with potential applications in biotechnology.

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**AMP013**

**Optimization and biochemical characterization of hydrogen formation from formate by the acetogen *Thermoanaerobacter kivui***

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Introduction: *Thermoanaerobacter kivui* is an anaerobic, thermophilic acetogen that uses the Wood-Ljungdahl pathway to produce acetate from $CO_2$ and $H_2$. The first enzyme in this pathway, the hydrogen-dependent $CO_2$ reductase (HDCR), catalyzes the reduction of $CO_2$ to formate with the concomitant oxidation of $H_2$ to protons. In times of climate change, the conversion of $H_2$ to formate is a promising way for $H_2$ storage.

Objectives: We aimed to further optimize hydrogen production from formate by *Thermoanaerobacter kivui* and to identify proteins that are important for HDCR activity.

Materials & methods: Cell suspension experiments with resting cells, mutant studies, growth studies and enzyme activities.

Results: The HDCR is one of the fastest known enzymes for the formation of hydrogen from formate. Optimal conditions for hydrogen production from formate by resting cells were high temperatures (70 °C) and low cell concentrations (0.3 mg/ml). The highest $H_2$ production was measured at a constant pH of 7, however, at lower pH (5.5-7) the initial $H_2$ production rate was increased[1].

The deletion of the gene *fdhD* resulted in significantly lower hydrogen production in cell suspension experiments. Furthermore, the formate:MV-oxidoreductase activity in crude extract of the *fdhD* deletion mutant was significant lower than in the wildtype. In sharp contrast, $H_2$:MV-oxidoreductase activity in crude extract of the *fdhD* mutant was significantly increased.

Conclusion: We were able to determine the optimal conditions for hydrogen production from formate by resting cells of *T. kivui*. FdhD is essential for formate dehydrogenase activity of HDCR.

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**AMP014**

**Structure and function of the nitrogenase-like reductase CfbC/D involved in coenzyme $F_{430}$ biosynthesis**

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The metallo-enzyme nitrogenase and its functional and structural homologs are involved in essential biological processes, such as nitrogen fixation, photosynthesis and biological methane formation (methanogenesis). Nitrogenase catalyzes the biological nitrogen fixation, and thus, the reduction of dinitrogen to ammonia. A structurally simpler, iron-sulfur cluster containing nitrogenase-like enzyme system called CfbC/D (Coenzyme $F_{430}$ Biosynthesis) is involved in the biosynthesis of coenzyme $F_{430}$, the essential cofactor of methyl-coenzyme M reductase in methanogenesis. During the biosynthesis of coenzyme $F_{430}$ the tetrapyrrole macrocycle of $Ni^{2+}$-sirohydrochlorin a,c-diamide (Ni-SHC-D) is reduced by CfbC/D to $Ni^{2+}$-hexahydrosirohydrochlorin a,c-diamide (Ni-H6-SHC-D) by the addition of 6 electrons and 7 protons. In homology to nitrogenase, CfbC/D consists of a reductase component and a catalytic component. The reductase component is a homodimer of CfbC carrying an intersubunit [4Fe-4S] cluster. The catalytic component is composed of a homodimer of CfbD bridged by an intersubunit [4Fe-4S] cluster, in contrast to the heterotetrameric catalytic component of nitrogenase.

CfbC and CfbD from the hyperthermophilic methanogen *Methanocaldococcus jannaschii* were produced in *Escherichia coli*. In contrast to nitrogenase, the reductase

$CfbC_2$ showed ATP hydrolysis activity in the absence of the catalytic component $CfbD_2$. However, ATP hydrolysis was stimulated by adding $CfbD_2$. Also, chimeric complexes of $CfbC_2D_2$ from *Methanosarcina barkeri* and *M. jannaschii* showed ATPase activity and the highest activity was observed with $CfbC_2$ from *M. barkeri*. Using microscale thermophoresis, the $K_d$ values for the interaction between $CfbC_2$ and $CfbD_2$ from *M. jannaschii* with and without nucleotides were determined. In the absence of nucleotides, a $K_d$ value of 123 ± 3 nM was determined. The addition of nucleotides (non-hydrolysable ATP analog or ADP) led to a substantial increase of the $K_d$ value. The exchange of leucine 125 to proline in the switch II motif of CfbC led to a slight increase in the affinity of the proteins. Further, this exchange led to a lower tetrapyrrole conversion but nearly the same ATPase activity. Structural analysis using Cryo-EM indicated two different conformations of the $CfbC_2D_2$ complex with or without substrate.

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**AMP015**

**Molecular architecture and electron transfer pathways in the Stn family of transhydrogenases**

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Introduction: Electron bifurcation is a fundamental energy coupling mechanism widespread in anaerobic microorganisms that thrive under conditions of extreme energy limitation. In acetogenic bacteria, electron-bifurcating transhydrogenases balance the nucleotide pool and provide correct electron carriers in the right stoichiometry for $CO_2$ reduction in the Wood–Ljungdahl pathway(1). Depending on the species, two very different electron-bifurcating transhydrogenases have been described, the Nfn- or Stn-type complexes that catalyse the endergonic reduction of $NADP^+$ with NADH and reduced ferredoxin as reducing agents(2,3). While the two-subunit Nfn is the most studied electron-bifurcating enzyme, nearly nothing is known about the three-subunit Stn complex which rather resembles the electron-bifurcating hydrogenase from anaerobic bacteria and archaea(3).

Objectives: We aimed to resolve the structure and functionally dissect the electron transfer pathway of the Stn class transhydrogenase from the acetogenic bacterium *Sporomusa ovata*.

Materials & methods**:** Protein purification, heterologous protein production, anaerobic Cryo-EM, mutational and functional studies.

Results**:** We solved the cryo-EM structure of the StnABC complex under anoxic and catalytic turnover conditions. It forms a tetrameric assembly with each protomer being the minimal functional unit. The StnAB subcomplex is similar to the electron-bifurcating subcomplex HydBC of the HydABC hydrogenase, while StnC is a very large protein possessing a NuoG-like domain and a GltD-like NADPH binding domain that strongly resembles NfnB of the NfnAB complex but cannot bifurcate electrons. A comparison now allows us to describe how the same fold, on the one hand, developed a bifurcation activity on its own and, on the other hand, was able to combine with an associated bifurcating module. This study exemplifies how modular evolution in anaerobic metabolism can produce novel activities critical for survival at the thermodynamic limit of life.

Conclusion: Our cryo-EM structure and functional analyses unravelled the structure, the electron transfer pathway, and the mechanism of electron bifurcation in the Stn transhydrogenase.

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**AMP016**

**Nitrogen fixation by the Iron nitrogenase is inhibited by $CO_2$**

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*Rhodobacter capsulatus* is able to fix molecular nitrogen ($N_2$) and convert it into ammonia catalysed by the nitrogenase enzyme. It possesses the conventional Mo nitrogenase and the alternative iron (Fe) nitrogenase.[1] The expression of the nitrogenases is tightly regulated. The Mo nitrogenase is expressed in the presence of molybdenum due to its higher efficiency, while the alternative Fe nitrogenase functions as a fail-safe enzyme under Mo limitation.[2] Despite their homology, the Mo and Fe nitrogenase differ in their metal content, quaternary structure as well as their activity and substrate profile. Besides their natural substrates $H^+$ and $N_2$, nitrogenases reduce $CO_2$, a common metabolite under heterotrophic growth conditions.[3] Hence, we set out to investigate the substrate competition between $CO_2$ and $N_2$ in *R. capsulatus* for the two nitrogenases.

At first, we constructed KO strains of *R. capsulatus* that exclusively express either the Mo or Fe nitrogenase. Their growth behaviour was evaluated in the presence and absence of additional $CO_2$ under diazotrophic growth. In addition, the products of the $CO_2$ reduction (HCOOH, $CH_4$ and CO) were quantified to monitor the promiscuous activity of the nitrogenases *in vivo*.

The diazotrophic growth rates of *R. capsulatus* strains expressing the Fe nitrogenase reduced by high $CO_2$ concentration, whereas no change was observed for the strains expressing the Mo nitrogenase. We therefore concluded that nitrogen fixation in *R. capsulatus* is inhibited by high $CO_2$ concentrations for strains expressing the Fe nitrogenase. Further, an increase of formate in the culture media and methane in the headspace was observed under $CO_2$.

The promiscuous activity of the Fe nitrogenase was characterized *in vitro*, showing a less stringent selectivity of the Fe nitrogenase compared to the Mo nitrogenase for $N_2$ over $CO_2$. Hence, the incorporation of Mo increased the nitrogenase efficiency and additionally the selectivity for $N_2$.

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**AMP017**

**Methane formation driven by reactive oxygen species across all living organisms and the early earth**

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Methane ($CH_4$), the most abundant hydrocarbon in the atmosphere, originates largely from biogenic sources linked to an increasing number of organisms occurring in oxic and anoxic environments. Traditionally, biogenic $CH_4$ has been regarded as the final product of anoxic decomposition of organic matter by methanogenic archaea. However, several plants, fungi, algae and cyanobacteria were shown to also produce $CH_4$. In our studies, we intended to (i) to identify the common mechanism behind these phenomena and (ii) expand these findings regarding the general environment outside living organisms with particular focus on the early Earth.

During these studies, we quantified $CH_4$ via gas chromatography that was formed in closed glass vials containing (i) buffer/media/cultures, (ii)iron and (iii) methylated sulfur compounds under variable further reaction conditions.

We demonstrate that $CH_4$ formation from over 35 model organisms from the Bacteria, Archaea and Eukarya domainss is triggered by free iron and reactive oxygen species (ROS), which are generated by metabolic activity and enhanced by oxidative stress. ROS-induced methyl radicals, which are derived from organic compounds containing sulfur- or nitrogen-bonded methyl groups, are key intermediates that ultimately lead to $CH_4$ production. Apart from $CH_4$ being formed inside all living organisms, we also demonstrate new routes towards abiotic $CH_4$ formation under early-earth conditions from organosulfur compounds with prebiotic origin via ROS produced by light and heat in aqueous environments. Organic iron chelators enhance reaction rates and recycle ferric to ferrous complexes via ligand-to-metal charge transfer (LMCT), thereby (i) establishing a light-driven iron redox cycle and (ii) also driving $CH_4$ formation via the LMCT-induced organoradical.

On both an intra- and extracellular level, this non-enzymatic reaction facilitates $CH_4$ and ethane formation across Earth"s humid realm, thereby contributing to atmospheric $CH_4$ emissions from the early Earth until today. The correlation between human $CH_4$ production and cellular stress levels may furthermore be used as diagnostic tool. Finally, our findings shed a new light on the importance of $CH_4$ as a biosignature on exoplanets.

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**AMP018**

**Tungsten enzyme catalyzed reductions**

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Molybdenum and tungsten are transition metals that are both present in biological systems. These metals occur in the active site of Mo- and W-dependent enzymes where they are coordinated to a metallopterin cofactor and are usually involved in the catalysis of various redox reactions. Although molybdenum and tungsten share similar chemical properties, they differ in the redox potentials of their biologically relevant oxyanions. Therefore, some reactions of this enzyme family have been found to be exclusively catalysed by tungsten-containing enzymes. This includes the direct reduction of carbonic acids to the corresponding aldehydes without any need of prior activation. One example for these W-dependent enzymes is the aldehyde oxidoreductase from *A. aromaticum* EbN1. This enzyme is a member of the bacterial subfamily of AOR and has been shown to catalyze the oxidation of various aldehydes as well as the reverse reaction. The reverse reaction provides a versatile tool in the reduction of various acids of interest to the corresponding aldehydes, especially in coupled reactions with other enzymes, like alcohol dehydrogenases or aminotransferases. Many organisms producing W-enzymes employ selective pathways for synthesising Mo- and W-containing enzymes simultaneously. Here we introduce potential applications of AOR as well as the potential mechanisms involved in the highly selective incorporation of tungsten during cofactor maturation of AOR.

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**AMP019**

**Characterization of the cobalamin-dependent *Radical* SAM methyltransferase QCMT involved in maturation of methyl-coenzyme M reductase**

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The enzyme methyl-coenzyme M reductase (MCR) plays an important role in the global carbon cycle due to its ability to catalyze both, the formation and consumption of methane in methanogenic archaea and anaerobic methane-oxidizing archaea, respectively [1]. This enzyme possesses several unusual post-translational amino acid modifications, including 2-C-(*S*)-methylglutamine [2]. The *Radical* SAM methyltransferase (QCMT) is responsible for this chemically challenging methylation of the unreactive $sp^3$ carbon atom at C2 of glutamine [3]. QCMT belongs to the subfamily of cobalamin-dependent Radical SAM methyltransferases and was initially characterized in a recent study.

In this project, we aim to solve the structure QCMT through crystallization and x-ray. Using the sitting drop method, first protein crystals were generated under anaerobic conditions, however, they have not been analyzed yet.

The second aim is the elucidation of the reaction mechanism. It has been demonstrated that QCMT catalyzes the transfer of a methyl group to the glutamine residue of a peptide substrate via a radical-based mechanism. During the methylation reaction, two molecules of SAM are consumed, and 5"-deoxyadenosine (DOA) and *S*-adenosyl-L-homocysteine (SAH) are formed as coproducts in a nearly 1:1 ratio. The methyl group itself originates from enzyme-bound methylcobalamin, which is re-methylated by SAM. It was also shown that peptides of different lengths can be used by QCMT. In further studies, the role of amino acid residnes within the active site as well as of the peptide substrate will be investigated.

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**AMP020**

**Hexanoate production with metabolically engineered *Acetobacterium woodii* strains using methanol**

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Introduction: Hexanoate is a platform chemical and is currently produced petrochemically. The anaerobic acetogenic bacterium *Clostridium carboxidivorans* is naturally capable of producing hexanoate from C1-carbon sources via reverse β-oxidation encoded by the *hcs* (hexanoyl-CoA synthesis)-operon [1]. Recombinant *Acetobacterium woodii* cells were constructed expressing this *hcs*-operon under control of the lactose inducible promoter P*bgaL*. Moreover, the greenFAST (fluorescence-activating and absorption-shifting tag) encoding gene was cloned downstream of the hcs cluster and served as expression control. Hexanoate production from methanol was analysed in combination with the gas phases H2 + CO2, H2 + N2, and N2 + CO2.

Objectives: The aim of this study was recombinant hexanoate production using metabolically engineered *A. woodii* strains, by performing batch experiments with methanol in combination with hydrogen containing gas phases.

Materials and Methods: The *A. woodii* [pMTL38251_P*bgaL*_hcs_greenFAST] strain was characterized in batch growth experiments using methanol in combination with H2 + CO2, H2 + N2, and N2 + CO2 regarding growth and formation of the products acetate, butyrate, and hexanoate. During growth of cells, the greenFAST mediated fluorescence was determined via spectral photometry to confirm expression of the *hcs*-operon.

Results: *A. woodii* [pMTL38251_P*bgaL*_hcs_greenFAST] utilized methanol and produced 3.9 mM hexanoate and 1.2 mM butyrate in combination of N2 + CO2 in the gas phase. With H2 + CO2, or H2 + N2 in the gas phase no methanol uptake or hexanoate and butyrate was determined. Green fluorescence indicated the expression of the *hcs*-operon.

Conclusion: *A. woodii* is an ideal model acetogen for production of higher value biocommodities or fuels from methanol, however, H2 might be a restricting factor for methanol uptake.

[1] Wirth and Dürre, 2021, doi: 10.1016/j.ymben.2021.04.010.

**AMP021**

**Expanding the repertoire of counterselection markers for markerless gene deletion in the human gut bacterium *Phocaeicola vulgatus***

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Introduction: The microbial order Bacteroidales exhibits strain-specific differences in their physiology, ecology, and impact on the intestinal tract. Of special interest are members of the genus Phocaeicola, which are closely associated with human health and were shown to affect the physiological state of the gut. *Phocaeicola* (*P.*) *vulgatus* (formerly *Bacteroides vulgatus*) is a highly abundant and ubiquitous member of the human gut microbiota, and therefore represents an important target for further investigations.

Objectives: The availability and diversity of tools for the genetic manipulation of *Phocaeicola* strains is quite limited. In this study a novel gene deletion method was developed for *P. vulgatus*, expanding the tools available for genetic manipulation of members of the microbial order Bateroidales.

Materials & Methods: A combination of bioinformatics and growth experiments in interaction with molecular cloning was applied to evaluate the applicability of SacB as a counterselection marker in *P. vulgatus*.

Results: In this study, the levansucrase gene *sacB* from *Bacillus subtilis* was verified as a functional counterselection marker for *P. vulgatus*, conferring a lethal sensitivity towards sucrose. Markerless gene deletion based on SacB was applied to delete a gene encoding a putative endofructanase (BVU1663). The *P. vulgatus* Δ*1663* deletion mutant displayed no biomass formation when grown on levan, inulin or their corresponding fructooligosaccharides. This system was also applied for the deletion of the two genes *bvu0984* and *bvu3649*, which are involved in the pyrimidine metabolism. The resulting *P. vulgatus* Δ*0984* Δ*3649* deletion mutant no longer showed sensitivity for the toxic pyrimidine analogon 5-fluorouracil, allowing a counterselection with this compound in the double knockout strain.

Conclusion: The genetic toolbox for *P. vulgatus* was expanded by a markerless gene deletion system based on SacB as an efficient counterselection marker. The system was employed to successfully delete three genes in *P. vulgatus* which all resulted in expected phenotypes as confirmed by subsequent growth experiments.

**AMP022**

**$CO_2$-based production of caproic acid by engineered a *Acetobacterium woodii* strain and *Clostridium drakei* WT applying a synthetic co-culture (CaproSyn)**

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Climate change is caused by increasing emissions of greenhouse gases such as $CO_2$ into our atmosphere. Acetogenic organisms such as *Acetobacterium woodii* are capable of autotrophic growth using $CO_2$ as carbon and $H_2$ as energy source, while producing acetate as a product. By knocking out the native genes encoding the confurcating lactate dehydrogenase complex and plasmid-borne expression of a D-lactate dehydrogenase (*ldhD*) from *Leuconostoc mesenteriodes,* new recombinant *A. woodii* strain emerged that can autotrophically produce lactate via the Wood-Ljungdahl pathway (Mook et al., Appl Microbiol Biotechnol 106, pp. 1447-1458 (2022)). The *A. woodii* strain was co-cultured with *Clostridium drakei* and the produced lactate served as a substrate for *C. drakei* cells to produce chain-extended carboxylic acids (Herzog et al., Eng LifeSci 23:e2100169 (2022)).

The goal of this work is to optimize the lactate production of new *A. woodii* strains for a higher production of lactate and caproate in the co-culture Therefore, the methylbranch of *A. woodii´*s Wood-Ljungdahl pathway will be bypassed using

the enzymatic reaction of the pyruvate-formate-lyase (PFL). PFL functions in a reversable manner in many anaerobic bacteria (e.g. *Clostridium pasteurianum*) and can convert acetyl-CoA together with formate into CoA and pyruvate. It depends on a PFL-activating enzyme (ACT). ACT activates PFL by introducing a glycyl radical in its Gly radical domain.

A plasmid containing the *pfl* as well as the *act* gene from *C. pasteurianum* was constructed. Thereby *act* is controlled by the P*pta-ack* promoter and *pfl* by the theophylline-inducible P*ackA*-theo promoter.

The *ldhD* gene is encoded on the plasmid pMTL83_PlctA_NFP and the *pfl* as well as *act* gene are encoded on the plasmid pMTL87_Ppta-ack_act_PackA-theo_pfl. Thus, a two-plasmid system is used for gene expression in the recombinant *A. woodii ΔpyrE ΔlctBCD* strain. Growth characteristics and product formation of the respective strain will be analyzed under heterotrophic and autotrophic conditions.

Bypassing the methylbranch of the Wood-Ljungdahl pathway presents a promising perspective for increasing the lactate production in *A. woodii* and in co-culture an increase of the butyrate and caproate production by *C. drakei*.

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**AMP023**

**Structure and function of a 1-MDa electron-confurcating/bifurcating, dearomatising enzyme complex from obligately anaerobic bacteria**

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Benzoyl-CoA reductases (BCRs) are key enzymes in the anaerobic microbial degradation of aromatic compounds, catalysing the reduction of benzoyl-CoA to 1,5-dienoyl-CoA. This reaction proceeds at a redox potential of $E^{\circ\prime} = -622$ mV, which is far below known physiological electron donors [1]. Two classes of structurally unrelated BCRs have been identified: While class I BCRs couple the endergonic reduction of benzoyl-CoA to ATP hydrolysis in facultative anaerobes, ATP-independent class II BCRs are proposed to catalyse this reaction in obligate anaerobes by flavin-based electron bifurcation [2]. Class II BCRs have been enriched from *Geobacter metallireducens* and *Desulfosarcina cetonica* and consist of eight subunits with a composition of ($Bam[(BC)_2DEFGHI]_2$) [3,4]. The subunits share similarities with aldehyde: ferredoxin oxidoreductases (BamB), electron-bifurcating heterodisulfide reductases (BamDE), and electron-bifurcating FeFe-hydrogenases (BamGHI). With 4 tungstopterins, 2 selenocysteins, 6 FADs and >50 FeS clusters, they are among the most complex metalloenzyme machineries known [3,4].

We applied cryo-electron microscopy to gain insights into the largely unknown structure and function of this complex. While obtaining a high resolution model for the $Bam[(BC)_2DE]_2$ subcomplex from both species, the $Bam(FGHI)_2$ components, though partially resolved, showed a high flexibility hampering structure determination. The arrangement of electron input and output modules suggests that class II BCRs achieve enzymatic dearomatisation by two consecutive electron confurcation/bifurcation processes: NADH and reduced ferredoxin act as donors for an electron confurcation at a flavin located in BamH. This is followed by an electron bifurcation at an FAD in BamE to benzoyl-CoA bound to BamB [1] and an unknown high potential acceptor. The latter is likely an electron transferring flavoprotein as predicted by AlphaFold.

[1] Kung et al. (2010), JACS, 132(28), 9850–9856

[2] Kung et al. (2009), PNAS, 106(42), 17687–17692

[3] Huwiler, Löffler, Anselmann et al. (2019), PNAS, 116(6), 2259–2264

[4] Anselmann et al. (2019), Environ. Microbiol., 21(11),4241–4252

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**AMP024**

**Production of substitutes for conventional plastics using greenhouse gases**

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Introduction: Due to environmental issues and shrinking petroleum resources substitutes for conventional plastics must be developed. Natural biopolymers seem to be an attractive candidate to substitute conventional plastics. The most common group of natural biopolymers is the group of polyhydroxyalkanoates, which are produced by microorganisms under unbalanced growth conditions as an intracellular carbon and energy storage. Poly-3-hydroxybutyrate (PHB) belongs to the group of polyhydroxyalkanoates and is naturally produced by *Cupriavidus necator* DSM 428, *Burkholderia thailandensis* DSM 13276, but also by the *Clostridium* species *Clostridium acetireducens* DSM 10703.

Objectives: In this study a synthetic pathway for production of PHB should be established in *Acetobacterium woodii* DSM 1030 to reach an autotrophic production using H2 and CO2 as energy and carbon source. Due to high effort cost to quantify PHB, an alternative quantification method using fluorescence measurements should be developed.

Material and Methods: For PHB production, the plasmid p83_PHB_Scaceti containing *thlA*, *hbd* and *crt* from *Clostridium scatologenes* 757 and *phaJ* and *phaEC* from *C. acetireducens* was transformed into *A. woodii*. Heterologous PHB production by metabolically engineered *A. woodii* was examined performing heterotrophic and autotrophic growth experiments. For fluorescence measurements an assay with the fluorescent dye LipidGreen2 was developed.

Results and Discussion: Heterotrophic production of PHB with recombinant *A. woodii* [p83_PHB_Scaceti] using fructose as substrate was successful and a production of 13 % PHB/cell dry weight (CDW) was reached. PHB could be also visualized using transmission electron microscopy and fluorescence microscopy in combination with LipidGreen2. Fluorescence intensity seems to increase in correlation with higher PHB content of the cells. For quantification exact correlation between PHB content and fluorescence intensity still needs to be elucidated.

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**AMP025**

**Anaerobic degradation of side chain-containing steroids: ATP-dependent and -independent mechanism to achieve hydroxylation of a primary carbon**

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Side chain-containing steroids such as cholesterol are highly hydrophobic, environmentally persistent compounds that can exclusively be fully degraded by bacteria. While the aerobic, oxygenase-dependent degradation pathways are well established, the degradation pathways in anaerobic bacteria have only recently been partially elucidated. The anaerobic degradation of cholesterol-like steroids of the isoprenoid side chain proceeds via hydroxylation at the tertiary C25 with water by a Mo-dependent steroid C25 dehydrogenase ($S25DH_1$). An enzymatic cascade then converts the tertiary C25 alcohol to a primary C26 alcohol via (1) ATP-dependent phosphorylation of the C25 alcohol, (2) phosphate elimination to a Δ24 steroid, and (3) hydroxylation of the terminal C26 at an allylic position. Recently, we have isolated an ATP-dependent 25-hydroxysteroid kinase (25-HSK) that phosphorylates various tertiary C25 alcohols with saturated side chains.[1] Here, we obtained three different crystal structures of 25-HSK. A monomeric one, in the absence of the steroid, and two different protein dimers in the presence of substrate alone or with substrate and ATP. We propose a mechanism in which the different forms of the kinase are involved in the capture of the hydrophobic substrate from the cytoplasmic membrane and in ATP-dependent phosphorylation.

We have recently revealed that steroids containing a Δ22-unsaturated isoprenoid side chain use an alternative degradation pathway. Here, a desaturase catalyses a direct dehydrogenation to a Δ22,Δ24-diene, which make C25-hydroxylation and the ATP-dependent dehydrogenation dispensable. We have isolated and characterised the α,β-heteromeric Δ24 steroid dehydrogenase (Δ24-SdhAB) from *Sterolibacterium denitrificans* with two covalently bound FMNs. UV/Vis and EPR analysis revealed that both flavins are in the red semiquinone state. We propose a radical-based mechanism in which each flavin abstracts a hydrogen atom from the substrate.

[1]Jacoby, C. et al., *J. Biol. Chem.* **297**, 101105 (2021)

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**AMP026**

**Adaptive metabolic strategies of autotrophic thermophilic bacteria**

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Microbial colonization of natural habitats relies on the ability of microorganisms to develop metabolic strategies that ensure their survival in the face of environmental changes. We studied metabolic strategies to cope with unstable environmental conditions in anaerobic autotrophic thermophilic bacteria *Ammonifex degensii* (Clostridia) and *Desulfurella* spp. (Deltaproteobacteria). *A. degensii* performs nitrate respiration to ammonium using hydrogen gas or formate [**1**]. Typically, hydrogen-oxidizing anaerobic, autotrophic prokaryotes are cultivated with a standard gas mixture composed of H2:CO2 (80:20, v/v), though hydrogen concentrations in natural environments are usually low and may fluctuate considerably. At 80% H2, the redox potential of hydrogen permits the reduction of ferredoxin ($E°'$ = ≈500 mV), and *A. degensii* operates the ferredoxin-dependent Wood-Ljungdahl pathway. In contrast, at 10% H2, *A. degensii* switches to the more ATP-demanding, ferredoxin-independent Calvin-Benson cycle. The sulfur-reducers *D. acetivorans* and *D. propionica* grow by means of acetate or propionate oxidation, or autotrophically with molecular hydrogen [**2, 3**]. In their metabolism, the tricarboxylic acid (TCA) cycle play a central role. In particular, *D. acetivorans* was one of the first organisms in which the operation of the reverse oxidative TCA cycle was described [**4, 5**]. This variant of the reductive TCA cycle relies on citrate synthase for citrate cleavage and can be operated in the reductive or oxidative direction without any enzyme substitution, a feature that allows this organism to adapt quickly to varying acetate and CO2 concentrations in the environment. Both organisms possess genes for the methylcitrate cycle of propionate oxidation in their genomes but still use the propionyl-CoA carboxylase pathway for growth on propionate. Interestingly, the further metabolism of succinyl-CoA produced after propionyl-CoA carboxylation is different in these two otherwise very similar species. Therefore, the studied bacteria, despite being able to use only a limited number of substrates, evolved elaborated strategies to adapt to their ecological niches.

1. Huber *et al.*, *Syst. Appl. Microbiol.* **19**, 40 (1996)
2. Bonch-Osmolovskaya *et al., Arch. Microbiol.* **153**, 151 (1990)
3. Miroshnichenko *et al., IJSEM* **48**, 475 (1998)
4. Mall *et al.*, *Science* **359**, 563 (2018).
5. L. Steffens *et al., Nature* **592**, 784 (2021).

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**AMP027**

**Structural and functional analysis of $tRNA^{sec}$ from different bacterial species**

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Selenium in the form of selenocysteine, the rare 21st amino acid, is an important component of proteins from all kingdoms of life, the so-called selenoproteins. Selenocysteine is incorporated cotranslationally at specific UGA (stop) codons by the specific $tRNA^{sec}$ (SelC) First, the specific $tRNA^{Sec}$ is loaded with serine by seryl synthetase, which is then converted to a tRNA-bound selenocysteine by selenocysteine synthase (SelA) with selenophosphate synthetase (SelD) providing the required selenol donor (1,2). Finally, the specific translation factor SelB is required, which simultaneously recognizes selenocysteine-loaded selenocysteyl-tRNAsec and a specific RNA structure close to the UGA codon that specifies the incorporation site of selenocysteine (3). This specific recognition sequence, the selenocysteine insertion sequence (SecIS), discriminates between UGA codons for selenocysteine insertion and chain termination (4). Bacterial selenoprotein synthesis has been studied mainly in *Escherichia coli* and a few other species, while selenoproteins appear to be present in many other taxonomic groups, often with divergent features in their selenocysteyl-tRNAs. Here, we investigate the functionality of several aberrant SelC-tRNAs from members of the *Bacillaceae*, Alpha- and Deltaproteobacteria, which have not been among the usual selenoprotein-forming model

organisms in earlier studies. This will provide valuable data on selenocysteine metabolism and what role different structural features of SelC play in heterologous systems.

(1) Forchhammer, K. et al. (1991) *Journal of Biological Chemistry*, *266*(10), 6318-6323.

(2) Leinfelder, W. et al. (1990) *Proceedings of the National Academy of Sciences*, *87*(2), 543-547.

(3) Forchhammer, K. et al. (1989) *Nature*, *342*(6248), 453-456.

(4) Heider, J. et al. (1992) *The EMBO journal*, *11*(10), 3759-3766.

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**AMP028**

**Itaconate degradation in aerobic and anaerobic bacteria**

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Itaconate is produced by mammalian macrophages during the inflammatory process where it controls immune responses. It also serves as an antimicrobial agent by inhibiting different reactions of carbon metabolisms [1, 2]. Some pathogens such as *Yersinia pestis* and *Pseudomonas aeruginosa* can metabolize itaconate, detoxifying it and using it as a carbon source. This pathway is catalysed by the itaconate coenzyme A (CoA)-transferase, itaconyl-CoA hydratase and (*S*)-citramalyl-CoA lyase and leads to the formation of acetyl-CoA and pyruvate [3, 4]. Even though the degradation of itaconate is not dependent on oxygen, the corresponding gene cluster could only be found in genomes of aerobic or facultative anaerobic bacteria. As itaconate is also produced by a broad range of fungi, the capability to degrade itaconate is wide spread in aerobic soil bacteria [2] but has not been detected in anaerobes so far. Here we isolated from soil samples a new strain of *Pelosinus fermentans* that was capable to grow anaerobically on itaconate as the only carbon and energy source. Its genome was sequenced, and the corresponding metabolic pathway was identified using enzyme activity assays, enzyme characterization and proteomics analysis. Itaconate degradation proceeds in this strain through its isomerisation to citraconate, hydration to (*R*)-citramalate, activation to (*R*)-citramalyl-CoA and its cleavage to acetyl-CoA and pyruvate. The corresponding pathway could be reconstituted *in vitro* with heterologously produced enzymes and compared to the aerobic itaconate degradation pathway from a soil bacterium *Cupriavidus necator* H16. The distribution of both pathways and their physiological role in the corresponding bacteria will be discussed.

[1] Michelucci A. *et al*., Proc. Natl. Acad. Sci. 110, 7820–7825 (2013)

[2] Cordes T. *et al*., Annu. Rev. Nutr. 35, 451–73 (2015)

[3] Sasikaran J. *et al*., Nat. Chem. Biol. 10, 371–377 (2014)

[4] Kronen M. *et al*., Appl. Env. Microbiol. 81, 5632-5638 (2015)

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**AMP029**

**Identification of enzymes responsible for anaerobic transformation of sulfamethoxazole in sulfate reducing bacteria**

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Introduction: Sulfamethoxazole has been frequently detected in the environment due to its extensive usage in animal farming and human healthcare as well as its recalcitrance to degradation. Anaerobic transformation of sulfamethoxazole into two transformation products (reduced or isomerized) has been observed in the sulfate reducer *Desulfovibrio vulgaris* Hildenborough. In addition to *D. vulgaris*, two other sulfate reducers *Desulfosporosinus meridiei* and *Desulfovibrio desulfuricans* were also proved to be capable to transform sulfamethoxazole into the same two transformation products as yielded by *D. vulgaris*.

Objectives: However, the underlying enzyme transformation processes are still unknown and the further investigation is required. Here, we investigate transformation of sulfamethoxazole in the above-mentioned sulfate reducing bacteria, using a combination of enzymatic, proteomics, and genomics approaches.

Methods: We developed *in vitro* activity assay, allowing for sensitive and reliable detection of sulfamethoxazole transformation activity in crude extracts from all three strains. Ultrafilters and fast protein liquid chromatography (FPLC) were applied to separate crude extract into fractions which were used in activity assay. Transformation of sulfamethoxazole was observed in some of the fractions. The proteome of the FPLC fractions was analyzed by shotgun proteomics via LC-MS/MS and label-free quantification.

Results: The comparison of protein abundances in the FPLC fractions with the corresponding sulfamethoxazole transformation rates revealed several protein candidates that may catalyze the transformation of sulfamethoxazole. Comparative genomics of the three strains also indicated the existence of functional genes that could be responsible for sulfamethoxazole transformation.

Conclusion: Findings obtained in this study are significant for comprehensive understanding on transformation of sulfamethoxazole and effective elimination of sulfamethoxazole in the environment.

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**ARP001**

**Unraveling the regulation of methoxydotrophic methanogenesis**

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Lignin is one of the most abundant organic polymers on Earth and is composed partially of methoxylated aromatic compounds. The conversion of these compounds has already been described rather extensively for bacteria. However, the archaeal degradation of methoxylated

aromatics has only been discovered recently: the methanogenic archaeon *Methermicoccus shengliensis* was the first archaeon shown to be capable to convert methoxylated aromatic compounds, also called methoxydotrophic growth [1]. In a recent study we showed that *M. shengliensis* uses an *O*-demethylation/methyl transfer (Mto) system that is more related to that of acetogenic bacteria than the methyl transfer system of methylotrophic archaea [2]. The most striking finding was that tetrahydromethanopterin instead of coenzyme M is the final methyl acceptor, which differs from the conventional methanogenic methyl transfer systems and might have implications for the energy metabolism during methoxydotrophic growth. In a next step we aim to unravel the regulation of the *mto* genes. Comparative transcriptomics using *M. shengliensis* has shown that these genes are only transcribed when methoxylated aromatics are present, but not when the methanogen is grown on methanol. By using the promotor of the *mto* gene cluster and adding *M. shengliensis* cell extract we will screen for the regulator of the *mto* operon and identify the protein binding to the *mto* promotor with MARLDI-TOF. In addition, we will test a transcription regulator that is located adjacent to the *mto* operon for its potential to act as a regulator, either activator or repressor, of the *mto* operon. This study will help us to understand how methoxydotrophy is regulated in archaea and how some methanogens such as *M. shengliensis* can regulate the conversion of a huge variety of methylated and methoxylated compounds.

[1] Mayumi D *et al.* (2016) Science 354: 222–225. DOI:10.1126/science.aaf8821

[2] Kurth J *et al.* (2021) ISME J 15: 3549–3565. DOI:10.1038/s41396-021-01025-6

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**ARP002**

**Determining the molecular function of bactofilin homologues in the archaeal model organism *Haloferax volcanii***

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The cytoskeleton as a network of intracellular filaments plays a major role in all domains of life. Besides actin and tubulin homologues another class of cytoskeletal proteins was recently discovered in bacteria, named bactofilins. The conserved bactofilin domain exhibits a characteristic β-helical structure enabling the polymerization of these proteins to non-polar filaments. The small proteins contribute to a variety of molecular processes, like stalk formation in *Caulobacter cresentus*, chromosome segregation and motility in *Myxococcus xanthus* and cell wall synthesis in *Helicobacter pylori*. In 2019 Deng *et al.* discovered that bactofilin homologues are also present in archaeal and even some eukaryotic genomes. Bactofilin domains were found in 80 % of the analysed Halobacteriota genomes alone. But to date functional studies of these genes are still missing.

Here we aimed to identify bactofilin homologues in the model organism *Haloferax volcanii* and evaluate their biological function.

To achieve our objective we used *in silico* analysis to identify promising candidates and subsequent knock-out and localization studies.

*In silico* analysis identified two bactofilin domain containing genes (*hvo_1237* and *hvo_1610*) in the *H. volcanii* genome as well as one *hvo_1610*-associated gene (*hvo_1611*) which expression is also effected by global transcriptional cell division regulator CdrS. The transmembrane domain-containing HVO_1237 showed a generally low expression level at the tested conditions, but overexpression of a fluorescently labelled construct demonstrated membrane localization predominantly at the cell poles. Localization studies of HVO_1610 revealed a localization at mid cell in moving patches, while HVO_1611 is found in the whole cytoplasm. Knockouts of *hvo_1237* or *hvo_1611* seem to have no effect on growth, cell shape or motility under the tested conditions. Knockout of *hvo_1610* on the other hand leads to a slightly reduced motility and elongated cells, with the cell shape defect resembling the effect of *ftsZ2*-knockout on *H. volcanii* cells, yet not as strong.

Overall, we concluded that bactofilin homologue HVO_1610 and the small hypothetical protein HVO_1611 are involved in cell division in *H. volcanii*, while the membrane protein HVO_1237 seems to possess a separate function. Further analyses involving pulldown experiments will reveal possible interaction partners of these proteins and shed more light on this unstudied part of the archaeal cytoskeletal network.

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**ARP003**

**Unraveling the dynamic interaction of ArlI and ArlJ in the archaellum motor complex**

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The three domains of life have independently evolved distinct motility structures, as flagella, archaella and cilia. Despite functional analogy, the structure and composition vary highly. In Archaea, the motility structure is part of the type four filament superfamily (TFF). In contrast to the other members, the archaellum is the only TFF dedicated to swimming motility.

The archaellum nanomachinery is composed of two elements, a filament and a motor complex, which includes the membrane protein ArlJ and the cytosolic ATPase ArlI. ArlJ and ArlI are homologous to the core complex of TFF. ArlI is essential for both, the biogenesis and the rotation of the archaellum. It is hypothesized, that the rotational movement of the filament is facilitated by the transmission of conformational changes through the ATP-hydrolysis of ArlI across the membrane by ArlJ. While the hexameric conformation of ArlI is well established, the oligomeric state of ArlJ has remained elusive. Using single-molecule total internal reflection microscopy (smTIRF), we were able to determine the oligomeric state of ArlJ in complex with ArlI. Enzymatic analysis of the ArlI-driven ATP hydrolysis, followed by single-molecule FRET and was performed to understand the dynamic interaction of ArlI and ArlJ assessing the rotary mechanism of the core machinery. We successfully established the purification of ArlJ and ArlI in complex. Initial screening with electron microscopy showed particles corresponding to the size of the complex. Cryo-electron microscopy of ArlJ and ArlI in complex will elucidate the dynamic interaction and lead towards understanding the rotary mechanism from biogenesis to function of the motor complex of the archaellum machinery.

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**ARP004**

**Analyzing selenium utilization in *Methanococcus maripaludis***

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The archaeal model organism *Methanococcus maripaludis* is known to utilize selenium in its primary energy metabolism, the hydrogenotrophic methanogenesis. To the present day, two selenium sources which can be utilized by *M. maripaludis* have been identified: (sodium) selenite and dimethylselenide. These selenium species get incorporated into selenocysteine-(Sec-) containing enzymes (selenoproteins) which are essential in methanogenesis. In case of a selenium-depleted environment, *M. maripaludis* employs a second set of enzymes containing cysteine (Cys) instead of Sec. So far, the genes coding for these Sec-/Cys-containing isoforms are the only genes known with expression influenced by the selenium status of the cell. Here, we aim to identify additional selenium sources utilized by *M. maripaludis*, and the selenium-responsive regulon.

To identify new selenium sources for *M. maripaludis*, a selenium responsive reporter strain was assessed with eleven environmentally available selenium species. These included selenocystine, selenourea and (potassium) selenocyanide. Selenocyanide was utilized by *M. maripaludis* at concentrations as low as 10 nM, thus appearing more effective than selenite. Surprisingly, selenocystine required rather concentrations, 10 µM, to be effective and selenourea appeared to not be used by *M. maripaludis* at all. Transcriptomic analysis revealed that approx. 7% of all genes are influenced directly or indirectly by the selenium status of the cell. Interestingly, while transcripts for Sec-isoforms were not depleted completely during selenium starvation, those for Cys-isoforms were barely detectable under selenium adequate conditions, which indicates preferential utilization of the selenoproteins. Some of the genes differentially expressed involve methanogenesis, accessory functions to nitrogen fixation, and active transport. Some transcripts for putative transporters increased under selenium-depleted conditions, which might be interpreted as the organism"s effort to tap into alternative sources of selenium. These omics-based observations inspired genetic and physiologic experiments aimed to broaden our understanding of microbial selenium metabolism.

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**ARP005**

**Investigating the nucleotide excision DNA repair system uvrABCD of the archaeon *Haloferax volcanii* by single-molecule microscopy**

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*Haloferax volcanii* is a well-established model organism for the phylum Archaea. While certain aspects of archaea resemble eukaryotes, such as their DNA organization involving histones, other features, like the nucleotide excision DNA repair system uvrABCD, share homology with their prokaryotic counterparts [1]. The DNA repair systems in *H. volcanii* are particularly intriguing as *H. volcanii* thrives in environments with high molecular salt concentration and elevated growth temperatures [2]. But despite significant interest, many functional details of this dynamic molecular machinery remain unclear. Therefore, our study aims to investigate the uvrABCD DNA repair system in *H. volcanii* utilizing single-molecule microscopy.

Recently, our group has successfully established single-molecule imaging to measure the dynamics of individual proteins in *H. volcanii* [3]. To visualize the proteins of interest (POIs) in vivo, we employ genetic tagging with fluorescent proteins using plasmid-based expression approaches due to their ease of use and controllability. However, the current expression systems employed in *H. volcanii* show substantial variability in protein expression levels among cells depending on several factors, i.e. also on the tagged POI. Consequently, only qualitative measurements of single molecules in *H. volcanii* have been feasible thus far, and obtaining reliable quantitative data has proven challenging.

In this work, we present our efforts to establish novel expression and fluorescent tagging systems for *H. volcanii*, with a specific focus on their application in studying the uvrABCD repair machinery. Through these advancements, we aim to enhance our understanding of the repair mechanisms at play in this archaeon, ultimately contributing to broader insights into DNA repair processes.

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[3] Turkowyd B, Schreiber S, Wörtz J, Segal ES, Mevarech M, Duggin IG, Marchfelder A, Endesfelder U. (2020) Establishing Live-Cell Single-Molecule Localization Microscopy Imaging and Single-Particle Tracking in the Archaeon *Haloferax volcanii*. Front Microbiol. doi: 10.3389/fmicb.2020.583010.

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**ARP006**

**Glycerol metabolism in *Sulfolobus acidocaldarius***

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Glycerol is an integral constituent of membrane phospholipids in all domains of life and also of storage lipids mainly known from bacteria and eukaryotes and is thus a highly abundant organic compound in nature. Accordingly, many organisms utilize glycerol as carbon and energy source. However, although the genetic capacity to grow on glycerol was reported for several archaea [ 1] detailed analyses of the glycerol degradation in this domain of life has so far only been reported for the halophile *Haloferax volcanii* [ 2].

In this study, the glycerol degradation in the thermoacidophilic crenarchaeal model organism *Sulfolobus acidocaldarius* was analyzed.

A combination of growth experiments, polyomics approaches and biochemical analyses has been used to unravel the underlying mechanisms. Key enzymes of the degradation pathway were recombinantly produced, purified and characterized. Furthermore, knockout mutants were constructed to gain further insights.

Although previously regarded as non-glycerol utilizer, we demonstrated that *S. acidocaldarius* is able to utilize glycerol as sole carbon and energy source. Glycerol degradation proceeds via phosphorylation to glycerol-3-phosphate followed by oxidation to dihydroxyacetone phosphate (DHAP) which then enters the common lower shunt of the ED and EMP pathway. The reactions are catalyzed by a glycerol kinase and a membrane associated, quinone reducing FAD-dependent glycerol-3-phosphate dehydrogenase (G3PDH), respectively. Whereas the GK showed high similarity to enzymes from Bacteria and Eukaryotes, the G3PDH represents an unusual bacterial GlpA subunit homologue of the GlpABC complex with a novel type of membrane anchoring by a small CoxG-like protein. Both, GK and G3PDH, are present in two paralogous copies which were shown to be functionally equivalent. To unravel the distinctive functions of the isoenzymes, single and double deletion mutants of the GK genes were constructed and comparative phenotype analyses will be presented.

In summary, we herein unraveled the glycerol degradation in the crenarchaeon *S. acidocaldarius* to proceed via the GK-G3PDH pathway involving a "classical" GK and a G3PDH which structurally differs remarkably in subunit composition and membrane association from those known in bacteria and haloarchaea.

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2. Sherwood KEet al. (2009). *J Bacteriol*, **191**(13):4307-4315.


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**ARP007**

**Unveiling the ecophysiology and genome complexity of *Candidatus* Altiarchaeum with high-quality genome recovery via nanopore sequencing**


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Introduction: Organisms of the Candidatus genus Altiarchaeum dominate multiple deep continental subsurface ecosystems across the globe, yet, standard genome reconstruction using short-read metagenomics or single-cell genomics have failed regularly. The, resulting highly fragmented genomes with low completeness and/or high strain diversity currently limit in-depth genome analyses.

Objectives: Here, we applied long-read sequencing to enable improved genome-recovery and overcome limitations of short-read sequencing to get a further insight into the ecophysiology of *Ca*. Altiarchaeum.

Material & Methods: Using Nanopore sequencing we were able to resolve the first closed, circular genome of *Candidatus* Altiarchaeum andernachense that dominates a subsurface aquifer in the Rhine Valley near Koblenz in western Germany (Geyser Andernach). We used metaFlye to reconstruct the metagenome and polished the assembly with racon and medaka. The obtained genome belonging to *Ca*. Altiarchaeum has a size of 1.68 Mb. To ensure an in-depth analysis of the metabolic potential we used multiple tools to annotate the genome (*e.g.,*PGAP and Genoscope). We used ISEScan to screen the genome for insertion sequence elements.

Results: Manual inspection of the annotated genome revealed transposases and a potential prophage, which likely contributed to the fragmentation of the population genome in short-read metagenomics. The plasticity of the genome is further increased by 19 complete insertion sequence elements. We also recognized a genomic region encoding hydrogenases, which probably provide the organism with energy from hydrogen oxidation.

Besides, having this complete genome as a basis, we were able to determine the genetic and genotypic differences of many *Ca*. Altiarchaea within individual populations of one site but also across sites from three different continents.

**Conclusion:**

The results of this study uphold the power of pure Nanopore metagenomics in reconstructing accurate genomes from metagenomes, provides in-depth insights into the architecture of an archaeal genome, and demonstrate the high complexity of *Ca*. Altiarchaea genomes across the globe.

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**ARP008**

**Investigating the biophysical properties of archaeal giant unilamellar vesicles derived from Haloferax volcanii membranes**


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In extreme environments, e.g. at high temperatures and under high salt concentrations, archaea thrive while other organisms struggle to survive. [t1] One key factor that sets archaea apart from prokaryotes and eukaryotes is their unique membranes, featuring inverted stereochemistry of glycerol compared to bacteria and eukaryotes [1]. Exploring the potential of liposomes derived from archaeal membranes opens up fascinating possibilities, as these non-toxic liposomes maintain their structural integrity even under extreme conditions. Consequently, they show promise as vehicles for drug delivery in acidic environments [2].

However, the biophysical properties of archaeosomes have been relatively unexplored thus far, in contrast to the well-studied membrane microdomains of bacteria and eukaryotes [3]. As a result, the characteristics and functions of archaeal membrane microdomains remain elusive.

In this study, we employed electroforming techniques to create Giant Unilamellar Vesicles (GUVs) [4] using isolated archaeal lipids from Haloferax volcanii. We conducted generalized polarization (GP) studies to investigate the organization, polarity, and fluidity of archaeal lipids [5]. Additionally, we utilized single-molecule tracking and confocal microscopy to visualize the archaeosomes and their dynamics. Here, we present our findings and provide an overview of future work.


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**ARP009**

**Use of fluorescent proteins for live imaging in *Sulfolobus acidocaldarius***

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Introduction: In vivo cell localization using fluorescent proteins (FP) represents a challenge in hyperthermophilic organisms. The high temperature required to grow them is a limitation, as it can affect proper folding and function of proteins derived from mesophilic organisms, such as most FP. In *Sulfolobus acidocaldarius*, a hyperthermophilic model archaeon, protein localization was accomplished so far using antibodies with fluorescent probes in fixed cells. No *in vivo* expression of FP or live fluorescent imaging has been done. Although some thermotolerant FP have been published during the years, the use of them in thermophilic archaea have not yet been described.

Objectives: Because live protein localization in the cell can help to better understand certain processes and the role of proteins in them, we aim to find a suitable FP to be used in the hyperthermophilic *S. acidocaldarius*. A suitable FP will then be used to perform live imaging of proteins belonging to the cell division machinery.

Materials and methods: Several plasmids were constructed carrying fluorescent proteins along with fusion proteins of interest and transformed into *S. acidocadarius* cells. Fluorescence of constructs was studied using Flow Cytometry. An Epifluorescent microscope was used to observe fluorescence, as well as localization in cells immobilized in agarose pads. For live imaging, a heating device was used to heat the cultures during microscopy.

Results: Of several tested proteins, the Thermal Green Protein (TGP) was found to be the most bright when expressed in Sulfolobus cells. However, fusions at the N-teminal of the TGP diminished the fluorescence greatly. With the addition of a linker between the two partners, we were able to fuse proteins successfully to the C-terminal side of TGP with minimal loss of fluorescence. Furthermore, we were able to successfully fuse TGP to CdvB, a cell division protein, and observe formation of CdvB rings during cell division.

Conclusions: We were able to design constructs with a fluorescent protein, TGP, that is suitable to be used for cell localization of proteins in *S. acidocaldarius*.

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**ARP010**

**The 3-hydroxypropionate/4-hydroxybutyrate pathway as a possible $CO_2$ fixation pathway in *Sulfolobus acidocaldarius*: Characterisation of relevant enzymes of the pathway**

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Introduction: $CO_2$ fixation via the 3-hydroxypropionate/4-hydroxybutyrate (3HP/4HB) pathway is best understood in the thermoacidophilic crenarchaeon *Metallosphaera sedula*, as well as in the mesophilic ammonia oxidizing Thaumarchaeon, *Nitrosopumilus maritimus* [1]. In *Sulfolobus acidocaldarius* a close relative to *M. sedula*, initially described as chemolithoautotroph capable of sulphur/hydrogen oxidation, all gene homologues of the 3HP/4HB cycle have been identified. However, current lab strains of *S. acidocaldarius,* have apparently lost the ability to grow autotrophically, leaving questions about the function of the 3HP/4HB cycle homologues.

Objective: The aim of this study is to get an insight into the function of the 3HP/4HB enzymes in *S. acidocaldarius.*

Materials and Methods: The genes, *saci_1078*, *saci_1633*, and *saci_0911* encoding putative homologues of the (S)3-hydroxybutyryl-CoA dehydrogenase (3HBD), promiscuous crotonyl-CoA hydratase/3-hydroxypropionyl-CoA dehydratase (CH/3HPD), and acryloyl-CoA reductase (ACR), respectively, in *S. acidocaldarius* were cloned and expressed in *E. coli*. The recombinant proteins were purified and biochemically characterized.

Conclusion**:** These results from biochemical analyses suggest that the 3HP/4HB cycle homologues might constitute a functional $CO_2$ fixation cycle in *S. acidocaldarius* and thus that a missing $CO_2$ fixation pathway might not be the reason for lack of autotrophic growth. Instead either regulatory implication [2] and/or problems in electron donor and energy conversion might be envisaged and will further be analysed to elucidate the autotrophic capacities of *S. acidocaldarius*.

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**ARP011**

**Intermolecular gene conversion in the polyploid haloarchaeon *Haloferax volcanii***

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Gene conversion is defined as the non-reciprocal transfer of genetic information between two homologous but not identical DNA sequences. It occurs in all three domains of life and can be part of different biological processes, like "antigenic variation" or "concerted evolution of gene families". We are focusing on "intermolecular gene conversion". This process results in an equalization of multiple genome copies and has hardly been studied in prokaryotes so far. Furthermore, a highly efficient correction of mutations via intermolecular gene conversion would be an escape from "Muller"s ratchet".

An experimental approach has been established to characterize unselected intermolecular gene conversion in *Haloferax volcanii*[1]. To this end, we made use of deleting genes encoding enzymes that are involved in carotenoid biosynthesis, resulting in white instead of the usually red cells. Protoplast fusions of red and white cells were used to produce heterozygous cells. Afterwards, unselected intermolecular gene conversion between different genomes

could be easily analyzed by screening of phenotypes or/and by PCR.

We could show that unselected intermolecular gene conversion is extremely efficient in haloarchaea. Notably, its frequency is independent from the extent of genome differences, i.e. even a one nucleotide difference triggers gene conversion. In addition, gene conversion also operates between genes of different species. It was verified that gene conversion tracts in haloarchaea are much longer than in antigenic variation or concerted evolution in bacteria and can extend to big parts of the genome. Furthermore, we could identify 16 proteins that are important for gene conversion in *H. volcanii*. Based on these data we analyzed proteins and their binding partners by co-affinity purification and subsequent peptide mass fingerprinting, resulting in a protein interaction network. Future work will concentrate on elucidating factors that determine the direction of gene conversion and on further characterization of the involved proteins and the molecular mechanism.

[1] Wasser et al. (2021) Frontiers Microbiol. 12:1477

**ARP012**

**Translational coupling via termination-reinitiation in *archaea* and *bacteria***

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Translational coupling occurs at many gene pairs of archaea and bacteria. Translational coupling means that translation of the downstream gene on a polycistronic transcript strictly depends on translation of the upstream gene. One specific mechanism of coupling is called termination-reinitiation (TeRe). In this case, the translating ribosome (at least the small subunit) remains on the mRNA after terminating translation of the upstream gene, and it directly continues translation by reinitiating at the downstream gene. This mechanism typically occurs at gene pairs that have overlapping stop/start codons or very small intergenic distances.

In a previous study we have shown that TeRe operates at several native gene pairs of *Haloferax volcanii* and *Escherichia coli*[1]. Translational efficiencies were quantified using reporter genes fused to native gene pairs, enzyme assays and northern blotting were used to determine protein and transcript levels. In the present study, this experimental approach was applied to unravel the correlation between the intergenic distance and the efficiency of translational coupling. Several native gene pairs of both organisms were chosen, and the intergenic distances were varied. As expected, efficient coupling required very short distances.

Furthermore, we investigated whether local mRNA structures at gene overlaps in *E. coli* are required to inhibit novel initiation at the downstream genes and guarantee coupling. To this end, we generated several constructs with truncated or mutated gene pairs, with the aim of destabilizing local mRNA structures close to the overlaps. The effects of these genetic perturbations on the efficiencies of translation and coupling will be reported. Additionally we created a random library with a 42 random nucleotide sequence directly upstream of the overlapping start/stop codon. In our two-reporter gene system, this library is used to select and screen for sequence motives that are important for efficient novel initiation at the downstream gene as well as translational coupling via TeRe.

Taken together, translational coupling via TeRe operates at many gene pairs in archaea and bacteria, and various aspects of the molecular mechanism could be characterized using native gene pairs fused to reporter genes.

[1] Huber et al. (2019) Nature Comm. 10: 4006.

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**ARP013**

**Enriching bathyarchaeota from freshwater sediments using genome-guided cultivation**

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Archaea represent one of the two primary domains of life and have been shown to inhabit almost every environment on Earth. Sequencing-based studies have uncovered a wide diversity of previously unknown archaeal lineages in a variety of habitats. However, the majority of archaeal phyla, including the ubiquitous Bathyarchaeota, lack cultivated representatives and information on their potential role in the environment is solely predicted based on genomic information. Here, we sampled ten sediment cores from Lake Erken (Sweden) for metagenomic sequencing and cultivation work in order to elucidate the diversity and lifestyle of Bathyarchaeota in freshwater sediments. With a relative abundance of up to 23% members of the Bathyarchaeota are dominating the microbial community in Lake Erken sediments. The analysis of 17 high-quality metagenome assembled genomes (MAGs) that were reconstructed from Lake Erken indicates a widespread potential of Bathyarchaeota to break down different carbohydrates including chitin, cellulose and mannan. Enrichment cultures of Bathyarchaeota were set up and monitored over a period of over two years. With Bathyarchaeota increasing up to a relative abundance of 29%, the response of Bathyarchaeota to different carbon sources allows to draw conclusions on the metabolic potential of these elusive archaea in freshwater sediments. Using a subsequent dilution to extinction approach the overall diversity of the enrichments could be further reduced, resulting in low-complexity enrichments of several Bathyarchaeota representatives. Their high abundance, diversity and ability to degrade a wide range of complex carbon sources highlights the importance of Bathyarchaeota in lake sediments and potentially global carbon turnover. The combination of genome-resolved metagenomics and classical cultivation work lay the foundation for further attempts to isolate and study Bathyarchaeota using high-throughput cultivation techniques.

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**ARP014**

**Analysis of the low salt transcriptome in *Haloferax volcanii***

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Archaea are well known for inhabiting areas of our planet that are usually hostile for any form of life, hence they are often termed as "extremophiles". For example, some species thrive on acidic or alkaline grounds, in submarine black smokers with temperatures surpassing 100°C or live in salt lakes where no other organisms can grow.

*Haloferax volcanii* is a halophilic and mesophilic archaeon that was first discovered in the 1930s and isolated from the hypersaline environment of the Dead Sea. Its optimal growth conditions are at 42 °C in 1.5-2.5 M NaCl and complex nutrient medium, however it can also grow in different synthetic minimal media and at a wide range of temperatures and NaCl concentrations. While NaCl concentrations as low as 0.7 M inhibit growth in synthetic glucose media, a concentration of 0.9 M is sufficient to sustain growth at a reduced rate.

Here we show for the first time a transcriptome-wide analysis of differential gene expression of cells grown under these low salt conditions. To this end, we cultivated cells at an optimal NaCl concentration of 2.1 M in glucose media and compared the expression profile of these cells to cells grown in 0.9 M NaCl after 26 and 68 h via RNA-Seq.

By these means, we identified a multitude of regulated genes and gene-clusters that seem to be of importance either as a fast "stress-response" (after 26 h) to the change of ion concentrations in the environment or to enable continuous growth under these conditions (68 h). We are furthermore establishing in-frame deletion mutants of selected genes/gene clusters and will investigate the impact of these deletions on growth under low salt conditions.

**ARP015**

**Characterization and localization of enzymes involved in amino acid biosynthesis in the hyperthermophilic archaeon *Ignicoccus hospitalis***

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*Ignicoccus hospitalis* is a hyperthermophilic anaerobic autotrophic sulfur-reducing archaeon isolated from a submarine hydrothermal vent (1). In this archaeon, a novel $CO_2$ fixation pathway, the dicarboxylate/4-hydroxybutyrate cycle was identified (2). Apart from the unusual carbon assimilation pathway, *Ignicoccus* cells exhibit an extraordinary ultrastructure with two membranes, an inner and an energized outer cytoplasmic membrane that separate peripheric and central cytoplasmic compartments (3). Whereas DNA replication and protein biosynthesis take place in the central cytoplasmic compartment, the peripheric cytoplasmic compartment contains at least some enzymes of the dicarboxylate/4-hydroxybutyrate cycle (4). Whereas all cellular carbon comes from $CO_2$ fixed in this cycle, the localization of the specific metabolic reactions leading to the formation of a variety of cellular building blocks is not known. Here we selected several yet uncharacterized enzymes involved in the biosynthesis of different amino acids, heterologously produced them and characterized them biochemically, confirming their participation in the corresponding processes. Furthermore, we used immunogold labelling of ultrathin sections to localize these enzymes in *I. hospitalis* cells. The physiological consequences of compartmentalization of metabolism and information processing in *I. hospitalis* will be discussed.

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(3) Küper U, Meyer C, Müller V, et al. Proc Natl Acad Sci U S A. 2010,107:3152-3156.

(4) Flechsler J, Heimerl T, Huber H, et al. Proc Natl Acad Sci U S A. 2021,118:e2022114118.

**ARP016**

**Molecular basis of methanogen-virus interactions**

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Methanogens are a group of archaea that produce methane gas as a byproduct of their metabolism. Like other archaea, they can be infected by viruses, and there is growing evidence to suggest that viral infection plays an important role in regulating their population dynamics and gene transfer. Despite their scientific importance, methanogenic viruses are still poorly understood compared to viruses that infect bacteria and eukaryotes, especially their infection cycles and host-range specificity. Therefore, further research is needed to understand better the factors that contribute to the host-range specificity of methanogenic viruses and to explore their potential biotechnological applications. We aim to understand the resistance of *Methanothermobacter thermautotrophicus* DH *(M.t.)* to virus ΨM2, in spite of its infectivity to the closely related *Methanothermobacter marburgensis* Marburg *(M.m.)*. *In-silico* analyses were performed to understand potential mechanisms for host-range specificity. Differences in the CRISPR loci in *M.t.* and *M.m.* suggest that *M.t.* has a stronger antiviral defense. For example, we found 7 spacers in the largest CRISPR array in the *M.t.* genome that align to the ΨM2 genome. We further follow the hypothesis that packaging multimers of the cryptic plasmid pME2001 from *M.m.* in the ΨM2 viral particles has an impact on host-range specificity. Wet-lab experiments, including interference assays with ΨM2 genome sequences based on our genetic tools for *M.t.*, as well as infection assays with plasmid-cured *M.m.* strains will provide insight into the hypotheses about host specificity and a potential function of the cryptic plasmid pME2001.

**BTP001**

**Metaproteomic approach to discovering novel biocatalysts from environmental sources**

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Biocatalysts are important for a wide range of industries, such as food processing, pharmaceuticals and biofuels. To increase cost-effectiveness and production capacities for these growing sectors, we need novel enzymes with high conversion rates and pH- and temperature stabilities suitable for the application.Our group has developed a functional metaproteomic method, in which we combine cultivation-independent -omics methodologies with the immediacy of activity screening [1]. It has the potential to discover all enzymes with a given activity in an environmental community and does not require prior assumptions about the biocatalyst"s structure. Combined with direct protein extraction, it will also be culture independent, broadening the search to biocatalysts from microorganisms, that are not cultivatable in lab conditions [2]. This procedure has been applied successfully to find novel heat stable lipases [3], as well as enantioselective esterases (published soon).For this method, proteins will be isolated from environmental samples collected in promising habitats, to find enzymes that have been naturally preselected to desired properties, like substrate specificity, temperature- and pH-optimum. We then use polyacrylamide gel electrophoresis to separate the metaproteome. After refolding, activity is directly assessed by in-gel zymography. First, 1D-PAGE as prescreen, then 2D-PAGE to increase the resolution of the zymogram. Activity can be screened directly with different methods depending on the enzyme.In this project we will apply this method to discover novel glycosidases, specifically amylases, cellulases and hemicellulases, as these enzymes are key to a sustainable utilization of renewable plant-based resources. We established two dye-based methods for activity detection. One based on the reaction of iodine with starch (for amylases), the other based on congo red, which can form a complex with cellulose and hemicellulose. For esterases, we used 4-Methylumbelliferone (MU) as a fluorescent substrate. We used enantiomeric forms of alanine, to find enantioselective esterases. Taken together, we can now screen multiple enzyme classes in environments that naturally select for desired enzyme properties, making them accessible for industrial or medical applications. Our method is versatile, as it can be adapted quickly to not only screen for different properties, but different enzyme classes entirely.

**BTP002**

**Investigation of exopolysaccharide formation and its impact on anaerobic succinate production with *Vibrio natriegens***

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*Vibrio natriegens* shows very high growth and substrate consumption rates, which render this bacterium a promising host for industrial biotechnology. First metabolic engineering approaches exploited its potential as a production strain for e.g. amino and organic acids such as succinate [1] and alanine [2]. In our group, we recently engineered *V. natriegens* for the production of succinate and established an anaerobic resting cell approach that yielded exceptionally high volumetric productivities. However, exopolysaccharides (EPS) were formed as a by-product, which negatively affected the process. To address these issues, several mutant strains carrying gene deletions associated with EPS formation were tested in fed-batch fermentations. Therefore, the EPS gene clusters *syp* [3] and *cps* [4] were targeted as well as the genes *cpsR* and *wbfF*. *CpsR* encodes for a transcriptional biofilm regulator [5] whereas *wbfF* is involved in the synthesis of capsular polysaccharides [6]. All strains still produced EPS in varying amounts. However, deletion of *cpsR* and *cps* resulted in consistently low viscosity of the reactor broths over the course of 33-hour fermentations.

Consequently, these genes were deleted in our succinate producer strain *V. natriegens* Δ*lldh* Δ*dldh* Δ*pfl* Δ*ald* Δ*dns::pycCg* (Succ1). No viscosity changes were observed for both strains throughout the process. Furthermore, higher yields, corresponding to 81% of the theoretical maximum, as well as increased volumetric productivities were achieved. Moreover, *V. natriegens* Succ1 Δ*cpsR* showed a maximum volumetric productivity of 19.4 gSuc L-1 h-1. Thus, it was successful to overcome challenges in the operation of bioreactors caused by EPS and the associated high viscosities while simultaneously improving succinate production.

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**BTP003**

**From natural to heterologous production of sakacin P in *Corynebacterium glutamicum***

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Bacteriocins are ribosomally synthetized, small and heat-stable antimicrobial peptides. The majority of bacteria produces bacteriocins some of which have the ability to kill or inhibit pathogens relevant to the food and healthcare industry. Nevertheless, only a few bacteriocins are well characterized and commercially available. To overcome this limitation, automation and microcultivation are used for accelerated strain construction and bioprocess development.

The heterologous production of bacteriocins can help with their characterization and production, since natural producers often only achieve low cell densities. The class IIa bacteriocin sakacin P, naturally produced by *Latilactobacillus sakei*, targets the mannose phosphotransferase system of susceptible bacteria leading to pore formation and cell death. The industrial production organism *Corynebacterium glutamicum* has GRAS status and lacks a mannose phosphotransferase system and could thus serve as a suitable host for the heterologous production of sakacin P. As heterologous production may be limited by secretion, different secretion signal peptides are tested for optimal production of sakacin P.

An automated molecular biology workflow for accelerated strain construction of heterologous bacteriocin producers harbouring different secretion signal peptides was established. With already existing automated tools for quantification of antimicrobial activity, the heterologous producers will be screened and antimicrobial activity determined using the indicator strain *L. innocua* LMG2785/pNZ-pHin2*Lm*, which expresses the pH-dependent ratiometric fluorescence protein pHluorin2 reporting membrane damage (Reich et al. 2022).

The molecular biology workflow was successfully automated for the construction of heterologous sakacin P producers harbouring different secretion signal peptides. The screening allowed the comparison of the heterologous producer strains.

The demonstrated methods enable a widely autonomous strain construction for the heterologous production and screening of bacteriocins in *C. glutamicum*. In the future, further strain constructs may be rapidly evolved and evaluated in a fast and robust manner.

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**BTP004**

**Exploiting the untapped potential of *Paracoccus* as a novel chassis organism**

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In this study, *Paracoccus*, a Gram-negative bacterium from the phylum Alphaproteobacteria and family Rhodobacteraceae, was investigated for its suitability to be a new microbial chassis due to its innate ability to utilize a diverse range of substrates including cheap and renewable feedstocks, $CO_2$, C1 and C2 compounds like formate and like

ethylene glycol respectively, and high tolerance to salt and pH (1,2).

In order to investigate the suitability as a new chassis organism, studies were performed exploring substrate utilization and tolerance, optimal growth temperature, and pH values covering over fifty strains from this genus. In addition to the generation of physiological data, genomic studies including core- and pan-genome analyses were performed offering insights into evolutionary relationships. Moreover, to elucidate the metabolic flux in vivo, an in-depth analysis of flux distributions was performed with the type-strain of the genus, *Paracoccus pantotrophus* DSM 2944 using $^{13}C$ labeled glucose.

The flux map showed that *P. pantotrophus* prefers the pentose phosphate and Entner-Doudoroff pathways over glycolysis offering a surplus co-factor regeneration coupled with energy generation. A Paracoccus-specific genetic toolbox was designed comprising promoters with tunable strengths, gene integration and deletion strategies, and compatible origins of replication. Highlights of genetic engineering include growth on the non-native carbon-source terephthalic acid along with native carbon-source ethylene glycol, the two monomers from polyethylene terephthalate (PET). Finally, tailor-made fermentation strategies were established, showcasing the applicability of *Paracoccus* in bioreactors.

The results show that the metabolically versatile Paracoccus deserves the title of being an upcoming chassis organism.

Keywords: *Paracoccus*, chassis organism, physiology, metabolic flux, genetic engineering, bioinformatics, fermentation

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**BTP005**

**Metabolic engineering of *Pseudomonas putida* for fermentative production of flavor compound from green tea**

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L-Theanine naturally occurs in green tea and gives rise to its umami flavor in particular in *gyokuro* shadow tea. L-Theanine is a *N5*-ethylated L-glutamine and is synthesized from L-glutamate. *N*-functionalized amines play important roles in natural metabolic pathways or can be used for industrial relevant products. Isolation of *N*-alkylated glutamate derivatives shows low efficiency and chemical synthesis results in a racemic mixture, which makes a biotechnological approach favorable. *N2*-methylglutamate (NMeGlu) is an intermediate in the monomethylamine (MMA) assimilation pathway of some methylotrophs. In the reactions of γ-glutamylmethylamide synthetase (GMAS, encoded by *gmaS*) and NMG synthase (NMGS, encoded by *mgsABC*) MMA and glutamate are converted to ammonium and NMeGlu. Here, we describe sustainable production of *N*-alkylated glutamate derivatives from alkylamine and glucose, glycerol, or xylose in a fermentation process using metabolically engineered *P. putida* KT2440.

By heterologous gene expression of *mgsABC* and *gmaS* from *M. extorquens*, production of NMeGlu by fermentation with added MMA was established in *P. putida.* The host-vector system safety level 1 certified (HV-1) strain KT2440 was used, and glycerol-based glutamate production was achieved by replacement of *glpR*, which encodes for the transcriptional repressor of glycerol catabolism GlpR, with the endogenous glutamate dehydrogenase gene *gdhA*[1]. Next, it was revealed that expression of only *gmaS* was sufficient to enable L-theanine production. For xylose-based production the xylose catabolic operon *xylXABCD* from *C. crescentus* was expressed additionally[2]. Production optimized in shake flasks containing a minimal medium with 20 g $L^{-1}$ carbon source and MMA or MEA as alkylamines was transferred to 2 L-scale bioreactor fermentations operated in fed-batch mode[1,2].

Excellent titers were achieved. Production in shake flasks resulted in a titer 3.9 g $L^{-1}$ of NMeGlu, which was scaled-up in fed-batch bioreactors to 18 g $L^{-1}$ [1]. For L-theanine, titers of 2.6 g $L^{-1}$ and 21 g $L^{-1}$ were obtained in shake flasks and fed-batch bioreactors, respectively. Production was possible with glycerol, glucose and/or xylose as carbon sources[2].

To the best of our knowledge, this is the first sustainable L-theanine process using *P. putida* and the first operational with alternative carbon sources[2].

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**BTP006**

**Low-biomass concept for industrial biotechnology with engineered *Vibrio natriegens***

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In recent years, *Vibrio natriegens* gained attention in biotechnology due to its very fast growth and associated high specific substrate uptake rate ($q_S$) [1]. To reach a high volumetric productivity ($Q_P$) most industrial production processes must operate at a high final biomass concentration, wasting significant amounts of carbon for biocatalyst generation. Therefore, the choice of future production processes will be low-biomass processes with non-growing but metabolically highly active catalysts.

To achieve this, we deleted the *aceE* gene encoding the E1 subunit of the pyruvate dehydrogenase complex (PDHC) in the prophage-free strain *V. natriegens* Δ*vnp12* [2]. This PDHC-deficient strain is acetate auxotroph with a decoupled catabolite repression, allowing the simultaneous consumption of glucose and acetate. Moreover, the biomass formation can be adjusted by the initial amount of acetate, splitting the whole process into a growth and a production phase in which pyruvate is secreted into the medium. Already during growth on 7.5 g glucose $L^{-1}$ and 1 g acetate $L^{-1}$ in shaking flasks, up to 4.0 ± 0.3 g pyruvate $L^{-1}$ were

excreted. Therefore, batch fermentations were performed and up to 22.2 ± 0.8 g pyruvate $L^{-1}$ were produced after 10 hours with a yield of 0.59 ± 0.04 g pyruvate $g^{-1}$ glucose, which is already in the range of other reported processes between 0.4 and 0.72 g $g^{-1}$ (Reviewed in [3]). To further enhance the process, an acetate feed to supply the cell"s energy demand in the non-growth production phase was added. An acetate feed of 8 mM $h^{-1}$ starting when the growth phase ends, increased the titer to 41 ± 2 g pyruvate $L^{-1}$ with a $c_{x,max}$ of 6.6 ± 0.4 g $L^{-1}$. Moreover, the $q_S$ of the non-growing cells increased to 3.5 g glucose $g^{-1}_{CDW}$ $h^{-1}$, close to the $q_S$ of exponentially growing cells [1] and therefore over twice as high as the $q_S$ of growing *E. coli*.

During this process, we noticed parapyruvate as side product formation and investigated the influence of an annotated parapyruvate aldolase. While the deletion showed no growth effects, overexpression of the enzyme significantly reduced the growth rate in shaking flasks from 1.15 ± 0.01 $h^{-1}$ to 0.63 ± 0.01 $h^{-1}$.

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**BTP007**

**A genetic toolbox to facilitate metabolic engineering and new biotechnological applications of *Zymomonas mobilis***

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*Zymomonas mobilis* is a promising organism for the production of bulk chemicals derived from pyruvate, as it has a very high yield and specific productivity for ethanol using glucose as a substrate. To make *Z. mobilis* a competitive chassis organism for products beyond ethanol several obstacles still need to be overcome: (i) increasing tolerance against inhibitory substances, (ii) broadening the range of utilizable substrates, and (ii) efficient synthesis of heterologous products. One major limitation in achieving these goals is the lack of efficient genetic tools for *Z. mobilis* as available for other biotech workhorses such as *E. coli* and *S. cerevisiae*. Additionally, many aspects of the basic biology of *Z. mobilis*, like its uncoupled growth phenotype are still not fully understood, hindering its optimization. A genetic toolbox would help in unraveling such fundamental information.

As a major step forward in the direction of efficient genetic engineering of *Z. mobilis*, we have developed Zymo-Parts, a toolbox of characterized regulatory elements (promoters, ribosome binding sites, terminators) and shuttle vectors. The different elements of Zymo-Parts can be freely combined using a modular cloning (MoClo) Golden Gate-based system. As a concrete application example, we present a tunable rerouting of glycolytic flux in *Z. mobilis* from ethanol towards the production of lactate. Using elements from our toolbox, we were able to achieve lactate yields of 1.65 mol per mol Glucose and specific productivities over 60 mmol/g DCW/h, which are much higher than values reported before for any heterologous product synthesized by *Z. mobilis*.

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**BTP008**

**Construction and characterization of a transcription factor – Based biosensor for chorismate detection in *Corynebacterium glutamicum***

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Metabolic engineering of microorganisms for the production of high-value compounds requires high-throughput screening approaches to minimize extensive laborious work. Over the last years, transcription factor-based biosensors for the detection of biotechnologically interesting compounds in single cells gained importance in the field of biotechnology as powerful high-throughput screening tools, in particular in combination with fluorescence-activated cell-sorting (FACS) 1.

In this work, a biosensor for the detection of chorismate in the shikimate pathway of *Corynebacterium glutamicum* was constructed based on the native LysR-type transcriptional regulator QsuR. QsuR controls the expression of the *qsu* operon responsible for shikimate and quinate utilization 2. After the initial construction, the dynamic and operational range of the biosensor was determined. For this, the shikimate pathway intermediate quinate was supplemented as an inducer during cultivations since *C. glutamicum* has no known import system for the biosensor ligand chorismate 3. An increased fluorescence signal was detected for quinate concentrations ranging from 250 µM to 64 mM. The highest induction with a 40-fold increase in specific fluorescence was measured in the presence of 64 mM quinate. The ligand spectrum of the biosensor was determined through the addition of molecules structurally similar to chorismate or various intermediates of the shikimate pathway. A fluorescence signal was only detectable in response to the supplementation of quinate, which is readily converted to chorismate. Other intermediates and products of the shikimate pathway downstream of chorismate did not activate the biosensor. The subsequent ability of the biosensor to discriminate between different *C. glutamicum* strains engineered for the production of aromatic compounds confirmed the applicability of the biosensors future FACS-based screening campaigns.

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**BTP009**

**Establishing enzymatic cascade reactions on multifunctional bacterial magnetosomes**

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Magnetic nanoparticles are of increasing importance for many applications in the (bio)medical and biotechnological field. Due to a high surface-to-volume ratio they can be used as efficient carriers for functional moieties, in particular enzyme proteins, thereby generating flexible nanocatalysts. However, usually applied surface chemistries lack selectivity, and the decoration of the particle surface can hardly be controlled. Moreover, for enzyme immobilization often harsh

reaction conditions are required that might abolish catalytic activity [1].

A promising alternative might be provided by bacterial magnetosomes, biogenic nanoparticles synthesized by magnetotactic bacteria. In the alphaproteobacterium *Magnetospirillum gryphiswaldense* they consist of a monocrystalline magnetite core enveloped by a biological membrane [2]. The latter provides sites for the covalent attachment of functional moieties and, as bacterial magnetosomes are accessible to genetic engineering, enables the expression of foreign protein cargo as translational fusion to highly abundant magnetosome membrane proteins. It has already been demonstrated that magnetosomes are suitable carriers for multiple enzyme proteins, thereby maintaining or even enhancing their catalytic activity. Although this technique is highly selective and allows the simultaneous display of several functional units at distinct stoichiometries [3], the particles are pre-determined to distinct functions. In contrast, the expression of molecular connectors can turn the magnetosome surface into a multimodal platform for the immobilization of any complementary-tagged structures.

Using genetic engineering, we expressed versatile coupling groups on the magnetosome surface for the immobilization of a distinct set of enzyme proteins constituting biotechnologically relevant cascade reactions. Using different colorimetric assays as well as HPLC analyses, we monitored substrate conversion, specifically the formation of intermediates and the final products.

Overall, our study demonstrates the potential of functionalized magnetosomes as a flexible platform for catalytically active moieties. As the particles can be easily magnetically immobilized, they might be used e.g. as nanocatalysts for biocatalytic processes in flow reactor systems.

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**BTP010**

**Metabolic engineering of *Corynebacterium glutamicum* for the production of anthranilate from glucose and xylose**

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Anthranilate and its derivative aniline are important basic chemicals for the synthesis of polyurethanes as well as various dyes and food additives. Today, aniline is mainly chemically produced from petroleum-derived benzene, but it can also be obtained more sustainably by decarboxylation of the microbially produced shikimate pathway intermediate anthranilate.

In this study, *Corynebacterium glutamicum* was engineered for the microbial production of anthranilate from a carbon source mixture of glucose and xylose. First, a feedback-resistant 3-deoxy-arabinoheptulosonate-7-phosphate synthase from *E. coli*, catalyzing the first step of the shikimate pathway, was functionally introduced into *C. glutamicum* to enable anthranilate production. Furthermore, modulation of the translation efficiency of the genes for the shikimate kinase (*aroK*) and the anthranilate phosphoribosyltransferase (*trpD*) improved product formation. Deletion of two genes, one for a putative phosphatase (*nagD*) and one for a quinate/shikimate dehydrogenase (*qsuD*), abolished byproduct formation of glycerol and quinate. However, the introduction of an engineered anthranilate synthase (TrpEG) unresponsive to feedback inhibition by tryptophan had the most pronounced effect on anthranilate production. Component I of this enzyme (TrpE) was engineered by following a biosensor-based *in vivo* screening strategy for identifying variants with increased feedback-resistance in a semi-rational library of TrpE muteins.

The best strain to date accumulates up to 5.9 g/L (43 mM) anthranilate from a mixture of glucose and xylose in defined CGXII medium in bioreactor cultivations. We believe that the constructed *C. glutamicum* strain is not only limited to anthranilate production but could also be suitable for the synthesis of other biotechnologically interesting shikimate pathway intermediates.

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**BTP011**

**Genetic engineering via CRISPR-Cas9 enhances PHB production in methanotrophs**

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Introduction: Methane ($CH_4$) is the second most important greenhouse gas (GHG) after carbon dioxide ($CO_2$) and the most powerful, with an impact 28 times greater than $CO_2$ over 100 years. Globally, up to 65% of the total $CH_4$ emissions originate from human activities. Currently, the only known biological sinks for $CH_4$ are obligate methanotrophic bacteria (methanotrophs), capable to use $CH_4$ as their sole energy and carbon source. This makes methanotrophs a potential crucial player in curbing methane emissions and mitigating global warming.

Objectives: In type II methanotrophs, the capability to metabolize $CH_4$ and to biosynthesize polyhydroxybutyrate (PHB), a biopolyester with plastic-like properties, mingle. In our project GasValor we aim at metabolic engineering type II methanotrophic strains to fuel PHB production.

Methods: In detail, CRISPR-Cas9 and double homologous recombination of linear DNA fragments are used to achieve targeted gene deletions in *Methylocystis parvus* OBBP and *Methylosinus trichosporium* OB3b. Methanotrophs are grown in nitrate mineral salt (NMS) medium supplemented with 10µM $CuSO_4 \cdot 5\ H_2O$ at 30°C. Plates are incubated in 20% $CH_4$, 10% $O_2$, and 1% $CO_2$ gas-phase in (gas-tight) jars; liquid cultivations are carried out in serum-bottles at 200 rpm.

Results: *In silico* genome analysis confirmed the presence of two *phaZ*-gene loci, each encoding different PHB depolymerases for both methanotrophs. The deletion of one depolymerase in each methanotroph was achieved via CRISPR-Cas9. Unfortunately, double homologous recombination of linear DNA fragments did not produce successful gene deletions. The gas-chromatographic analysis verified that the deletions outcome was as expected and described for other species, i.e. an increase in PHB accumulation.

Conclusion: Bioconversion of methane to PHB is a notable example of killing two birds with one stone: it is an attractive solution to tackle global warming by mitigating $CH_4$

emissions and reducing oil-based material dependency. Hence, solutions to increase PHB production and optimization of recovery techniques urge to be investigated and researched further.

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**BTP012**

**Stirred-tank reactor bioleaching of cobalt and nickel from brazilian laterite ores**

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Laterite ore deposits in tropical countries, including Brazil, contain approximately 70% of the world's cobalt and nickel resources. Both metals are of interest due to their commercial importance, e.g. their use in lithium batteries of electrical vehicles. The recovery of cobalt and nickel via pyrometallurgy or high pressure acid leaching is usually incurred with high reagent and/or energy costs. Therefore, the development of an integrated low-energy and environmentally benign biohydrometallurgical process for the recovery of both metals from Brazilian laterite ores is the aim of the project BioProLat. On laboratory scale, bioleaching of laterites was carried out in 2 L stirred-tank reactors using autotrophic, acidophilic bacteria able to couple the oxidation of sulfur to the reduction of ferric iron, and thereby dissolving nickel- and cobalt-bearing mineral phases. Biogenic sulfuric acid production during the process ensured the acidic milieu needed to keep iron and other metals in solution. Mineralogical and geochemical analyses were used to identify attacked mineral phases and to estimate released metal portions by bioleaching, while microbiological analyses were used to monitor microorganisms during the leaching process. Results of laterite bioleaching with a consortium of different *Acidithiobacillus thiooxidans* strains under oxic conditions with and without pH control showed a recovery of up to 88% and 56% for cobalt and nickel, respectively. Eventually, the optimized process will be upscaled and reach pilot scale, transforming unexploited ores and limonite stockpiles into valuable resources, unlocking new reserves of raw materials through increasing recovery of metals from existing mines.

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**BTP013**

**Membrane manipulation by free fatty acids improves microbial plant polyphenol synthesis**

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Microbial synthesis of nutra- and pharmaceutically interesting plant polyphenols represents a more environmentally friendly alternative to chemical synthesis or plant extraction. However, most polyphenols are cytotoxic for microorganisms as they are believed to negatively affect cell integrity and transport processes. To increase the production performance of engineered cell factories, strategies have to be developed to mitigate these detrimental effects. We examine the accumulation of the stilbenoid resveratrol in the cell membrane and cell wall during its production using *Corynebacterium glutamicum* and uncover the membrane rigidifying effect of this stilbenoid experimentally and with molecular dynamics simulations. A screening of free fatty acid supplements identify palmitelaidic acid and linoleic acid as suitable additives to attenuate resveratrol"s cytotoxic effects resulting in a 3-fold higher product titer. This cost-effective approach to counteract membrane-damaging effects of product accumulation is transferable to the microbial production of other polyphenols and may represent an engineering target for other membrane-active bioproducts.

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**BTP014**

**Sucrose utilization in recombinant *E. coli* strains for efficient cofactor regeneration**

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Introduction**:** Many oxidoreductases such as Baeyer-Villiger monooxygenases (BVMO) depend on expensive cofactors such as NADPH, which impairs their industrial application and sustainability of the process. Efficient cofactor regeneration can be coupled to the cellular metabolism, however this might require additional carbon sources, lowering the atom economy. Alternatively, a cheap and abundant carbon source such as sucrose can be utilized. The photoautotrophic cyanobacterium *Synechocystis* sp. S02 produces sucrose from carbon dioxide, light and water and is able to secrete it into the medium. In a proof-of concept study the photosynthetically derived sucrose was utilized by co-cultivated *E. coli* W ΔcscR to regenerate NADPH for conversion of cyclohexanone to ε- caprolactone *via* recombinantly produced BVMO [Tóth et al., 2022].

Objectives: Herein, our goal was to expand the co-cultivation onto standard laboratory strains, which are natively unable to utilize sucrose and to use a faster BVMO variant isolated from *Bulkoderia xenovorans.* [Erdem et al., 2021]. Additionally, we investigated essential genetic features needed for *E. coli* strains to successfully utilize sucrose in a whole-cell biotransformation.

Materials & methods: Liquid flask cultivation was used to grow the microorganisms and *Syn* sp. S02 was immobilized into alginate beads. Whole-cell biotransformation was performed using 5 mM of substrate cyclohexanone at 25°C, with addition of 1 mM sucrose, 2 mM glucose or no sugar for a comparison. GC-FID was used to quantify the ε-caprolactone formation.

Results: In the presence of 1 mM sucrose we obtained specific activity of 7.45 U $g_{CDW}^{-1}$ using *E. coli* W ΔcscR whereas using *E. coli* BL21 (DE3) resulted in a specific activity of 3.35 U $g_{CDW}^{-1}$. Finally, the co-cultivation of sucrose-producing *Syn* sp. S02 and the sucrose-utilizing *E. coli* W ΔcscR reached full conversion of substrate within two hours.

Conclusion: We conclude that a coupled-cultivation system consisting engineered *Synechocystis* sp. and *E. coli* expressing recombinant genes expands the current application of light-driven production of biochemicals where photosynthetically produced sucrose is efficiently used to regenerate NADPH in whole cells.

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**BTP015**

**Screening approaches to identify bottlenecks in carotenoid production in *Corynebacterium glutamicum* through CRISPRi and application of structurally different lycopene β-cyclases**

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Carotenoids are yellow to red colored pigments with important roles in their biological hosts, but are also used in the feed, cosmetics and health industry. *Corynebacterium glutamicum* naturally synthesizes the rare yellow C50 carotenoid decaprenoxanthin and its glycosides, making it a potential host for carotenoid production [1].

A CRISPRi-based library was created to repress 74 genes in *C. glutamicum*, including those related to central metabolism, regulatory genes, and carotenoid biosynthesis [1] and used to screen for gene targets affecting production of the native carotenoid decaprenoxanthin.The CRISPRi screening identified 14 genes that affected natural decaprenoxanthin biosynthesis and revealed new bottlenecks in the central metabolism of *C. glutamicum*.

In a second approach, we aimed to convert the intermediate of decaprenoxanthin biosynthesis lycopene to β-carotene. To this end, the native genes *crtEb* and *crtYE/F* for decaprenoxanthin biosynthesis were deleted and genes for heterologous lycopene β-cyclases (EC 5.5.1.19) expressed. First, we systematically analyzed the phylogeny and transmembrane structures of this enzyme from all kingdoms of life by bioinformatics approaches. The phylogenetic tree showed five distinct clusters of lycopene β-cyclases and the DeepTMHMM [2] scan revealed four structurally different groups distinguishing membrane-bound from cytosolic enzymes. Nine representative lycopene β-cyclases from five different phylogenic lineages were chosen and tested for β-carotene and astaxanthin production in *C. glutamicum* [3]. New lycopene β-cyclases were identified that allowed increased carotenoid production. Notably, higher astaxanthin production and less feedback inhibition on total carotenoids were achieved with CrtL from *Synechococcus elongatus* and CrtYcd from *Brevibacterium linens*. There were indications of different feedback-inhibition mechanisms between the candidates.

Our combined approach identified limitations of precursor supply of carotenogenesis as revealed by a CRISPRi screening and new lycopene β-cyclases enabling efficient β-carotene and astaxanthin production by *C. glutamicum.*

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**BTP016**

**In vitro functionalization of bacterial magnetic nanoparticles by SpyTag-SpyCatcher "click biology"**

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Magnetic nanoparticles are of increasing importance for many biomedical and biotechnological applications as they can be used as a versatile platform for the immobilization of various molecules, ranging from simple drugs to complex protein cargo. Functionalization of synthetic nanoparticles is usually based on chemical crosslinking reactions, however, such procedures lack flexibility and cannot be controlled precisely. An alternative might be provided by magnetosomes, membrane-enveloped magnetic nanoparticles biosynthesized by magnetotactic bacteria like the model organism Magnetospirillum gryphiswaldense. Because of a genetically strictly controlled biomineralization process, magnetosomes exhibit superior physicochemical properties compared to their chemically synthesized counterparts. Furthermore, as magnetosome biosynthesis is accessible to genetic engineering, multifunctional particles can be produced by expression of foreign protein cargo as translational fusions to abundant magnetosome membrane proteins [1].

Although the functionalization of the particle surface by genetic engineering is highly selective and controllable, the process is demanding and time-consuming. In order to establish a more rapid but still highly specific (multi-)functionalization procedure, the display of molecular connectors has been explored. Specifically, the SpyTag-SpyCatcher system [2], has been adapted to magnetosomes and investigated with regard to specificity and functionality. Thereby, SpyCatcher display on the magnetosome surface enabled the effective covalent immobilization of SpyTag-equipped fluorophores and enzymatic proteins [3].

However, the interchangeability of constituting coupling partners has not been investigated so far. In our study we demonstrate that the SpyTag peptide can be efficiently expressed on the magnetosome surface, thereby generating a flexible adapter platform that enables the simultaneous coupling of SpyCatcher bearing proteins such as antibodies and fluorophores, as well as SpyCatcher decorated beads. Overall, the magnetosome-adapted SpyTag-SpyCatcher system greatly enhances the existing toolkit for magnetosome functionalization, turning the particle surface into a multimodal platform for functional moieties.

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**BTP017**

**A temperature dependent pilin promoter for production of thermostable enzymes in *Thermus thermophilus***

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Introduction: Thermostable enzymes from thermophiles have gained significant attention for their stability and high catalytic efficiency. However, their production in mesophilic hosts, such as *Escherichia coli*, often results in inactive enzymes, incorrect incorporation of cofactors, and aggregation. To overcome these problems, thermophilic hosts such as the transformable *Thermus thermophilus* have been studied as potential production platform. However, the pool of systems for protein overproduction in *Thermus* is still limited.

Objectives: We aimed to develop a novel expression system for the overproduction of thermostable enzymes in *T. thermophilus* using the promoter of the gene encoding the structural subunit of type IV pili (PilA4p).

Materials & methods**:** *E. coli* and *T. thermophilus* strains were cultivated in appropriate media. Plasmid construction was carried out to introduce the putative *pilA4* promoter into the pMKE2-*bglT* plasmid, which carried the β-glucosidase gene *bglT.* The characterization of *PpilA4* was performed with the plasmids, pMKE2-PpilA4-206 and pMKE2-PpilA4-499. Furthermore, β-glucosidase reporter gene assays as

well as Western blot studies were performed to assess promoter activity and protein production.

Results**:** The *PpilA4* promoter was successfully used to overproduce active β-glucosidase in large amounts in *T. thermophilus* at an optimal growth temperature of 68 °C. The longer 499 bp fragment mediated a higher promoter activity than the shorter 206 bp fragment. Highest protein production was observed at 68 °C in *Thermus* minimal medium. Increase of the growth temperature to 80 °C completely abolished *PpilA4*-mediated enzyme production.

Conclusion: We successfully expressed a thermostable β-glucosidase as active form in *T. thermophiles* and show that the *pilA4*-promoter based expression system can be used for temperature dependent production of thermostable enzymes.

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**BTP018**

**Artificial conductive biofilms in *Shewanella oneidensis***

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The transition to a sustainable future depends heavily on investigating alternative ways to produce power and platform chemicals. For this purpose, exoelectrogenic organisms can be utilized as catalysts in bioelectrochemical systems. In this process, chemical energy is directly converted into electrical energy or valuable products by anodic biofilms. Since the biofilm thickness is a limiting factor when it comes to current generation, we aim to establish a system forming a thick conductive biofilm. To increase the conductive biofilm volume the Spytag/Spycatcher system was introduced into the extracellular *c*-type cytochrome MtrC. The split protein domains Spytag/Spycatcher form a covalent bond *in vitro* spontaneously at physical interaction and thus can be used to crosslink cells when integrated in proteins displayed at the cell surface. In addition, MtrC has been shown to move across the outer membrane and to transfer electrons intermolecular1. Hence, it is the ideal candidate for the suggested artificial biofilm approach.

As the C-terminus integration of the linker peptide hindered the secretion of MtrC, a novel technique for the determination of the optimal integration site was developed. Therefore, we aimed to identify a functional MtrC version which has the linker incorporated. A transposon integration was combined with an isothermal *in vitro* assembly to generate random integration events of our insert. We obtained a mixed culture with the linker integrated every six amino acids. After screening of over 3,000 colonies in a ferric reduction assay, the remaining clones were tested for the accessibility of the insert. Thus, magnetic beads were functionalized with Spytag or Spycatcher followed by incubation with the respective MtrC-linker cells. Fluorescence microscopy revealed that the cells were able to form an artificial biofilm on the functionalized bead surface. Oxford nanopore sequencing showed that only a few integration positions led to a functional MtrC with an accessible linker on the outside of the cell. In our next step, we will use the three most abundant versions to investigate whether we can increase the conductive biofilm volume in a microfluidic system to consequently improve current density output.

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**BTP019**

**Metabolic engineering of *Hydrogenophaga pseudoflava* for fatty acid production**

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Introduction: More efficient and sustainable production of chemicals is becoming increasingly important in times of climate change and global pollution. A promising approach is the aerobic gas fermentation using carboxydotrophic Knallgas-bacteria. The Gram-negative β-proteobacterium *Hydrogenophaga pseudoflava* DSM 1084 shows comparable high growth rates heterotrophically as well as on CO, $H_2$ and $CO_2$, and mixtures thereof under aerobic conditions. Moreover, a basic genetic engineering toolbox is available which renders this bacterium an interesting host for biotechnological application [1, 2].

Objectives: Fatty acids (FA) have a wide range of applications, including cosmetic products, lubricants or food [3]. In this study, we engineered *H. pseudoflava* for FA production by deregulating FA biosynthesis, increasing precursor availability by avoiding polyhydroxybutyrate (PHB) synthesis, preventing the degradation of FAs by deleting putative *fadD* genes and optimizing the cultivation conditions.

Materials & methods: All strains were cultivated under phosphate-limiting conditions (0.178 g $Na_2HPO_4$ $L^{-1}$ and 0.075 g $KH_2PO_4$ $L^{-1}$) using 10 g fructose $L^{-1}$ as sole carbon source. FAs were quantified via GC-FID (Agilent 8890 GC System) equipped with a DB-FATWAX UI (30 m x 250 µm x 0.25 µm).

Results: *H. pseudoflava* Δ*fadD2* Δ*fadD3* produced 49 mg $L^{-1}$ extracellularly FAs, which is 39 % more than the wild type (WT) strain. Deletion of both *phbA* genes avoided PHB synthesis but negatively affected the growth on fructose. Compared to the WT, the mutant strain Δ*phbA1* Δ*phbA2* reached a lower final biomass concentration (3.68 ± 0.18 g $L^{-1}$ vs. 1.64 ± 0.21 g $L^{-1}$) and consumed less of the fructose (8.0 ± 0.4 g vs. 5.3 ± 1.2 g) after 48 h. However, the mutant strain produced 96 % more FAs per biomass and had a 39 % higher product yield.

Conclusion: We engineered *H. pseudoflava* for fatty acid overproduction for the first time. Future studies have to verify if the introduced genetic modifications also lead to an overproduction of FAs under autotrophic conditions.

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**BTP020**

**Pre-treatment strategies enhance aromatic degradation in a synthetic actinobacterial consortium**

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Introduction: To fully harness the potential of microorganisms, several strategies are being done such as

using a consortium. The use of microbial consortium may lead to improved activities due to production of different enzymes and functional redundancies. Actinobacteria are metabolic powerhouses that can degrade a plethora of harmful compounds such as styrene, phenols, and synthetic dyes. Therefore, it could be promising to investigate the potential of an actinobacterial consortium.

Objectives: We present a synthetic actinobacterial consortium comprised of *Gordonia* and *Rhodococcus* species. Our aim is to develop a functional consortium that can degrade a variety of pollutants with a focus in the application of wastewater systems.

Methods: To determine the best degraders, about 100 isolates from our strain library were screened using a minimal medium plate with different compounds. The best degraders were narrowed down and were subjected to a series of liquid culture assays with just the compounds as the energy source. UV-vis spectrophotometer and HPLC were used to analyse the samples.

Results: Experiments showed that careful construction and pre-treatment of the consortium is necessary to reach the best results. Moreover, how these organisms can uptake and destroy compounds can differ depending on the pre-treatment beforehand. It was shown that even with the 100% uptake of the synthetic dyes tested, a lack of pre-treatment of simpler aromatics can only lead to dye adsorption.

When pre-treated with simpler aromatics such as phenols, this led to higher biomass of the actinobacterial consortium - suggesting it can use the simpler aromatics as the carbon source. When the biomass of this pre-treated consortium was used on different types of dyes, it led to improved and faster degradation of compounds – showing about 100% degradation for some dyes in a shorter period. When compared with the untreated consortium, it also displayed better results in decolorizing dyes that were not accepted before such as reactive red 120.

Conclusion: Mixing different bacteria to achieve a functional consortium is not as easy as just throwing microbes in one pot. The use of pre-treatment strategies is necessary to maximize the potential of such consortium. Moreover, this strategy can be applied for adaptive laboratory evolution of individual isolates or consortium to turn-on certain pathways and effectively degrade xenobiotics.

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**BTP021**

**Linker mutagenesis: Changes in fusion protein activities by varying linker length and properties**

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Introduction: The peptide linker and its length have an influence on the biocatalytic activity of the separate protein domains of a fusion protein. The fusion of a formate dehydrogenase from *Candida boidinii* and azoreductase from *Rhodococcus opacus* 1CP was reported to have changes in the activities and overall biochemical properties when linked together with histidine linkers for NADH regeneration. Therefore, it is of great interest to investigate the influence of different linkers on the properties of the protein domains.

Objectives: The objectives of this research include the investigation and biochemical characterization of the previously reported FDHC23S+AzoRo fusion protein with flexible, helical, and non-helical rigid linkers with varied length under different conditions, to determine changes in overall properties, enhancement of enzymatic activities and changes in substrate scope.

Materials and Methods: To determine the extent of linker properties and lengths on substrate acceptance and thus the structure of the fusion protein, different dyes were examined for degradation by the linker variants. Moreover, kinetic parameters for the most promising linker constructs were determined by examining the degradation of Methyl Red and Brilliant Black BN.

Results: The improved substrate promiscuity was not limited to Brilliant Black BN, but the fusion protein linker constructs also accepted other azo dyes. Overall, the fusion proteins showed improved activities against the dyes tested. Moreover, the results showed higher Km-value of FDHC23S+AzoRo for Methyl Red and but lower for Brilliant Black BN, indicating changes in the affinity to the proteins. Also, linker constructs showed differences depending on linker and length, indicating structural impact on biocatalysis.

Conclusion: Overall, the studies show promising results regarding the effect of the peptide linkers with different properties and lengths on the biochemical activity of the fusion protein. Structural information like structural resolution or the dimerization state and methods such as LC-MS and HPLC may be helpful to understand the reaction mechanism and the influence of altered structure generated by the different linkers on the enzymatic activity of the FDHC23S+AzoRo fusion protein.

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**BTP022**

**Streamlining bioprocess optimisation for bifunctional protein production in *Corynebacterium glutamicum***

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Bifunctional proteins (BiFuProts) are a promising tool for a more sustainable industry. Their bipartite structure consists of an adhesion-promoting peptide and a functional domain. This enables specific targeting and functionalisation of surfaces, such as crop leaves or packaging foils. The wide variety of prospective applications requires bioprocess optimisation workflows that can easily be adapted to new target proteins. Two key aspects here are the accelerated creation of new strain libraries and a generalizable, activity-independent protein quantification assay.

Secretion of heterologously produced target proteins by the host organism streamlines the production process by eliminating several downstream processing steps. It is, however, not yet possible to predict under what conditions secretion efficiency will be optimal, requiring the screening of larger strain libraries under different process conditions.

The objectives of this work are to create a library of *C. glutamicum* secretion strains expressing target proteins fused to different Sec-type signal peptides (SP) and screening the influence of these SP on secretion efficiency. To accelerate strain library creation, relevant molecular biology steps have to be automated. For the quantification of secreted proteins, a fast and reliable activity-independent assay is needed.

Strain libraries of *C. glutamicum* expressing BiFuProts fused to 24 Sec-type SP were created using automated protocols for the Opentrons OT-2 lab robot. Strains were cultivated in a BioLector Pro microcultivation system and automatically processed using a TECAN Freedom Evo lab robotic platform. Secreted proteins were purified and characterised in a split GFP assay and by SDS-PAGE.

Central molecular biology steps were automated using an open-source lab robot, which reduced sample processing time and manual work. Different activity-independent protein quantification assays were compared and a suitable assay for quantification of the secreted BiFuProts was identified. The effects of different Sec SP on the secretion efficiency of *C. glutamicum* for selected proteins were evaluated.

BiFuProts have great potential for multiple applications in industry. With the parallelised strain construction workflow and an improved protein quantification method, bioprocess optimisation was accelerated. The production of sufficient quantities of newly designed BiFuProts for field studies and commercialisation is now possible in a shorter timeframe.

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**BTP023**

**From waste to bioplastic – The genetic modification of bacterial isolates from waste to force PHB production during growth on waste**

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Conventional plastic is a rapidly increasing problem for the environment, yet it is impossible to imagine everyday life without it. For this reason, alternatives for this material are needed and one such alternative could be polyhydroxybutyrate (PHB). PHB is a biodegradable polymer that is formed in various microorganisms as a carbon storage. It could replace common plastics if it were more cost-effective to produce. Industrially, the bacterium *Cupriavidus necator* is mainly used for PHB synthesis and is considered the most suitable organism for this purpose. However, it requires expensive carbon sources for synthesis of the biopolymer, which can account for up to 50% of the total cost. Therefore, a new strategy is needed in which the production expenses are reduced by using agricultural waste as a carbon source.

The idea is to isolate *Acetobacter fabarum* from untreated apple juice residues obtained from an industrial source and to modify it by using the plasmid pWBT-PT5-*phaABC*, which carries the genes for PHB-synthesis. This way it is possible to utilize the natural ability of bacterial isolates that have already adapted to the environment of agricultural waste and force them to produce PHB.

For this work it was necessary to design a plasmid with the relevant genes and good expression patterns in bacteria isolated from agricultural waste and to introduce it into *A. fabarum*. The PHB-production was monitored via Nil-Red staining, measurement of fluorescence and PHB-extractions.

We managed to produce 0.345 g/L polyhydroxybutyrate with apple juice residues as carbon source for *A. fabarum* + pWBT-PT5-*phaABC* which is a bit lacking in comparison to industrial strains but an interesting new way to address the problem of high cost in PHB-production and might bring great results in the future.

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**BTP024**

**Fate and microbial transformation of sulfonamide antibiotics during bankfiltration column experiments**

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Introduction: Wastewater treatment plants do not sufficiently remove sulfonamide antibiotics and their transformation products (TPs) and hence discharge considerable amounts into surface water. In order to effectively pre-treat surface water, bankfiltration represents a well-established process to potentially mitigate the risk of antibiotic resistance propagation to groundwater as highly vulnerable drinking water source. Despite the essential role of microbial processes to attenuate sulfonamides during bankfiltration, the redox conditions and the responsible microbial community governing their fate and the fate of their TPs are not fully revealed to date.

Objectives: The objective of the presented study was to mimic the bankfiltration process on lab-scale and establish sulfonamide transformation activity by testing different redox conditions. Thereby we aimed to enrich and describe transforming microbial populations and link revealed transformation pathways with the respective metabolism of the enriched communities.

Material and methods: We have operated lab-scale sediment columns in replica under oxic and anoxic conditions and varying antibiotics concentration levels (0.001 to 10 mg L-1). Tandem mass spectrometry was employed to investigate the formation and persistence of TPs. Additionally, we are using 16S-rRNA gene based microbial community fingerprinting methods (d*enaturing gel gradient electrophoresis*, DGGE) and later amplicon sequencing to identify key populations involved in the transformation of sulfonamides.

Results: We observed the disappearance of sulfonamides in 0.001 and 0.1 mg L-1 columns under anoxic but not oxic conditions, detected the formation of several TPs and hypothesized transformation pathways. In addition, we described the shift of microbial populations fingerprint within the columns by DGGE and identified microbes that were potentially involved in the sulfonamide transformation process.

Conclusion: The conducted column experiments indicated the suitability of bankfiltration under certain redox conditions to promote the natural attenuation of antibiotics and thereby protect groundwater as essential drinking water resource.

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**BTP025**

**Expansion of plasma-driven biocatalysis using the unspecific peroxygenase from *Collariella virescens* and a capillary plasma jet**

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Plasma is a complex mixture of various components, including electrons, ions, radicals, and excited species. Especially, reactive oxygen and nitrogen species (RONS) are of interest for biological applications. One of the long-living species is hydrogen peroxide ($H_2O_2$), which we aim to

utilize in biocatalysis applications to convert substrates into more valuable products using suitable enzymes. The enzyme class of peroxygenases carries out one-electron oxidation reactions and stereoselective oxyfunctionalizations using $H_2O_2$.Their industrial application and the use of $H_2O_2$ in biocatalysis is very challenging, since high concentrations of $H_2O_2$ result in enzyme inactivation. We previously reported a non-invasive approach for *in situ* $H_2O_2$ production for biocatalysis using non-thermal atmospheric pressure plasma [1]. It has already been shown that plasma-driven biocatalysis with recombinant unspecific peroxygenase from *Agrocybe aegerita* (r*Aae*UPO) is possible using the µAPPJ as plasma source and immobilization of the enzyme as a protection strategy [2]. For biotechnological applications, it is necessary to produce enzymes in large quantities in a cost-effective manner. *Escherichia coli* (*E. coli*) is a commonly used host organism for overproduction since it can be handled safely and multiplies quickly. Todate, r*Aae*UPO cannot be produced in *E. coli*. Therefore, it appears attractive to employ other enzymes to perform oxidation reactions. The unspecific peroxygenase from *Collariella virescens* (*Cvi*UPO) can be heterologously produced in *E. coli* [3] and could thus present a promising alternative. In this study, we investigated the utilization of *Cvi*UPO for plasma-driven biocatalysis. To this end, *Cvi*UPO was produced in *E. coli* and plasma-driven biocatalysis was performed with purified *Cvi*UPO using a capillary plasma jet. Like for the model enzyme r*Aae*UPO enzyme stability was improved by immobilization. We tested different immobilization types for their performance under plasma treatment. The residual activity after 15 min plasma treatment ranged from 5 to 80% depending on the type of carrier used. To evaluate reusability of the immobilized enzyme, conversion rates were tested over several cycles. The enzyme remained active for 390 min when the reaction solution was exchanged every 10 min and the enzyme was immobilized onto amino beads. In conclusion, we were able to expand the plasma-driven biocatalysis by using heterologously produced *Cvi*UPO and a capillary plasma jet.

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**BTP026**

**Conidia-based process for the scale-up of optimized fermentative L-malic acid production**

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Global conflicts and advancing climate change are driving the need for regional and environmentally friendly sources of raw materials. As part of the FNR project MALUM, the aim is to promote the transition from an oil-based to a more sustainable economy. The project seeks to establish the biotechnological production of L-malic acid using agro-industrial by-products. Unlike chemical synthesis, the production of L-malic acid using the fungus *Aspergillus oryzae* promises a pure racemate end product. L-malic acid and its polymers are used in the food and beverage industry as well as in pharmaceuticals and cleaning agents.

The objective is to establish a conidia-based process for the scale-up of optimized fermentative L-malic acid production based on the previously established process published by Kövilein et al. (2022) and Kubisch & Ochsenreither (2022). Initially, a conidia-based inoculation method is established, using conidia from *A. oryzae* directly for the inoculation of the production fermenter without prior biomass production in a preculture. Since significantly higher local power peaks are generated in the fermenter than in shaked cultivation vessels, parameters such as stirrer speed and stirrer distance must be set to ensure suitable homogenisation, oxygen supply, maintenance of morphology and prevention of biofilm formation while protecting against fragmenting shear forces. The most promising process window is used for scale-up into a 30 L fermenter considering important aspects of scale-ups. In parallel, a purification strategy, including reactive extraction, is being developed.

Spore recovery is performed on agar plates, and spore concentrations are determined via Thoma counting chambers. The process optimization is carried out in 2 L laboratory fermenters. Enzyme kits and high-performance liquid chromatography are used to determine the substance concentrations of the substrates consumed and the products formed. Parameters like pH, dissolved oxygen tension, oxygen transfer rate, carbon dioxide transfer rate, electrical capacity and conductivity are measured online. The osmolality of selected samples is measured using an osmometer. The biomass concentration is determined by weighting the dried mycelium.

First results on using conidia from *A. oryzae* directly for the inoculation of the production fermenter will be presented.

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**BTP027**

**The FAST way to assess promoter strength, riboswitches and inducibility in *Clostridium cellulovorans***

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The transformation to a sustainable industry is one of the major tasks of our society. Renewable resources such as lignocellulosic plant material offers great potential as raw material for the biotechnological production of chemicals or biofuels without competing with food production. To develop sustainable production processes from lignocellulosic substrates, the anaerobic *Clostridium cellulovorans* is an interesting organism due to its ability to degrade cellulosic material at a moderate temperature. However, there is a need to establish and improve tools for genetic modification of this microorganism.

One key requirement for strain engineering and biotechnological applications is the ability to monitor the promotor strength in order to control the level or time point of protein expression. Unfortunately, the establishment of suitable reporter systems are still challenging in *Clostridia* because of oxygen requirement or low signal strength of most reporter proteins. Recently the application of the fluorescence absorption-shift tag (FAST) system was described for different anaerobes.

In this study, we use our rapid and easy-to-use protocol for the introduction of DNA into *C. cellulovorans* by triparental conjugation and demonstrate the successful use of the fluorescence absorption-shift tag (FAST) system to characterize the strengths of different promoters.

In this way, we characterized a set of promotors and riboswitches for controlled gene expression in *C. cellulovorans* that can be used for various applications such as the development of *codBA* and CRISPR/Cas9 based markerless gene editing systems for *C. cellulovorans*.

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**BTP028**

**Establishment of a biobased upcycling process of hemi- and lignocellulosic biomass**

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Introduction: Anaerobic fungi with their cellulosomes can hydrolyse hemi- and lignocellulosic grasses and subsequently metabolize them to form hydrogen, carbon dioxide, ethanol, acetate, formate, lactate and succinate. While the hydrogen formed can be used effectively as a green energy source and energy storage to avoid fossil fuels, the other metabolites, especially acetate and lactate, can be used for the production of basic chemicals. Oleaginous yeasts, on the other hand, are ideally suited for the production of oils from acetate and other residual streams. The produced oils can be extracted on a large industrial scale via the Pulsed Electric Field (PEF) process and can thus be used in a wide range of applications.

Objectives: The aim of this work is the establishment of a biorefinery concept based on hemi- and lignocellulosic raw materials, anaerobic fungi and oleaginous yeasts. As the primary C-source for the later established process the metabolite production of anaerobic fungi is therefore analyzed and improved. At the same time the oil production by oleaginous yeasts and the use of residual streams as C, N and P-source with subsequent establishment of the biorefinery process is being investigated.

Material & methods: Batch cultivation in serum flasks and shake flasks, upscale to bioreactor cultivations and cascade-like coupling of the anaerobic fungi and aerobic yeast fermentation. Full analyses of the nutrients and metabolites by GC, HPLC and enzymatic assays.

Results: Anaerobic fungi with their cellulosomes were able hydrolyse wheat straw and subsequently metabolize this to form hydrogen, carbon dioxide, ethanol, acetate, formate, lactate and succinate. Lipid content of up to 40 % of cell dry weight has been produced with several oleaginous yeast grown in batch bioreactors.

Conclusion: Anaerobic fungi can be a tool for the utilisation of a wide variety of previously untapped C sources and in combination with other organisms, can enable a variety of environmentally friendly platform chemicals.

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**BTP029**

**Characterization of a multi-modular xanthanase from *Cohnella* sp**

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*Cohnella* sp. is a good xanthan-degrader, which was revealed by bacterial growth in xanthan minimal medium. Degradation of xanthan was confirmed by congo red staining after growth of the strain on plates containing xanthan. After comparing the secretome of *Cohnella* sp. from different media (lysogeny broth, glucose minimal medium, xanthan minimal medium), a xanthanase designated as *Csp*Xan9 was found and its gene was successfully expressed in *E. coli* Rosetta2. *Csp*Xan9 could efficiently degrade xanthan after previous removal of pyruvylated mannose residues from the ends of the native xanthan side chains by xanthan lyase treatment. Compared with a known xanthanase from *Paenibacillus nanensis*, the modular xanthanase *Csp*Xan9 had a different module composition at the N- and C-terminal ends. The results of thin layer chromatography and high-performance anion-exchange chromatography (HPAEC-PAD) showed that *Csp*Xan9 could putatively produce tetrasaccharides and octasaccharides as primary products from xanthan lyase-pretreated xanthan. In order to explore the functions of the N- and C-terminal regions of the enzyme, deletion derivatives (*Csp*Xan9-C, *Csp*Xan9-N, *Csp*Xan9-C-N) lacking some of the non-catalytic domains were produced in recombinant *E. coli* strains. After protein purification, enzyme assays indicated these deletions resulted in a shift of the specific activity from 10.31 ± 0.23 U/µg (*Csp*Xan9-C) to 1.38 ± 0.04 U/µg (*Csp*Xan9-C-N). Deletion of the C-terminus (*Csp*Xan9-C) resulted in an enzyme that retained xanthanase activity but no longer showed retarded mobility in native polyacrylamide gel eletrophoresis containing different concentration of modified xanthan.

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**BTP030**

**Oxygenic photosynthesis-driven hydrogen formation by the oxygen-sensitive [NiFe] hydrogenase of *Synechocystis* sp PCC6803**

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Introduction: To enable a transition towards sustainable energy production, hydrogen has gained attention as a renewable energy carrier. Cyanobacteria have emerged as a promising candidate for biological hydrogen production through oxygenic photosynthesis, utilizing sunlight. In fact, the native [NiFe] hydrogenase of the unicellular cyanobacterium *Synechocystis* PCC6803 is capable of photohydrogen production during dark light transition. In the genetically modified strain M55, deficient in the *ndhB* gene of the NDH-1 complex, cyclic electron flow around PS1 is disrupted, resulting in prolonged hydrogen production. However, enzymatic activity in both WT and M55 is inhibited during extended exposure to light, potentially due to the inhibitory effects of oxygen produced during oxygenic photosynthesis.

Objectives: The aim of this study was to develop a system for extracellular oxygen removal without interfering with cyanobacterial metabolism or the supply of electrons to the hydrogenase. Additionally, cytosolic components for hydrogenase protection against oxygen *in vivo* should be identified.

Materials & methods: Therefore, a cocultivation system was established using *Pseudomonas taiwanensis* VLB120, which efficiently removes oxygen through respiration using citrate as carbon source. Hydrogen formation in light was quantified in different reaction setups. Additionally, enzymatic activity in crude extracts was determined after exposure to oxygen, employing different buffer additives to evaluate protective effects of cytosolic components.

Results: Notably, biological extracellular oxygen removal resulted in prolonged hydrogen production over a period of days and weeks, utilizing electrons derived from oxygenic photosynthesis. The strain M55 achieved hydrogen production rates of up to 0.42 ± 0.01 U/gCDW. Furthermore, the potential protective effects of the intracellular environment, particularly focussing on the redox status will be addressed.

Conclusion: Our findings indicate that photohydrogen production using an oxygen-sensitive hydrogenase is feasible when oxygen is removed from the cytoplasm exclusively via the extracellular environment. Cellular homoeostasis contributes significantly to the protection of the [NiFe] hydrogenase against intracellular oxygen generated during photosynthesis.

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**BTP031**

**Generation of a genetic toolbox for protein production in actinobacteria**

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Introduction: With biotechnology gaining more traction in an effort towards more sustainable processes in the chemical and pharmaceutical industries, focus is mostly set on established systems for obtaining suitable biocatalysts. However, the established systems are generally not suitable for the expression of genes with an elevated Guanosine (G) and Cytosine (C) content, and thus pose as difficulties for application. Little attention was devoted to actinobacteria, which have elevated G and C contents, and would therefore be suitable for the expression of such genes. Unfortunately, there is no established genetic toolbox for manipulation of and expression in actinobacteria, with only some useable plasmid-systems being published. Hence, the generation of a genetic toolbox for actinobacteria is of interest to harness the potential of biocatalysts that originate from genes with elevated G and C content. In addition, the use of actinobacteria is also of interest, due to their inherent resistance to xenobiotics.

Objectives: We present a genetic toolbox for a set of different actinobacteria, *Rhodococcus*, *Gordonia*, and *Kocuria*. Available plasmid-systems will be compared with respect to their transformation efficiency and expression levels of a reporter gene (sfGFP) to give an overview of suitable plasmid-systems with respect to the different actinobacteria. As a proof of concept, promising systems will be used to produce a set of biocatalysts.

Methods: A list of plasmids that are useable in actinobacteria was derived from literature. As a reporter gene, sfGFP was cloned into the respective plasmids. Creation of competent cells and transformation via electroporation was optimized, and then performed using generalized protocol. Transformation efficiency and expression levels were compared.

Results: The reporter gene was successfully integrated into the respective plasmid-systems. Experiments showed that there are differences in transformation efficiency of the selected plasmid-systems between the different actinobacteria. It was observed that different plasmid-backbones are not well suited for certain strains, and that there are also differences in expression levels, depending on the used promoter (constitutive or inducible).

Actinobacteria are suitable hosts for the expression of genes with an elevated G/C content. While literature does not provide an overview of useable plasmid-systems, we have summarized usable systems for the expression of such genes.

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**BTP032**

**Marine fungi as a source for mycoremediation of manure and sewage sludge**

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The application of manure and sewage sludge on farmland is a key element in building up soil organic matter and contributes to soil carbon sequestration. However, these organic fertilizers are often contaminated with anthropogenic pollutants that can harm ecosystems and persist for a long time. Bioremediation approaches have gained attention as clean-up treatment in recent decades due to their cost-effectiveness and environmental friendliness. Besides phytoremediation and bacterial bioremediation, mycoremediation has also been successfully applied for pollution control. While land-based fungal species, especially white-rot fungi, have been extensively studied for mycoremediation, marine fungi are underrepresented but have adaptive traits (e.g., halophilic, anaerobic) that could be beneficial under certain conditions and may expand the degradation portfolio. In this study, marine filamentous fungi from the Flensburg strain collection of marine fungi are screened for their ability to tolerate and degrade organic pollutants, such as pharmaceuticals, PCBs, PAHs, and PFAS, in a simple growth medium. In a second step, favorable fungal candidates are employed to degrade organic contaminants in manure and sewage sludge, and degradation rate and products are assessed using LC-MS, with kinetics determined. This study provides an example of an applied bioremediation technique as a sustainable, eco-friendly, and inexpensive way to deal with pollutants and thereby promoting the use of organic fertilizers in agriculture to scale up soil carbon sequestration.

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**BTP033**

**Evaluation of dynamic stress responses of *Pseudomonas putida* with fluorescent dual reporters**

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During large-scale biotechnological fermentation in bioreactors, inhomogeneous areas appear due to imperfect mixing and foam formation. Cells passing the emerging inhomogeneities experience alternating conditions which can induce stress and influence the performance of a fermentation process. Investigation of inhomogeneity effects in large-scale reactors is often performed in scale-down models. Analyzing the cells responses and adaptations to these inhomogeneities is a crucial part for the establishment of a complete model. Here, plasmid based dual reporter systems with rapidly maturing fluorescence reporter proteins were generated to investigate the activity of specific stress inducible promoters during heterologous rhamnolipid production in *Pseudomonas putida* KT2440. Relative fluorescence reporter intensities were measured to analyze stress responses of *P. putida*, as a first step towards mimicking large-scale inhomogeneities in scale-down experiments. The results will contribute to the general view of microbial stress responses and help to evaluate the effects on process performance during rhamnolipid production.

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**BTP034**

**Phage-assisted synthetic evolution of cytosine deaminases for genome editing**

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Introduction: Base editing is a genome editing technique that allows targeted changes of single DNA bases. Fusions of TALEs (transcription activator-like effectors) and the cytosine deaminase DddA (double-stranded DNA deaminase A) can be used for C-to-T base editing on chromosomes, mitochondria and chloroplasts. The efficiency of the native DddA is strongly restricted to cytosines in a 5''-TC context, but the enzyme has previously been evolved to also recognize 5'-AC and 5'-CC, but not 5'-GC targets. In contrast, SsdA is a cytosine deaminase with broad specificity for the target C context, but it prefers ssDNA over dsDNA. Both, DddA and SsdA are toxins and their activity needs to carefully controlled.

Objectives: We use phage-assisted evolution to develop DddA variants for efficient cytosine base editing in all contexts. In addition, we aim to evolve SsdA as a novel cytosine deaminase for genome editing.

Materials & Methods**:** DddA is split into two non-toxic fragments which together regain activity at a target site. The N-terminal fragment (DddA-N) is cloned into a continuously evolving M13 phage to function in *coli* with a TALE base editor pair in a selection circuit that favors 5'-GC target specificity. The activity of DddA and SsdA is further tested in a plant-based reporter system.

Results: To set up the selection system, coiled-coil tags were successfully established to recruit DddA to the TALE protein at the target site. This system now allows to evolve the DddA part independently of the TALE. Phage propagation and activation of reporter constructs on suitable target sequences was achieved. In the next steps, these components will be used to evolve DddA-N variants. For this, phage populations will be passaged through non-continuous *coli* cultures with selection circuits for the different contexts. Further progress on DddA evolution will be reported. SsdA was tested first as CRISPR/Cas9 fusion in a beta-glucuronidase plant reporter system and we could thereby establish this enzyme as a novel cytosine deaminase for base editing. Further steps to evolve this enzyme for use in a TALE base editor will be reported.

Conclusion: Phage-assisted evolution is a powerful technique to domesticate enzymes and optimize their activity in genome editing tools.

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**BTP035**

**Accelerated secretion efficiency screening for the production of microplastic-binding peptides in *C. glutamicum***

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Micro- and nanoplastic particles (MP/NP) are becoming increasingly prevalent in the food and drink humans consume. Health risks have been associated with the substances these particles may carry. Therefore, an approach is needed by which MP/NP can be detected in and ultimately removed from foodstuffs. Adhesion promoting peptides (APs) present an elegant solution by binding specifically to different types of polar and nonpolar MP/NP. They can be used to label MP/NP with fluorescent dyes for specific detection or to bind MP/NP to magnetic beads or crossflow membranes in enrichment techniques. Their secretory production in *Corynebacterium glutamicum* facilitates easy recovery and purification.

Protein secretion in *C. glutamicum* can be utilised in heterologous production by expressing a fusion protein consisting of a secretion signal peptide (SP) and the target protein. Since no a priori predictions can be made regarding the efficiency of different SPs, secretion efficiency needs to be optimised for the secretory production of APs. For this, strain libraries expressing different SPs are generated for two variants of the AP LCI. Supported by automated cultivation, production and product quantification, these libraries are then screened for secretion efficiency in high throughput. Promising candidates are selected for further bioprocess development.

Two secretion strain libraries expressing 24 different SPs from *Bacillus subtilis* were generated for LCI and LCI F16C using automated molecular biology workflows. *C. glutamicum* was cultivated in a microbioreactor and secreted APs were harvested by an automated liquid handling platform. For the quantification of secretion efficiency, a split GFP assay was automated and optimised on the same platform.

Automated molecular biology workflows were successfully established for the accelerated construction of secretion strain libraries. Libraries with 24 different SPs were generated for LCI and LCI F16C. The split GFP assay was optimised for automated liquid handling to ensure reproducible data generation and analysis. In an automated screening experiment, first results were obtained regarding the secretion efficiency of different SPs for the production of LCI and LCI F16C.

With the optimised workflows, strain construction and secretion efficiency screening can be conducted in high throughput for selected APs.

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**BTP036**

**Production of chitin and chitosan in *A. niger***

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Chitin is the second most abundant polymer in nature and is found in various sources, e.g., insects, crustaceans, as well as fungi. Therefore, chitin can be extracted from by-products in the production of crab meat, insect protein or fungal biomass waste from industrial productions streams and processed into further functional derivates. One of these derivates is chitosan, which is derived from chitin by deacetylation. The biodegradable chitosan and chitin have antimicrobial, wound-healing and antioxidant properties, among others, which depend on their physicochemical properties. According to the literature, these properties and the availability from fungal origin are superior to those from animal origin. The application of chitosan in various industrial processes as well as the high demand reflects the great potential of this raw material of fungal origin. However, the efficient extraction and optimisation of chitin and chitosan from fungal origin has not been clearly described.

It is intended to show possibilities for the analysis and production of chitin as well as chitosan from fungal biomass of the industrial cell factory *Aspergillus niger* for further applications.

For the extraction of chitin and chitosan from *A. niger* biomass (BM), a consensus reaction was established by literature search. Fungal BM was first lyophilised and then ground to powder. Subsequently, the BM is heated under alkaline treatment with NaOH. Afterwards, the alkalinised BM is heated again and centrifuged to obtain the alkalised insoluble material (AIM), which is treated with acid after pH neutralisation. Acid treatment of the AIM is carried out using acetic acid at high temperature under reflux cooling. Crude chitin is separated from chitosan by centrifugation and dried after washing steps with deionised water, ethanol and acetone.

Quantitative measurements via absorption showed that the extracted crude chitin consists primarily of chitin and might reach yields of up to 30% of applied BM whereas the chitosan yields reaches merely 0,2%.

The established extraction of chitin and chitosan was able to reproduce the chitin content described in literature. As this study is still in progress more insights into the optimization of chitosan extraction as well as the development of chitin overproducing *A. niger* strains will be presented.

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**BTP037**

**Exploring the potential of water-dependent steroid C25 hydroxylases for 25-OH-vitamin $D_3$ synthesis**

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Vitamin $D_3$ is an important regulatory component of calcium and phosphate metabolism, which ensures optimal cell function and bone mineralization. Deficiency of physiologically active 25-OH-vitamin $D_3$, which appears to be common in populations worldwide, is associated with osteoporosis, fractures and osteomalacia[1]. Here, we demonstrate the potential of water-dependent steroid C25 hydroxylases (S25DHs) for the efficient enzymatic hydroxylation of vitamin $D_3$ to its clinically relevant 25-OH form, using water as hydroxyl donor and ferricyanide as an artificial and regenerable electron acceptor[2].

While the genetic inaccessibility of *Sterolibacterium denitrificans* limits the use of this enzyme in industrial processes, the use of *Thauera aromatica* K172 as a heterologous production platform has opened up further applications for S25DHs[3]. We have established a *T. aromatica*-based whole-cell system that allows the production of up to 1.24 g $L^{-1}$ 25-OH-vitamin $D_3$ within 22 h in the presence of hydroxypropyl-β-cyclodextrin as solubilizer. In addition, no byproducts such as 1α,25-di-OH-vitamin $D_3$, which are commonly found in cytochrome P450-dependent reactions, were detected, demonstrating the potential of S25DHs for 25-OH-vitamin $D_3$ synthesis.

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**BTP038**

**Yeast as novel vehicle for targeted drug delivery**

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In recent research the delivery of biologically active agents to specific cells or tissues represents a growing therapeutic approach. Particularly the targeting of phagocytic cells is a promising tool for preventing infections as well as treating a wide variety of diseases (e.g. Alzheimer's disease, arthritis and cancer) using active immunotherapy. In the field of non-viral carrier systems, nanoparticles have already been extensively studied as vehicle for immunotherapy. But, even though both uptake and retention of nanoparticles in target cells can be enhanced by modifying the surface with particular ligands or varying their physicochemical properties, there is still a need for improvement to eliminate off-target accumulation and uptake by undesired cell types.

Yeast cells, however, are naturally taken up by phagocytic cells and have been successfully used as a vehicle in many studies. Consequently, the interaction between different yeast genera and chitosan-coated PLGA particles was characterized and a nano-in-micro carrier was established, which allows a targeted delivery of nanoparticles into macrophages. This novel combination of nanoparticles and yeast cells significantly expands the possibilities of delivery systems used so far with regard to specificity, capacity and efficiency.

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**BTP039**

**Caproate production from enset fibers in two step fermentation using anaerobic fungi *(Neocallimastix cameroonii G341)* and *Clostridium kluyveri***

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The use of renewable resources is crucial for sustainable development and minimizing environmental impact [1]. An enormous amount of byproduct known as Enset biomass is thrown away during traditional Ethiopian Enset food processing [2]. This study aimed to produce caproate from Enset fibre using *Neocallimastix cameroonii G341* and *Clostridium kluyveri* in two-step fermentation. The process started by growing *N. cameroonii G341* on Enset fibre as a carbon source for 7 days, then the fungal culture was inoculated with active *C. kluyveri* preculture and incubated. The results showed that *N. cameroonii* grew on 5 g/L Enset fibre and produced 1.11 mmol acetate, 1.34 mmol formate and 0.49 mmol hydrogen, respectively. In addition, lactate, succinate, and ethanol were detected in small amounts, 0.17 mmol, 0.08 mmol and 0.7 mmol, respectively. After inoculating with *C. kluyveri*, 0.29 mmol of caproate and 0.48 mmol of butyrate were produced, and hydrogen production also increased to 0.94 mmol compared to *N. cameroonii* fermentation. Moreover, after the culture was supplemented with 43.35 mmol of ethanol during *C. kluyveri* inoculation, caproate and hydrogen production increased to 1.19 and 1.35 mmol, respectively. The growth of *C. kluyveri* was not inhibited by *N. cameroonii G341* growth and caproate could be produced from Enset fiber. However, further research is required to gain the maximum amount of acetate and ethanol possible from *N. cameroonii G341* fermentation.

Keywords: Enset fibre, caproate, hydrogen, *N. cameroonii G341, C. kluyveri,* Anaerobic fungi,

References:

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**BTP040**

**Genomic comparison of two *Aspergillus niger* isolates identifies differences in critical genes involved in polysaccharide degradation and metabolism**

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Fungal hydrolysis promises to be a sustainable and economical solution for the holistic use of agricultural waste residues. The filamentous fungus *Aspergillus niger* is an efficient producer of pectinases and hemicellulases for this purpose. We previously observed strong secretion of pectinolytic enzymes in the strain ATCC 11414. On the downside, ATCC 11414 requires amino acid supplementation for proper growth (esp. glutamine) and displays lower xylan and arabinan degradation efficiencies compared to the general *A. niger* lab strain NRRL3. To better understand the phenotypic differences between ATCC 11414 and NRRL3, and to screen for candidate genes underlying these, a genomic comparison was performed. To this end, a *de novo* assembly of the ATCC 11414 genome was executed and analyzed for single point mutations, indels and gene losses potentially involved in polysaccharide hydrolysis. Only few carbohydrate-active enzyme-encoding genes (CAZys) were affected by genomic variants in *A. niger* ATCC 11414. However, within two genomic deletions, six genes were found to be absent compared to NRRL3, including two transcription factors. One of the transcription factors is a homolog to ClrC in *Penicillium oxalicum*, where it is involved in the stress response and production of CAZymes. This finding could therefore potentially explain the lower hemicellulose degrading activity of ATCC 11414. The second transcription factor missing in ATCC 11414 is encoding a homolog of the acridine resistance gene *acr-2* from *Neurospora crassa*. To unravel the function of these genes, knock-in (in ATCC 11414) and knock-out strains (in NRRL3) were created. By studying the phenotypes of these strains *clrC* was found to regulate the production of hydrolytic enzymes in *A. niger* and *acr-2* influences growth on minimal medium. The results of this study will help to find regulators involved in the production of carbohydrate-active enzymes and transporters to create a strain combining high hydrolase production with high growth rate independent of amino acid supplementation. These modifications could find applications in the production of hydrolytic enzymes to degrade plant waste residues for the release of valuable cell wall monomers with interesting properties.

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**BTP041**

**Structure-function characterization of antifungal peptides and development of high-level production chassis strains**

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Fungal infections claim more human casualties than both, tuberculosis and malaria. Cysteine-stabilized antifungal peptides (AFPs) from filamentous fungi contain several promising future lead drugs. These AFPs form a peptide family of more than 100 members. Extensively studied members of this family are AFP from *Aspergillus giganteus*, PAF from *Penicillium chrysogenum* and AnAFP from *A. niger*. These peptides act as antifungals, exert a specific target spectrum and are considered promising molecules for use in medical or agricultural applications to combat human- and plant-pathogenic fungi. AFP was found to exclusively act on filamentous fungi without affecting the growth of bacteria, yeast, mammalian or plant cells. Although similar in amino-acid sequence, 3D structure and amphipathic character, this ribosomally synthesized peptide differs considerably in its antifungal mode of action, specificity, and efficacy compared with AnAFP and PAF.

To investigate the underlaying moieties that are responsible for the differences in specificity we apply recombinant chimeric peptides of AnAFP and PAF. Their 3D structures and antifungal activities deliver insights to further elucidate interaction models.

In parallel, we are interested in the regulation of AFPs to increase their production in filamentous fungi by design of high-level production chassis strains. Thereby, we consider two aspects: i) genetic regulation in the case of AnAFP in *A. niger* and ii) enzymatic processing on the peptide-level in case of AFP in *A. giganteus*.

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**BTP042**

**Construction of *CodAB*-based vectors for a markerless genetic deletion system in *Clostridium kluyveri***

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Decreasing the environmental impact has become one of the major challenges and responsibilities of our present-day society. The need for reducing CO2 emissions and the demand for regenerative bulk chemicals and fuels cannot be met by petrochemical production processes. The co-culture of the acetogenic, gas-fermenting organism *C. carboxidivorans* with *C. kluyveri* performing a carbon chain elongation is a promising approach to produce medium-sized alcohols. The supply of the substrate synthesis gas is almost unlimited due to its occurrence as industrial off gas or generation by gasification of municipal and food waste. A stable synthetic co-culture between both organisms has already been established. Unfortunately, the increasing performance of *C. carboxidivorans* at high CO partial pressures competes with a reversible growth-inhibition of *C. kluyveri*. To overcome this issue and to increase the co-culture"s conversion rate it is necessary to engineer *C. kluyveri* genetically for better CO-resistance and improved synthesis of longer chained alcohols.

Recently, protection against restriction by a dominant type II restriction system was achieved using *in vivo* methylation in a cell-free extract assay. This allowed development of a protocol for triparental conjugation, though a vector and a

protocol for markerless genetic modification is not yet available. The current work is focusing on creation of potential deletion-vectors based on the *CodAB* counter-selection system. Conjugation of potential deletion vectors was successful and their counter-selectability was evaluated. The strain *C. kluyveri* DSM555T meets the requirements for using the *CodAB*-system, because it has a high 5-fluorocytosine (5-FC) tolerance of up to 500 µg/ml and is very sensitive for 5-fluorouracile (5-FU), with a sensitivity below 30 µg/ml. Testing the *CodAB* mediated 5-fluorocytosine sensitivity under thiamphenicol selection pressure, a minimal inhibitory concentration of 2.5 µg/ml 5-FC was determined. Furthermore, after counterselection with 5 µg/ml 5-FC, a complete loss of the plasmid was confirmed. In a next step, origins of replication that are suitable for a deletion system in *C. kluyveri* were identified.

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**BTP043**

**Determination of plasmid stability and copy number in *C. cellulovorans* 743B**

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The bioconversion of renewable resources into fuels and commodity products has become a global concern to achieve an environmentally friendly industry and sustainable management of natural resources. *Clostridium cellulovorans* represents a promising microorganism for the bioconversion of lignocellulosic materials into biofuels and commodity products. Though it is highly cellulolytic, *C. cellulovorans* cannot naturally produce solvents in a desired concentration. However, the cellulose-degrading *C. cellulovorans* could be used as part of a synthetic co-culture, e.g., together with a solventogenic *Clostridium* spp. To achieve a stable co-culture, the different organisms should be engineered to be dependent on each other. Unfortunately, the lack of tools for the genetic engineering of *C. cellulovorans* impairs the construction of the required strains. For the development of aforesaid tools, the functionality and stability of different Gram-positive origins in *C. cellulovorans* were investigated. For this, different test plasmids from the pMTL80000 modular plasmids series were transferred into *C. cellulovorans* via transconjugation. The pBP1, pCB102, pUB110, p19 and pAMβ1 replicons were identified as suitable Gram-positive origins of replication (ori). Subsequently, the plasmid stability was evaluated to find the most unstable replicon among them, which is an important requirement for an ori of a deletion vector used for allelic exchange. It was found that the pUB110 ori was the most unstable ori in *C. cellulovorans*, while the pCB102 ori was the most stable one. Besides, the plasmid copy number was estimated using qPCR, using the *catP* gene as target gene, and the chromosomal *Clocel_3734* gene as reference. Cells with the plasmid containing the pUB110 ori had a lower copy number of the target gene when compared with cells containing another test plasmid. These results contribute to establish a markerless gene editing system for genetic engineering of *C. cellulovorans*.

Keywords: Clostridia, shuttle vector, "pseudo-suicide" replicon, synthetic biology, genetic engineering

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**BTP044**

**Shaping spatiotemporal oxygen landscapes in microbial single-cell analysis**

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(1) In nature, oxygen exists in various spatial patterns or landscapes such as gradients, and the availability fluctuates dynamically over time, thus influencing microbial physiology. To investigate the complex interplay between spatiotemporal oxygen availability and microbial behavior, single-cell analysis in microfluidics is promising as it allows precise spatiotemporal control of the local microenvironment. Such a level of control is not possible in conventional cultivation methods. Although microfluidic devices have been demonstrated to create constant oxygen environments, dynamic control of oxygen in the order of seconds has not yet been implemented in microbial single-cell analysis.

(2) Here, we developed a double-layer microfluidic chip enabling to create various oxygen landscapes with a fast switching capability in the range of seconds. With our new device, we aim to study microbial physiology at the single-cell level under controlled oxygen availability. For example, cell growth could be studied under fast alterations of oxygen availability and the spatiotemporal organization of microbial communities could be investigated under defined oxygen landscapes.

(3) The double-layer microfluidic chip comprised two layers, a 3 mm-thick top layer incorporating the gas supply channels and a bottom cultivation layer of only 50 µm including the fluid channels and cultivation regions of micrometer height. Both layers were fabricated of elastomer polydimethylsiloxane (PDMS). The top layer was fabricated by PDMS molding using a 3D-printed mold. The bottom layer was fabricated by spin-coating PDMS on a silicon wafer containing microfluidic structures to obtain a thin PDMS layer acting as an oxygen-permeable membrane after chip assembly. The gas channels on the top layer were connected to mass flow controllers for oxygen control. The fluid channels were connected either to a solution of the oxygen-sensitive dye RTDP for device characterization or a culture medium.

(4) To verify the gas exchange times, oxygen in the fluid channels was imaged by FLIM microscopy using RTDP. The result showed the gas exchange in the range of 10 seconds. In addition, the top layer was designed with two separate channels to create an oxygen gradient in the fluid channel to demonstrate oxygen landscape patterning.

(5) We presented the double-layer microfluidic chip for microbial single-cell analysis under a confined spatiotemporal oxygen environment.

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**BTP045**

**The evolution and diversity of PETases in the bacteroidota phylum**

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Introduction: *Bacteroidetes* is globally distributed in most ecological niches due to their ability to decompose a wide variety of polymers and polysaccharides. Recently we identified potential PET esterase that are mainly affiliated with the *Bacteroidetes* and provided the first evidence that they code for the active enzymes associated with the degradation of PET.

Objectives: A handful of different bacteria produces hydrolase enzymes that can degrade PET. However this

synthetic substance might not be the natural substrate for these enzymes. We aim to look for more putative PET degraders from *Bacteroidetes* and to provide evidence that marine *Bacteriodetes* secrete PET active enzymes in a model biofilm under artificial marine conditions.

Materials and methods: The aquatic microbial communities were enriched in R2A media with PET powder for *Bacteroidetes* isolation. Their genomic DNA were then sequenced. HMM model was constructed to identify putative PET esterases which were then expressed in *E. coli* BL21 and used for the activity assay. Further, the promotor fusion constructs of selected genes were generated and analyzed with a confocal microscopy.

Results: We have isolated 19 *Bacteroidetes* species. The genome sequences for these isolates have been established. Using functional screening we identified two isolates, UHH-5R5 and UHH-Hm9b that are highly active on BHET and MHET which are the degradation intermediates of PET. These two isolates also formed thick biofilms on PET foils. Using PETase specific HMM model, the partial genomes of these 2 isolates were mined for potential PET hydrolases. Two dienelactone hydrolase (DLH) that are highly abundant and found in many different marine isolates were identified. In addition to the previous published enzymes, these two DLHs are not secreted via T9SS secretion system, implying that *Bacteroidetes* harbor different types of PETases. Both genes were designated PET93 and PET94 and heterolgously expressed. Activity assays confirmed that both purified proteins were able to hydrolyze PET powder and foil at low rates.

Conclusions: PET93 and PET94 identified from two *Bacteroidetes* isolates were successfully expressed in *E. coli* cells. With the activity assay, the enzymes show relatively high hydrolytic activity on MHET and BHET. They can also degrade PET but at low rate, therefore further works are directed towards searching for possible natural substrates that induce the transcription of the respective PET active enzymes

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**BTP046**

**Screening *Bacteroidetes* type strains and new isolates for utilization of plant-based polymers and formation of SCFAs**

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Anaerobic members of the *Bacteroidetes* phylum produce short chain fatty acids (SCFAs) like propionate in their metabolism, which is an important precursor chemical in industry [1]. Beyond that, they are equipped with a large repertoire of carbohydrate-active enzymes that target plant polymers [2]. These abilities may allow to develop *Bacteroidetes* sp. toward the utilization of plant biomass as a substrate for renewable propionate production.

The objective of this work is to identify strains that efficiently degrade plant polymers and convert them to SCFAs.

In order to obtain new polymer degraders, we set up enrichment cultures using selective conditions for polymer-using *Bacteroidetes*. From that, we isolated 82 *Bacteroidetes* strains and screened them for their product formation on complex media. Based on these results, the isolates were sorted into groups from which representative strains were selected. Subsequently, we compared the ability of 13 isolates and 14 type strains to produce SCFAs with xylan, pectin, starch, inulin or cellobiose as substrate.

Of all tested conditions, the highest product formation was observed for the isolate *D. gadei* BGG-A1 on starch, indicating an efficient conversion of this substrate. On potatoe starch, 34 mM of SCFAs were produced within 48 h, and again, propionate was the main product. Since this isolate did not only show high product concentrations but could also use all of the tested plant-based substrates, it was chosen for further investigation.

Genetic accessibility is crucial for investigating gene functions as well as for optimizing the strain"s metabolism towards an industrial application. For this reason, we tested electroporation with the shuttle vector pG108 [3]. We confirmed the successful transformation into BGG-A1 via colony-PCR and re-isolation of the plasmid. Additionally, the genome of BGG-A1 has been sequenced.

Summing up, we gained an overview about the polymer utilization of 27 anaerobic *Bacteroidetes* strains and obtained their product profile on the respective substrates. Furthermore, isolate BGG-A1 turned out to be the most promising candidate for polymer conversion to SCFAs and will be studied in more detail. Currently, the genome of BGG-A1 is analysed for genes of the energy metabolism as well as for glycosyl hydrolase containing gene clusters.

[1] Gonzalez-Garcia *et al.* 2017, *Fermentation*

[2] Thomas *et al.* 2011, *Front Microbiol*

[3] Jones *et al.* 2020, *Mol Oral Microbiol*

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**BTP047**

**Broadening the power-to-X platform: Conceptualizing a two-stage bioreactor system for bioindustrial syngas processing**

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Due to their unique metabolic capabilities, methanogenic archaea offer the potential for a wide range of biotechnological applications. To meet the ever-increasing demand for low-cost renewable energy, the power-to-x platform exploits the metabolic properties of biotechnologically relevant microbes. In this context, *Methanothermobacter thermautotrophicus* ΔH has already been successfully established as a biocatalyst in commercial power-to-methane processes. In this project, we aim at implementing a lab-scale two-stage bioreactor system for bioindustrial syngas processing to optimize syngas conversion to methane by the two methanogenic microbes *Methanosarcina acetivorans* and *M. thermautotrophicus* ΔH. In addition, we seek to investigate isopren(oid) production yields of metabolically engineered *M. acetivorans* strains in chemostat bioreactors. To gain insights into the fermentation process, $OD_{600}$ measurements and gas measurements were conducted to investigate microbial growth and to assess gas production and consumption, respectively. *M. acetivorans*, which is capable to grow on high partial pressures of carbon monoxide (CO), depleted this component of syngas completely, while leaving the hydrogen ($H_2$) portion of it

unaffected. For *M. thermautotrophicus* ΔH, CO concentrations during $H_2$-dependent growth were successively increased to 5, 10, and 20 vol%. Preliminary results indicate that methane production proceeded, even at an applied CO concentration of 20 vol%. Moreover, the data suggest that *M. thermautotrophicus* ΔH did not consume CO. Ultimately, upgrading syngas via biomethanation and assessing the production of value-added products, such as isoprenoids aligns with the power-to-x concept, and thus, would contribute to creating the framework for a more sustainable and climate-friendly economy.

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**BTP048**

**Laccase expression in the dung fungus *Coprinopsis cinerea* with 17 natural laccase genes**

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Laccases are phenoloxidases that can oxidize phenolic and aromatic compounds and occur in bacteria, fungi, insects, and plants. Among fungi, wood-rotting Basidiomycetes are considered to be main producers of laccase in nature that under different environmental conditions secrete various forms of this enzyme, being either laccase isoforms encoded by the same gene, isoenzymes encoded by different laccase genes or allozymes encoded by different alleles of a gene. The ink-cap mushroom *Coprinopsis cinerea* has 17 different laccase genes divided into 2 subfamilies, which are differentially expressed during growth on distinct media and at different temperature regimes, during fungal differentiation and as defense in confrontation with other microbes. Different monokaryotic strains of the fungus can have inactivated copies of some of these genes. Most often, laccase gene *lcc15* is affected from gene inactivation. Monokaryotic strains usually express Lcc1 and Lcc5 as main laccases, under stress at lower temperature of 25-28 °C much higher than at 37 °C as the best growth temperature. Lcc9 can also be expressed in traces while full expression is encountered as response in presence of competitors. Further, mutants in *lcc* overexpression can be identified by brown pigment secretion into the growth medium. Laccases of *C. cinerea* are of interest for biotechnological applications, which requires good production rates of properly glycosylated enzymes. Enzymes can be overexpressed in *C. cinerea* upon cloning their genes behind highly active promoters, transformation of constructs into suitable monokaryotic strains and cultivation of transformants under favorable environmental conditions, with yields up to 30 U/ml culture supernatant depending on the gene used for cloning. Crossing of transformants can further enhance laccase yields with dikaryons expressing a single enzyme or mixtures of laccases when transformants of different laccase genes were mated.

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**BTP049**

**Accelerated bioprocess development for peroxidase producing *K. phaffii* strains with automated sample processing and microscale cultivation**

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The polymerization process of unsaturated polyester resins to manufacture compound materials for, e.g., boat construction is currently accelerated with cobalt species. Because of the carcinogenic nature and limited availability of cobalt, a bio-based alternative would be beneficial in industrial applications. A promising approach is the utilization of peroxidases, which have been shown to catalyze the same reaction as cobalt species and can therefore substitute cobalt in the polymerization of polyester resins. A suitable host for the heterologous production of these peroxidases is the yeast *Komagataella phaffii*.

Because there is no a priori prediction possible for optimal process conditions under which peroxidases can be produced in *K. phaffii*, bioprocess development and optimization need to be performed to analyze most suitable process parameters. To enable sufficient exploration of process parameters, tools for cultivation and sample analysis need to be developed in high throughput.

Reproducible and efficient sample processing in high throughput can be realized with liquid handling platforms. On these fully automated devices, robotic arms can transfer liquids and labware across a distinctive deck with implemented devices (e.g. centrifuges, incubators and photometers). Automated sampling and analysis unlocks the ability to analyze process conditions with regard to key performance indicators, for example enzymatic activity, productivity and yield, in high throughput with a decrease in hands-on labor. Additionally, microcultivation systems such as the BioLector allow for up to 48 parallel cultivations with online data monitoring of biomass, oxygen and pH value. With this device, process parameters such as carbon-source concentration, different induction strategies and media compositions can be analyzed in a thorough manner.

Workflows for automated sample processing with a microtiter plate based ABTS enzymatic assay were developed, validated and applied to analyze samples from lab-scale cultivations under glycerol batch and fed-batch conditions to asses the effect on enzymatic activity per gram of cell dry weight. Furthermore, micro-scale cultivations were performed with different glycerol-concentrations under batch conditions to examine glycerol metabolism and assess optimal conditions.

These results are a first step in the implementation of an automated, miniaturized workflow for bioprocess development of peroxidase production processes in *K. phaffii*.

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**BTP050**

**Enhanced removal of sulfamethoxazole through sequential anaerobic-aerobic microbial treatment**

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Sulfamethoxazole (SMX) is a widely used antibiotic that typically remains unaltered during conventional wastewater treatment plant (WWTP) processes. Notably, under anoxic conditions, SMX can be transformed by sulfate-reducing bacteria. However, the fate of the anaerobic transformation products (TPs) of SMX and the potential occurrence of these

anaerobic biotransformation processes in WWTPs remain largely unknown. In this study, we investigated the anaerobic formation and aerobic degradation of TPs generated from SMX. Analysis of microbial populations revealed that families such as *Desulfomicrobiaceae*, *Desulfovibrionaceae*, *Desulfobacteraceae*, and *Syntrophobacteaceae* could potentially play a crucial role in SMX biotransformation under sulfate-reducing conditions. In our enriched cultures, we tentatively identified nine TPs of SMX, with eight of them exhibiting alterations in the heterocyclic moiety and only one in the *N*4-arylamine moiety. Abiotic oxic incubation of SMX with sulfate-reducing culture filtrates led to further degradation of the two major anaerobic TPs and the corresponding increase of two secondary TPs. After re-inoculation of the abiotic incubations under oxic conditions, all anaerobically formed TPs, including the secondary TPs were degraded and removed. Three of the anaerobically formed TPs of SMX, along with its acetylated human metabolite, were detected in samples collected from different stages of a full-scale municipal WWTP. Concentrations of these three anaerobic TPs were higher in the effluent of the primary clarifier and the digested sludge units, where anoxic conditions were prevalent. Contrarily, concentrations of these TPs were lower in the final effluent as well as the effluent of the activated sludge unit. These results suggest that anaerobically formed TPs were effectively eliminated during the aerobic treatment stages, similar to our observations in batch biotransformation experiments. More generally, our findings emphasize the significance of varying redox states on the fate of SMX and its TPs in engineered environments.

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**BTP051**

**Microbial production and application of double-stranded RNAs as innovative bio-agents in agri- and horticulture**

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Introduction: Chemical crop protection methods to control plant parasites in agri- and horticulture can be problematic or are not permitted anymore due to high environmental toxicity. Hence, there is a need to develop new methods to control plant parasites for sustainable and environmentally friendly production of plants for food and feed. The use of double-stranded RNAs (dsRNAs) for non-chemical crop protection strategies represents a promising opportunity to control plant parasites *via* RNA interference (RNAi).Objectives: Plant-parasitic nematodes (PPNs) such as *Meloidogyne* penetrating plant roots and leading to root galls, deformed growth and reduced plant health, are a major problem in crop production. The objectives are microbial production of dsRNAs and exploring their applications for practical control of PPNs *via* RNAi to protect crops and improve plant health.Materials & methods: Literature was searched about use of RNAi in the PPN *Meloidogyne*. Reported target sequences and primer information were used to select RNAi targets. To obtain template DNA, *M. incognita* was cultivated and eggs were isolated from roots of infected tomato plants. Then, hatched second stage juveniles (J2s) were used to purify total RNA. Oligo dT primers were used to generate cDNA which was used to generate DNA fragments for construction of dsRNA expression plasmids. Constructed expression plasmids were electroporated into an RNase III-deficient strain of the model microorganism and industrial workhorse *Corynebacterium glutamicum*. The resulting strains were used to produce selected dsRNAs.Results: Literature search revealed RNAi experiments almost exclusively for *M. incognita*. More than 20 proven RNAi targets of different functional subgroups were found. With total RNA obtained from hatched *M. incognita* J2s almost all described DNA fragments were obtained and could be used for construction of dsRNA expression plasmids. Deletion of the RNase III gene *rnc* in *C. glutamicum* MB001(DE3) was successful and allowed microbial dsRNA production.Conclusions: Isolation of total *M. incognita* RNA of the laboratory strain and sequencing of generated DNA fragments revealed RNAi target sequence variations and allowed construction of dsRNA expression plasmids. Microbial production of dsRNAs based on T7 RNA polymerase in *C. glutamicum* MB001(DE3) Δ*rnc* can result in a very high dsRNA content providing sufficient material for future *in vitro* and *in vivo* RNAi studies with plant/nematode pathosystems.

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**BTP052**

**The L-rhamnose-dependent regulator RhaS and its target promoters from *Escherichia coli* expand the genetic toolkit for regulatable gene expression in the acetic acid bacterium *Gluconobacter oxydans***

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Introduction: The acetic acid bacterium (AAB) *Gluconobacter oxydans* serves as a cell factory for oxidative biotransformation and as a model organism to study metabolism in AAB. For *G. oxydans*, only recently the first two regulatable expression systems became available[1,2,3]. Hence, there is still need to expand the opportunities for regulatable gene expression in this model organism.Objectives: The objectives were to evaluate the suitability of the L-rhamnose-dependent regulators RhaS and RhaR and target promoters $P_{rhaBAD}$, $P_{rhaT}$, and $P_{rhaSR}$ from *Escherichia coli* for regulatable gene expression in *G. oxydans*.Materials & methods: pBBR1MCS-5-based expression plasmids were constructed to test RhaSR-, RhaS-, and RhaR-dependent reporter gene expression with $P_{rhaBAD}$, $P_{rhaT}$, and $P_{rhaSR}$ in *G. oxydans* 621H. mNeonGreen was used as fluorescence protein reporter.Results: In contrast to the responsiveness in *E. coli*, in *G. oxydans* RhaS increased expression from $P_{rhaBAD}$ without L-rhamnose and repressed $P_{rhaBAD}$ with L-rhamnose[4]. Inserting an additional RhaS binding site downstream at the *E. coli* $P_{rhaBAD}$ −10 region generating $P_{rhaBAD(+RhaS\text{-}BS)}$ almost doubled apparent RhaS-dependent promoter strength. Plasmid-based $P_{rhaBAD}$ and $P_{rhaBAD(+RhaS\text{-}BS)}$ activity could be reduced up to 90% by RhaS and L-rhamnose, while a genomic $P_{rhaBAD(+RhaS\text{-}BS)}$ copy appeared fully repressed. RhaS-dependent repression was tunable by L-rhamnose between 0% and only 0.3%. $P_{rhaT}$ was almost inactive without RhaS, was weak with RhaS and without L-rhamnose, and inducible up to 10-fold by RhaS with L-rhamnose, resulting in a moderate expression level. Insertion of an additional RhaS binding site downstream at the *E. coli* $P_{rhaT}$ −10 region increased non-induced expression strength and reversed the regulation by RhaS and L-rhamnose from inducible to repressible. The $P_{rhaSR}$ promoter appeared to be positively auto-regulated by RhaS and expression was increased by L-rhamnose.Conclusions: *E. coli* RhaS-$P_{rhaBAD}$ represents the first repressible expression system in *G. oxydans* providing new opportunities for gene regulation. The RhaS-$P_{rha}$ systems are also expected to enable simultaneous repression and activation of target genes in metabolic engineering approaches including dynamic redirection of carbon fluxes.
[1]Fricke et al. (2020) Appl Microbiol Biotechnol 104(21):9267;[2]Fricke et al. (2021) Appl Microbiol Biotechnol 105(18):6835;[3]Bertucci et al. (2022) Peer J 10:e13639;[4]Fricke et al. (2022) Front Microbiol 13:981767

**BTP053**

**Isolation and characterisation of *Sphingopyxis* strains using droplet-based microfluidics**

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The number of microbial species on Earth is estimated to be 1011-1012. [1] Modern metagenomic analysis methods have identified many new species, but not even 1% of prokaryotes have been successfully isolated and cultured. [2] Among these, soil microbiomes are highly diverse communities that provide tools for a variety of biotechnological and ecological applications. For example, *Sphingopyxis chilensis* is known to be able to degrade chlorophenol compounds. The metabolism of chlorophenol is accompanied by an accumulation of polyhydroxyalkanoate (PHA), a renewable, sustainable and biodegradable polymer of great importance to industry. [3]

The aim was to use microfluidic droplet cultivation of microbial communities to achieve statistical formation of small consortia through low cell numbers and enrichment of rare or specially adapted species. The simultaneous application of gradually increased stressors created serial microniches that allowed a richer selection of adapted strains in a high-throughput procedure. The isolated strains were then screened for growth potential by dose-dependent serial droplet screening.

Using microfluidic-based cultivation, we were able to find, various *Sphingopyxis* strains that are capable of degrading phenolic compounds under certain conditions. Our data from high-resolution dose-response experiments with different heavy metal ions using micro-segmented flow show that despite great genetic similarities within the strains, different stress responses were observed in the form of tolerance traits. The results showed different sensitivities to heavy metal ions. The incorporation of neutral lipids also varied when different phenolic compounds were used.

These results show that the methodology used is well suited to identify new strains or subtypes of *Sphingopyxis* that may also be more efficient in metabolising halogenated hydrocarbons in general.

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**BTP054**

**Production of L-arabinonic acid by biotransformation of L-arabinose using the acetic acid bacterium *Gluconobacter oxydans***

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Introduction: The acetic acid bacterium *Gluconobacter oxydans* harbors the beneficial ability of regio- and stereoselective incomplete oxidation of sugars, sugar alcohols and other substrates in the periplasm by membrane-bound dehydrogenases and release of resulting products into the cultivation medium. In *G. oxydans* 621H, the membrane-bound glucose dehydrogenase GdhM (GOX0265) can also oxidize L-arabinose in the periplasm yielding L-arabinonic acid. In the literature, this sugar acid was described as interesting compound that could serve a variety of purposes including its use for production of erythrose and erythritol, and for medical applications.

Objectives: The objectives were to test high-level production of L-arabinonic acid from L-arabinose by biotransformation when using the native GdhM activity level of *G. oxydans* 621H and when GdhM was additionally overexpressed using a plasmid.

Materials & methods: *G. oxydans* 621H and 621H/pMM4a-mGDH constitutively overexpressing *gdhM* from plasmid pMM4a were grown in shake flasks with 60 mL d-mannitol medium and in fed-batch pH 6-controlled DASbox® mini-bioreactors with 100 mL d-mannitol medium. In shake flasks, 10 g/L, 40 g/L, and 80 g/L L-arabinose were supplemented to the medium. In fed-batch mini-bioreactors, initially 40 g/L and 80 g/L L-arabinose were supplemented and every 24 h 40 g/L and 80 g/L were fed three times. Growth and metabolites where observed and analyzed over 114 h.

Results: In shake flasks without pH control, 17.4 g/L L-arabinonic acid and 5.7 g/L L-arabino-1,4-lactone were obtained. A large fraction of L-arabinose remained unused since acidification of the medium prevented further substrate oxidation. In pH 6-controlled fed-batch mini-bioreactors, up to 120 g/L L-arabinonic acid and 13 g/L L-arabino-1,4-lactone could be obtained with strain 621H (144 h). Constitutive *gdhM* overexpression did not increase product titers and resulted in strongly reduced biomass formation, yet *gdhM* overexpression enabled a higher biomass-specific production (169 g/L/gcdw) compared to 621H (131 g/L/gcdw)1.

Conclusions: Already without strain optimization and further process improvements, the high combined product titer (133 g/L, 814 mM) was more than three-fold above the titers reported for engineered *S. cerevisiae* and *E. coli*, making *G. oxydans* a very promising host for high-level production of L-arabinonic acid from renewable L-arabinose. [1]Fricke et al. (2022) Bioresource Technology Reports 17:100965

**BTP055**

**Centrifuge-Free Clarification of Bacterial Lysates using Alluvial Filtration with Diatomaceous Earth**

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The clarification of bacterial lysates is a crucial step to isolate target molecules from bacteria and enable further purification procedures such as affinity chromatography. Conventionally, the separation of cell debris and target molecules requires tedious and time-consuming steps such as centrifugation and subsequent filtration. In our newly developed method, we use a combined alluvial and membrane filtration with porous diatomaceous earth, which eliminates the need for a centrifuge. The alluvial filtration is based on the use of porous diatomaceous earth, and different grades have been tested in combination with different E. coli lysis methods. Our results show that diatomaceous earth grades C65 and C100 at ratios of 20 g/L to 80 g/L of lysate provide efficient

clarification without the need for centrifugation. This innovative method offers a time-saving and efficient alternative to the conventional clarification of bacterial lysate and facilitates the subsequent purification of target molecules for various applications.

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**BYFP001**

**Molecular identification of two endophytic filamentous fungi and in-vitro antibacterial activities of their secondary metabolites**

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Question: Increasing demand for new drugs necessitates the exploitation of endophytic fungi for potent natural products. Herein, the antibacterial activities of secondary metabolite produced by two endophytic moulds were studied.

Methods: Fungal strains were identified using phenotypic and molecular techniques. Metabolite production and extraction were followed using standard methods. Antimicrobial sensitivity, minimum inhibitory concentration (MIC) of single and combined extracts, and minimum bactericidal concentration (MBC) of single extracts were carried out against selected Gram-negative and Gram-positive typed bacterial isolates. The mode of action, fractional inhibitory concentration index (FICI), and phytochemicals present were also determined.

Results: Endophytes were identified as *Aspergillus flavipes* (OP341429) and *Curvularia akaii* (OP341430). Extract from *A. flavipes* (MAF) was 1.57 g/100 µL while that from *C. akaii* (MCA) yielded 3.82 g/100 mL. The single and combined metabolites were active against the test bacterial isolates except for *Bacillus stearothermophilus* (NCIB 8222). The most sensitive extract was MAF with a zone of inhibition ranging between 27.00±1.00 mg/mL (*Micrococcus luteus* (NCIB 196)) and 40.00±0.01 mg/mL (*Serratia marcescens* (NCIB 1377)). MIC and MBC ranged from 15.63 to 500 mg/mL, and 500 to 1000 mg/mL respectively. The extracts exhibited a bacteriostatic effect on the isolates while FICI reveals antagonism in the combined extract. Phytochemical screening detected the presence of saponins, proteins and fats, and oils, in MAF and MCA, while phenolic compounds (flavonoids and cardiac glycosides) were detected only in MAF.

Conclusions: Our findings revealed that secondary metabolites from *A. flavipes* (OP341429) and *C. akaii* (OP341430) are potential bacterial therapeutic agents with broad-spectrum activity.

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**BYFP002**

**Microbial production of polymalate by the black yeast *Aureobasidium pullulans* NRRL 62031**

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Introduction: Polymalate is a linear biopolyester with potential applications as a novel drug delivery system and in the production of other advanced biomaterials.

Objective: The draft whole genome of *Aureobasidium pullulans* NRRL 62031 sampled from a leaf was sequenced and annotated to predict functional genes. A non-ribosomal peptide synthetase gene was likely to be implicated in polymalate production, which should be confirmed. A furthermore goal was to identify the genes involved in metabolic byproduct formation as targets to enhance polymalate biosynthesis.

Materials & methods: The genome sequence of *A. pullulans* NRRL 62031 was annotated mainly based on KEGG, GO, and KOG databases, followed by employing Cre/loxP-mediated gene knockout technology to verify the putative polymalate synthetase gene and find key genes that contribute to the higher production of polymalate.

Results: The 25.05-Mb genome of *A. pullulans* NRRL 62031 with a GC-content of 50.1% encodes 9,241 predicted proteins and 55 rRNA genes, 235 tRNA genes, and 47 other non-coding RNA. The mutant in which a putative polymalate synthetase gene was abolished completely lost its ability to produce polymalate, while the cell biomass was higher than that of the wild type and still maintained the yeast cell-like growth as the wild type displayed. The concentration of polymalate produced by the mutant strain where a polyketide synthase gene regarding liamocin production was removed was about 26.8% higher than that of the wild type. Furthermore, the mutant did not secrete any amounts of liamocins and accumulated less cell biomass than the wild type.

Conclusion: The putative polymalate synthetase gene played a significant role in the biosynthesis of polymalate in *A. pullulans* NRRL 62031. The disruption of the polyketide synthase gene regarding liamocin biosynthesis contributed to the elevated production of polymalate, and the resulting strain could be used as a potential microbial chassis for large-scale polymalate production.

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**BYFP003**

**In vivo proximity labeling with biotin to identify protein-protein interactions in sordaria macrospora**

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Introduction: Proteins are essential building blocks of life and interact with other proteins through binding or modification to form complex functional networks. The analysis of these interactions provides important insights into the function of proteins and their contribution to the molecular mechanisms of cellular processes. With the biotin identification (BioID) method presented here, it is possible to investigate the molecular environment of proteins *in vivo*, including transient and weak interactions. This method is widely used in yeast, plants, and animals, but lacks application in filamentous fungi.

Objectives: In this project, we aimed to establish the BioID method in the filamentous fungus *Sordaria macrospora*.

Material & Methods: The BioID method relies on the *in vivo* labeling of proximal proteins through a promiscuous biotin

ligase that is fused to the protein of interest. Biotinylated proteins are enriched through biotin affinity capture and subsequently identified by liquid chromatography coupled to mass spectrometry (LC-MS).

Results: For the establishment of BioID in *Sordaria macrospora*, we used the well-characterized striatin-interacting phosphatase and kinase (SmSTRIPAK) complex as an example. This multiprotein complex is highly conserved from yeast to man and regulates cellular pathways by phosphorylation/dephosphorylation. Deletion of SmSTRIPAK subunits causes sterility and developmental defects in hyphal fusion and vegetative growth. For the proof of concept, one component of the complex, the SmSTRIPAK interactor 1 (SCI1), was fused to a codon-optimized TurboID biotin ligase. Expression of this SCI1-TurboID fusion construct controlled by the native *sci1* promoter in the Δsci1 deletion strain complemented the deletion strain phenotype and fertility was restored. In the following SCI1-BioID experiments, the SmSTRIPAK components PRO11, SmMOB3, PRO22, and SmPP2Ac1 were captured.

Conclusion: Through the significant enrichment of already known SmSTRIPAK components, we were able to demonstrate the successful application of BioID in the filamentous fungus *Sordaria macrospora*. We hope that the BioID proximity labeling approach will provide a powerful proteomics tool for other fungal biologists.

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**BYFP005**

**Cell surface expression of enzymes using different cell wall anchorage systems in yeast**

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(1) Cell surface display of enzymes is a widespread tool for the immobilization of various enzymes aiming to catalyze biologically relevant reactions. Numerous types of display systems in different species evolved, whereas most are focussing on bacteria and yeast. Owing to its GRAS status and the possibility to anchor foreign enzymes via cell wall proteins (CWP), yeast was extensively used as a display platform in the past. Previously, the monomeric esterase A (EstA) from *Burkholderia gladioli* was displayed on *Saccharomyces cerevisiae* by two CWPs and its activity was confirmed. (2) The major aim of this work is the improvement of yeast surface display systems which can be used as biocatalysators in a variety of reaction cascades by expressing enzymes via different cell wall anchoring systems. Based on these previous studies fusion constructs of EstA with Sag1p and Sed1p were expressed in *S. cerevisiae* and *Pichia pastoris*. Additionally, two multi-component systems for the immobilization of EstA were designed, which are based on the interaction of the protein A derived ZZ-domain and the Fc-fragment of IgG antibodies as well as cohesin and dockerin derived from *Clostridium cellulovorans*. (3) To reach these objectives the multi-component systems will be coexpressed in yeast and validated by western blotting, fluorescence microscopy and FACS. Enzymatic activity is determined by photometric assays, which are based on the turnover of enzyme specific substrates. (4) The anchorage of EstA by Sag1p and Sed1p in wildtype strains of *S. cerevisiae* and *P. pastoris* was successfully shown in activity assays. The coexpression of ER-chaperones led to an increase in esterase activity. Concerning the multi-component system consisting of ZZ and the Fc-fragment, ZZ antibody interaction was verified by fluorescence microscopy and FACS. (5) These results demonstrate the efficiency of direct EstA anchorage by the CWPs Sag1p and Sed1p and can be enhanced by chaperone coexpression, which form a basis for the anchorage of other biotechnologically relevant enzymes. By using multi-component systems the generation of reaction cascades through cell wall display of multiple enzymes is possible, which could be applied to regenerate cofactors or obtain therapeutically useful substances.

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**BYFP006**

**Investigating the influence of altered wee1 expression levels on kinetochore architecture in *Schizosaccharomyces pombe***

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The fission yeast Schizosaccharomyces pombe is a well-established model organism for investigating the regulation of the eukaryotic cell cycle [1]. As such, it is an ideal system for studying the structure and function of the kinetochore - a multi-protein complex responsible for connecting spindle microtubules to the centromeric region of chromosomes during mitosis. Given its significance in maintaining genome stability, malfunctioning of the kinetochore can result in aneuploidy-related diseases such as cancer, and various disorders [2, 3]. In a recent publication, we established a multi-color single-molecule localization microscopy imaging protocol capable of quantitatively assessing the molecular positioning and stoichiometry of key proteins within the kinetochore [4].

The objective of this study is to comprehensively characterize the effects of altered protein expression levels of the wee1 kinase on both the structure and function of the kinetochore. Wee1 kinase serves as a critical regulator of the G2/M checkpoint in the cell cycle by inhibiting the activity of the Cdc2 kinase. Previous research has demonstrated that altered expression of wee1 can significantly influence the regulation of this checkpoint, thereby affecting cell size and leading to uncontrolled cell division and cancer. It has also been shown to regulate histone synthesis [5, 6, 7]. Consequently, it is of great interest to investigate the impact of wee1 protein levels on the architecture of the kinetochore, given that the kinetochore relies on the centromere-specific H3 histone variant cnp1CENP-A.

To address this question, we introduced wee1 deletions into selected strains from our existing multi-color kinetochore strain library marking key kinetochore proteins, and quantitatively imaged the resulting strains. We further employed spot tests and flow cytometry as supplementary techniques to assess the overall strain health. Here, we present the preliminary findings and provide an overview of future experiments.findings and provide an overview of future experiments.

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**BYFP007**

**Evolution of chromosomal regions with *A* mating type loci in agaricomycetes**

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(1) The Auriculariales with ear-shaped gelatinous fruiting bodies are an early-diverging clade of the Agaricomycetes, whereas the Agaricales with lamellate basidiocarps

represent the largest euagaric clade of the Agaricomycetidae. Fruiting bodies typically form on a fertile dikaryotic mycelium obtained by mating of two compatible monokaryotic mycelia germinated from sexual basidiospores. (2) Mating is controlled by mating-type genes which are subject to evolutionary forces like balancing selection, gene duplication, and gene conversion, which can further shape genetic diversity and dynamics of populations. Here we explore the evolution events on the mating-type loci of selected Auriculariales in comparison with species of the Agaricales. (3) The *MAT-A* loci are defined by presence of two types of homeodomain transcription factor genes (*HD1* and *HD2*). Other genes in the chromosomal regions are structurally defined and annotated by homologies to genes of other species, using software like the MCScanX toolkit and manual corrections. Gene synteny maps are generated by ChromoMapper. (4) Species of Auriculariales differed in number of *MAT-A* genes with two complete transversely transcribed *HD1-HD2* gene pairs in *Auricularia suglabrata*, one typical *HD1-HD*2 gene pair and one pair of genes with a transversed *HD2* gene in *A. heimuer*, and two complete gene pairs plus one extra *HD2* gene in *Exidia glandulosa*, suggesting duplications and inversions in the evolution of *MAT-A* in the Auriculariales. Duplications as well as sometimes inversions and deletions in *HD1-HD2* genes pairs were known before from the Agaricales. In these species, *MAT-A* loci are usually flanked by the conserved genes *mip* and *β-fg*. These genes are also found closely linked to each other in the vicinity of *MAT-A* genes in the Auriculariales, but in *E. glandulosa* in mirrored symmetry. Many other genes conserved in chromosomal *MAT-A* regions in the Agaricales were found also back in the chromosomal environment of the *MAT-A* genes in the Auriculariales, suggesting an ancient accumulation of these genes. Between species in the Auriculariales were blocks of synteny with inversions and translocations, as well as to species of the Agaricales. Phylogenetic analyses of *HD1* and *HD2* genes are performed to define temporal events in production of paralogous gene pairs to increase mating type numbers. (5) Research on *MAT-A* loci will benefit the understanding of the long-term survival and evolution of these fascinating fungi.

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**BYFP008**

**Unconventional suppression of plant defence responses by the signal peptide peptidase Spp1 in the *Ustilago maydis* – Maize interaction**

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Secretion of effector proteins is essential for communication between biotrophic fungi and their host plants. In the fungal plant pathogen *Ustilago maydis,* the unfolded protein response (UPR) is specifically activated during biotrophic growth generating an optimised intracellular infrastructure, crucial for efficient processing and secretion of effectors. To gain insight into the virulence contribution of individual UPR regulated factors, we used a combined RNAseq/ChIPseq approach. Screening of more than 40 deletion strains identified the signal peptide peptidase Spp1 as a novel key factor essential for virulence. SPPs are ER-membrane localised aspartic proteases that cleave type II oriented transmembrane domains, including remnant signal peptides, which have previously been processed by the signal peptidase complex. Spp1 is dispensable for vegetative growth, filament formation and ER stress resistance, but required to supress plant defence responses upon infection of the host plant maize. Broad transcriptomic changes in both the fungus and the plant suggest an important role of Spp1 in the fungal plant interaction. Importantly, the essential virulence function requires Spp1 catalytic activity but can neither be attributed to known physiological roles of SPPs, such as ER-associated degradation (ERAD) or hypoxia adaptation, nor to the altered secretion of effectors. Thus, Spp1-mediated suppression of plant defence responses likely involves a previously unknown mechanism of fungal plant communication, which is currently further explored. To this end, we utilise proteomics approaches for the identification of potential Spp1 substrates and combine dual RNAseq with plant metabolomics to further our understanding of the underlying mechanism.

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**BYFP009**

**Genetic analyses of a pleiotropic fruiting body mutant of *Coprinopsis cinerea***

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(1) The life cycle of *Coprinopsis cinerea* starts through germination of meiotic basidiospores growing into sterile monokaryons. Compatible strains of different mating types fuse to form a fertile dikaryon having haploid nuclei of both parents. The dikaryon responds to light, temperature, nutrients, CO2 and humidity to form fruiting bodies with basidiospores. Differentiation initiates with the formation of loose hyphal aggregations (primary hyphal knots, Pks). In dark, Pks will grow into multicellular brown resting bodies (sclerotia). A light-signal alternatively leads to compact aggregations in which stipe and cap tissues differentiate. This development culminates in light-induced karyogamy and meiosis in basidia on gill surfaces. Basidiospores are formed parallel to the fruiting body maturation. Monokaryons do not produce fruiting bodies, unlike the self-fertile mating-type-mutant AmutBmut that mimics a dikaryon by clamp cells on hyphal septa, light-induced mitotic spore (oidia) formation, and fruiting body production. (2) Mutations can be induced in haploid AmutBmut oidia and will be expressed in germinating mycelia, regardless of whether dominant or recessive. (3) Mutant Proto159 was isolated from AmutBmut. It grows slightly slower and thinner with reduced amounts of aerial mycelium than AmutBmut. Hyphae still carry clamp cells at their septa. Oidia formation is light-induced, but at 6-fold lower concentration. However, Proto159 excretes pigmented droplets on the culture surface and stains the agar, likely by an unusual high laccase expression. Importantly, Proto159 is unable to produce Pks (a defect called *pkn1*), and thus neither sclerotia nor fruiting bodies. The pleiotropic phenotypes suggest Proto159 to carry a defect in a regulatory gene. (4) In an attempt to complement the fruiting defect, a gene *NWD2* for an NTPase with a NACHT-domain was detected. Transformation of the gene restored mycelial phenotype, blocked pigmentation, and allowed sclerotia and fruiting body formation. The *NWD2* allele in Proto159 is not mutated, indicating that it acts by extra inserted copies as a suppressor. *C. cinerea* has a total of 36 different *NWD2* genes clustering into 7 different subgroups. Other *NWD2* genes showed also suppressor effects in transformed Proto159. Many other Agaricomycetes have no related *NWD2* genes which are thus unlikely central to the regulation of fruiting. (5) To identify the actual *pkn1* defect, we will next sequence the complete mutant genome.

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**BYFP010**

**Adding missing pieces to the puzzle of *Aspergillus fumigatus* sexuality: TomA is required for cleistothecia formation and an a-factor-like pheromone exist**

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The filamentous ascomycete *Aspergillus fumigatus* is the major cause of a variety of fungal infections commonly termed aspergillosis. Besides vegetative growth and asexual spore formation, this mold is capable of fruiting body formation accompanied by ascosporogenesis under specific environmental conditions. Knowledge about the sexual phase of its lifestyle and regulatory determinants is, however, incomplete. *A. fumigatus* sexuality is governed by a bi-polar mating-type system comprising an idiomorphic region that contains one of the master regulator-encoding genes *MAT1-1-1* or *MAT1-2-1*. Transcriptional profiling data from strains overexpressing the mating-type regulators turned out to be a rich source shedding light on a variety of fungal traits, such as secondary metabolism, antimicrobial defense, or cleistothecia formation. For the latter, an annotated gene encoding a conserved hypothetical protein and assigned as *tomA* (target of MAT1-1-1) was investigated. Transcription of *tomA* strictly depends on the *MAT1*-encoded master regulators and putative MAT1-1-1 binding sites are present within the corresponding upstream regulatory region. Preliminary inspection of strains expressing GFP-tagged TomA indicates its nucleolar localization, supporting functionality of a deduced nuclear localization sequence in the *tomA* coding region. To explore any cellular function of the *tomA* gene product, deletion strains in either mating-type background were generated to reveal that the absence of TomA in the heterokaryon results in sterility, indicating that *tomA* is crucial for *A. fumigatus* sexual reproduction. Moreover, we made use of the *MAT1* overexpressing strains to produce *A. fumigatus* pheromones. Using sensitive yeast strains as chassis to express the respective pheromone receptor, existence of the so far elusive a-factor-like peptide could be demonstrated; current efforts are focused on its identification and functional characterization.

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**BYFP011**

**Mode of action Penicillium steckii IBWF104-06 as biological control agent**

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Organisms are increasingly developing resistance to conventional pesticides. More and more of those are being banned due to their harmful impact on the environment. So alternatives need to be found. Biological control agents (BCAs) are therefore in high demand because they are more sustainable and environmentally friendly than conventional broad-spectrum pesticides. Because of its activity against different plant pathogens like Phytophthora infestans, Fusarium graminearium, Alternaria solani and Botrytis cinerea, the Penicillium steckii strain IBWF 104-06 (P. steckii) was patented as such a potential biological control agent.

Tanzawaic acids, secondary metabolites from P. steckii, were identified as antifungal compounds. A gene cluster was found to contain an essential polyketide synthase (PKS1) gene for their production. In addition, a potential compound (AB1) with activity against Phytophthora infestans, seemingly connected to the sporulation, was found. In order to identify which further genes in the potential gene cluster are also relevant for the Tanzawaic acid production, in addition to the corresponding PKS1-gene, loss-of-function transformants will be generated. Therefore, the current system of transformant generation is in optimization by implementing a CRISPR-Cas9 driven transformation system. Furthermore, the production of AB1 will be optimized and the compound identified. Changing solid-state fermentation from water-agar to a rice-based medium, the production of AB1 was significantly increased and the specific mass and potential formula could be identified.

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**BYFP012**

**Studying fungal partners of temperate forest tree species**

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Mycorrhizal symbiosis is a mutualistic interaction between plants and fungi. Many trees in temperate forests show colonization of fine roots by mycorrhizal fungi. The trees benefit from this symbiosis by easier access to water and mineral nutrients, and the fungi acquire carbohydrates from the trees. Many studies have focused on mycorrhizal interaction, and for example transcriptomic analysis has identified a huge number of genes as differentially regulated during the onset and / or maintenance of symbiosis. Yet, molecular analysis of mycorrhizal interaction partners in laboratory model systems as well as functional analysis of genes remains scarce.

To establish such models, we morphologically and genetically analyze mycorrhizal fungi from different tree roots, concentrating on beech and pine. Microscopic analysis is complemented by DNA isolation and sequencing of different morphotypes. We aim to establish lab cultivation methods for selected fungal interaction partners to use these partners in further studies. For instance, we further analyze the ability of these fungi to colonize fine roots of genetically tractable plant model species. Through studying different stands in different seasons, we will also gain insight into mycorrhizal fungal diversity at the community level.

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**CMP001**

**Long-term investigation of the interaction between the microbial and viral communities in a biogas reactor**

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Changes in ammonia concentrations are one of the most frequent disturbances observed in regular biogas reactor operations where microbial communities have been shown to respond accordingly. In this study, we explored the diversity and changes of the microbial, archaeal and viral community of a mesophilic, full-scale biogas reactor (477 kWh h−1) fed with maize silage, dried poultry manure, and cow manure undergoing initial process disturbance by increased ammonia concentration. Over a time period of 587 days, the community of the reactor was sampled on a monthly basis. Using a genome-centric metagenomic approach, we

assembled 298 Medium-quality (< 50% complete, <10% contamination) and High-Quality (>90% complete; <5% contamination) Metagenome-Assembled Genomes (MAGs) and identified 49 bacteria and archaea MAG species representatives. We identified the reactor viral community and putative auxiliary metabolic genes carried by high-quality viruses. We predicted putative MAG hosts for each identified virus using in-silico methods where we predicted viruses with a wide host range that can likely infect bacteria and archaea hosts. Lastly, we examined changes in the relative abundance of virus-host pairs over our time series sampled dataset.

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**CMP002**

**StrainInfo – A central database for resolving microbial strain identifiers**

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Microbial strains can be known by a myriad of different strain designations, culture collection numbers and sequence accessions, which poses a challenge to the communication of research findings, as well as the comparison and reuse of data. Culture collection numbers, like DSM 5522 or ATCC 43876 are the most commonly used strain identifiers for microorganisms. They have the advantage of being unique, stable and subject to the high quality standards of culture collections. Nevertheless, each collection receiving a culture of the same strain assigns their own number at deposition. Different designations are thus used throughout publications and databases, making it difficult for scientists to draw connections between them.

Here we present the StrainInfo database, a service that collects and curates culture collection numbers as well as their relations and links them with different sources of information, such as publications and sequence accession numbers. This facilitates the connecting of data describing the same strain. The database builds on the idea of the defunct StrainInfo.net web services, which were developed at the LMG (Ghent University) between 2000 and 2015. It uses archived data and functionalities from the original database combined newly collected data and additional features. The information is provided through a modern and intuitive web user interface, which enables users to easily find corresponding strain identifiers and links to associated data, and through a web API, that allows for direct integration of strain identity resolution into workflows and other databases: In the future, StrainInfo additionally aims to provide a central registry service for cultures, allowing microbiologists to register strain designations and receive persistent identifiers prior to deposition and publication.

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**CMP003**

**BakRep: A searchable web repository for bacterial genomes and standardized characterizations**

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Introduction: The large amount of bacterial genomic data in public genome databases is an important resource for research in various fields. However, most of this data has been processed differently, making accurate comparisons challenging. *Blackwell et al.*, used a uniform approach to assemble and characterise 661,405 bacterial genomes retrieved from the European Nucleotide Archive (ENA) in November of 2018. On the one hand, this revealed the highly uneven taxonomic composition, with just 20 of the total 2,336 species making up 90% of the genomes. On the other hand, new genomes of 311,006 isolates were added, which had not been assembled before. This resource of sequence data has been published by *Blackwell et al*., with the intention to be used as a comprehensive basis for further analyses.

Objectives: We build up on this resource and further analyse the assembled genomes in a standardised manner. We will conduct a robust taxonomic classification using the Genome Taxonomy Database (GTDB) and furthermore subtype all eligible genomes via multilocus-sequence typing. In addition we will annotate all genomes assigning functional categories, e.g. COG and E.C., and database cross references to public databases. The overarching goal is to make this standardised resource available to non-bioinformaticians via an interactive website. This website will provide researchaers with a flexible search engine to query the repository. For example, researchers could query for all genomes of a species that belong to a given sequence type containing a particular set of AMR genes and virulence factors.

Methods: This compute-intensive data processing workflow was implemented in Nextflow to ensure flexible modularity and maximum scalability. This workflow is currently conducted in the de.NBI compute cloud taking advantage of more than 2000 CPU cores.

Results: So far about one third of the data has been processed. We estimate that the entire workflow will be completed within the upcoming 3 months. A first batch has already been uploaded to a website prototype, which is currently under active development.

Conclusion: The BakRep project conducts comprehensive and standardised characterisation of one of the largest collections of bacterial genomes worldwide. We envision it as a high-quality open resource for microbial researchers all over the world.

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**CMP004**

**Exploring provenance gaps between lab and computer analysis in genomics and metagenomic studies, a part of the NFDIMicrobiota consortium**

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There is a growing need to make data from life sciences findable, accessible, interoperable, and reusable (FAIR), which is incredibly challenging in metagenomics. This field deals with the functional potential of microorganisms in natural, host-associated, and constructed environments using high-throughput sequencing from total DNA isolated from microbial communities. Currently, there is often a disconnection between wet and dry labs leading to provenance issues in collaborations in metagenomics studies.

This study aims to improve FAIR principle usage in metagenomics by creating guidelines to connect the wet and dry lab parts of projects contextualized by data provenance. To this end, we explored provenance in over 100 projects involving more than 5000 samples from multiple sources (e.g., bioreactors, agricultural and forest soils, freshwater, wastewater, and gut microbiome). We used short and long-read sequencing to explore the data provenance of wet lab sample preparation (storage and DNA extraction), library preparation and sequencing, and sequencing data preprocessing before data analyses. We organized the guidelines into five parts: sample preparation, sequencing logistics, data downloading, integrity and quality of sequences, and preprocessing the sequenced data until assembly.

First, we analyzed provenance from sample preparation (sample storage and DNA extraction) for metagenomics. Our data indicated that particular attention is necessary to plan sampling campaigns for DNA yield and quality (particularly if interested in long-read sequencing). DNA quality check must be performed in every sample in BioAnalyzer-like machines. We also highlight that provenance should include memory configurations and resources required for sequencing projects to facilitate reproducibility, particularly for sequencing data preprocessing, as it demands a high usage of resources. To improve the interoperability and reuse of metagenomics data, reviewing and commenting needs to be implemented on automatic reports in existing data processing pipelines, and a massive yield in yield in samples can lead to failed library preparation.

In Conclusion, small research groups and data stewards responsible for organizing data in local or large sequencing facilities may use our guidelines to bridge the gap between wet and dry lab researchers. Our guidelines may help to improve FAIR usage of metagenomics as we concentrate on data interoperability and reuse aspects of provenance.

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**CMP005**

**Current developments in SubtiWiki: Structure prediction, genomic neighborhood, and metabolites**

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The massive acquisition of novel biological data requires the development of dedicated databases to make the information accessible in an intuitive way. We have developed *Subti*Wiki, the most popular freely available database for the model bacterium *Bacillus subtilis* (1). It holds a wealth of up-to-date curated information as well as datasets from relevant publications. In addition, it features many interactive displays to intuitively explore the available data. To keep up with newest scientific findings, *Subti*Wiki is constantly being expanded. Currently, several features are in development, which will provide the *B. subtilis* research community with new tools to develop research hypotheses. Recently, AlphaFold has revolutionized computational structural biology with its unprecedented accuracy in protein structure prediction (2). To reflect these advances, we included structure predictions for all proteins in the database. We also added complex predictions from a recent global interaction study on *B. subtilis* (3). To help with their interpretation, we developed an interactive visualization of the predicted aligned error (PAE), an important metric of accuracy (4). Identifying the function of proteins in *B. subtilis* is still a big challenge. However, the analysis of gene order conservation across different organisms can provide important first hints about the role of a gene. For this reason, we have added an interactive implementation of the FlaGs tool (5), which allows users to compare gene neighborhoods among selected representative bacteria. Another extension is the introduction of dedicated pages for metabolites and their integration into the interaction browser. With this, we hope to give a more complete picture of the inner organization of the *B. subtilis* cell. We are confident these additions will make *Subti*Wiki even more useful for the *Bacillus* research community.

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**CMP006**

**gSpreadComp – A novel open-source, user-friendly workflow – Showed plasmid-mediated horizontal transfer enrichment of antimicrobial resistance in vegans and higher virulence in ketogenic-related bacteria**

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Introduction: Horizontal gene transfer (HGT) allows bacteria to adapt to fast environmental changes. Plasmid-mediated conjugation (PMC) is among the most impactful HGT mechanisms, as it can make pathogenic bacteria resistant to antimicrobials.

Objective: We developed gSpreadComp to detect PMC in entire microbiomes without reference genomes reducing the technical barrier for non-bioinformaticians looking for ecological insights in HGT.

Methods: The gSpreadComp is a modular workflow integrating genome quality estimation, taxonomic assignment, plasmid and pathogen bacteria identification, virulence factors (VF) annotations, gene spread, plasmid-mediated HGT analysis and antimicrobial resistance genes (ARGs). This workflow configures all dependencies and databases during installation. We use weighted average gene prevalence (WAP), normalized mutual information to estimate spread, and the Smillie heuristic to identify HGT. We tested our workflow to identify ARGs in human gut metagenomes with different diets (17 ketogenic, 158 omnivores, 10 vegans, and 44 vegetarians) and 45 ancient samples (1300-5300 years old). We recovered metagenome-assembled genomes (MAGs) using MuDoGeR and used them as input for gSpreadComp.

Results: We recovered 3659 MAGs dereplicated into 400 Operational Taxonomic Units. We annotated 356 unique ARGs from 24 resistance types. All diets spread glycopeptide and multidrug resistance (WAP > 0.7) ubiquitously. However, bacitracin resistance is statistically more prevalent ($p < 0.05$) in Vegans than in Ketogenic or Omnivores. Fosmidomycin is less prevalent in Vegans than

in all other diets. In addition, phenicol resistance was more prevalent in Ancient samples. Following, *Bacteroidota* phylum had more VF in Ketogenic diet. Moreover, pathogens found in Ancient samples seem to have less VF than in modern diets. Plasmid-mediated HGT at Family level from ARGs happens more often in Vegans, 24.3% of all potential plasmid-mediated HGT events, while Ketogenic had 11.7% and Ancient had the lowest (1.8%). Finally, modern diets had an average of 39.2 unique ARGs in highly pathogenic species and 12.1 in other species.

Conclusions: We showed enrichment of specific ARGs in different diets, a concerning high resistance level in pathogens, a higher risk of ARGs plasmid-mediated HGT in Vegans and higher virulence in ketogenic-related bacteria. The gSpreadComp can be used in any other system and help design strategies to control plasmid-mediated transmission.

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**CMP007**

**Comparative analysis of functional categories for pan-genomes on the EDGAR platform**

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Introduction: The significant increase of whole genome sequence data availability, made possible by next generation sequencing methods, has caused the emergence of a variety of tools, concerned with the comparative analysis of related genomes. This approach aims to gain insight into genomic functions by comparing grouped subsets, highlighting similarities and differences, conserved and divergent regions within the sequence data. The web-based EDGAR platform, which is under constant development for more than a decade, is one of the most established tools in the field. It offers an ever-increasing suite of analysis features for pan-genomic and phylogenomic analyses, aiming to enable users to gain a comparative perspective of their data.

Another approach for sequence analysis is represented by functional categorization, which seeks to order genes with related functions into distinct groups. Long-running database projects like KEGG, COG and GO can be used to first annotate genes and subsequently group them according to higher-level functions.

Objectives: The project goal was to extend the existing capabilities of EDGAR, integrating subset and functional category information into one package.

Materials & methods: Functional category annotation of all EDGAR projects is carried out using the fast alignment tool Diamond for COG and GO, as well as hmmserach for KEGG, respectively. As KEGG and GO both feature regular changes of their annotations, the corresponding data is automatically updated to ensure the best possible annotation quality. In case of COG, the most recent 2021 update is used.

Results: Utilizing the generated functional information, EDGAR now enables users to explore subset- or organism-specific categorizations via interactively navigable graphs. The provided interface facilitates data transformation, allowing for various different views on the data. Quantitative information of functional categories can be easily exported from the visualizations. Additionally, the export of sequence data enriched with and filtered according to functional annotations is supported.

Conclusion: Functional categories were computed for more than 80,000 genomes available in EDGAR, and the results are available to the scientific community via the web application. Functional categories are now part of the default EDGAR pipeline and will be computed automatically for all future projects.

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**CMP008**

**Computational prediction of pH changes during bacterial growth**

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Introduction: Changes in pH due to bacterial metabolism play a key role in diverse areas such as tooth decay and food production, and are also important for detection of bacterial contamination and infection. Flux Balance Analysis is a well-established method for computational prediction of metabolism, but it has rarely been used to predict pH changes.

Objectives: We aimed to develop a computational framework to predict pH changes associated with bacterial growth, and to compare its results to measurements for diverse bacterial species under a range of growth conditions.

Materials and Methods: A mathematical modelling framework for predicting pH changes associated with bacterial growth was developed by combining Flux Balance Analysis with the pH calculation software CurTipot. Experimental measurements of changes in pH during bacterial growth were made for lab strains of *Escherichia coli* and *Staphylococcus epidermidis*, on LB medium with and without glucose and under aerobic and anaerobic conditions.

Results: Our computational predictions were in good agreement with our experimental measurements for both *E. coli* and *S. epidermidis*, under the various growth conditions. We observed strong differences between the pH changes mediated by *E. coli* and *S. epidermidis*. Our computational approach also allowed us to identify the key metabolites and metabolic pathways that mediate the effect of microbial growth on pH for different microorganisms.

Conclusion: We developed and verified a computational framework to predict pH changes associated with bacterial growth, by combining metabolic modelling (flux balance analysis) with speciation methods for calculating pH. The ability to predict pH changes associated with bacterial growth based on genomic information could have broad applications, from dental health to early detection of infection in wounds and on medical implants.

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**CMP009**

**SymBLAST – A browser-based user-friendly solution for custom reciprocal ortholog identification using BLAST**

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Identifying orthologous genes in large sequence databases can be challenging, especially for anyone without

programming skills. SymBLAST (Symmetric-BLAST) simplifies this process in a stand-alone platform, allowing users to search for putative orthologs on their computers without programming knowledge, making sophisticated and customized BLAST analyses accessible to a wider range of researchers. The state-of-the-art web interface guides users through the entire pipeline, from BLAST database creation, protein sequence search and download to data deployment, Reciprocal Best Hit (RBH) inference, and post-processing.

SymBLAST uses the Basic Local Alignment Search Tool (BLAST) command-line program in combination with the workflow engine Snakemake, the web framework Django, and various other bioinformatic tools.The application is packaged in a Docker container network that allows it to be installed on any system where Docker is installed. SymBLAST is a web application that, once installed and launched, is accessible via common browser applications. Due to web-based data deployment, pipeline execution, and monitoring, the application enables easy and user-friendly customization of reciprocal BLAST analyses and downstream result processing.

Prior to conducting the SymBLAST pipelines, the tool allows users to customize BLAST databases. Users can upload their own proteome FASTA-files, or they can download sets of proteomes by applying taxonomic and completeness filters to GenBank or RefSeq databases. SymBLAST operates on those databases. The reciprocal BLAST pipeline consists of several workflow steps to infer RBHs. Based on identified RBHs SymBLAST conducts various post-processing steps, involving multiple sequence alignments, phylogenetic tree inference and synteny analysis.

With its user-friendly customization options, intuitive web interfaces, and efficient workflow engine, SymBLAST has the potential to transform the way researchers approach ortholog identification and analysis.

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**CMP010**

**TaDReP: Targeted detection and reconstruction of plasmids**

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Introduction: Bacterial plasmids carry a wide range of genes including antimicrobial and metal resistance determinants. As a key driver of horizontal gene transfer they play an important role in the genetic network of bacteria and therefore are subject to genomic epidemiology. However, because of sequencing quality and costs, most routine surveillance systems depend on short-read sequencing platforms, which are notoriously incapable of generating closed replicons. Therefore, the automated identification and analysis of plasmids via short-read sequencing data remains challenging and is often complemented by partial hybrid sequencing. In particular the targeted detection and reconstruction of plasmids from complete genomes within draft genomes of the same cohort, as for example in outbreak analyses, often remain subject to time-consuming and error-prone manual analysis .

Objective: We address these issues by automating the many common steps involved in the targeted analysis of known plasmids within bacterial cohorts by a modular bioinformatics workflow.
Results: Here we present TaDReP, a new semi-automated bioinformatics software tool providing a comprehensive toolkit for the extraction, targeted detection, reconstruction and analysis of plasmids within cohorts of bacteria. TaDReP, allows researchers to extract closed plasmid sequences from closed and semi-closed genomes originating from long-read or hybrid sequencing approaches. Extracted plasmid sequences are then subject to a sequence-based clustering process also taking into account various characterizations, e.g. sequence length, GC content and incompatibility groups to split heterolog clusters and to select appropriate representatives for each plasmid group. Afterwards, draft genomes are screened for these plasmid groups and detected instances are reconstructed via an automated contig-alignment approach. Finally, a presence/absence matrix of all detected plasmid groups for the entire cohort is provided and each reconstructed plasmid is visualized.

Conclusion: TaDReP is a versatile software tool streamlining the targeted analysis of plasmids within bacterial cohorts. Its semi-automated approach makes it a flexible toolkit for researchers studying the distribution of plasmids.

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**CMP011**

**Large scale comparative genomics and phylogenomics using EDGAR3.2**

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Introduction**:** EDGAR3.2 provides precomputed orthology databases for more than 80,000 microbial genomes in public as well as private databases. The platform allows rapid identification of the differential gene content of kindred genomes, i.e., the pan genome, the core genome, or singleton genes. Furthermore, EDGAR3.2 provides a wide range of analyses and visualization features required for phylogenomic inter- and intraspecies taxonomic analyses. EDGAR calculates core-genome-based phylogenetic trees as well as amino acid identity (AAI) and average nucleotide identity (ANI) matrices. Since the last update, it also offers a functional categorization of genes based on the databases KEGG, COG and GO. Thus, the software enables a quick survey of evolutionary relationships and simplifies the process of obtaining new biological insights.

Objectives**:** Over the last decade, the average number of genomes analyzed in EDGAR projects constantly increased. Consequently, with EDGAR version 3.0 new storage infrastructure was developed using a file-based high-performance storage backend which ensures timely data handling and memory efficient calculation of orthologs. Parallelization has led to drastically reduced processing times. The adaptation of the EDGAR platform for larger genome numbers is still an ongoing task, though. Building on previous improvements, further optimizations of the platform resulted in the current version EDGAR3.2.

Materials & methods: The backend for the calculation of orthologs and genomic subsets has been rewritten in Rust, received performance optimizations, and is integrated using a gRPC API. It is deployed in Kubernetes and uses autoscaling features along with an improved scheduling mechanism to ensure optimal scaling behavior for large datasets. In the alignment workflow, BLAST will be replaced by the much faster Diamond tool, and results will be stored in an S3 object storage. The whole process is streamlined to avoid unnecessary data transfers between cloud and local environments.

Results: In EDGAR3.2, functional category data was added for all genomes together with respective visualization features. The technical infrastructure was further optimized to be scalable with increasing query sizes, and is currently used to process more than 30,000 genomes per year. The optimizations ensure that EDGAR3.2 stays a convenient platform for comprehensive microbial gene content analysis. The web server is accessible at: http://edgar3.computational.bio

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**CMP012**

**Genomic and molecular analysis of siderophores and biosurfactans**

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Siderophores and especially biosurfactants play a major role in different industrial fields. Due to the amphiphilic nature of biosurfactants and marine siderophores they are especially interesting for the cleaning and pharmaceutical industry. Usually, these compounds are obtained from the petrochemical or oleochemical sector, and therefore have a negative impact on our environment. The increased demand for these compounds incremented the interest in acquiring surfactants and siderophores from a renewable source in order to lower the impact on our environment and the dependency on fossil resources. Usually, all genes needed to produce these natural products are located in BGCs (Biosynthetic Gene Clusters) which are part of the secondary metabolism. The overreaching goal of the EU funded project SECRETed (**S**ustainable **E**xploitation of bio-based **C**ompounds **R**evealed and **E**ngineered from na**T**ural sources) is to produce new-to-nature tailor-made compounds with specific properties by combining different components of genomic clusters together. The combination of clusters has the potential to increase the diversity of these molecules and increment the possible use cases in different industrial sectors. To reach this goal a database was build, containing all known biosurfactants and siderophores, for whom the BGC is known. This database includes the basic chemical class and producing BGC of the compound as well as the predicted biosynthetic pathway. Currently, the database contains 65 Siderophores and 101 Biosurfactants. This compounds where analysed, to identify common grounds and sub-clusters. Information about similarities between the clusters and the function of sub-clusters can then be used down the line to synthetically design a molecule by "Mixing and Matching" the sub-clusters together. Furthermore, available genomes of producer strains were analysed using antiSMASH where no cluster is linked to the produced compound. Already collected data and defined cluster rules were then used to identify the possible cluster, which could be responsible in the production of the compound. Using the rules defined during the comparison of the clusters additional 87 possible clusters were linked to a compound. All the data collected in this part of the project will be used to identify possibilities to genetical design new to nature compound with desired properties.

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**CMP013**

**A new foundation for *Subti*Wiki: Introducing *Core*Wiki**

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The exponentially increasing amount of biological data can only be handled and provide useful insights if the data are organized and presented in integrated and meaningful databases. Our group has developed *Subti*Wiki, the most popular freely available database for any bacterium. It holds information about more then 6000 genes, 280,000 protein interactions and more than one million gene regulations in *Bacillus subtilis*. It contains a vast amount of current, hand-selected data and datasets sourced from relevant publications. Furthermore, it offers numerous interactive tools that allow for easy exploration of the data. *Subti*Wiki has several thousand accesses each day, and the number is growing. Here we introduce *Core*Wiki, a new foundation for *Subti*Wiki, which will accelerate development of new features and further improve functionality of the website. In addition, *Core*Wiki will function as a generalized framework for model bacterium databases, and will also be used a a platform for other databases maintained by our group, such as *Myco*Wiki (2) and *Syn*Wiki (3). To be able to respond more rapidly to feature requests in the future, to ensure better availability of data for research groups, and to implement the integration of the different databases, we are working on a major transformation of the *Subti*Wiki universe: we are migrating the application to a three-layer architecture, separating the data layer from the web application. This offers a full stack of new opportunities: it is now possible to access the data via a REST API, a modern, easy-to-use interface; the web application is modernized and available both as a website and a mobile app; and several new features for improved usability are added. Here, we present the current state of development, give an idea about the new features and how they can support microbiological research, and discuss the next steps for future development.

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**CMP014**

**Evaluation of the zoonotic potential of *Streptococcus agalactiae* – A comparative analysis of cattle and human isolates**

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Introduction: *Streptococcus agalactiae* – often referred to as group B streptococcus (GBS) – is one of the most important pathogens in cattle breeding regularly associated with mastitis. Moreover, GBS are also commonly isolated from other animal species and frequently identified as colonizer of the human female genital tract. GBS are also considered as one of the most important neonatal pathogens, associated with pneumonia, meningitis, and septicemia. Recent reports mention GBS as source of foodborne infections. In Singapore, a cluster of severe infections induced by consumption of raw fish could be linked to GBS. However, the zoonotic potential of GBS is not well understood. Even though, cases of human and cattle infections associated with the same sequence type are described, it needs to be clarified, whether this is induced by direct transmission, or linked to a common environmental source of bacterial origin.

Objectives: To elucidate similarities as well as differences between GBS strains of bovine and human origin, we performed a comprehensive comparative genome sequencing approach including isolates from cattle and human infections. Previous studies mostly focused on non-holistic methods, sequencing only genomic regions of particular interest. In contrast, our bioinformatic analysis on genome level will provide much higher resolution to shed light on host specificity and in that way address current questions regarding epidemiology and zoonotic potential of GBS.

Materials & methods: A comparative genome sequencing approach was carried out including cattle strains isolated from mastitis milk samples submitted to the Hessian State Laboratory (n=263) and human isolates from invasive infections provided by the National Reference Center for Streptococci, University Hospital RWTH Aachen (n=255).

Results: First results show a very broad distribution of capsule-serotypes and multilocus sequence types in human as well as cattle isolates. Ongoing analyses will focus on virulence factor distribution, mobile genetic elements, and global gene composition.

Conclusion: This comparative genetic analysis will not only identify a yet underestimated number of virulence associated genes that might enable new treatment options for human and animal infections but will also provide highly valuable insight in the epidemiology and general zoonotic potential of GBS.

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**CMP015**
**Predicting microbiome life history traits**
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Organisms face evolutionary and ecological trade-offs while allocating limited resources when adapting to environmental conditions. Life history theory outlines strategies comprising functional traits that result from choosing between often conflicting objectives such as growth, maintenance, and reproduction. Although concepts like oligotrophes/copiotrophes or Grime's CSR framework exist [1], microbial life history theory is less developed, especially regarding more complex communities. In my talk, I will conceptualize life history theory for microbiomes and apply computational approaches (for example, metabolic modeling [2]) to predict relevant traits. In previous works on *C. elegans*, we found inferred life history traits associated with microbial colonization success [3,4]. Our ongoing research encompasses the microbiome of both mice and humans. Finally, the prediction of life history traits not only improves the understanding of microbiome diversity and coexistence but allows evolutionary-informed treatments to tackle, for example, antibiotic-resistant strains.

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**CMP016**
**Genomic exploration of prokaryotic diversity in the culture collection of the Archaea Centre Regensburg**
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Almost four decades of expertise in anaerobic cultivation and single-cell isolation, combined with specialized equipment and the efforts of numerous scientists throughout the years, culminated into an extensive and worldwide unique strain collection at the Institute of Microbiology and Archaea Centre Regensburg. Our collection currently contains approximately 2250 different archaeal and bacterial isolates and enrichments from highly diverse and unique habitats (such as submarine solfataric springs, deep sea hydrothermal vents, hypersaline environments, anoxic freshwaters, terrestrial hot springs, acidic mines, etc.), with most of them being available as pure cultures. Only a tiny fraction of the isolates in the BBR were sequenced using third-generation sequencing approaches. Consequently, this collection represents a "treasure trove" of truly unexplored genomic diversity especially within the domain Archaea.

In this study, we focused on isolates from the order *Thermococcales* and sequenced 24 strains using Oxford Nanopore"s long-read sequencing technology. The taxonomic placement of the isolates was confirmed using GTDB-Tk. For all sequenced isolates, we were able to generate a circular, closed chromosomal element. All assemblies placed within the order Thermococcales, but half of them are to be considered novel species within. Comparisons at the sequence- and the annotation-level further reinforce this taxonomic assessment.

Additionally, we show the analysis of the regulatory network of the euryarchaeal archaeellum regulator A (EarA) surveying *Thermococcales* genomes available from databases and our sequences. We found that the genomic arrangement of EarA binding sites close to the promoter of the archaeellum operon differs between the genus *Thermococcus* and the genera *Pyrococcus* and *Palaeococcus*. These data demonstrate how the survey of genomic diversity based on an extended set of genomes of closely related strains provides new insights into archaeal biology.

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**CMP017**
**Efflux pumps and nutrient availability: The impact on *Escherichia coli* susceptibility to tetracycline**
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Introduction: Efflux pumps (EP) are important in bacterial antibiotic resistance by expelling antibiotics from bacterial cells, leading to reduced antibiotic effectiveness and promoting resistance. Understanding the functionality of EPs, their impact on bacterial physiology, and their influence on

antibiotic susceptibility is essential. This knowledge enables the development of novel treatment strategies.

Objectives: We evaluated the impact of EPs on antibiotic susceptibility by comparing the growth rate, lag phase extension, and maximum OD. We gained insight into better antibiotic treatment that affects bacterial physiology, using microscopy techniques. We developed a mathematical model that considers the use of EP to provide insight into how cells can overcome the threats of antibiotics.

Materials & Methods: The growth profiles of 7 knockout variants of the arcAB-tolC EP operon were examined under various concentrations of Tetracycline. Evaluation of cell physiology via staining, image analysis with Fiji with BiofilmAnalyzer, and curve fitting was carried out using Matlab.

Results: Differences in growth rate, lag time, maximum OD600, and resistance were change over time based on antibiotic concentration and parent strain's genetic background. AcrA and AcrB deletion mutants were highly susceptible to tetracycline, while overexpressors and the TolC mutant showed diminished susceptibility. In nutrient-poor media, lag phase extensions of the exponential growth were observed. Fluorescence LIVE/DEAD staining revealed in the lag phases very high death rate but also filamentation. A linear correlation between tetracycline concentration and lag phase extension was found, indicating that cells in nutrient-poor media can survive in higher concentrations. Mathematical modelling allowed to better understand and predict the impact of the EP of the arcAB-tolC operon under the influence of tetracycline.

Conclusion: We investigated the impact of EP of the acrAB-tolC operon on *E. coli's* antibiotic susceptibility to tetracycline through growth curves, microscopy techniques, and mathematical modeling. We revealed filament formation and lag phase extension as a response to tetracycline. This study will inform the development of mathematical models for the functional role of EP and their impact on bacterial physiology and antibiotic susceptibility. Additionally, the data sets can be used to train mathematical modelling systems to predict reaction of EPs to antibiotics.

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**CMP018**

**An inexpensive FHOTOBOX for high-resolution imaging**

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Introduction: Good scientific practice considers it as one of its most important principles that research methods and results should be documented accurately and comprehensively, especially while imaging of results becomes increasingly important. In the diverse research areas of microbiology, a cost-effective documentation system that enables universal documentation of bacteria on agar plates, time-lapse films, protein and DNA gel documentation in high resolution is not yet available on the market.

Objectives: Our goal was to develop a universal and standardized illuminated photo documentation station for agar plates and DNA gels in the high quality range that is easy to reproduce, inexpensive to build and adaptable to your own needs.

Materials & Methods: Standardized aluminum plates and profiles were purchased and brought to the desired size by drilling and milling. Other materials included various light-emitting diodes (LEDs), a Raspberry Pi4 Model B, a Raspberry Pi High-Quality Camera. Using three-dimensional printing, the individual building blocks were manufactured according to plans created with FreeCAD software.

Results: The newly designed photo box consists in the base of a light box with front and lid flap and an inner dimension of 310x310x363 mm. The basic light box contains white light LEDs dimmable by pulse width modulation on all four walls. For taking microbial colony images on agar plates, a diffuser module is used, which provides uniform reflection-free illumination. The photobox is controlled via a Raspberry Pi4 Model B. Images are captured using the Raspberry Pi High-Quality Camera and its 12.3 megapixel Sony IMAX477 foto sensor and Raspberry's official 16 mm telephoto lens. For macroscopic photography, the lens can be operated in retro position as a low cost macro lens. For documentation of DNA gels, the diffuser module is exchanged for a UV light stage module. A UV filter is positioned in front of the lens. On the software side, a phyton script with graphical user interface and light profiles that can be stored in the csv file format. The FHOTOBOX has been used to photograph a series of agar plates with different colored media, as well as DNA agarose gels. In each case images were obtained at high-resolution.

Conclusion: A universal, customizable and affordable FHOTOBOX was designed that delivers high-resolution images for documentation and publication purposes in the laboratory.

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**CMP019**

**Modelling the host specificity of a biopesticide**

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Cry-toxins originally found in strains of Bacillus thuringiensis are the main component of most biopesticides used worldwide in agriculture. Currently the Bacterial Pesticidal Protein Resource Center (BPPRC) comprises 1060 proteins from 13 bacterial species. In previous research, we optimized profile hidden Markov models (HMMs) to identify all members of every toxin class according to the BPPRC nomenclature. Our HMMs are implement in the IDOPS software tool, which helps in the identification and characterization of known and novel pesticidal proteins encoded by Bacillus thuringiensis and other bacterial species. The group of Cry toxins are active against diverse organisms, including pests from the orders Diptera, Lepidoptera, Coleoptera and Rhabditida.

The toxins and their mode of action are a focus of investigation. However, knowledge on what determines the particular target of a given toxin is still under research. Here we present an HMM based analysis to study determinants of host specificity in Cry toxins.

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**EMEP001**

**Soil type and irrigation water quality influence variety and abundance of antibiotic resistance genes and mobile genetic elements in gram positive soil bacteria**

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Wastewater is utilized for irrigation in agricultural production in dry and semiarid regions to alleviate water shortages. The coexistence of antibiotics, pathogens, and antibiotic resistance determinants in wastewater raises concerns that antibiotic resistance genes are disseminated into the environment and mobilized from the environmental resistome and finally transferred to potential human pathogens.

In this context, community DNA from three soil types (Phaeozem, Leptosol, and Vertisol) from the Mezquital Valley in Mexico was examined using quantitative real-time PCR to determine the relative abundance of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in Gram positive bacteria. In an incubation experiment conducted in microcosms, these soils were irrigated for 8 weeks with either untreated or treated wastewater and spiked or not with antibiotics and disinfectants. The soil samples were collected after four days and four weeks, and the DNA was then isolated from those samples.

TaqMan qPCR was used to assess the relative abundance of Inc18 plasmids, multiresistance Rep A_N-type *Enterococcus* plasmids, multiresistance *Staphylococcus* plasmids of pSK1 and pI258 families as well as *ermA*, *dfrD*, *dfrG* resistance genes, which encode for resistance to erythromycin and trimethoprim, respectively.

Regardless of the soil type, an increase in the relative abundance of plasmids belonging to pSK1 family was detected in soils irrigated with spiked wastewater. The relative abundance of *ermA*, *dfrD*, *dfrG* resistance genes, and plasmids of the pI258 family only increased in soils treated with unspiked wastewater. The occurrence of Inc18-type and pI258-like plasmids, as well as the presence of the trimethoprim and erythromycin resistance genes (*dfrD*, *dfrG*, and *ermA*) were significantly and positively intercorrelated. In contrast, pSK1-like plasmids were negatively correlated with the *ermA* and *dfrG* genes in all soil types.

Our findings therefore imply that antibiotics and disinfectants can enhance the abundance of some ARGs in wastewater-irrigated soils and shed light on the possible co-selection of ARGs and MGEs in Gram positive bacteria.

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**EMEP002**
**The application of rhizosphere competent actinobacteria as a tool to increase corn ability to ameliorate drought stress in the United Arab Emirates**
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In the United Arab Emirates (UAE), several management strategies have been adopted to help crops cope with the deleterious effects of water scarcity and drought. Plant growth promoting actinobacteria (PGPA) have the ability to colonize the seeds and/or the plant rhizosphere, and play a significant role in the alleviation of drought stress in plants. Date pits (DP) are by-product wastes of many date-processing industries in the UAE. We propose -for the first time- a seed coating/bacterization method that consists of corn seeds, gum Arabic (adhesive material), UAE locally-produced DP powder (DPP; carrier) and PGPA inocula as biological fertilizers for potential field applications. This agro-biotechnological application aims at: (1) evaluating growth promotion activities of PGPA using DPP carrier compared with other commercial carrier formulations; (2) assessing growth performance and drought tolerance in corn using PGPA carried on DPP or other carriers under water scarcity conditions; and (3) determining the role of 1-aminocyclopropane-1-carboxylic acid deaminase-producing PGPA in *relieving* corn from the adverse effects of drought. Our results showed that combining PGPA with DPP (as a carrier) on seeds effectively performed as growth promoters for corn irrigated with minimal amount of water. There is clear evidence that DPP, as a seed coating/food-base combination, ensures successful seed and root colonization by the root-colonizing PGPA. In our greenhouse experiments, there was a five-fold increase in corn biomass in DPP+PGPA treatment when limited water of 20% field capacity (FC) was supplied compared to that with PGPA only or PGPA combined with other carriers. We also noticed that water use efficiency increased by 12-fold in the severe water scarcity regime (20% FC) when DPP and PGPA were coupled, compared to PGPA alone. This study is the first to demonstrate a novel, environmentally-friendly technique through using DPP+PGPA to promote the growth of drought stressed-seedlings and boost plant production in "water scarcity" ecosystem in the UAE or elsewhere. The outcomes will help develop large-scale strategies to utilize unused nutrient-poor dry lands in the UAE as a primary source for animal feed and a potential practice in horticultural plants with minimal water consumption. This will enable UAE farmers to grow plants using limited water resources; thus, solving the water scarcity issue in the UAE agricultural sector.

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**EMEP003**
**Application of halotolerant plant growth promoting actinobacteria along with fish emulsion further improves growth and development of mangrove (*Avicennia marina*)**
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In the United Arab Emirates, the combination of commercial fish emulsion (FE) and plant growth-promoting actinobacteria capable of producing plant growth regulators (PGRs) was studied as a biofertilizer to increase the growth and development of grey mangroves (Avicennia marina). *Streptomyces griseorubens* UAE1 (Sg) was shown to be rhizosphere-competent, capable of solubilizing phosphorus and producing auxins, cytokinins, gibberellic acid, polyamines, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Growth promotion, nutrient contents and PGR levels in tissues of mangrove plants treated with FE and/or *Sg* were further assessed *in vivo*. Under greenhouse and natural open-field nursery conditions, sediments amended with FE only (+FE/-*Sg*) were found to effectively support growth promotion of mangrove compared to those inoculated with *Sg* (-FE/+*Sg*) only. Plant growth promotion by *Sg* was more pronounced in the presence of FE (+FE/+*Sg*) than in any of the individual application. Our data showed that *Sg* which produced PGRs appeared to use the biostimulant FE as a source of nutrients and precursors for plant growth promotion. Thus, *in planta* PGR levels following the combined +FE/+*Sg* were significantly ($P$<0.05) induced over other treatments. This was evident when the maximum velocity of rubisco carboxylation (Vcmax) and triose phosphate utilization rate (TPU) increased three-fold in +FE/+*Sg* treated plants compared to control. This is the first report of its kind, to our knowledge, on the use of the

biostimulant FE as a nutrient-base for soil microorganisms, including the bioinoculant Sg, to stimulate mangrove growth in a coastal agricultural setting.

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**EMEP004**

**Endophytic 1-aminocyclopropane-1-carboxylic acid deaminase-producing *Streptomyces* spp. as a tool to alleviate salt stress in tomatoes in the United Arab Emirates**

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Salinity is a significant factor limiting crop productivity worldwide. Utilizing plant growth-promoting microorganisms, an eco-friendly technology can be used to increase crop yield in salty regions. Endophytic actinobacteria that produce the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD) are capable of modulating ethylene concentrations within a plant. The purpose of the current study was to determine if endophytic actinobacteria can reduce endogenous ethylene levels in tomato plants, hence improving the plants' resistance to salt stress. From halophytic plant roots, 43 isolates of streptomycete actinobacteria and non-streptomycete actinobacteria with ACCD activity were isolated. Three of the most promising and excellent ACCD generating isolates with the strongest internal root colonization were also examined for their potential to produce plant growth regulators (auxins such as indole-3-acetic acid, polyamines such putrescine, spermidine, and spermine). The application of the three isolates resulted in the reduction of the endogenous levels of ACC, which is the immediate precursor of ethylene, in roots and shoots, as well as lowered ethylene levels and boosted plant growth in comparison to control plants grown under greenhouse conditions. The application of the three isolates at various salinity levels in a greenhouse environment significantly accelerated tomato plant growth after eight weeks and increased the dry weights of roots and shoots and the lengths of roots and shoots in comparison to control plants grown at the same salinity level but without the application of the ACCD-producing actinobacteria. In conclusion, we report the production of ACCD by endophytic actinobacteria and its ability to enhance tomato growth under saline conditions compared to control plants. This was accomplished by a reduction in the *in planta* levels of endogenous ACC and a subsequent lowering of endogenous ethylene levels in plant tissues. In addition, we found that the production of ACCD by endophytic actinobacteria was associated with an increase in tomato yield. This investigation is anticipated to contribute to the development of strategies for utilizing high salinity underground water for primary production in the UAE, including the cultivation of vegetables, thereby permitting local farmers to utilize the highly saline groundwater for irrigation purposes.

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**EMEP005**

**In vitro assessment of fungal volatile organic compounds: A two-chamber system for evaluating respiratory health**

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Introduction: Previous epidemiological studies suggest that volatile organic compounds from fungi (fVOCs) affect human health and cause respiratory irritation and other symptoms. To date, only a few studies have investigated the causal relationship of individual substances on human health. *In vitro* tests are an important alternative to e.g. animal experiments to shed more light on this issue. However, reproducible and standardized in vitro methods for testing fVOC mixtures produced from fungal cultures are not currently available due to the high technical and biological challenges involved.

Objectives: To close the gap between epidemiological and missing *in vitro* data on toxic effects of fVOCs by providing a feasible device for evaluation of these substances in private or occupational areas there is a need to

i. develop a controlled growth chamber for single/multiple fungi to generate fVOCs

ii. design a modified exposure chamber for the exposure of respiratory tract models with fVOCs

ii. enabling periodic, short-term or long-term exposure to sterile fVOCs through controlled air flow

iv. analyse the produced fVOCs independently/parallel to the exposure to ensure proper amount of fungal VOCs is exposed to the cell cultures

Methods: The described work uses a two-chamber system to test fungal VOCs on respiratory tract models. The first chamber optimizes fungal growth and fVOC production, with monitoring of temperature, CO2 and humidity. FVOCs are collected and pushed downstream to the exposure chamber. The fVOC mixture is analyzed using Tenax tubings and GC-MS before exposing lung cells using an adapted exposure chamber. The effects of fVOCs on the respiratory tract models are characterized by analysing viability markers, barrier integrity, live-dead analysis, gene expression, and cilia behaviour.

Results / Conclusions: The two-chamber system is readily build and first experiments are planned. We successfully developed standard operating procedures for the cultivation and differentiation of human respiratory cells and established growth conditions for evaluating different fungi. We also established a monitoring device and an experimental process for evaluating cilia beating. Overall, the setup is promising and has the potential to contribute to the understanding of the health effects of exposure to fVOCs. Future work will focus on simulating conditions in occupational settings to determine the risk of health effects caused by fungal VOCs.

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**EMEP006**

**Colonized 3D skin models – An advanced test system to study toxicity modulation of pesticides by the human skin microbiome**

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Toxicity evaluation in human risk assessment relies on animal models and human cell culture. However, one major aspect of the human body has been neglected – the microbiome. Trillions of microbes inhabit the body and contribute to human health. They harbor an enormous metabolic potential and thus could influence a substance"s toxicity. Several studies with the gut microbiome indicate that environmental pollutants can induce dysbiosis, which may lead to different diseases. At the same time, the microbiome

can modulate the toxicity and bioavailability of the former. Although the skin is a site of first exposure, toxicological studies on environmental pollutants and the skin microbiome are scarce. In our previous work, we demonstrated that skin bacteria are capable of degrading the environmental pollutant benzo[*a*]pyrene (B[*a*]P). Application of B[*a*]P to commensally colonized skin models led to the formation and accumulation of partly genotoxic B[*a*]P-metabolites.

We now want to investigate the impact of the skin microbiome on host health in the context of exposure to pesticides that are likely to affect the metabolism of tryptophan or tyrosine. The latter two are also microbial metabolites, which play a crucial role in host-microbe communication. Changes in their bioavailability thus might affect the host"s health.

To assess microbiome-mediated effects of Mesotrione (MT), an active agent of herbicides, in skin, we developed a co-culture system based on MatTek's EpiDermFT, a human skin tissue model. These 3D skin models were colonized with a defined bacterial community (DBC) in order to emulate the situation of healthy skin *in situ*. Concomitantly we set up used carbon-limited batch cultures selecting for MT-degrading commensals. Microbially produced metabolites of MT will be analyzed by LC-MS using targeted and non-targeted screening. Currently, more than 20 MT-degrading bacteria are being verified on the basis of their metabolites.

Preliminary colonization studies indicate the co-culture system to be biologically stable and show that following initial signs of cytotoxicity there is a strong adaption by skin cells. The generation of skin models colonized with a DBC composed of MT-degrading bacteria, now enables us to investigate modulated toxicity as well as changes in skin physiology after MT treatment.

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**EMEP007**

**Bacteria in stress: Effect of multiple stressors on microbial degradation of organic carbon in river ecosystem as revealed by mesocosm experiments**

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Rivers play a substantial role in the global carbon cycle through their high rates of carbon respiration and sequestration. Dissolved organic carbon (DOC) in such ecosystems is an integral part in biogeochemical cycling. Microorganisms are the key organism group responsible for DOC turnover providing a crucial ecosystem function. However, rivers are impacted in multiple ways by anthropogenic pollution and climate change whereas salinization and temperature rise is a global trend. Hence, the goal of this research is to investigate the effects of salinity and temperature on microbial communities and their ecosystem function to degrade DOC in river water and sediments. The influence of these stressors is studied on an urban river in laboratory microcosms and in outdoor mesocosms. The degradation rates of DOC to CO2 were quantified via a novel Reverse Stable Isotope Labelling method measuring isotope ratios in CO2 with an isotope analyzer. Our results showed that raising the salinity by 250 mgCl/L and temperature by 3 deg. Celsius significantly increased degradation rates in river sediments from 0.34 to 1.34 mgC/L/d after stress release, while having no significant effect during stress application. In the water column an increase of salinity by 1.5 gCl/L and temperature by 5 deg. Celsius tripled the degradation rates in the recovery phase where the stress was released. Carbon degradation rates in water were 30 times lower than in sediments presumably due to lower organic matter quantity and the 10-fold lower organism density as revealed by flow cytometric analysis.

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**EMEP008**

***Roseobacter* bacteria alter their morphology if in touch with dead eukaryotes**

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Members of the "*Roseobacter* group" are highly abundant in marine environments. Due to their physiological and metabolic versatility, they successfully adapt to different ecological niches. Furthermore, morphological heterogeneity is a common trait observed for strains belonging to this group. As effective biofilm formers and colonizer of marine surfaces, they attach either as rod -shaped single cells or as aggregates in form of rosettes. However, the triggers and ecological effects of morphology changes during attachment in marine ecosystems remain enigmatic.

To shed light on this conspicuous behavior, we investigate rosette-forming *Roseobacter* group members with a focus on *Phaeobacter inhibens* DSM 17395.

We found that morphological changes did not occur in chemically defined media, but only if remains of dead eukaryotes such as yeast extract or algal extracts were present in the growth broth. Such changes include extreme longitudinal cell elongation of a *P. inhibens* subpopulation. We hypothesize -that *P. inhibens* controls buoyancy and vertical positioning in a nutrient- rich zone via this morphological changes. Such nutrient- rich zones can relate for example to algal bloom decay. Filamentation allows the rosette to remain longer in the nourishing area and to take up as many nutrients as possible. The resulting aggregates exhibit a larger surface area for attachment and cells are more resistant towards stress.

We validate our hypothesis with the construction of *P. inhibens* deletion mutants, phase contrast microscopy, Image analysis and various growth experiments.

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**EMEP009**

**Hydrogen-driven microbial redox reactions in deep geosystems**

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In the subsurface, biotic and abiotic processes can generate and consume hydrogen. Hydrogen has a low reduction potential and is thus a highly energetic electron donor when involved in sulfate, carbon dioxide or ferric iron reduction. Although known as important drivers for the deep biosphere, the contributions of different processes to hydrogen turnover in different geosystems still are not well understood. In context with the ongoing transformation to renewable energy resources, underground H2 storage (UHS) in deep porous or salt cavern systems came into focus. In situ microbial and geochemical reactions that consume H2 are highly relevant topics in deep biosphere research, and also are still a major uncertainty during UHS.

Consequently, we studied the potential microbial hydrogen oxidation rates – combined with the possible production of metabolic products like H2S, acetic acid or CH4 - in formation fluids from natural gas fields and salt caverns, thereby considering the importance of *in situ* pressure and temperature conditions, fluid chemistry and mineral composition. In addition, more defined experiments were conducted with selected pure cultures representing important metabolic groups of deep biosphere microorganisms.

Several original formation fluids showed immediate H2 consumption. Microorganisms oxidized hydrogen at relevant *in situ* pressure conditions (up to 100 bar) and tolerated dynamically changing pressure and temperature conditions. The microbial hydrogen oxidation rate was strongly dependent on H2 partial pressures and the availability of e.g. sulfate as a terminal electron acceptor. High-throughput sequencing of 16S rRNA gene amplicons indicated hydrogen oxidation by sulfate reducing bacteria to be the presumed process in the studied porous rock reservoir fluids. In addition, hydrogen turnover by methanogenic and acetogenic as well as iron-reducing microorganisms was investigated. Also, the importance of biotic reactions in relation to abiotic hydrogen turnover processes at mineral surfaces will be discussed.

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**EMEP011**

**Effect of fungicide tebuconazole on the mice microbiome**

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One of the most commonly used fungicides in agriculture is Tebuconazole, a triazole group fungicide. It has already been studied and confirmed that tebuconazole induces a cascade of toxic reactions associated with oxidative stress in rats (oral)[1] and earthworms Eisenia fetida (soil-introduced) [2]. But the issue of the interaction of pesticide molecules with the microbiome inside the animal body is still not disclosed. How does a pesticide affect bacteria at the molecular level, which genes and then proteins are modified under the influenced by tebuconazole.
An artificial mouse microbiome (miBC) consisting of 21 major mouse microbiome bacteria called Oligo-Mouse Microbiota (OMM) is used for the study. Samples were received from the German Collection of Microorganisms and Cell Cultures (DSMZ). Grown at 37 °C in AF media. All samples were treated with tebuconazole at various concentrations. For the study, a modified and specialized method for testing the toxicity of the pesticide on bacterial cultures using 96 well plates was established.
First results of the study showed that tebuconazole in maximally used concentration (100 mg/L) did not affect the growth of Enterococcus faecalis, and Escherichia coli. Finally, studies are being carried out to find bacterial strains that use tebuconazole as carbon source, to establish the pathway for the degradation of tebuconazole in nature.

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**EMEP012**

**Microbial diversity and metabolic potential in sandstone reservoirs for thermal energy storage**

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Microbial processes such as biofilm formation (clogging) and mineral precipitation (scaling) can affect the effectiveness of aquifer thermal energy storage (ATES) systems. They can reduce the permeability of potential reservoirs and compromise the efficiency of ATES facilities on the long term. In addition, microbial processes can influence dynamics of toxic trace elements in the subsurface *e.g.* by releasing arsenic through iron mineral dissolution. It is therefore crucial to identify the microbial key players and the metabolic processes involved to estimate the microbial impact on ATES and the clogging potential.

Here, we analyze the microbial abundance, community composition and their functional potential in relation to the thermo-hydrogeochemical conditions of bulk sediment and formation water of a sandstone aquifer of the North German Basin. The study focusses on the application of DNA-based approaches such as qPCR, high throughput sequencing and metagenomics. Bulk sediments and fluids were obtained from Jurassic sandstone in Berlin, Adlershof from a depth of 200-450 m.

The aquifer is characterized by an *in-situ* temperature of 17-22°C, Na and Cl dominated fluids (TDS ~20 g L-1) and DOC including acetate (~3.5 mg L-1), propionate and valerate. First results show that the fluid microbial community is adapted to saline and alkaline conditions. The community is highly dominated by the two taxa *Alkaliflexus* and *Defluviitaleaceae* UCG-011, but also contains sulfate reducing bacteria.

Results of this study together with a flow-through experiment analyzing geochemical, hydrochemical, mineralogical and microbiological processes under different conditions typical for ATES, will help to develop prediction tools for potential system operational failures and appropriate countermeasures.

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**EMEP013**

**Community analysis of complex environments using 3D macroporous materials**

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Introduction**:** Microorganisms are found in almost any conceivable habitat and their isolation has served numerous applications in the industry. However, their isolation and subsequent cultivation is a challenge and more than 99% of microorganisms have not been cultured or characterized yet. This vast majority is known as Microbial Dark Matter (MDM). Advances in sequencing technology and bioinformatics have made the identification and characterization of new microorganisms much easier and less expensive, especially when combined with selective enrichment.

Objective: Development of a workflow using macroporous elastomeric silicone foam (MESIF) as an enrichment matrix in combination with genomic sequencing to effectively capture a broader range of uncharacterized microorganisms.

Method: A custom developed macroporous silicone chip for the cultivation of MDM was employed for the cultivation of previously uncultured microorganisms.1 The applicability was demonstrated by placing the chips in three different complex habitats so that the microorganisms living there could colonize the chip by penetrating the macroporous silicone matrix. After cultivation, DNA was extracted, sequenced and bioinformatically analyzed to determine the taxonomy of the entire community.

Results: After incubation of the cultivation platform in the various habitats, a broader community composition was found, possibly enriching slow-growing bacterial phyla and hindering the growth of well-characterized, community overtaking phyla. Furthermore, since the chips contained a reservoir for nutrient supply, it was possible to select for specific microbial groups that could metabolize the stressor glyphosate.

Conclusion: The concept presented in this poster enables the identification and characterization of novel organisms from diverse habitats. Furthermore, it can be used to select for specific microbial groups thanks to a reservoir chamber. This opens the way to enrich and cultivate such novel organisms and better understand the composition of natural environments.

Literature:

Zoheir AE, Meisch L, Velaz Martín M, Bickmann C, Kiselev A, Lenk F, Kaster A-K, Rabe KS and Niemeyer CM.: Macroporous Silicone Chips for Decoding Microbial Dark Matter in Environmental Microbiomes. *ACS Applied Materials & Interfaces* (**2022**) *14* (44):49592

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**EMEP014**

**Environmental controls of dark $CO_2$ fixation in wetland microbiota**

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Introduction: Rising atmospheric concentrations of $CO_2$ and $CH_4$ are the primary drivers of climate change and became a major concern to society because of their global warming potential. From a historic view the light driven photosynthesis is well understood and plays an obvious role in the climate system. Nevertheless, in recent years the importance of non-photosynthetic greenhouse gas fixation pathways i.e. methanotrophy has become a research focus. Methanotrophs prevent up to 90% of the methane produced in soils from entering the atmosphere. By contrast, non-photosynthetic microbial $CO_2$ fixation or so-called dark $CO_2$ fixation has received much less attention. However, it could be similarly important as methanotrophy for balancing the climate system.

Objectives: The objective of this work was to elucidate the environmental factors that determine occurrence and magnitude of dark $CO_2$ fixation pathways along environmental gradients. The estuarine environment is ideally suited to understand environmental constraints on these pathways because estuarine wetlands are characterized by steep environmental gradients in $O_2$ availability, organic matter (OM) availability, and salinity.

Materials & methods: To cover the $O_2$, salinity and OM gradient, soil samples were collected from the marshes along the Elbe estuary at the salt marsh, brackish marsh and freshwater marsh to a depth of 50 cm below the soil surface in each of the high marsh, low marsh and pioneer zones. The samples were analysed based on metagenome- and metatranscriptome analyses and supported by metabolite- and phylogenetic analyses.

Results: We observed that transcript abundance was confined to specific environmental niches and that the gene expression level did not correlate with the gene abundance. The transcription of key genes of the reductive tricarboxylic acid cycle, the Calvin cycle and the carbonic anhydrase were favored by low salinity and $O_2$ and OM rich niches. The Wood-Ljungdahl pathway and dicarboxylate/4-hydroxybutyrate cycle were favored by low $O_2$ niches.

Conclusion: Variations in the expression of specific functional genes across environmental gradients were more prominent than variations in microbial groups or genes related to dark $CO_2$ fixation, suggesting that functional diversity plays a crucial role by allowing microorganisms to adapt to their specific environment, leading to niche formation. Our results suggest that $O_2$, salinity and OM control dark $CO_2$ fixation in wetland soils.

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**EMEP015**

**Biodegradable plastic in forest soil: Dominant tree species and forest types drive changes in microbial community assembly, influence the composition of plastisphere, and affect poly(butylene succinate-co-adipate) degradation**

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Bio-based and biodegradable plastics offer microbial degradation in various environments. Poly(butylene succinate-co-adipate) (PBSA) is one of the promising polymers for microbial degradation. While its degradation and plastisphere microbiome in cropland soils have been studied, such knowledge in forest soils is still lacking. Thus, we investigated: i) the impact of forest types (conifer and broadleaved forests) on the plastisphere microbiome and its

community assembly, ii) their link to PBSA degradation, and iii) the identities of potential microbial keystone taxa. Microbial richness ($F$ = 5.26–9.88, $P$ = 0.034 to 0.006) and fungal community composition ($R2$ = 0.38, $P$ = 0.001) of the PBSA plastisphere microbiome were significantly impacted by forest type. However, no effect of forest type was found on microbial abundance and bacterial community composition. The bacterial community was governed by stochastic processes (mainly homogenizing dispersal), whereas the fungal community was driven by both stochastic and deterministic processes (drift and homogeneous selection). The highest molar mass loss was found for PBSA degraded under *Pinus sylvestris* (26.6 ± 2.6 to 33.9 ± 1.8% (mean ± SE) at 200 and 400 days, respectively), and the lowest molar mass loss was found under *Picea abies* (12.0 ± 1.6 to 16.0 ± 0.5% (mean ± SE) at 200 and 400 days, respectively). Potential important keystone taxa include fungal PBSA decomposers (*Tetracladium*) and atmospheric dinitrogen (N2)-fixing bacteria (symbiotic: *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Methylobacterium* and non-symbiotic: *Mycobacterium*). The present study is among the first to determine the plastisphere microbiome and its community assembly processes associated with PBSA in forest ecosystems. We detected consistent biological patterns in the forest and cropland ecosystems, indicating a potential mechanistic interaction between N2-fixing bacteria and *Tetracladium* during PBSA biodegradation.

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**EMEP016**

**Drivers of deadwood decay of 13 temperate tree species are similar between forest and grassland habitats**

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Deadwood provides an important carbon source in forests and wooded ecosystems and, accordingly, forest management strategies discuss the enrichment of deadwood amount and diversity by different tree species. To investigate the decomposition processes of enriched deadwood, we simultaneously placed 3,669 size-standardized and gamma sterilized wood specimens of 13 tree species (*Populus tremula, Tilia cordata, Prunus avium, Betula pendula, Carpinus betulus, Fraxinus excelsior, Quercus robur, Fagus sylvatica, Acer platanoides, Larix decidua, Pinus sylvestris, Picea abies*, and *Pseudotsuga menziesii*) at a total of 300 forest and grassland plots in three regions in Germany covering large gradients of management intensity and environmental conditions. After 1 year, mass loss was calculated and its relationship with wood traits and environmental conditions was assessed to determine the most important factors. Mass loss was overall higher in forest compared to grassland habitats, with wood traits as the most important driver, followed by region and environmental factors related to microclimate. However, management intensity was less relevant to explain the mass loss in both habitats. Our results suggest that decomposition of enriched deadwood, even after removal of endophytes, is influenced by the same drivers (positively by moisture and abundance of macronutrients, negatively by lignin and phenol concentration) as naturally occurring wood. Furthermore, due to the immense and standardized experimental setting, our study contributes to a better understanding of the important drivers of mass loss in different tree species and thus provides the basis for predictions of the carbon cycle in a changing world.

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**EMEP017**

**Bacterial degradation of polymer plasticizers on the example of diethyl phthalate (DEP)**

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The continuing reports of plastic pollution in various ecosystems highlight the threat posed by the ever-increasing consumption of synthetic polymers. Plastics are frequently associated with additives such as phthalic acid esters. Such compounds are used as plasticizers to provide flexibility to plastic products and as common additives in various consumer products, and suspect to cause endocrine disruption in animals.

The bacterial degradation of diethyl phthalate (DEP) as a model compound for plasticizers was studied. In order to isolate bacterial DEP degraders, samples from a former landfill (Nahleberg, Leipzig) rich in brittle plastic waste were taken. In addition, a biofilm present on a polyurethane tubing was scratched off. From this biofilm, a bacterial strain identified as *Xanthobacter* sp. was isolated. This strain was characterized being capable to grow in mineral medium with DEP as sole carbon energy source. The complete degradation of up to 4 mM DEP was confirmed by UPLC analysis Furthermore, the substrate spectrum of the isolated strain was assessed by testing different carbon source like trihydroxybenzene, phenol and other aromatics. Based on that, a degradation pathway of DEP was suggested.

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**EMEP018**

**Influence of fertilisation strategies on the endophytic seed microbiome of barley**

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Legacy effects in the plant soil system have been so far mainly linked to interactions between decomposing roots and the surrounding soil. Recently also the role of root exudates has been considered. Another component is the endophytic seed microbiome, which is acquired during plant growth by not yet well understood filtering processes and transferred to the next generation. How changes in the environmental microbiomes influence the structure of the seed microbiome is so far unclear.

We investigated the impact of two different fertilizers and a foliar amendment on the structure of the endophytic seed microbiome of barley in a greenhouse experiment. We used surface sterilised barley seeds (cult. Fantex) which were planted in prepared pots containing two different soils obtained from an agricultural field site close to Regensburg, Germany. One soil was characterized as clay loam with

higher nutrient contents (C & N) compared to the other soil which was classified as loam. Amendments included a control without additional fertilization, NPK fertilizer with or without foliar application of a silicon spray (Si) as well as compost-charcoal-mushroom pellets with or without Si. Plants were grown for about four months until seed ripening. The seeds were surface sterilised and subsequently subjected to DNA extraction, followed by a 16S rRNA gene based metabarcoding approach to assess bacterial community structure.

Our data indicates no differences in alpha diversity between treatments. The bacterial core microbiome shared between all treatments contained members of the genera *Cutibacterium*, *Enterococcus* and *Escherichia*, which account for up to 30% of the relative abundance. Soil effects on the seed microbiome were visible for several of the highly abundant genera. These varied in the number of ASVs in response to the soil type including *Erwinia* or *Rhizobium*. Effects of the fertilizers were mostly found for the less abundant taxa, including *Massilia*, *Paracoccus* and *Methylobacterium,* which were more prevalent in NPK treatment. *Xanthomonas* was more abundant in pellet treatments. The genus *Niveispirillum* was found especially in the NPK+Si treatment.

Overall, our data shows shifts in the structure of the seed microbiome in response to differences in environmental conditions in the first generation of plants indicating potential short-term legacy effects, which may become more pronounced when plants are grown for longer under contrasting environmental conditions.

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**EMEP019**

**Microbial transplants as a tool to improve soil quality from technical origin**

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Urban biodiversity needs space and the fastest way to implement it is the use of already existing areas, such as rooftops. Here, a mixture of artificially modified and recycled materials with organic substances, known as technosols, is used as an soil-like substrate for plant growth. However, the microbiome associated with technosols often has low diversity, biomass, and activity, resulting in poor soil quality with limited growth opportunities for plants. Since strategies to improve the microbiome of these artificial soils are necessary, we present the results of our study in which the microbiome of different grassland soils was used to improve the quality of technosols.

We performed a greenhouse experiment using three different technosols with varying materials and compositions planted with (1) *Dactylis glomerata* L. only or (2) *D. glomerata* L., *Festuca pratensis* Huds. and *Trifolium pratense* L. The technosols were used as produced or mixed with 5 % of two different soils (*D. glomerata* L. grassland or grassland with diverse plants). Sampling was performed after 0, 78 and 136 days of plant growth. We measured leave biomass, pH, $C_{mic}$, $N_{mic}$, dissolved soil carbon and nitrogen. DNA was extracted with a commercial kit followed by 16S rRNA gene qPCR and metabarcoding with primers targeting the V3-V4 region of the 16S rRNA gene. Raw sequencing data were processed with DADA2, and taxonomy was assigned using SILVA v138. Microbial diversity, and community composition were analysed in R Studio (4.2.2) with different packages.

Our data confirm that technosols have low diversity and dominant taxa are oligotrophic phyla like *Acidobacteria*. The inoccula from diverse grasslands increased the alpha diversity of the technosols especially after 78 days of plant growth, which further resulted in higher plant biomass. The effect of diverse plant community on microbial alpha diversity was more pronounced after 136 days, when the root system was well developed. Overall, our data indicates clear positive feedbacks of increased microbial diversity in technosols on plant performance, which might induce legacy effects also for subsequent plant generations.

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**EMEP020**

**Beyond plant diversity: Drivers of microbial diversity in a temperate grassland buffer zone**

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Plant diversity is considered the major driver of the soil microbiomes' structure and function. Grassland buffer zones are an interesting showcase to study which other factors influence soil microbiomes. These stripes act as a filter and have been established to avoid nutrient and material input from croplands into aquatic ecosystems. In the present study, we examined a temperate grassland buffer zone, which is strongly affected by an agricultural site and its parallel floodplain grassland, which is less affected by factors like nutrient input or transport of microorganisms.

The studied site is located next to the Otterbach River in Süßenbach, Germany. Samples were taken along two catenas. One catena included three sampling points in a managed grassland and one at the floodplain, just before the creek. The other catena included three sampling points in a slope barley cropland and the associated grassland buffer zone. Five topsoil (0-5 cm) replicates were taken at each location at three time points over the year (03/2022, 06/2022 and 11/2022)

To characterize the composition and total abundance of bacterial communities, 16S rRNA gene amplicon sequencing and 16S qPCR were carried out. Briefly, sequencing results were processed with DADA2 v1.22.0 and QIIME2 v2022.8, taxonomically classifying the amplicon sequence variants (ASVs) against the Silva v138.1 database.

Our results show that the buffer zone was the site with the highest number of 16S rRNA gene copies per g dry soil. The floodplain however showed a higher diversity of ASVs than the buffer zone in March and November. Furthermore, the percentage of common ASVs of the buffer zone and the floodplain declined during the year, probably influenced by nutrient and material input. Some bacterial taxa that were significantly more abundant at the buffer zone and might be coming from the cropland were *Nitrosomonadaceae* and *Burkholderiales*, which include important ammonia oxidizers, in spring, *Vicinamibacteraceae* in summer, and *Bacillaceae* and *Gaiellales* in autumn.

In conclusion, our data indicate that in grasslands which are characterized by high nutrient input, like buffer zones,

bacterial biomass increases over the seasons, but at the same time these sites become less diverse, which may result in less resilience, stability and functionality. It is still to be tested in further experiments if a regeneration is possible at the vegetation free period in winter.

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**EMEP021**

**Determinants of the activity of soil bacteria and their contribution to key ecosystem function**

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Introduction: The precise and comprehensive identification of metabolically active or cellularly replicating bacterial taxa in highly complex microbial communities is essential to understand their functions in the ecosystem. (2) Objective. Methodologically, a high-throughput determination of active bacteria still represents a major challenge, particularly in habitats such as soils. (3) Materials & methods. Here we apply an improved approach using 16S rRNA/rDNA ratios obtained from amplicon sequencing in which a novel statistical procedure reliably distinguishes false-positives caused by stochastic sequencing noise (false-positive rate 0.3%). (4) Results. Contrary to the current perception, rare as well as dominant taxa were identified as active and constituted only up to 1% of all bacterial taxa present in the sample. Using 60 soil samples from German grasslands (https://www.biodiversity-exploratories.de/en/), we investigated a wide set of physicochemical soil properties, microbial biomass, land use intensity values and traits of the accompanying plants and soil animal communities, to identify drivers of the activity status (high rRNA/rDNA ratios) of individual bacterial sequence variants. We show that counts of active taxa had the strongest statistical explanatory power for soil respiration and enzymatic activities related to the carbon cycle (glucosidase, xylosidase and chitinase) but not for enzymes of the nitrogen or phosphorus cycle. Using qPCR for the analysis of laboratory cultures of representative taxa from these soil samples, we determined that high rRNA/rDNA ratios typically occur at the peak of exponential growth rates, suggesting cellular replication in soil. 5) Conclusion. In the era of easily accessible high-throughput sequencing, our approach provides a feasible way to detect active sequence variants in high throughput and offers the possibility to elucidate particular soil functions that cannot be done based on custom alpha- or beta-diversity estimates alone.

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**EMEP022**

**Significant, but not biologically relevant: *Nosema ceranae* infections and colony winter losses**

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Question: Managed and wild insect pollinators play a key role in ensuring that mankind is adequately supplied with food. Among the pollinating insects, the managed Western honey bee (*Apis mellifera*), which provides about 90% of commercial pollination, is of special importance. Hence, diseases as well as disease causing pathogens and parasites, that threaten honey bees, have become the focus of many research studies. The ectoparasitic mite *Varroa destructor* together with deformed wing virus (DWV) vectored by the mite have been identified as the main contributors to colony losses, while the role of the microsporidium *Nosema ceranae* in colony losses is still controversially discussed. In an attempt to solve this controversy, we analyzed a unique data set on honey bee colony health including information about colony mortality*, V. destructor* loads and *Nosema* spp*.* prevalence.

Methods: In the course of a 15-year longitudinal cohort-study on *Nosema* spp. epidemiology and honey bee health, data on colony performance and survival as well as bee samples for pathogen/parasite detection were collected from autumn 2005 to spring 2020. Advanced statistical analyses such as classification tree analysis, linear regression models, and chi square tests giving Pearson residuals were performed to investigate the relationship between colony mortality, the parasite *V. destructor*, and the pathogen *N. ceranae*.

Results: Our multivariate statistical analysis confirmed that *V. destructor* is the major cause of colony winter losses. When using cumulative data sets, we also found a significant relationship between *N. ceranae* infections and colony losses. However, determination of the effect size measure Cohen"s ω revealed that this statistical significance has no biological relevance, since the deleterious effects of *N. ceranae* infections are usually masked by the more severe effects of *V. destructor* infestation on colony health and therefore detectable only in the few colonies that are not infested with mites or are infested at very low levels.

Conclusion: With these results, we end a long-standing controversy about whether or not *N. ceranae* is capable of killing entire honey bee colonies. Under certain circumstances, *N. ceranae* can be a threat to individual honey bee colonies, but generally it is not, as infestation with *V. destructor* is still the predominant health problem.

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**EMEP023**

**Bacterial microbiome structure of corbicular pollen of honey bees is related to the collection month**

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Bee-microbe interaction is a non-negligible facet of honey bee health. The depletion of microbes from pollen provisions can lead to weight loss, prolonged development time and declined survivorship of larvae. Honey bees also harbor a highly conserved core microbiome that functions importantly in various physiological activities, and upon disruption, can have detrimental effects on bee health. However, corbicular pollen, as an intermediate product during foraging activity, has so far attracted little attention. In this study, we aim to examine the composition of bacterial microbiome of corbicular pollen in a hive- and time-dependent manner. As honey bees forage a wide variety of plants and for a long

period in a year, diverse and differential microbiome structures might be present.

Corbicular pollens were collected from two hives in the apiary of Justus-Liebig-University Giessen three days per month from June to September. On each sampling day, corbicular pollens were collected from sunrise to sunset at one-hour intervals. Bacterial microbiome composition was examined by Ion Torrent sequencing of the 16S rRNA gene (V4-V5) from corbicular pollen metagenomic DNA. Alpha, beta diversity, differential abundance analysis, core microbiome, and correlation between collected environmental variables and ASVs were performed with specialized R packages (phyloseq, MeanRarity, ALDEx2, vegan).

The significant differences of alpha diversity of corbicular pollen microbiome are time-dependent. The most prominent difference is observed between June and September in all indices (richness, Hill-Shannon and Hill-Simpson), and September tends to have the highest bacterial diversity. Principle component analysis based on Aitchison distance matrix indicates that the microbial community is clearly separated in terms of both month and hive (*p*-value 0.001). Comparing June and September, 15 bacterial genera are differentially abundant. *Duganella* is the most increased genus in September (60-fold), and *Spiroplasma* in July (68-fold). No bacterial genus is differentially abundant between two hives. Temperature tends to be the dominant environmental variable that correlates with some genera.

In conclusion, the microbiome structure of corbicular pollen changes over time. Such differences shall be further considered with honey bee indigenous microbiome composition and the expression of immunological markers to potentially reflect the health condition of this globally valuable pollinator.

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**EMEP024**

**Microbial degradation of organophosphonates in context of lake constance oligotrophication**

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The organophosphonate (OP) and herbicide glyphosate is still approved in the EU [1] despite its rising occurrence in freshwater environments because of its extensive usage [2]. Microbial degradation of xenobiotic glyphosate and of other, natural OPs has been reported [2,3]. The microplankton of Lake Constance might have adapted to utilize OP as alternative phosphorus sources, specifically OPs such as ciliatine, methylphosphonate and glyphosate, in response to the lake"s increasing oligotrophic state and P-limitation of phytoplankton growth. The objectives of this study are to characterize OP degradation by microorganism of Lake Constance and how the OP-phosphorus might support phytoplankton growth. Tests with a selection of microalgae and cyanobacteria including many Lake-Constance specific species with OP as sole P-sources, were negative thus far. Twelve OP-degrading heterotrophic bacterial strains were isolated *via* enrichment cultures, and a representative strain, *Brucella cytisi* DNF1 completely degrading ciliatine, methylphosphonate and glyphosate, is further being investigated. Differential proteomics identified proteins *phn G, H, I, J, L, F, M* and a phosphate transporter upregulated in *B. cytisi* DNF1, suggesting OP degradation *via* the C-P lyase pathway. A stable phototrophic enrichment culture from Lake Constance containing microalgae with bacteria, which are currently being identified by 16S- and 18S-rDNA amplicon sequencing, utilizes ciliatine as sole P-source. Overall, the results at its present state corroborate the notion that heterotrophic bacteria may be the main contributors to the mobilization of OP-phosphorus in the planktonic food web of Lake Constance.

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**EMEP025**

**Genomic adaptation of *Burkholderia anthina* to glyphosate uncovers a novel herbicide resistance mechanism**

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The herbicide glyphosate (GS) was brought to the market for agricultural use under the trade name Roundup [1]. GS kills plants, fungi, bacteria, and other organisms by the specific inhibition of the 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, which converts phosphoenolpyruvate (PEP) and shikimate-3-phosphate to EPSP, the precursor for the biosynthesis of aromatic amino acids, folates and quinones. Thus, GS-dependent inhibition of the EPSP synthase depletes the cell of these essential metabolites, resulting in cell death [1]. In the past years, a variety of GS resistance mechanisms have been described in bacteria, among them target modification, altered transport and GS degradation [2]. Recently, we have identified *Burkholdera anthina* and *Burkholderia cenocepacia* isolates in a commercially available Roundup solution. Both isolates are resistant to high amounts of GS [3]. To assess whether high GS resistance could be a basic property of the members of the genus *Burkholderia,* we evaluated the GS resistance of the *B. anthina* strain DSM 16086. We found that the strain is GS sensitive, but quickly develops resistance to the herbicide by mutating *ppsR*, encoding the PEP synthetase regulatory protein PpsR. Usually, PpsR binds to and inhibits the gluconeogenic PEP synthetase PpsA. Characterization of the evolved strains revealed that GS-resistance is due to the mutational inactivation of the *ppsR* gene and enhanced PpsA-dependent synthesis of PEP that competes with GS for binding to PEP synthetase. Moreover, GS resistance could be further enhanced by the loss of three amino acids in the phosphoglycerate mutase PgmA, which converts 3-phosphoglycerate to 2-phosphoglycerate. Thus, the overproduction of PEP and rewiring of central carbon metabolism rapidly enhances GS resistance in *B. anthina* [4].

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**EMEP026**

**Biodegradation of novel polyethylene-like bioplastic by natural soil microbial communities under mesophilic conditions**

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Most conventional plastics are virtually non-biodegradable under environmental conditions. Biodegradable plastics promise reduced persistence, however, their degradation rates vary enormously depending on the specific receiving environment. For example, while thermophilic composting studies may suggest a principle biodegradability, the rates observed are not transferable to mesophilic settings. Polylactic acid (PLA) for instance is a well-known thermophilic-compostable bioplastic, however, to date no meaningful degradation at ambient temperatures has been observed. Polyester materials synthesized from plant oils and exhibiting polyethylene-like properties, were recently developed at the University of Konstanz[1] and shown to biodegrade within 60 days under thermophilic composting conditions[2]. These polymers show great potential for replacing non-biodegradable plastics like high-density polyethylene (HDPE), but their degradation in natural environments and under mesophilic conditions is yet to be tested. We studied the biodegradation of these materials in a forest-soil setting, both *in situ* and under controlled, mesophilic laboratory conditions. To this end, polymer films were incubated in forest soil for one year and subsequently analyzed by scanning electron microscopy (SEM) for signs of degradation. Furthermore, soil from the same location was used for degradation studies under laboratory conditions at 30°C. Degradation was assessed by tracking CO2-evolution in polyester-spiked soil samples in comparison to non-spiked samples and those spiked with reference polymers such as cellulose and HDPE. Bacterial and fungal communities associated with the respective samples were analyzed through sequencing of the 16S rRNA gene and the internal spacer region 2 (ITS2), respectively. The results showed the biodegradation of the tested polymers by natural microbial communities at ambient temperatures, with polymer dependent differences in community composition. Interestingly, a full degradation was only achieved under laboratory conditions, following the supply of additional nitrogen, phosphorus and trace elements. The results highlight the relevance of nutrient availability for efficient polymer degradation in the tested soil environment. We propose strategies to address the issue of nutrient limitation in plastic biodegradation through the use of new hybrid materials.

1. Häußler, M. *et al. Nature* 590, 423–427 (2021)
2. Eck, M. *et al. Angew. Chemie Int. Ed.* (2022)

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**EMEP027**

**Phosphatidylcholine biosynthesis in *Rhizobium leguminosarum* and its importance for symbiosis with clover**

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The lipid composition of bacterial membranes is a critical determinant of the bacteria's interaction with eukaryotic hosts. Phospholipids are the primary constituents of bacterial membranes. The phospholipid phosphatidylcholine (PC) is commonly found in eukaryotic cells but not typically present in bacterial membranes. However, several bacteria that interact with eukaryotic hosts contain PC, including *Rhizobium leguminosarum*, a nitrogen-fixing symbiont of legumes [1, 2, 3].

In this study, we aimed to identify and characterize the enzymes involved in PC biosynthesis in *R. leguminosarum*, as well as determine the importance of PC for the symbiotic interaction with clover. We found that *R. leguminosarum* uses multiple pathways and substrates for PC biosynthesis. In the methylation pathway, phosphatidylethanolamine (PE) is methylated in three steps to form PC via the intermediates monomethyl-PE (MMPE) and dimethyl-PE (DMPE). The methylation pathway in *R. leguminosarum* involves four different phospholipid *N*-methyltransferase enzymes (PmtS1, PmtS2, PmtS3 and PmtR1) with distinct substrate specificities. However, PmtS2, which catalyzes all three methylation steps, is sufficient to produce PC. In the PC synthase (Pcs) pathway, cytidine diphosphate diacylglycerol (CDP-DAG) is condensed with choline to form PC. Choline can be obtained from the environment or derived from CDP-choline or glycerophosphocholine. *R. leguminosarum* can also synthesize PC by acylating lyso-PC.

Our findings reveal that the production of PC in *R. leguminosarum* is a complex process involving multiple enzymes and substrates. Most importantly, we demonstrate that sufficient amounts of phosphatidylcholine are important for a functional symbiosis of *R. leguminosarum* with clover plants.

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**EMEP028**

**Earthworm gut microbes stimulate the slow environmental degradation of polylactic acid**

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Introduction: Wastewater irrigation, plastic mulching and also inadequate plastic disposal can lead to soil microplastic pollution. Although there is a trend in replacing non-biodegradable with biodegradable polymers, e.g. polylactic acid (PLA), the actual biodegradation is limited in soil, as environmental conditions are unfavourable. Nevertheless, the influence of soil macrofauna on the actual PLA degradation has rarely been investigated.

Objectives: Here we hypothesise that soil-dwelling earthworms enhance PLA degradation due to stimulation of gut microbial activities.

Materials & methods: The compost worm *Eisenia fetida*, a model organism in ecotoxicology, was exposed to uncontaminated (control) or PLA-supplemented soil to test our hypothesis. Subsequently, life history parameters were assessed and amplicon high-throughput sequencing of 16S rRNA genes and short-chain fatty acid concentrations derived from the guts were analysed. PLA mineralisation rates were elucidated in an additional 13C-PLA-tracing experiment.

Results: The earthworm"s reproduction was positively affected by PLA. Bacterial communities in the gut of earthworms exposed to PLA differed significantly compared to the control. Amongst others, taxa such as Phycisphaera-like WD2101, known for their ability to ferment complex carbohydrates, were more prominent in PLA-treatments. Higher lactate concentrations in the guts of earthworms exposed to PLA than those in controls indicated enhanced microbial activities and potential PLA degradation. This was indeed confirmed, as ~0.07 µmol CO2 d-1 were derived solely from PLA in presence of earthworms. Earthworms enhance PLA mineralisation rates relative to controls without earthworms by about 5 times.

Conclusion: PLA affected *E. fetida* positively likely due to enhanced microbial metabolism during PLA degradation in the gut. The findings provide strong evidence that conditions inside the earthworm guts are favorable for PLA degradation and suggest potential mitigation strategies for microplastic pollution.

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**EMEP029**

**Chromatic acclimation in the cryptophyte alga *Hemiselmis cryptochromatica***

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In cyanobacteria chromatic acclimation (CA) is a well-studied process in which the organism adapts its light-harvesting machinery to varying light qualities. Therefore, the phycobilisomes (PBS) get restructured, depending on the type of CA used (Sanfilippo *et al*, 2019). A similar process was recently described for the cryptophyte alga *Hemiselmis cryptochromatica,* where the organism changes its cellular absorption upon different light exposure (Heidenreich *et al*, 2020). Cryptophytes are microalgae, which originated from a secondary endosymbiosis event of a red alga cell and an unknown eukaryote, capable of living in a variety of aquatic habitats. Most of them are performing oxygenic photosynthesis, making them important primary producers. For light harvesting, phycobiliproteins (PBPs) are used, which in contrast to cyanobacteria are not organized in PBS, but are present as soluble proteins in the thylakoid lumen. Color differences amongst cryptophytes depend on the type of PBP pigment. In total, nine different PBPs have been identified so far and each different strain only harbors one. They can either be phycocyanin (PC) or phycoerythrin (PE), designated by the maximum absorption wavelength. Cryptophyte PBPs are soluble α-/β-dimers, with the β-subunit highly homologous to the ancestral red algal one. The α-subunit, in contrast, evolved separately with no found homologs. Overall, multiple α-subunits are encoded on the nucleus genome, while only one β-subunit is encoded on the plastid genome. α-subunits are bound to one light absorbing chromophoric group, the phycobilin, and β-subunits have three phycobilins attached. The project aim is to get a deeper understanding for the molecular mechanisms of the observed CA. The objective is to isolate phycobilins from cultures grown under different light conditions, as well as looking at their biosynthetic pathway. Synthesis of phycobilins starts with the linearization of heme by heme oxygenase, resulting in the formation of biliverdin (BV) IXα. This precursor is then further processed by an enzymatic class called ferredoxin-dependent bilin reductases (FDBRs) resulting in the production of the individual bilins, which are then linked to the corresponding PBPs (Overkamp & Frankenberg-Dinkel, 2014). For this purpose, five FDBRs were bioinformatically identified and are now subject for investigation in a recombinant protein approach. Preliminary results are shown and discussed in relation to cyanobacterial CA.

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**EMEP030**

**Microplastic impacts archaeal soil communities: Journey to a blind spot of plastisphere research in africa**

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Introduction:Over the last decade, various studies focused on microbe-microplastic (MP) interactions in different terrestrial environments, and reported impacts of MP on microbial community composition and physiology. Enrichment of putative pathogens in the plastisphere is one of the possible threats associated with MP pollution. Despite the high pace in this research field, the African continent and Archaea are largely neglected. Considering its rapidly increasing population, prosperity with rising plastic-consumption, and the importance of Archaea in soils, we aim to close this knowledge gap.

Objectives: (i) Examine plastisphere prokaryotic microbiome formation in Sub-Saharan plastic-polluted soils, (ii) study effects of MP on the archaeobiome and the connectivity of microbial communities, (iii) elucidate proportions of mechanisms driving the community assembly, and (iv) characterize the physiology of communities from soil and plastic compartments.

M&M: Plastic fragments and adhering soil from 5 different sites in a Sub-Saharan environment were separated and DNA was extracted. Resulting 16S rRNA gene amplicons were sequenced via Illumina iSeq. Community data were then analysed regarding general composition and co-occurrence networks. *In silico* analyses were applied to estimate the pathogenic (16SPIP), xenobiotic biodegradation (PICRUSt2) and plastic biodegradation potential (PlasticDB).

Results: Sequencing data revealed a significant difference between plastisphere and soil communities. Differences for certain pathogenic genera were non-significant. The results indicated an increased degradation potential for plastic-derived compounds. Furthermore, we determined a strong repulsive effect on Archaea, more precisely on Nitrososphaeraceae, the dominant archaeal family in the sampled environment. Our results showed that stochasticity is the main driver of community assembly, though plastisphere communities are slightly more influenced by deterministic processes. Finally, the connectivity in co-

occurrence networks substantially decreased in the plastic compartment, indicating a possible increased vulnerability of ecosystems after plastic-pollution.

Conclusion: Sub-Saharan MP reduces the abundance of ammonia-oxidizing archaea and generally alters terrestrial prokaryotic communities. Our study provides first insights into this largely neglected ecosystem and emphasizes the risk of plastic-derived ecosystem disruption in a fast-changing environment.

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**EMEP031**

**A year of microbial dynamics in the Warnow estuary and the Baltic Sea coast in high spatiotemporal resolution**

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1. Estuaries are outstanding study objects for microbial ecology. In them, one can observe a transition between a river and a marine habitat on a manageable size scale, together with regular and irregular, weather-dependent mixing events. As such, estuaries harbor multiple ecologically relevant gradients, such as salinity, temperature and chlorophyll. Furthermore, many estuaries are the site of cities or smaller settlements, making them social-ecological coupled systems. But neither the spatio-temporal dynamics of nor anthropogenic impacts on microbial communities are particularly well understood.

2. The objective of this study is to study natural and anthropogenic gradients as well as the temporal dynamics of the microbial community along the Warnow estuary and the Baltic Sea coast close to Rostock, Germany.

3. From April 2022 to May 2023, we sampled the the above mentioned area twice a week at 14 sampling stations. Using 16S and 18S rRNA amplicon sequencing, we identified the microbial as well as parts of the eukaryotic community composition from approximately 1500 samples. Furthermore, we measured the concentrations of 40 different anthropogenic trace substances of pollutant classes relevant in corresponding estuaries, namelyherbicides, pharmaceuticals, and UV filters.

4. In this presentation, we will provide the first insights gained from a year of sampling the Warnow estuary with high spatiotemporal resolution. Among these are seasonal patterns that strongly diverge between the brackish Baltic Sea and the freshwater Warnow sampling points and a clear spatio-temporal stratification of samples pointing to the large impact of seasonality and the salinity gradient along the estuary. Furthermore, we will discuss the machine learning approaches we will employ to use the microbial community composition as bioindicator for anthropogenic trace substances.

5. This study represents, to our knowledge, the first high-resolution examination of microbial dynamics in an estuarine environment. Taken together, our results point to environmental microbiomes acting as highly differentiated indicators of general ecosystem state.

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**EMEP032**

**Chloroplast and photosystem in the marine dinoflagellate *Prorocentrum cordatum***

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Introduction: Marine photosynthetic organisms have a major impact on multiple biogeochemical cycles and are responsible for generating more than 45% of the global net primary production. This group represents only 1% of the earth photosynthetic biomass, and consists of diatoms and dinoflagellates, small eukaryotic algae and photosynthetically active cyanobacteria. One of these keystone organisms is the bloom-forming dinoflagellate *Prorocentrum cordatum* CCMP 1329, standing out with its distinct cell-biological features and a photosynthetic machinery, atypical to well-known systems of other photosynthetic organisms.

Objectives: Here we apply microscopic and proteogenomic approaches to obtain new insights into the cell-biological structures and photosynthetic machinery of *P. cordatum*.

Material & Methods: High-resolution FIB/SEM analysis was performed for 3D reconstruction of subcellular structures. For comparative analysis, enriched thylakoid membrane protein complexes of *P. cordatum* and well-studied *Arabidopsis thaliana* were co-separated by 2D BN DIGE. For protein identification, enriched thylakoid membrane protein complexes were separated by 2D BN-SDS PAGE and subjected to LC-ESI MS/MS. Proteogenomic mapping of photosynthesis proteins of *A. thaliana* with proteins of *P. cordatum* was conducted by sequence-based *in-silico* comparison and integration of experimentally identified proteins to the photosynthetic apparatus.

Results: FIB/SEM analysis revealed one large, continuous barrel-like chloroplast, which completely lines the inner face of the cell envelope and comprises ~40% of the total cell volume. Further, a single, reticular mitochondrium, the Golgi apparatus as well as diverse storage bodies could be reconstructed from *P. cordatum*. The constituents of the thylakoid membrane protein complexes of *A. thaliana* were almost all present in the genome of *P. cordatum*. Around half of them could also be identified from the protein data. Moreover, high amounts of pigment-binding proteins (PBPs) of four different categories could be detected. The distinct gene structures and arrangements in *P. cordatum*, e.g., multi-codon genes or multiple genes with similar functions, hampers the allocation of these PBPs to the photosystems.

Conclusion: Overall, core elements of the thylakoid membrane protein complexes could be identified from *P. cordatum*, while the antenna complex and other interacting constituents seem to be different and highly adapted to its environment.

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**EMEP033**

**Co-selection for biocide and antibiotic resistance in microbial wastewater communities upon exposure to the biocide didecyldimethylammonium chloride (DDAC)**

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Biocides are used for a wide range of purposes, including disinfectants or preservatives. They play a major role in the prevention of microbial infections in healthcare and animal husbandry. The use of biocides often leads to the discharge of active biocidal substances into wastewater streams, causing the exposure of wastewater microbial communities to subinhibitory concentrations. In turn, it is known that wastewater treatment plants (WWTP) are hotspots for antibiotic resistant bacteria. Since similar mechanisms confer resistance to biocides and antibiotics, exposure to biocides can result in co-selection of antibiotic resistant bacteria in WWTP

Here, we want to investigate co-selection processes of antibiotic resistance in natural WWTP microbial communities upon biocide exposure. Microbial communities were sampled at the WWTP Ruhleben in Berlin and characterized regarding their susceptibility against different clinically relevant antibiotics. To investigate the link between biocide exposure and antibiotic resistance, changes in the susceptibility level after exposure to environmentally relevant concentrations of the commonly used biocide didecyldimethylammonium chloride (DDAC) will be determined by enumerating resistant and non-resistant *E. coli* on selective plates with and without antibiotics and DDAC. In case of antibiotics, clinical breakpoint concentrations according to EUCAST will be used to discriminate between susceptible and resistant strains. In case of DDAC (and biocides in general), clinical breakpoints do not exist. Therefore, we determined a cut-off concentration at which the majority of naturally-occurring *E. coli* strains cannot grow anymore based on (I) the MIC (minimal inhibitory concentration) distribution, and (II) by plating wastewater communities onto selective indicator agar plates loaded with increasing DDAC concentrations. Additionally, antibiotic cross-resistance will be determined by spotting single colonies, isolated from DDAC-selective plates onto antibiotic plates. The results of our experiments will help to determine selective concentrations and to estimate the risk of antibiotic co-selection and cross-resistance in microbial WWTP communities upon biocide exposure.

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**EMEP034**

**Anaerobic cell extraction of live biomass from marine sediments**

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Advancements in imaging and cultivation techniques have led to the successful ecophysiological characterization and isolation of novel prokaryotes from natural environments. Especially anaerobic marine sediments, which were challenging to access became available for cultivation through an increase of sampling expeditions exploring subsurface habitats. The retrieved sediment cores can now be used for enrichment cultures using the native sediment matrix.

However, methodologies used for high throughput characterization such as fluorescence activated cell sorting and imaging techniques like electron microscopy are susceptible to sediment particles, which can adsorb fluorescent dyes and probes, cover microorganisms and cause misidentifications. Consequently, it is important to separate the microbial cells from the sediment particles during sample preparation. Previously described methods to separate microbial cells from sediment particles have used fixatives or focused on the enumeration of cells and did not regard the viability of these cells for further cultivation or microscopic purposes.

The aim of this project is to evaluate and optimize various cell extraction techniques for the retrieval of live biomass in an anaerobic environment. The primary objective is to preserve the viability of the cells while simultaneously removing a significant portion of sediment particles.

In this study various cell detachment techniques, including sonication, the addition of mild detergents and shaking followed up by a cell separation step using nycodenz or polytungstate gradient centrifugation are used. To evaluate and improve the cell extraction methods, viability and activity assays such as Syto9/propidium Iodide (PI), Redox sensor green/PI and propidium mono azide (PMA) in combination with flow cytometry, microscopy, digital PCR and 16S rRNA gene amplicon sequencing are utilized.

Preliminary results indicate that different cell extraction techniques influence both cell count and community composition of the extracted cell fraction. We observe that density centrifugation employing nycodenz yielded high cell counts sufficiently removing particles for downstream analysis.

The obtained results allow us to generate an adaptable and effective cell extraction protocol that can be used to efficiently characterize and isolate viable microorganisms of interest from marine sediment and other complex matrices, while highlighting the bottlenecks of live cell extractions.

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**EMEP035**

**Exploring the diversity of Nitrospira infecting viruses**

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Industrialization is a significant stress factor for global aquatic systems, causing pollution, temperature increases, and changes in salt and nutrient concentrations. The effects of these stressors on eukaryotic organisms are fairly well understood, yet little is known about the impact of combinations of stressors on microbial communities with key roles in biochemical cycling. Here, we genomically resolved aquatic biofilms naturally grown at an outlet discharging highly ferruginous mine effluent (Friedlicher Nachbar, Germany) with increased salt concentrations to investigate the bacterial, archaeal, and viral community thriving under these stressors. Biofilms were dominated by *Nitrospira* and iron oxidizing *Gallionella*, with five out of six recovered *Nitrospira* MAGS encoding for complete ammonia oxidation (COMAMMOX). We also identified CRISPR-Cas systems in all six *Nitrospira* genomes, whose spacers matched 22 previously taxonomically unrecognized viral particles, indicating either past and/or current viral infections of *Nitrospira*. These results demonstrate that biofilms in this ecosystem impacted by multiple stressors have likely adapted by living off ammonium and reduced iron contained in the mine effluent. Our bioinformatics approach also provided the first evidence for viral infection of *Nitrospira* populations, rendering this ecosystem a treasure trove for exploring *Nitrospira* virus-host relationships. We conclude that aquatic ecosystems impacted by multiple stressors can

bear ecological information on key metabolisms like COMAMMOX controlling prevalent biogeochemical processes on Earth.

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**EMEP036**

**Genome-resolved metaproteogenomics of gracilibacteria from cold groundwater**

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Introduction: Bacteria of the Candidate Phyla Radiation (CPR), constituting 25% of the bacterial biodiversity, are characterized by streamlined genomes without key metabolic pathways such as lipid or protein biosynthesis. Consequently, CPR are mostly considered symbionts. Despite CPR bacteria being ubiquitous, they are poorly characterized, in particular when referring to subsurface dwellers.

Objectives: We studied temporal metaproteogenomic dynamics of the microbial community in the cold-water geyser Wallender Born, and elucidated both symbiotic relationships as well as key community members.

Materials & methods: We coupled genome-resolved metagenomics to metaproteomics to elucidate community composition and determine their activity in a 12-day time-series. Groundwater was collected and sequentially filtered on 0.2µm and 0.1µm filters to fraction CPR bacteria and potential hosts. Using proportionality networks, we investigated whether CPR have specific interaction partners. In-depth analyses of genome and metaproteomic annotations for Gracilibacteria, who represent the most abundant CPR bacteria in the geyser, were used to unravel their lifestyle.

Results: We recovered 751 high-quality genomes, which represented 123 population genomes after dereplication. Co-occurrence networks indicated two sources of groundwater based on two distinct clusters, with one cluster enriched in CPR bacteria. Gracilibacteria were the most abundant CPR bacteria but showed no co-occurrence with potential hosts. Annotation showed that Gracilibacteria MAGs encoded for many proteins related to scavenging (*e.g.*, secretion systems type II and IV, cell-cell interaction mechanisms and broad-range transporters).

Conclusion**:** This metaproteogenomic investigation sheds light on the repertoire of proteins of scavenging bacteria and their expression in deeply sourced groundwater.

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**EMEP037**

**A novel GFPs-nanoluciferase reporter system to identify natural products mediating cross-kingdom microbial interactions**

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In all habitats on earth microorganisms form consortia with many different species closely living together in the soil [1]. The interspecies communications in these communities are decisive for function of microbial communities and further lead to the induction of otherwise silent natural product biosynthesis gene clusters. One prominent example is the interaction of the bacterium *Streptomyces rapamycinicus* with the fungus *Aspergillus nidulans*. Upon co-cultivation, the streptomycete is able to activate the otherwise silent *ors* biosynthesis gene cluster in *A. nidulans* [2]. Recently, we discovered that the compound family of arginine-derived polyketides including azalomycin F produced by *S. iranensis* (and *S. rapamycinicus*) serve as the long sought-after bacterial signals for this induction [3].

To measure the induction of silent gene clusters, we developed a fungal reporter system encoding the gene for the green fluorescence protein (GFP) coupled with the nanoluciferase gene. It allows to measure qualitatively and quantitatively the transcriptional activation of genes. This construct was translationally fused to the *orsA* gene of the orsellinic acid biosynthesis gene cluster of *A. nidulans* that is silent when the fungus is cultivated in monoculture. Transformants of *A. nidulans* with the reporter system showed fluorescence and luciferase activity upon addition of *S. iranensis* or of azalomycin F to the culture. The sensitivity of the reporter system allowed detection of azalomycin F concentrations as low as 10 nanoMolar. In line, the reporter was induced even when soil extract was added to the culture medium indicating that arginoketides are indeed present in the soil.

Further, with this reporter we were able to identify several bacterial strains that induce green fluorescence in the fungus [3]. Arginoketides can be found around the world and seem to play an important role in mediating microbial interactions in the soil.

1. Coban et al. Science 2022 ;
2. Schroeckh V, et al. PNAS 2009
3. Krespach MKC and Stroe MC, *et al.* Nat. Microbiol, accepted

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**EMEP038**

**Phenotypic and genomic comparison of spaceflight-relevant *Staphylococcus capitis***

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Previous studies have reported that spaceflight-specific conditions such as microgravity can lead to changes in bacterial physiology and resistance behavior, including increased expression of virulence factors and biofilm formation, and decreased susceptibility to antibiotics. We compared three spaceflight-relevant *Staphylococcus capitis* strains (DSM 111179, International Space Station (ISS); DSM 31028, clean room; DSM 113836; artificial gravity bed rest study) to the type strain (DSM 20326T) to investigate spaceflight-induced physiological and genomic changes. The strains were tested for growth, colony morphology, metabolism, fatty acid and polar lipid patterns, biofilm formation and susceptibility to antibiotics. Furthermore, we tested survival under various stress conditions such as

treatment with hydrogen peroxide, desiccation, irradiation with X-rays, and UV-C. We examined the genomes of all four strains for possible genetic determinants of the phenotypic differences. In general, we found that all four strains had similar metabolic patterns, the same sensitivity to antibiotics, and similar radiation tolerance. Physiological differences were observed mainly compared to the type strain and minor differences between the other three strains. The strain isolated from the ISS and the strain isolated from the bed rest study exhibited delayed yellow pigmentation, which is absent in the other two strains. The phenotypic and genomic differences between the strains observed in this study do not indicate increased virulence of the spaceflight isolate. However, the enhanced growth rate, higher weighted average melting temperature of the fatty acid profile, and colony pigmentation of the spaceflight isolate are relevant phenotypes that need to be further explored in the context of human spaceflight.

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**EMEP039**

**The T6SS membrane-disrupting effector Tme1 of *Photorhabdus luminescens* deploys antibacterial activity**

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Secretion systems are known to play a central role in modulating interactions between bacteria and their hosts. The Type VI secretion system (T6SS) is a widespread bacterial nano weapon and translocates toxins, so-called effector proteins, into directly neighbouring target cells. T6SSs play a key role in interbacterial competition and in bacterial interactions with eukaryotic cells. *Photorhabdus luminescens* is a Gram-negative, entomopathogenic enterobacterium living in symbiosis with nematodes or plants. Due to the complex biology of the *P. luminescens* life cycle, which is characterized by different host switchings, the bacteria represent a perfect model organism to study biotic host interactions. The *P. luminescens* genome encodes more than 20 T6SS effectors, however, the role of the effectors for the biology of the bacteria is unclear. The effector arsenal consists of various effectors with pore-forming, lipase or amidase activity. Here we focussed on the *in silico* predicted pore-forming effector Tme1. To determine the toxicity, *in vivo* toxicity assays were performed. Thereby, Tme1 was confirmed to inhibit growth and induce cell death of *E. coli* cells. For *in vitro* studies, deletion mutants of *tme1* were generated in *P. luminescens* and interbacterial competition assays were performed. The increased survival of prey cells upon deletion of the effector gene indicates a role of Tme1 in *P. luminescens* interbacterial competition. To get more insight in the molecular mechanism of Tme1, protein-protein interactions of Tme1 and other effector proteins were analysed by BACTH analysis. To solve, whether the effector toxicity could be neutralized, the interaction with putative immunity proteins was determined and the interaction confirmed. Subsequent effector-immunity protein functionality assays were performed and a neutralizing effect of the immunity proteins Tmi1 could be shown. To gain first insight into the putative delivery mechanism of Tme1, we analysed the putative interaction of Tme1 with the T6SS-HcP core components using BATCH. Thereby we suggest a delivery of Tme1 into the target cell along the T6SS HcP tube. Considering these results, we conclude that the *P. luminescens* T6SS effector Tme1 is important for interbacterial competition, leading to prey cell death which can be neutralized by the Tmi immunity proteins. The conditions under which Tme1 is deployed and whether the effector plays a role e.g. in interkingdom competition is currently under study.

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**EMEP040**

**Aquifer microbes – Not yet cultivated, but full of potential**

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Deep down in the groundwater, microbes face harsh conditions, but some adapted strains manage to survive. Nearly isolated from the outside world, only limited amounts of nutrients reach deep aquifers by seepage waters or are supplied by chemolithoautotrphy and decaying biomass. With only a handful of nutrients available microbes might use different biochemical defense mechanisms (e.g. antibiotics) than those occupying surface habitats. And to make matters worse, oxygen is highly limited in such ecosystems. The project focuses on the cultivation of groundwater microbes, especially (but not limited to) members of the phylum *Planctomycetota*. Enrichments or isolated axenic strains will be cultivated using different bioreactor setups, e.g. Semi-Continuous Stirred Tank Reactors (CSTR) or Fixed Bed Reactors (FBR). Since strains from aquifers probably require either no oxygen or only low concentrations most cultivation experiments are executed under microoxic or anoxic conditions, thereby covering microorganisms normally falling off the grid.
Species of high interest are anaerobically ammonium oxidizing (anammox) Planctomycetes that can grow autotrophically by converting ammonium and nitrite (both toxic in higher concentrations) to dinitrogen gas. As the Semi-CSTR system has already been proven to be well-suited for the cultivation of anammox-performing Planctomycetes the effluent of this reactor is used to prove the concept and efficiency of the FBR. Both reactor types were established with the Anammox model planctomycete *Kuenenia stuttgartiensis*. The FBR can be run under various conditions and partly shows cell accumulation. The fine-tuning of cultivation-related parameters will be continued to increase the efficiency of this reactor type.
The project is highly relevant for biotechnological applications as Anammox Planctomycetes are essential for the global nitrogen cycle and play a key role during nitrogen elimination processes in wastewater treatment plants. But with their high growth temperature of approx. 30 °C, so far characterized strains are unfavorable for large scale applications. In contrast, Anammox-performing strains originating from the groundwater with an average temperature
of 10 °C might be better suited for an application in wastewater treatment plants due to higher cost- and energy efficiency.

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**EMEP041**

**Unveiling microbial communities in granite deep subsurface environments along a climate gradient in Chile**

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The terrestrial deep subsurface is a vast and enduring habitat, representing one of Earth's most extensive, unexplored, and consistent ecosystems. Microorganisms thriving within this deep biosphere are pivotal in maintaining the planet's biodiversity and preserving genetic diversity crucial for the adaptation and evolution of organisms. However, our understanding of microbial colonization and persistence in terrestrial systems, particularly in igneous rocks like granite, remains limited. Unlike sedimentary rocks that form on the surface, granite systems lack autochthonously produced organics. Consequently, it remains unclear whether subsurface communities in granite receive photosynthetically produced carbon through transport along pore space and fractures or in situ-produced chemoautotrophic carbon.

This study aims to characterize a deep regional biosphere in Chile by sampling granite rock cores from three different sites, encompassing a climate gradient spanning from arid conditions in the north to humid conditions in the south. High-throughput DNA sequencing, combined with geochemical and mineralogical analysis of the samples, unveils the biodiversity of the deep biosphere at different depths.

The findings reveal a diverse microbial community in deep granites predominantly composed of organisms belonging to the phyla Proteobacteria, Actinobacteria, and Firmicutes. Several Amplicon Sequence Variants (ASVs) exhibit affiliations with hydrocarbon-associated organisms (e.g., *Arthrobacter sp.* 11/16c, *Pseudomonas stutzeri*, uncultured *Acinetobacter sp.*), indicating heterotrophic conditions at greater depth.

Furthermore, we identified a core community across all three sites, consisting of 30 ASVs, including *Enhydobacter*, *Pseudarthrobacter*, and *Rhodococcus*, accounting for 21.9% of the overall sequences. Comparative analysis indicates that samples do not cluster distinctly based on geographical sites but rather based on depth sections. This observation, coupled with phylogenetic analysis, suggests the existence of a highly specialized subsurface community experiencing significant selective pressure and remaining largely unaffected by ongoing surface processes.

Our study underpins the adaptability and resilience of deep subsurface communities colonizing the nutrient-limited underground. Future metagenomic analyses of these samples will further explore their functional potential and, thus, unravel the importance of these communities in Earth's ecosystem.

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**EMEP042**

**Polyphasic characterization of *Lacunimicrobium album* gen nov., sp. nov., a novel member of the phylum *Planctomycetota***

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Bacteria inhabit diverse environments and play a vital role in nature. Despite the high number of bacteria predicted to live on Earth, only about 1% is believed to have been cultured, leaving 99% of the suspected diversity undiscovered. Despite the advancement of technology in metagenomics and bioinformatics, the cultivation of microorganisms remains fundamental for microbial research and new technologies should rather improve than replace classical cultivation techniques.

Members of the ubiquitous bacterial phylum *Planctomycetota* are often found to be associated with the surfaces of macroscopic phototrophs mostly in aquatic ecosystems. The phylum comprises species with uncommon morphology and physiology, and many characterized members have large genomes and show complex lifestyles. High numbers of genes with unknown functions and biosynthetic gene clusters (BGCs) make them a promising and yet for the most part untapped source for bioactive small molecules.

Since the 1970s, many planctomycete strains have been brought into culture by Heinz Schlesner (Institute of General Microbiology, Kiel University). His collection harbours numerous novel strains isolated from limnic and marine environments in Northern Germany, ca. 60% of them being uncharacterized members of the phylum *Planctomycetota*.

Here, we investigated three limnic strains from this collection, SH203, SH248, and SH280, that were isolated from two different ponds in Kiel and water from a gypsum mine. Phylogenetic analyses based on the 16S rRNA gene sequence and the genome support the assignment of the three isolates to a novel species of a novel genus in the family *Planctomycetaceae*. With a generation time of 7-9 hours, the strains belong to the faster-growing planctomycete strains. All three are unpigmented and divide by a for the most part uncharacterized type of asymmetrical division followed by all members of the family. In addition to the polyphasic characterization of the strains including morphological and physiological properties, we also analyse their metabolome with a focus on the discovery of novel small molecules with potentially antimicrobial or other health-promoting activities.

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**EMEP043**

**Impact of climate change on the diversity and abundance of marine *Vibrio* species**

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*Vibrio* is a ubiquitous bacterial genus that is particularly prevalent in marine environments. Members of the genus play a major role as colonizers of marine surfaces and opportunistic pathogens. An overall rise in *Vibrio* abundance has been linked to higher water temperatures as a consequence of climate change. Alongside the rise in seasurface temperatures, an increasing number of vibriosis outbreaks in both humans and marine animals has been recorded over the past years. This is of particular relevance to the Baltic Sea, which is subject to some of the highest warming rates globally. We aim to understand whether *Vibrio* from the water column and endemic inhabitants of the Kiel Fjord show climate related changes in their relative abundance or genomic makeup.

Here we assess diversity, abundance, as well as genomic and physiological characteristics of *Vibrio* spp. on the surfaces of different marine hosts as well as in the free-living community along the Kiel Fjord. Hosts include two *Fucus vesiculosus* spp. and the moon jelly *Aurelia aurita.* The marine models systems were monitored with regard to simulated global warming and ocean acidification scenarios. The overall *Vibrio* community was recorded using marker gene analysis, while individual isolates were subjected to a classical characterization approach combined with genomic

analysis. To provide greater depth to diversity estimates, 16S rRNA amplicons are analysed together with *hsp60* amplicons, which provide a higher taxonomic resolution of the *Vibrio* genus.

Our data show, that *A. aurita* harbour a significantly higher proportion of *Vibrio* than the *Fucus* species. *Vibrio* isolation from hosts and water column yielded less diverse isolates during a summer heatwave with water temperatures above 20°C than in the following autumn. Most isolates from the water column belong to a yet unclassified *Vibrio* species, while host-associated *Vibrio* belong to the *V. anguillarum* or *V. harveyi* clade. Comparative genome analysis of the isolates from from both the host-associated and free-living conditions, will provide further information on the adaptations involved in the different life-styles. Overall we aim to assess the status quo of *Vibrio* in the Kiel Fjord in order to establish a base-line for further research on climate-change related developments in the Baltic.

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**EMEP044**

***Legionella* in the water cycle of Berlin – Analyzed with established and new methods**

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Introduction: Waterborne pathogens and associated diseases are a major public health problem worldwide. The impact of these pathogens is not only associated with mortality and morbidity, but also with social impact and economic losses from prevention and treatment. One of the pathogenic bacteria in Berlin's water cycle is the ubiquitous *Legionella*, which can be transmitted to humans through inhalation of water droplets and can cause severe pneumonia or Pontiac Fibre. Legionnaires' disease is fatal in about 8% of cases and in patients treated in an intensive care unit it is as high as 30%. Due to the high mortality, the rising temperatures and the consequentially enhanced possibility for the bacterium to multiply, fast and accurate monitoring methods become very important.

Objectives: We aimed to find a robust method for monitoring *Legionella* in the different parts of the water cycle of Berlin.

Material & Methods: We took samples from surface water, raw water, drinking water, process water and sewage water and analyzed Legionella with different methods: according to DIN EN ISO 11731, flowcytometry after immunomagnetic separation (COUNT) and dPCR.

Results: The culture-based method was unable to detect *Legionella* especially in higher contaminated samples because of the accompanying bacterial flora. In addition to that led the required heat and acid treatment to significant reduction of *Legionella*. The other tested methods showed valid results, so that an evaluation of the different parts of the water cycle was possible. Although the dPCR showed higher values both new methods nearly led to the same ranking. The contamination was extremely high in sewage water and decreased from surface and process water to raw and drinking water.

Conclusion: The results showed that the culture method according to DIN EN ISO 11731 took very long and was not sensitive enough compared to the new methods. The culture method failed especially testing highly contaminated water like sewage water despite pre-preparation due to the accompanying bacterial flora. In contrast, the new methods showed fast feasibility, easy handling and higher sensitivity.

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**EMEP045**

**Identification of polyester-degrading esterases from deep-sea samples**

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The continuing increase in the amount of plastic waste confronts today's society with a steadily growing problem. The use of polymer-active enzymes represents a promising solution for breaking down polymers into their individual components before they end up in the environment. In particular, enzymes acting on polyesters have received pronounced attention in science and society during the last years. We tried to extent the available collection of polyesterases and to get insights activity under marine conditions (high salt/low temperature/high pressure) by recovering polyesterases from marine microbes that are found in plastic-polluted habitats and may have begun to adapt to the degradation of plastics. For this purpose, sediment samples from the deep sea of the North Atlantic were used to inoculate enrichment cultures with polyester-polyurethane as carbons source. Indeed several marine organisms were isolated from these cultures that exhibited polyesterase activity. Analysis via partial 16S rDNA sequencing showed that these were predominantly unknown strains of *Pseudomonas*. Their genomes were sequenced and Motif hidden Markov model search algorithms were applied to identify novel potential polyesterases, complemented by automated gene function prediction to search for members promising enzyme classes like cutinases. Subsequently, nine genes were cloned and expressed in *E.coli;* initial assessment of the strains on indicator agar plates confirmed active esterase expression in five strains. Of these, three exhibited polyesterase activity and were therefore identified as new polyester hydrolases from *Pseudomonas sp.* and *Rhodococcus sp.* and are currently characterized with regard to substrate spectrum, temperature activity profile and salt tolerance.

These new insights into the diversity of polyester-degrading enzymes of marine organisms have created a new starting point for the screening and characterization of polyesterases, thereby improving the understanding of this still poorly studied class of enzymes. This finding holds significant promise for addressing the issue of plastic waste and reducing its impact on the environment.

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**EMEP046**

**The buried threat below? – Investigating the impact of microplastics on rhizosphere microbial communities in agricultural plants**

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Microplastics (MP) are considered as the most pervasive global pollutants of terrestrial ecosystems. In particular, arable soils are prone to plastic deposition due to agricultural

practices or unintentional dispersal. In order to derive consequences for crop production, a comprehensive understanding of MP-induced effects on physical, chemical, and microbial processes in agricultural soils is urgently needed.
In the rhizosphere, root-associated microorganisms fulfill crucial functions to promote plant growth and maintain plant health. Our study aims to give insights on the impacts of MP on microbial communities and microbially mediated biogeochemical processes in the rhizosphere of two different agricultural plants.
We conducted a controlled greenhouse experiment with maize (*Zea mays*) and strawberries (*Fragaria* × *ananassa*), grown in soils spiked with MP particles (75-400 µm) to assess the influence of MP on plant growth, soil properties, and microbial communities. Over a three-month growth period, plants were exposed to either conventional (low-density polyethylene, LDPE; poly(ethylene terephthalate), PET; polystyrene, PS) or biodegradable (poly(butylene adipate-*co*-terephthalate), PBAT) MP at an environmentally relevant concentration (1% w/w). In our ongoing work, the microbial community composition is elucidated by state-of-the-art 16S rRNA gene and ITS metabarcoding. The activity of selected soil enzymes relevant for carbon and nutrient cycling is assessed with fluorometric 4-methylumbelliferyl (MUF)-based enzyme assays. Root colonization with arbuscular mycorrhizal fungi is estimated via a microscopy approach.
Our preliminary results indicate that MP did not directly affect aboveground plant growth and root colonization with arbuscular mycorrhizal fungi throughout our trial. However, microbial communities tend to be more responsive to biodegradable than conventional plastics in bulk soils. Whether this trend can be extended to rhizosphere soil communities is currently under evaluation. Ultimately, the findings of this study will add to our comprehension of the impacts of MP on soil microbial communities and the potential implications for agroecosystems.

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**EMEP047**

**Bacterial populations in different parts of domestic drinking water systems are distinct and adapted to the given ambient temperatures**

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Background: The energetic optimization of buildings plays an important role to achieve the German climate targets in 2030. More than 14 percent of the total $CO_2$ emissions in Germany originate from the building sector with the energy required for heating drinking water in buildings with central heating circles playing a significant role. Lowering the temperature from currently 60°C to a temperature that is more compatible with modern energy-efficient heat pumps however is in conflict with drinking water hygiene. The effect of temperature on the composition of bacterial communities in plumbing systems is hereby typically unknown.

Objective: The objective of this study was to examine the bacterial communities at different locations within plumbing systems. For this purpose, cold and warm drinking water were sampled in four buildings in different regions in Germany. The buildings had central heating circles and were equipped with ultrafiltration modules. Bacterial communities were characterized based on their regrowth behaviours at different temperatures and by sequencing.

Results: Bacteria from cold water propagated best at 22°C, but poorly at 36°C and not at all at 50°C. In contrast, bacteria from hot water grew best when incubated at 50°C, whereas growth at 22°C was poor with a long lag phase. Bacteria from taps at periphery locations retrieving both cold and hot water showed an intermediate growth behaviour.

These results corroborate the existence of distinct bacterial communities within domestic drinking water systems, which are shaped by the conditions and temperatures prevalent at different points of plumbing systems.Sequence data confirmed the differences in the microbiomes of cold and hot water samples. In hot water, the abundant bacterial groups included Deinococci, Kryptonia, Ignavibacteria, Nitrospiria,Gemmatimonadetes and Gammaproteobacteria. Cold water samples contained apart from Gammaproteobacteria high relative abundances of Alphaproteobacteria. Incubation of hot water at 50°C, 55°C or 60°C furthermore shaped the microbiome in different ways indicating that small temperature differences can have a substantial impact on the bacterial communities.

Outlook: Further strategies that allow a reduction of the hot water temperature while at the same time ensuring good drinking water hygiene are currently being pursued.We thank the German Federal Ministry of Economic Affairs and Climate Action for financing this study (ULTRA-F grant no. 03ET1617D).

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**EMEP048**

**Impact of a high-altitude temporary lake on microbial communities along a moisture transect in the Barrancas Blancas plain (Chile)**

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The high mountain deserts are harsh environments characterized by extreme aridity, intense solar UV radiation, and extreme shifts in daily temperature. The Barrancas Blancas Plain in the high Atacama Andes of Chile (~5000 m a.s.l, 68°39' W, 27°02' S) is a unique place that combines the characteristics of the mountain deserts with the presence of a temporary lake, which presumably can impact microbial life. Therefore, to better understand how microbial life can survive and thrive under these extreme conditions and what is the role of the lake water in shaping the microbial community, we sampled three transects along a moisture gradient, from the lake sediments to a reference site with no water impact. We used a novel protocol to separate the intracellular DNA (iDNA), which represents the living community, from the extracellular DNA (eDNA), which represents dead or past communities. Since this study was focused on the potentially active microbial community, only the iDNA pool was considered. Our results showed high microbial abundance in all samples of the moisture transect compared to other desert environments and a highly specialized and adaptative psychrophilic microbial community, mainly composed of Proteobacteria and Gemmatimonadota, which followed a distinctive decreasing and increasing pattern along the moisture transect, respectively. Particularly, the most abundant ASVs were

related to the family Gemmatimonadaceae and were closely related to *Gemmatimonas phototrophica*, a microaerophilic and facultative photoheterotroph and a possible key microorganism in maintaining microbial life in this ecosystem. Multivariate analysis showed a clear separation between the water-affected samples from the reference site and explained that around 65% of the variation in the microbial community is mostly related to the water content. Additionally, the functional annotation of prokaryotic taxa (FAPROTAX) showed that heterotrophy is the main function, but phototrophy and photoheterotrophy functions are present and showed a decreasing pattern along the transect. Altogether, our study provides insights into how this specialized microbial community can thrive in extreme environments and how water is shaping it, indicating that the temporary lake is a hotspot for life in this desertic region and plays a key role in maintaining the microbial community potentially active by promoting a base metabolism.

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**EMEP049**

**Experimental and mathematical optimization of energy efficiency in microbial electrolysis cell dealing with hydrolysate**

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Electroactive microorganisms have the ability to oxidize organic material and generate electrical current while growing on an electrode surface as the electron acceptor. Fermentation hydrolysate is a suitable substrate to produce hydrogen via microbial electrolysis cell (MEC); however, its complexity limits the relevant application. We here aim to construct a mathematical model where optimal operation of MEC dealing with fermentation hydrolysate can be achieved. The model takes into account the composition of hydrolysate, the maximum measured current and corresponding hydrogen production, the coulombic efficiency, the applied anodic potential, measured cell voltage, and calculated power consumption. Experiments using different applied potential and hydrolysate dilutions were performed where an anodic potential of 0.15 vs SHE had the optimal measured power consumption at the peak hydrolysate concentration of 50%. Then, concerning the optimal hydrogen production at minimal power consumption as the objective function, the experimental data at different conditions were used for parameters fitting in a Matlab model consisting of ordinary differential equations, mass balances, and kinetic rates. Parameter estimation was conducted to determine the sum of the least squared error between measurements and corresponding simulation results values for the kinetic model. Meanwhile, the optimal MEC performance, when dealing with different compositions of hydrolysate, can be mathematically predicted by selecting the optimal applied anodic potential and hydrolysate concentration.

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**EMEP050**

**The Schlesner strains collection expands the diversity of the phylum *Planctomycetota***

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Members of the phylum *Planctomycetota* occur ubiquitously and receive attention due to various cell biological peculiarities including an uncommon form of asymmetric cell division without canonical cell division proteins such as FtsZ. Research in the phylum is currently in a transition phase from the pure description of novel members to the exploration of potential biotechnological applications. In line with their large genomes and complex lifestyles, *in silico* genome mining analyses of planctomycetal strains yielded several putative biosynthetic gene clusters which might be responsible for the production of novel small molecules with potential antimicrobial or other health-promoting activities. However, for a long time, *Planctomycetota* were considered as unculturable. Due to pioneering work of Heinz Schlesner (Kiel University), who focused on the development of cultivation media and isolation techniques for enrichment and isolation, many strains belonging to the phylum have been brought into culture in the 1970s-90s. The Schlesner collection of microbes contains more than 250 strains of "budding bacteria" morphologically resembling members of the phylum *Planctomycetota* that were isolated mainly from aquatic habitats. We have now set out to re-cultivate and analyze the strains from this collection.

In total, more than 180 planctomycetal strains were revived from the Schlesner collection, that belong to at least 15 novel genera and 58 novel species within the phylum *Planctomycetota.* First, the phylogenetic most diverse strains were selected for in-depth polyphasic characterization based on 16S rRNA gene sequencing. Typical genome sizes of 7-10 Mb and up to 10 secondary metabolite-associated biosynthetic gene clusters per genome were obtained. The Schlesner collection will be an important driving force to expand the currently described diversity of the phylum. Speeding up and streamlining the throughput of the strain isolation and description pipeline will have major benefits for the transition to an application of the strains, for example in bioprospection studies.

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**EMEP051**

**Impacts of $CaO_2$ treatment on the biogeochemistry and microbiology of freshwater sediments**

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In the advent of climate change and increased anthropogenic influences, small lakes and ponds are at the risk of increased eutrophication, impairing their ecological and economical functions. Eutrophication management is challenging, expensive and often ephemeral. One novel approach claims to provide a cheap and sustainable method for eutrophication management using the oxygen slow-releasing agent calcium peroxide ($CaO_2$). Oxygen release at the sediment-water interphase as well as the precipitation of phosphate to apatite has been proclaimed to activate microbial mud reduction and limit algal growth. $CaO_2$ has previously found successful applications in soil remediation and wastewater treatment. Still, the impacts of its application in lake eutrophication management are not yet well understood.

To address this knowledge gap, our study aims to generate insights into biogeochemical as well as microbial changes after $CaO_2$ amendment.

Sediment microcosms (MC) of a hypertrophic small lake were incubated with $CaO_2$ for up to 77 days. Biogeochemical microprofiles of oxygen availability, redox potential and pH were measured using noninvasive and spatially high resolute microsensors. DNA was extracted from sediment subsections. 16S rRNA gene metabarcoding was used to characterize the taxonomic composition of treated and untreated MC for a spatially explicit community profiling.

Further C/N ratios and total phosphorus concentrations were quantified from the sediment sections.

Treated MC were characterized by higher concentrations of available oxygen in deeper sediment layers. Coincidingly in sediment depths with augmented oxygen, we identified significant shifts in microbial community compositions. Taxa within the *Bacteroidetes* and *Verrucomicrobia* were enriched, while *Archaea* appeared depleted. The C/N ratios also increased. Thus, $CaO_2$ treatment seemed to stimulate aerobic organoheterotrophs, resulting in higher levels of microbial detritus turnover, and increased C/N ratios. Other aspects of the application, especially presumed phosphate precipitation capacities and redox potential shifts remain to be addressed.

Ultimately, we aim to elucidate the applicability of $CaO_2$ as a potential treatment against eutrophication of small lakes.

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**EMEP052**

**The influence of *Zostera marina* on pelagic and benthic bacterial communities correlates with its growth characteristics**

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Aquatic macrophytes are ecosystem engineers that influence their shallow water environments and have been suggested to shape the bacterial community composition in the sediment and water. In this study we characterized the impact of *Zostera marina* on bacterial communities in the Baltic Sea during summer conditions between a salinity range 4-15, using massive parallel 16S rRNA gene sequencing. To investigate the combined influence of salinity and *Z. marina* on the microbial community, we collected tripled samples from the inside, edge, and outside meadows of surface sediments, as well as from the water and epiphytic biofilm of *Z. marina* leaves. The bacterial community in the three different substrates (water, sediment and epiphytic biofilm) differed clearly in their bacterial community composition. Independent of the presence of *Z. marina*, sediment had the highest bacterial diversity of the investigated habitats. Within a substrate, salinity was the most important factor in structuring the bacterial community composition. At alpha-mesohaline (salinity 9-15) conditions, a significant influence of *Z. marina* was detected on the benthic bacterial community composition and its Chao1 diversity. A co-occurrence network of the alpha-mesohaline samples indicated a stronger influence of eukaryotic microorganisms, a higher modularity and more negative connections. The results are congruent with longer *Z. marina* leaves above salinity 9 which has been ascribed to higher productivity and carbon storage capacity. Hence the reduced growth characteristics of *Z. marina* described for lower saline regions of the Baltic Sea also reduces its impact on bacterial community composition. The results have implications for *Z. marina* management at different salinities.

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**EMEP053**

**Citizen-Science in action: Discovery of untapped magnetotactic bacteria**

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Magnetotactic bacteria (MTBs) form a phylogenetically and morphologically heterogeneous group of bacterial strains. It is thought that these bacteria orient themselves on the earth"s magnetic field to navigate effectively through complex environments. This way, MTBs potentially shuttle electrons from anaerobic to aerobic habitats. The magnetic field orientation is achieved by specialized organelles named magnetosomes. These organelles are positioned in chain-like structures and can be found across many bacterial phyla in various sizes, numbers, and forms.

The process of magnetosome assembly is complex and requires the interplay of various proteins. Over the years, the function of many proteins involved in magnetosome formation has been elucidated[1]. However, proteins determining magnetosome morphology are still waiting for their discovery[1]. To identify and characterize these magnetosome-shaping proteins, more high-quality genomes and axenic cultures of MTBs with different magnetosome characteristics need to be made available, since only approximately 27 species are validly published (but not all are available as axenic cultures) and 25 are in candidate status. Therefore, more MTBs from various habitats must be located, isolated, and described.

To identify sampling locations and ecosystems inhabited by potentially interesting MTBs, a Germany-wide Citizen-Science project was conducted in the framework of the highly-competitive Hochschulwettbewerb 2022. More than 225 samples were collected from various limnic and marine habitats including shores, rivers, moors, creeks, ponds, alpine lakes, and many more. All of them were screened for the presence of MTBs, which could surprisingly be found in approximately 60% of all samples. The morphology of those MTBs ranged from ovoid over small cocci, to long spirilla, and even multicellular magnetotactic cells could be found. Some of the most promising locations were re-sampled and MTBs were enriched. Currently, we work on establishing cultivation-dependent as well as -independent pipelines for their further characterization. The project is an important contribution to unveil the phylogenetic distribution of magnetotactic bacteria and the molecular basis for magnetosome assembly and shape determination.

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**EMEP054**

**Establishment of dark septate endophytes (DSEs) as supplements in peat-free substrates**

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Dark septate endophytes (DSEs) are a sub-group of endophytic fungi and are featured by their melanized and septate hyphae. They produce conidial as well as sterile hyphae that colonize roots intracellularly or intercellularly. Peat is a fossil material that contributes to greenhouse gas emissions and has been utilized as an ingredient in horticultural growth substrates in Europe for decades due to its low cost, great availability, and unique physico-chemical properties. Reducing peat use is critical for greenhouse gas lowering in horticultural production. As a result, it is important to use ecologically friendly replacements for peat in horticulture growth substrates, but this has been shown to cause particular problems as th the immobilisation of nitrogen. We had previously found that DSEs *Periconia macrospinosa*, *Cadophora* sp., and *Leptodontidium* sp. are tolerant to abiotic stress and could be successfully further *in vitro* acclimatized to salt stress. This process was not attributed to their melanised hyphae. Moreover, DSEs can mobilise nitrogen from organic resources and increase nutrient uptake in tomato plants and under salt stress. This prompted us to investigate the use of the DSE *Leptodontidium* sp. Me07 model in two types of peat-free substrate, sterile and non-sterile, to determine how the melanised hyphae impact the interaction and colonization with petunia roots, and how this interaction affects plant growth and nutrition.

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**EMEP055**

**Isolation of antarctic actinomycetes and investigation of their natural product biosynthetic potential**

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Unique habitats are known to be a prolific source for hitherto unknown actinomycetes that produce novel natural compounds. Thus, in recent years, several research projects have focused on isolating strains from extreme environments. The Antarctic region is still one of the most unknown environments, especially in terms of bacterial benthic ecosystems. Despite the extreme climate, it is considered to be a biodiversity hotspot. The aim of this study is to isolate novel filamentous actinomycetes from marine Antarctic sediment samples and investigate their biosynthetic potential to produce bioactive natural compounds.

Marine sediment samples from Admiralty Bay, Hope Bay, Collins Bay, Deception Island, and Bransfield Strait in Antarctica were sampled and utilized for the isolation of actinomycetes. The isolation protocol was stepwise adapted by testing different parameters, such as pH, salinity, and temperature regarding cultivation conditions. Pre-treatments for isolation included a gradual acclimatization of the sediment samples, as well as a heat shock, followed by a pre-incubation at 30°C to promote spore germination. Multiple selective and minimal culture media were tested with the addition of sea salts.

The isolation approach revealed that slow acclimatization of the samples coupled with a pre-incubation step are appropriate to increase the number of actinomycete isolates from marine sediment samples. In total, 16 actinomycetes strains were identified based on their morphological features. Among them, two have been classified by 16S rDNA sequencing belonging to the genera *Streptomyces* and *Micromonospora* and will be genome sequenced. In parallel, an additional set of Antarctic strains has been selected from the DSMZ strain collection, including the genera *Micromonospora*, *Pseudonocardia*, and *Streptomyces*, to be included in the list of actinomycetes to be analyzed.

In the further course of the study, genomes from Antarctic strains will be analyzed for the abundance of biosynthetic gene clusters to determine their natural compound production potential. This will be exemplarily shown for a potential novel angucycline producer strain *Streptomyces* sp. 33D.

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**EMEP056**

**Access to the uncultured microbial life: A functional metagenomic RubisCO screen reveals a novel ribulose-1,5-bisphosphate converting enzyme**

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Most of Earth's microbial diversity resists cultivation in the laboratory, which makes physiological characterizations hard, if not impossible. Consequently, metabolic activities and possible contributions to global elemental cycles have to be predicted from genomics and environmental characteristics, and many new metabolic enzymes and pathways remain undiscovered. To close this knowledge gap, we are using functional metagenomics to access and elucidate the unknown potential of numerous novel enzyme functions and metabolic pathways.

Here, we report on the establishment and successful use of one of our function-based metagenomic screens that allows us to mine and investigate RubisCOs (Ribulose-1,5-bisphosphate carboxylase/oxygenase) from the environment independent of the culturability of the native host. RubisCO is well known as a key enzyme in the biological carbon cycle. This enzyme mediates the fixation of more than 90% of the world's inorganic carbon into biomass by catalyzing the carboxylation of ribulose-1,5-bisphosphate (RuBP), leading to the formation of two molecules 3-phospho-D-glycerate (3-PGA). Screening of 15,000 fosmid clones from deep-sea hydrothermal vents resulted in the recovery of 41 recombinant RubisCO active fosmid clones originating from distinct phyla. Next to identifying various active RubisCOs from uncultured microbes and gaining new insights into RubisCO regulation and activation processes, we found one clone that was particularly striking. This clone converted RuBP not only exceptionally fast (up to 20 mM is converted within a few seconds), but also does not form 3-PGA. Indeed, biochemical characterization and crystallization of the overexpressed novel enzyme confirms that it is a novel RuBP converting phosphatase. This is surprising as substantial RuBP conversion in bacteria has so far only been observed in the context of autotrophic CO2 fixation via RubisCO. Thus, this novel phosphatase has the potential to change our current understanding of RuBP associated metabolism, as it possesses a yet unknown function in a previously unrecognized RuBP pathway.

**EMEP057**

**Identification and growth characteristics of PE-degrading bacteria**

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Polyethylene (PE) is the most abundant plastic type worldwide with a total amount of 26,9% of all plastic types being globally produced in 2021. Due to the favourable physicochemical properties as high density (HD), low density (LD) or linear low density (LLD) PE blends, it is used for a great variety of applications ranging from pipelines to single use packaging and therefore majorly contributes to the current plastic pollution crisis. Biodegradation of PE in the environment is limited because of the inert properties of the material itself.

The aim of this study is to identify potential PE-degrading bacteria and their growth characteristics for future applications in the industry, bioremediation or waste management.

The biodegradation of LDPE film particles (FKuR Kunststoff GmbH) was researched using *Rhodococcus opacus* and two wild type bacterial strains, isolated from the film particles. The isolates were identified via MALDI-Tof measurement. Growth and degradation were initially examined with Ethylene glycol as a carbon source. Additionally, the utilization of paraffin oil was assessed. To analyse whether hydrolytic enzymes are involved in the degradation process, a clear zone assay was performed. Finally, the degradation of the above-mentioned PE-particles was tested in liquid culture, by means of the released CO2 volume measured via gas chromatography (Multiple gas analyzer with TCD-detector, SRI instruments Europe GmbH). Residual fragments in the supernatant of culture broths were either quantified using HPLC- or GC-MS measurements.

All three cultivated bacterial strains showed growth on paraffin oil, which confirms that growth in the latter experiments does not solely occur on additives. Furthermore, all strains grew on PE film particles, indicating the utilization of the polymer. One of the isolates could be identified as *Bacillus vietnamensis*. The identification of the second strain as well as further results are still pending.

The identification of plastic-degrading bacteria is key for a future, sustainable waste- and resource management. Both *Rhodococci* and *Bacillus species* have previously been described to break down PE, and are known to form biofilms, which aids the degradation process. However, microbial PE-degradation is only partial, as the polymer backbone remains. Building on the results of this study, PE-metabolization to CO2 should be confirmed using isotope-labelled PE.

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**EMEP058**

**Metatranscriptomics of microbial biofilm succession on HDPE foil: Uncovering plastic-degrading potential in soil communities**

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In soils, microbial communities are living in complex systems securing soil health, nutrient cycling and the degradation of natural and xenobiotic substances. Plastic pollution is increasing worldwide, but very little is known about microbial processes taking place once plastic debris gets incorporated into the soil matrix.

For the first time, we studied metatranscriptomes of polyethylene (PE)-associated biofilm communities in a highly polluted landfill soil and compared their gene expressions to those of forest soil communities over a period of 53 days. The soil samples had been incubated in microcosm experiments with PE foil and class slides.

Surprisingly, the microbial communities from an undisturbed forest soil contained a diverse array of plastic-associated genes (PETase, alkB etc.), indicating the presence of an enzymatic machinery capable of plastic degradation. Plastic-degrading taxa were upregulated in the early stages of biofilm and the PE-degrading enzymes and known important transporters and fatty acid β-oxidation pathway were active during the maturation of the biofilm. We also found an increase in nitrogen fixation genes in the plastic soil community but not in forest soil, indicating an essential metabolic adaptation of biofilm communities in the plastisphere.

With our study, we address the underlying patterns of gene expression during biofilm development of a PE-associated plastisphere in soil and address the question of the potential of natural microbial communities to biodegrade petrochemical-based plastic in soil environments.

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**EMEP059**

**Exploring the antibiotic resistomes in activated sludge and wastewater treatment plants via genome-centric analyses**

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Antimicrobial resistance (AMR) has been recognized as a global challenge threatening human and animal health. However, we are far from knowing all the consequences of AMR on human, animal and ecosystem health. The present study aimed to use publicly available metagenomes from activated sludge and wastewater treatment plants to decipher Antibiotic resistance gene (ARG) compositions and abundance in genomes. We also determined the potential of ARGs to be horizontally and vertically transmitted through plasmids and chromosomes. A total of 5916 MAGs of high and medium quality were recovered, covering 68 phyla and 279 genera. MAGs were dereplicated into 1204 genome operational taxonomic units (gOTUs) as a proxy for species. The most prevalent resistance mechanisms detected in our data were antibiotic target alteration, antibiotic efflux and antibiotic inactivation. The dominant resistance gene classes were bacitracin, multidrug, macrolide-lincosamide-streptogramin (MLS), glycopeptide and aminoglycopeptide. *Escherichia*, *Klebsiella*, *Acinetobacter*, *Gresbergeria, Mycobacterium* and *Thauera* were the dominant genera and were determined as resistome's main hosts. [JPLFS1] Our results showed that ARGs are more mediated by chromosomes than plasmids indicating the potential for horizontal and vertical gene transfers. We determined 784 virulence factor (VF) genes in the genomes, which indicate

potential human pathogens carry ARGs. [JPLFS2] This study provides a comprehensive overview of ARGs in different wastewater treatment plants, highlighting the prevalence of ARG-carrying human pathogens. The outcome of this research may help to design measures to reduce AMR transmission and evolution.

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**EMEP060**

**"Midi-Metagenomics": A novel approach for cultivation independent microbial genome reconstruction from environmental samples**

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Introduction: Less than 1% of all prokaryotic species have been successfully cultured to date. The remaining majority is referred to as "microbial dark matter" (MDM). The standard methods to study MDM are metagenomics and single cell genomics. However, in metagenomics, discrete genome assembly is sometimes unfeasible, especially for complex communities and organisms of low abundance. In turn, SCG requires whole genome amplification methods (WGA), which can cause uneven sequence coverage that results in incomplete genomes. "Mini-metagenomics" - an attempt to combine those two methods - still requires WGA and is thus suboptimal.

Objectives: Here we present an alternative strategy called **"midi-metagenomics"** that uses cell sorting of different microbial fractions and avoids the necessity of WGA before sequencing.

Materials & methods: Fluorescence-activated cell sorting was used to selectively enrich and deplete various cell types, resulting in large enough cell fractions to avoid WGA and use them for co-abundance variation-based binning. Sorting was based on cell size, complexity and autofluorescence. DNA was extracted from each sorted fraction as well as the original unsorted samples and directly sequenced using shotgun and 16S rRNA amplicon approaches. All samples were then bioinformatically analyzed regarding their taxonomic composition, and the diversity and quality of metagenomic assembled genomes (MAGs).

Results: The reconstructed MAGs from the midi-metagenomics fractions showed higher completeness and lower contamination compared to conventional MAGs of the control samples. Additionally, the fractions were compared to each other and to the corresponding unsorted samples, based on 16S rRNA amplicons. The sorted fractions of each sample displayed higher beta-diversities than between the original samples themselves, indicating that sorting achieves notable enrichment or depletion of different taxa. This enhanced diversity proved beneficial for co-variance based binning approaches.

Conclusion: Compared to current methods, midi-metagenomics enabled a more accurate reconstruction of MAGs with higher quality than co-assembly of multiple distinct samples, and has the potential for targeted enrichment and sequencing of uncultivated organisms of interest*.

*Vollmers et al. *bioXriv* (2023). doi:10.1101/2023.01.26.525644

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**EMEP061**

**A simple, reliable and inexpensive method for the identification of lactic acid bacteria**

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Introduction: Identification of bacterial species is nowadays realized by sophisticated technologies such as gene amplification followed by DNA-sequencing and database analysis, or by microbial detection systems based on Matrix Assisted Laser Desorption/Ionization (MALDI). Both procedures deliver results with the highest possible accuracy within one or two days. However, the methods require expensive equipment as well as microbial and bioinformatic expertise, which may not always be available in every laboratory.

Objectives: The goal was to develop a rapid, reliable, and cost-effective method for identifying lactic acid bacteria (LAB) based on colony morphology and cell shape.

Materials & Methods: Environmental samples were plated onto DeMan-Rogose-Sharp (MRS) agar plates with or without bromphenol blue (MRS-BPB). LAB were identified by 16S rRNA sequencing followed by application of the Basic Logical Alignment Tool (BLAST) service from the National Center for Biotechnology Information (NCBI, USA). Colony images were taken in a self-designed photo box giving defined lighting conditions. Cell shape was inferred from light microscopy. An easy-to-use identification tool was developed using Excel software.

Results: We isolated LAB from milk and teat canal biofilms to find novel strains be used as probiotic or protective cultures in food. Based on the observation that LAB species differ in colony morphology and bluish color on MRS-BPB agar, we aimed to establish a method to identify LAB at a species level. Therefore, 27 LAB species of the genera *Aerococcus, Enterococcus, Lactiplantibacillus, Lactobacillus, Lactococcus, Latilactibacillus, Lentilactobacillus, Leuconostoc, Pediococcus*, and *Weissella* were selected. For data collection, colonies were examined for shape and bluish color on agar plates and in high-resolution photographs. Cell shape was inferred by microscopy. The data were then entered into the Excel analysis tool. Of the 27 species, 24 could be unambiguously identified. Analysis for the remaining three revealed two possible species.

Conclusions: Species of LAB were identified with an accuracy of 89% solely by looking at colony morphology and cell shape. We are currently extending the system by including more LAB with the ultimate goal to achieve 100% identification for all LAB species.

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**EMEP062**

**From science to market: Potential use of *Pediococcus pentosaceus* strains as starter, probiotic or protective cultures**

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Introduction: *Pediococcus pentosaceus* is a lactic acid bacterium used in fermentation of milk, sausages, wine and beer. Furthermore, health-promoting effects have been attributed including microphage stimulation, anti-inflammation, anti-cancer, antioxidant, detoxification, and lowering the cholesterol levels.

Objectives: Our aim was to isolate *Pedioccus pentosaceus* strains from different locations to determine their potential application in food products.

Materials & Methods: Samples were plated onto DeMan-Rogose-Sharp agar plates supplemented with bromphenol blue (MRS-BPB). *Pediococci* DNA was subjected to sequencing of the gene encoding 16S rRNA followed by application of the Basic Logical Alignment Tool (BLAST) service from the National Center for Biotechnology Information (NCBI, USA). Subspecies were identified by Random-Amplified-Polymorphic-DNA analysis (RAPD). The point inoculation assay was used to determine inhibition capacity against various pathogenic bacteria. In addition, formation of hydrogen peroxide and acid production was recorded.

Results: We isolated more than one thousand LAB from milk samples and teat canal biofilms from fifteen milk farms located in the vicinity of Münster. Among them, we found 40 *Pediococcus pentosaceus* strains. Binary matrices obtained from RAPD experiments revealed that most of them could be assigned to different subspecies. Inhibition of pathogens was monitored for 13 *P. pentosaceus ssp.*. While most of them effectively inhibited *Pseudomonas aeruginosa* and *Salmonella typhimurium*, some were less potent in killing *Listeria monocytogenes* or *Klebsiella pneumoniae*. In addition, inhibition of the sporulating bacterium *Bacillus subtilis* was observed. Hydrogen peroxide formation was only observed in only one of the 40 strains tested. Acid production was detected on agar plates from between pH values of 4,1 to 6,1. Interestingly, this was dependent on the test strains used in the inhibition assay.

Conclusions: Isolation of the *Pediococcus pentosaceus* strains from different dairy farms exhibits high biodiversity at the subspecies level. All isolates show inhibitory activity against pathogenic bacteria making them applicable in food products as starter, probiotic or protective cultures.

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**EMEP063**
**Biodiversity of lactic acid bacteria isolated from dairy cattle**

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Introduction: Lactic acid bacteria (LAB) are of outstanding importance for the use in food products as starter or protective cultures. They also play a pivotal role as probiotics conferring a multitude of health-promoting effects. The isolation of new strains could help to further extend the application in foods and nutraceuticals.

Objectives: Autochthonous LAB bacteria from diverse dairy farms will be isolated, identified and subjected to environmental biodiversity analysis.

Materials & Methods: Fore milk samples and samples from teat canal biofilms were collected. LAB were identified by plating onto modified DeMan-Rogosa-Sharp agar plates supplemented with bromphenol blue (mMRS-BPB). Gene sequences of the gene encoding 16S rRNA were uploaded into the entry form of the Basic Logical Alignment Tool (BLAST) service from the National Center for Biotechnology Information (NCBI, USA). Subspecies were identified by Random-Amplified-Polymorphic-DNA analysis (RAPD). Alpha- and beta-biodiversity were analyzed according to Shanon-Wiener and Jaccard, respectively.

Results: Over one thousand LAB strains were isolated from milk samples and teat canal biofilms from dairy cattle farms located in the Münsterland area. Therefore, an equal number of five farms were selected that pursue stable farming, stable farming combined with open grazing, and organic farming. A variety of more than 20 genera was identified of which the species *Pediococcus pentosaceus*, and the genera *Enterococcus*, *Weissella*, and *Aerococcus* were the most abundant. A thorough analysis of the alpha- and beta-biodiversity of the found LAB was conducted. It appeared that biodiversity of LAB can vary considerably within each farming type varies. Nevertheless, a slightly higher biodiversity was observed in organic farmed dairy cattle.

Conclusions: The study provides a comprehensive dataset on LAB communities present in milk samples from dairy cattle with respect to the farming type.

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**EMEP064**
**Global environmental surveillance of human fungal pathogens via metagenomics**

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Introduction: Human fungal infections are a significant threat to human health but receive less attention than other infectious diseases. Of the species recognized by the World Health Organization as priority pathogens, the majority have their primary habitat in the environment. However, we are lacking a comprehensive understanding of their geographic distribution, as well as the ecology of these pathogens in their native niche.

Methods: To define global patterns of human fungal pathogens in the environment, we retrieved ITS rRNA amplicon sequence data from >8,000 air and soil samples from across the globe.

Results: The significant human pathogen Aspergillus fumigatus was globally prevalent, being present in 24% of all soil samples using a relative abundance cutoff of 0.01%. Interestingly, A. fumigatus was detected at significantly higher abundances and prevalence in Asia compared to North America and Europe, despite this pathogen causing substantial disease in all three regions. We also observed that Fusarium and Exophiliala spp were globally prevalent at high abundances. Despite this, they are infrequent causes of human disease, suggesting that their rarity in the clinic is likely not due to lack of exposure but to an intrinsic low virulence of these species. Co-abundance analysis indicated that most human pathogenic fungi have their own distinct ecological niche, with little interconnectivity between each pathogenic species. However, most human fungal pathogens played key ecological roles, as evidenced by their significantly higher closeness and betweenness centralities compared to non-pathogenic fungal genera. Altogether, this work illuminates the global exposome of humans to fungal pathogens, as well as their ecology.

Conclusions: This knowledge can be used to better understand the link between environmental exposure and subsequent clinical infection, as well as informing "One Health" approaches for combatting antifungal resistance by identification of specific habitats enriched for human pathogens.

**IBP001**

**Unexpected diversity and evolutionary advantage of antimicrobial heteroresistance in *Pseudomonas aeruginosa***

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Introduction: Antimicrobial heteroresistance describes the phenomenon of high antimicrobial resistance in only a small subpopulation of a bacterial culture and likely represents a selectively advantageous bet hedging strategy. Heteroresistance is common in human pathogens and often causes underestimation of the antimicrobial minimum inhibitory concentration (MIC), potentially leading to treatment failures. To date, the exact characteristics and evolutionary advantages of heteroresistance are unexplored for most pathogens, including the high-risk pathogen *Pseudomonas aeruginosa*.

Objectives: The objectives of our study are to enhance our understanding of the distribution of antimicrobial heteroresistance in *P. aeruginosa*, its diversity and also its selective advantage under antibiotic therapy.

Materials and Methods: Here, we performed a systematic analysis of heteroresistance for a representative *P. aeruginosa* strain panel (i.e., the mPact panel) that covers the entire genomic diversity of this pathogen species. For our analysis, we combined heteroresistance tests, mathematic modelling, a series of evolution experiments, and whole genome sequencing.

Results: Our systematic analysis revealed: (i) a surprising diversity of heteroresistance profiles across the mPact strain panel of *P. aeruginosa*, (ii) a high prevalence of beta-lactam, especially carbapenem heteroresistance, (iii) a clear fitness benefit of heteroresistance in treatments with continuously high antimicrobial concentrations, but (iv) absence of an evolutionary advantage of heteroresistance in alternative regimens with initially below-MIC drug doses. We further demonstrate that fast switches between antimicrobials (i.e., fast sequential therapy) can constrain heteroresistance-promoted adaptation.

Conclusion: The highly problematic pathogen species *P. aeruginosa* expresses an unexpected diversity of distinct heteroresistance types across the representative strain panel and across antimicrobial drugs. Moreover, heteroresistance is not universally beneficial but only provides a selective advantage under specific conditions, thereby pointing to treatment strategies that remain effective in the face of resistance heterogeneity.

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**IBP002**

**The role of pneumococcal extracellular vesicles on the pathophysiology of the kidney disease Hemolytic Uremic Syndrome**

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Introduction*: Streptococcus pneumoniae*-induced hemolytic uremic syndrome (Sp-HUS) is a kidney disease characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute kidney injury. Sp-HUS predominantly occurs in children under the age of two, having an estimated mortality rate of 12.3%. This disease is frequently underdiagnosed and its pathophysiology is poorly understood.

Objectives: In this work we compared clinical isolated strains from infant Sp-HUS patients to a reference pathogenic strain D39 for host cytotoxicity and further explored the role of Sp-derivate extracellular vesicles (EVs) in the scope of a HUS infection.

Methods: The isolated Sp-HUS EVs were characterized by performing dynamic light scattering microscopy and a thorough proteomic analysis. Interaction of Sp-EVs with the host was evaluated by hemolytic assays, cell viability assays and proteome arrays.

Results: Pneumococcal HUS strains caused significant lysis of human erythrocytes and increased the release of hydrogen peroxide, in comparison with the WT strain. Sp-HUS strain released EVs at a constant concentration during growth, yet the size of the EVs varied and several sub-populations emerged at later time points. The cargo of the Sp-HUS EVs included choline-binding proteins at high abundance, e.g. PspA, LytC and sugar utilization proteins as GlgD. Sp-HUS EVs lacked cytotoxicity towards human erythrocytes and endothelial cells but, instead, elicited the release of pro-inflammatory cytokines (e.g. IL-6) and chemokines (e.g. CCL2) by human monocytes.

Conclusions: These findings shed a new light into the overall function of Sp-EVs in the scope of infection-mediated HUS and open new doors to research on the usefulness of Sp-EVs as therapeutic and diagnostic targets.

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**IBP003**

**Carbon utilization of *Salmonella* Typhimurium during colonization in different mouse models**

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*Salmonella* Typhimurium (*S*. Tm) is a common foodborne pathogen and a well-established pathogenic Enterobacterium to study colonization and infection of mammalian hosts. *S*. Tm invades the gastrointestinal tract and causes inflammation, which drastically changes the gut luminal environment in terms of available inorganic electron acceptors. *S*. Tm uses these electron acceptors to bloom and outcompete the resident microbiota (1). However, it is largely unknown which carbon sources *S*. Tm utilizes at the onset of invasion before triggering inflammation. For this reason, mutant pools representing the metabolic capacity of *S*. Tm specifically sugar degradation were constructed, with each mutant labeled with a wild-type isogenic standardized hybrid (WISH) tag. The WISH tag is unique for each mutant and can be quantified by qPCR or Illumina sequencing, allowing us to study the fitness of several mutants deficient in carbon utilization in a mouse model. To modulate niche

competition, the mutant pool will be studied in different mouse models and challenged in the presence of different competitors. Host physiology, e.g., colonization resistance (2) and microbiota composition (3) are known to impact *S*. Tm colonization. This will provide valuable data on which metabolic pathways are important during initial growth and how changes in mutant fitness depend on the environment.

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**IBP004**

**The electrophilic immunometabolite itaconate causes an acid stress response as well as S-bacillithiolation and S-itaconation in the thiol proteome of *Staphylococcus aureus***

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Introduction: *Staphylococcus aureus* is a major pathogen, which has to defend against reactive oxygen and electrophilic species encountered from the host immune defense. Activated macrophages produce the immunometabolite itaconate as potent electrophile and antimicrobial upon pathogen infection. *S. aureus* was shown to adapt to itaconate by enhancing biofilm formation (1). Objectives: In this work, we aimed to investigate the mode of action and specific redox modifications caused by itaconate in *S. aureus*. We were further interested to elucidate novel defense mechanisms against itaconate in *S. aureus*. Materials & Methods: Using RNA-seq transcriptomics and Northern blot transcriptional analyses, we analysed the specific stress responses caused by itaconate. Shotgun proteomics was applied to identify the targets of itaconation and S-bacillithiolation by itaconate in *S. aureus*. Phenotype analyses of mutants were used to analyse the role of specific defense mechanisms against itaconate stress. Results: In the RNA-seq transcriptome, itaconate caused predominantly an acid stress response as revealed by the induction of the GlnR, KdpDE, CidR, SigB and GraRS regulons and the urease-encoding operon in *S. aureus*. The urease and urea supplementation were found to protect *S. aureus* from itaconate-induced acid stress. The generation of ROS and oxidative protein damage by itaconate was indicated by the up-regulation of the PerR, CtsR and HrcA regulons. Using shotgun proteomics, itaconate was shown to cause widespread *S*-bacillithiolation and *S*-itaconation of redox-sensitive antioxidant and metabolic enzymes, ribosomal proteins and translation factors in *S. aureus*, supporting the oxidative and electrophilic mode of action of itaconate in *S. aureus*. In phenotype analyses, the catalase KatA and the low molecular weight thiol bacillithiol (BSH) were found to provide protection against itaconate-induced ROS in *S. aureus*. Conclusion: Our results revealed that the antimicrobial mode of action of the itaconate in *S. aureus* is mediated by acid stress as well as oxidative and electrophilic stress, leading to *S*-bacillithiolation and itaconation. Due to its redox properties, itaconate could be a promising antimicrobial against multi-resistant *S. aureus* isolates.

Reference:

(1) Tomlinson KL, et al., (2021) *Staphylococcus aureus* induces an itaconate-dominated immunometabolic response that drives biofilm formation, Nat. Commun. 12(1): 1399. doi: 10.1038/s41467-021-21718-y.

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**IBP005**

**Studying the molecular basis of interaction and physiological effect for *Staphylococcus aureus* adhesion factor SdrC in the mammalian retina**

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Introduction: The Gram-positive opportunistic bacterium *Staphylococcus aureus* is responsible for a plethora of community and nosocomial infections worldwide. Surface associated cell wall adhesion (CWA) proteins contribute to the success of *Staph. aureus* in bacterial virulence, attachment, biofilm formation and subsequent colonization of target host matrix substrates. These CWA adhesins comprise the microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) featuring conserved regions, immunoglobulin IgG-like folded domains (N2 and N3 regions) and ligand binding domains. We are interested specifically in the SdrC protein belonging to the serine-aspartate repeat (sdr) subfamily of MSCRAMMs. It has been reported that SdrC interacts with the mammalian neuronal adhesin family of Neurexins localized at the presynaptic photoreceptor synapses.This raises the possibility that synapses could be important targets of adhesion upon bacterial infection, thus enabling such interactions to influence synaptic physiology.

Objectives: The details of molecular mechanisms for bacterial attachment to synapses remain elusive, which hinders novel pharmaceutical development and intervention strategies. Our study focuses on identifying novel receptors for SdrC at the synapse, and also exploring effects of SdrC-Neurexin interaction on synaptic physiology.

Materials and Methods: (I)Cloning and expression of bacterial virulence factor SdrC and respective mammalian receptor Neurexin in protein expression systems, followed by investigating putative interaction (protein pulldown assays, and immunofluorescence colocalization assays). (II) Characterization of the functional impact of the binding on synaptic physiology - synaptic vesicle cycling, $Ca^{2+}$ influx, using genetically modified SypHy and SyGCaMP mouse line respectively. (III)Further identification of novel SdrC receptors in wild type mouse brain lysate via global protein pulldown, and Mass Spectrometry analyses.

Results: (I) We reconfirmed SdrC-Neurexin interaction, though we didn"t observe any enrichment of Neurexin in our pulldown assays or Mass Spectrometry analyses. (II) Our physiological studies are currently ongoing. (III) An interesting find from our global pulldown assays was identifying synaptic glycoprotein Neuroplastin65 as a novel

binding partner to SdrC. Is it needless to say that essential controls were performed with housekeeping proteins like β-actin, fodrin, and GAPDH.

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**IBP006**

**Identification of targets and function of the *Yersinia pseudotuberculosis* type VI secretion system**

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The type VI secretion system (T6SS) is a powerful weapon used by many Gram-negative bacteria to deliver a plethora of toxic effector proteins into neighbouring target cells. While most of the characterised effector proteins are employed for interbacterial competition, some were shown to possess anti-fungal activity or to be targeted at eukaryotic host cells to enhance pathogenesis. The enteropathogen *Yersinia pseudotuberculosis* encodes four full sets of T6SS clusters (T6SS1-4) in its genome which are expressed independently from each other. However, expression and firing of T6SS1-4 are tightly regulated under standard laboratory conditions making it difficult to investigate their purposes. In recent studies, the T6SS4 of *Y. pseudotuberculosis* was shown to be activated by the transcriptional regulator RovC at moderate temperatures in the stationary phase. This provides an important tool for studying the function and potential targets of the T6SS in *Y. pseudotuberculosis*.

In this study, a T6SS4 secretion assay was established by using a *Y. pseudotuberculosis* strain overexpressing *rovC* to upregulate T6SS4 gene expression, and applying osmotic stress to enforce a firing event. Based on this assay, the secretomes of an actively firing strain and a D*vipA4* mutant strain that is unable to fire its T6SS4, were compared via mass spectrometry to identify potential effector proteins of the T6SS4.

As the T6SS was demonstrated to act as a bactericidal and fungicidal machinery, investigating its function and targets might lead to new opportunities in the research and development of therapeutic agents against bacterial or fungal infections.

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**IBP007**

**Regulation of the hydrogen peroxide sensing protein perR in *Clostridioides difficile***

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*Clostridioides difficile* is a gram positive, sporogenic bacterium, which can cause severe colitis due to toxin production. For the preferentially facultative anaerobically growing organism, an increased oxygen tolerance of the laboratory strain *C. difficile* 630Δ*erm*, an Erythromycin-sensitive derivate of the parental isolate *C. difficile* 630, was recently demonstrated. This phenomen is based on a mutation in the binding domain of the repressor protein perR, which controls the oxygen stress response in various of bacteria.

The aim of our studies is to characterize the function and regulation of the mutated perR in *C. difficile* 630Δ*erm* more precisely. To get a better understanding, we use different biochemical, molecular and bioinformatic methods, such as transcriptomics, proteomics and metabolomics.

At first, we performed anaerobic growth experiments with a *perR* knockout mutant of our laboratory strain *C. difficile* 630Δ*erm* in minimal-defined medium (MDM). In addition to salts and glucose, this medium contains a defined amino acid composition from leucine, isoleucine, valine, cysteine, methionine, proline and thryptophane. The results showed a significant growth deficit of the *perR* mutant in the lag as well as in the log phase compared to *C. difficile* 630Δ*erm* and the parental strain 630. In order to analyze the regulation of perR in detail, the concentrations of the MDM components where changed to determine whether certain components have an influence on the regulation and whether cell growth can be induced again. Therefor we were able to reinduce biomass production of the mutant by reducing the leucine concentration and remove threonine and glucose from the medium.

With the aim to analyze the function of perR regulation, we showed that perR is potentially regulated by adaption to carbon starvation. Futher omic techniques are currently conducted.

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**IBP008**

**Paenilamicin – A secondary metabolite from *Paenibacillus larvae*: Defense against microbial competitors and self-resistance mechanism**

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The Gram-positive, spore-forming bacterium *Paenibacillus larvae* is the causative agent of the honey bee brood disease American Foulbrood (AFB). Due to the very resistant spores of *P. larvae*, there is no cure to date, and burning of diseased honey bee colonies to prevent further spread of the disease is often considered as the only effective measure. This is why AFB is classified as notifiable disease in many countries. The infectious form of *P. larvae* are the spores which are ingested by the young honey bee larvae via the food. In the larval midgut, the spores germinate, whereupon the vegetative bacteria colonize the entire gut lumen causing a shortage of nutrients. The invasive phase starts with the bacteria breaching the gut epithelium, which causes larval death. Subsequently, *P. larvae* invades the hemocoel and decomposes the entire larval cadaver into a ropy mass which dries down to the characteristic foulbrood scale containing the spores. A pure culture of *P. larvae* is usually isolated from the ropy mass and scale. Secondary metabolites produced by *P. larvae* like Paenilamicin (Pam), a linear cationic polyketide-peptide hybrid that contains unconventional hydroxylated and N-methylated building blocks, D-amino acids, and a spermidine moiety have previously been shown to play a role the elimination of competitors like bacteria and fungi.

The first objective of this study was to investigate the antimicrobial activity of both synthesized and purified Pam against the entomopathogen *Bacillus thuringiensis* (*Bt*) as potential competitor of *P. larvae* in honey bee larval gut by exposure bioassays with honey bee larvae. Second, we examined the acetyl-CoA-dependent N-acetyltransferase PamZ as a putative self-resistance factor. Therefore, we generated a *pamZ*-knockout mutant of *P. larvae,* which was tested in an agar diffusion assay.

The exposure bioassays with *Bt*-infected honey bee larvae identified *Bt* as honey bee brood pathogen. Moreover,

feeding with Pam helped to reduce the larval mortality significantly, which demonstrates the potency of Pam as an antimicrobial secondary metabolite produced by *P. larvae*. Furthermore, our results confirmed the role of PamZ in the self-resistance mechanism of *P. larvae*. The knockout mutant *P. larvae* Δ*pamZ* was not able to grow in the presence of purified Pam.

These findings show that Pam is active against the honey bee pathogen *B. thuringiensis* and shed light on the mechanism underlying the self-protection of *P. larvae* against Pam.

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**IBP009**

**Molecular classification by multi locus VNTR analysis for epidemiological analysis of *Paenibacillus larvae***

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The Gram-positive bacterium *Paenibacillus larvae* (*P. larvae*) is the causative agent of American foulbrood of honey bees (AFB). This disease of honey bee brood is widespread worldwide and is a notifiable epizootic in many countries. AFB is caused by oral ingestion of the infectious spores and is invariably fatal to the infected larva.

Several methods for molecular typing of the species *P. larvae* exist. Repetitive element PCR (repPCR) using Enterobacterial Repetitive Intergenic Consensus (ERIC) primers led to the differentiation of several so-called ERIC-genotypes, which differ from each other at the genome and protein levels and thus in various phenotypic characteristics, including virulence. The genotypes ERIC I and ERIC II are of practical relevance because these two are responsible for current AFB outbreaks worldwide. The ERIC-classification scheme was confirmed by another genotyping method, the Multi Locus Sequence Typing (MLST) that is based on the partial sequence analysis of seven housekeeping genes of *P. larvae*. The MLST scheme resulted in an improved resolution of genetic variability within the species *P. larvae* and partitioned the ERIC genotypes into a total of 34 MLS types. Another method that promises even better resolution of genetic variability is Multiple-Locus VNTR Analysis (MLVA), which is based on the analysis of the number of certain tandem repeats (VNTR, variable number of tandem repeats) in the genome of the microorganism under study and seems to be particularly suitable for large-scale epidemiological studies of *P. larvae*.

Adaption and optimization of the MLV analysis for its application in *P. larvae* epidemiology led to the identification of eleven suitable DNA sequence segments that allow typing of isolates based on different VNTR patterns. Using this optimized MLVA scheme, we analyzed about 1000 *P. larvae* isolates from six continents and classified them into more than 300 MLVA types. The number of MLVA types in the two clinically relevant genotypes ERIC I and II is about the same, suggesting that the two genotypes do not differ in their genetic variability. The MLVA types of the non-ERIC I/II-isolates (ERIC III-V) branch off from the ERIC I cluster.

In summary, a method has now been established that provides a comprehensive picture of the relationships between different *P. larvae* isolates. Direct connections between individual disease outbreaks can now be elucidated and the epidemiology of AFB can be comprehensively studied.

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**IBP010**

**Fast and reliable purification method of non-tagged S100A8/A9 to assess its antimicrobial properties**

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S100A8/A9, commonly known as Calprotectin is a damage-associated molecular pattern in the mammalian innate immune system. It displays antimicrobial properties by sequestering transition metal ions like Mn(2+) from the site of infection [1].This starves bacteria of essential nutrients, resulting in the so-called nutritional immunity. In-depth studies of the function of S100A8/A9 and its contribution to the nutritional immunity have been conducted primarily for human S100A8/A9. However, data of S100A8/A9 of other species is still lacking, due to limited availability of the respective protein. We developed an easy and reliable expression and purification method for the production of recombinant human and porcine S100A8/A9 [2]. By immobilized metal ion affinity chromatography, this method takes advantage of the intrinsic, high-affinity metal ion binding properties of S100A8/A9. Using this method, we purified porcine and human S100A8/A9 in high purity and quantity. Growth studies with the purified S100A8/A9 proteins confirmed antimicrobial properties against pathogenic bacteria of porcine S100A8/A9. Our protocol is assumed to be applicable for the purification of S100A8/A9 of several mammalian species, due to the high sequence conservation of the S100A8/A9 metal binding sites. In general, availability of S100A8/A9 of different species allows the assessment and comparison of its antimicrobial properties. This helps the overall understanding of S100A8/A9 and its significance as antimicrobial agent in the context of host-pathogen interaction, particularly in veterinary medicine.

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**IBP011**

**The role of flagella in swarming motility, biofilm formation and virulence of *Paenibacillus larvae***

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The most devastating bacterial honey bee brood disease is American Foulbrood caused by the Gram-positive, spore-forming, peritrichously flagellated bacterium *Paenibacillus larvae* that can be classified into different genotypes using

enterobacterial repetitive intergenic consensus (ERIC) primers. Infection with *P. larvae* begins when the ingested spores of *P. larvae* germinate in the midgut lumen of the honey bee larvae. After massive proliferation of the vegetative bacteria in the midgut, *P. larvae* attacks the midgut epithelium with the help of various virulence factors and thus manages to invade the larval hemocoel, which leads to the death of the diseased larva. *P. larvae* then decomposes the larval carcass into a ropy mass that eventually dries into a scale tightly adhering to the lower rim of the brood cell, consisting of billions of spores. During the pathogenesis of *P. larvae* infection, not only molecular mechanisms of the single bacterial cells play an important role, but also cooperative multicellular behaviours.

For the *P. larvae* genotype ERIC II, we recently demonstrated the cooperative behaviours of swarming and biofilm formation. Since swarming motility is generally driven by the filamentous appendages of bacteria, flagella, which have also been shown to play a role in biofilm formation, we aimed to investigate the role of flagella in swarming motility, biofilm formation, and virulence of *P. larvae* ERIC II.

The flagellin coding gene *flaA* of *P. larvae* was disrupted by intron insertion using the TargeTron™ system (Sigma). Successful construction of the knockout mutant was confirmed at both the protein and cellular levels. The mutant *P. larvae* Δ*flaA* was then compared with wildtype *P. larvae* with respect to their swarming motility and biofilm formation. In addition, an *in vivo* exposure bioassay was performed using honey bee larvae to test whether the *P. larvae* mutant exhibited altered virulence.

We here show that the flagellin-deficient *P. larvae* ERIC II mutant was no longer able to form flagella, and consequently lost the ability to swarm and exhibited altered biofilm formation. Although the bald, flagellin-deficient *P. larvae* ERIC II mutant was still able to kill honey bee larvae, its virulence potential was significantly reduced. We therefore conclude that the flagella of *P. larvae* ERIC II play a key role in multicellular behaviour of the bacteria during pathogenesis, but that these behaviours are not essential for *P. larvae* to kill the host.

**IBP012**

**Staphylococcus aureus maximizes killing of phagocytes by a coordinated biogenesis of purine deoxyribonucleosides**

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*Staphylococcus aureus* is a dangerous pathogen that causes fatal diseases in humans. During infection, *S. aureus* secretes different virulence factors including the key virulence factor adenosine synthase A (AdsA). Together with a secreted nuclease, AdsA generates deoxyadenosine (dAdo) from neutrophil extracellular traps to eradicate phagocytes by targeting the mammalian purine salvage pathway. Here, we used a multi-facetted approach to investigate the enzymatic properties of AdsA in an abscess-like microenvironment and show that the activity of AdsA is not limited to the formation of dAdo. Specifically, we demonstrate that *S. aureus* uses AdsA to generate deoxyguanosine (dGuo), a cytotoxic deoxyribonucleoside that eliminates phagocytes. Based on a genome-wide CRISPR-Cas9 knock-out screen, we further show that dGuo-mediated killing of phagocytes involves uptake of dGuo via human equilibrative transporter 1 (hENT1) and subsequent targeting of the mammalian purine salvage pathway to stimulate caspase-3-dependent cell death. Moreover, we illustrate that synchronous targeting of this route by AdsA-derived dGuo and dAdo boost macrophage cell death. These results suggest that a coordinated biogenesis of purine deoxyribonucleosides by AdsA may maximize staphylococcal survival during persistent infections thereby offering new therapeutic strategies to fight antibiotic-resistant *S. aureus.*

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**IBP013**

**Interaction of emerging *Staphylococcus pseudintermedius* with neutrophil extracellular traps**

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*Staphylococcus pseudintermedius* is an important zoonotic pathogen that causes severe diseases in companion animals. Moreover, this microbe can cause skin, catheter-, or implant-associated infections as well as life-threatening diseases in humans. However, bacterial and host determinants that may affect the pathogenesis of *S. pseudintermedius* infections in animals or humans are undefined. Specifically, the interaction of *S. pseudintermedius* with antimicrobial neutrophil extracellular traps (NETs) remains elusive. Here, we seek to explore mechanisms of *S. pseudintermedius* to escape from NET-mediated entrapment and killing. Our results indicate that exposure of *S. pseudintermedius* to primary human neutrophils triggers the release of NETs which are subsequently degraded by a major secreted nuclease of *S. pseudintermedius*. Consequently, nuclease-deficient *S. pseudintermedius* were more susceptible to extracellular killing by activated neutrophils. Thus, *S. pseudintermedius* requires nuclease secretion to escape from NET-mediated killing, presumably supporting pathogen survival during infections. Further, these results may help to develop new therapeutic approaches to combat infections caused by methicillin-resistant *S. pseudintermedius.*

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**IBP014**

**The TAL-effector TalAO from *Xanthomonas oryzae pv. oryzae* induces a phosphate exporter to enable bacterial growth during the infection of rice**

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Introduction: Rice-pathogenic *Xanthomonas oryzae pv. oryzae* (*Xoo*) bacteria infect the xylem of rice leaves and cause bacterial blight disease and severe harvest loss. Virulence of *Xoo* depends on so- called TALEs (transcription activator-like effectors), which are delivered by a type III secretion system and act as transcriptional activators in plant cells. TALEs exhibit a unique repetitive DNA- binding domain with a clearly predictable binding specificity. Based on this region, TALEs can be grouped into classes. The TALE class TalAO is present in more than 80 % of sequenced *Xoo* strains and constitutes one of the core classes, but its role in virulence is not clear.

Objectives: We aim to decode TALE repertoires, identify their targets and understand their contribution to bacterial virulence. For this, we established Nanopore sequencing to read large repetitive clusters of TALE genes. We further analyzed the activity of the core TALE TalAO.

Materials & Methods: High molecular weight genomic DNA of Xoo was prepared using magnetic beads and sequenced on a Nanopore flow cell (Erkes et al, 2023). Deletion of the OsPHO1;3 gene in rice was achieved by *agrobacterium*-mediated transformation of rice calli with a construct containing Cas9 and sgRNAs targeting upstream and downstream regions of OsPHO1;3 simultaneously. Rice plants were grown in hydroponic solution with or without phosphate and infected with *Xoo* via leaf-clipping.

Results: We decoded complex TALE clusters successfully, by implementing Oxford Nanopore sequencing in combination with an algorithm to correct sequencing errors in TALE genes. TalAO was shown to induce expression of the rice gene OsPHO1;3 encoding a phosphate exporter. To understand how this contributes to virulence, we deleted the OsPHO1;3 gene in rice using CRISPR/Cas9. Bacterial proliferation in this mutant plant was normal under standard conditions, but severely restricted when the plants were grown under phosphate starvation.

Conclusion: Here, we show that *Xoo* are limited in multiplication in rice leaves if they can not induce expression of a phosphate exporter under phosphate limiting conditions. We conclude that the virulence function of TalAO is to supplement the bacteria with phosphate during the infection.

Erkes, A., Grove, R.P., Žarković, M. *et al.* Assembling highly repetitive Xanthomonas TALomes using Oxford Nanopore sequencing. *BMC Genomics* **24**, 151 (2023). https://doi.org/10.1186/s12864-023-09228-1

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**IBP015**

**Balance in plant bacterial recruitment affects resilience to fungal invasion of the phyllosphere**

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Introduction: Plant health and productivity depend on the interactions between plants and their microbial colonizers. Disruptions to the stability of the plant microbiome can lead to disease, while a stable and diverse microbiome can have protective effects against pathogens through plant-microbe-microbe interactions. The necrotrophic fungal pathogen *Sclerotinia sclerotiorum* (Ssc) is one example of a pathogen that can cause significant damage to plants, underscoring the importance of understanding the processes governing microbiome assembly and stability.

Objectives: In this study, we investigated how host barriers to bacterial colonization shape microbial interactions contributing to plant resilience against a fungal pathogen invasion.

Methods: A mutant (CLLF) of a local *A. thaliana* ecotype (NG2) was created through EMS mutagenesis. CLLF leaf bacteria were tested for their ability to inhibit Ssc *in vitro*. Amongst the tested isolates, the most inhibiting strain, *Stenotrophomonas* sp. SrG, was selected for *in planta* biocontrol experiments using a pseudo-gnotobiotic setup. The effects of live and heat-killed bacterial inoculums on Ssc leaf lesion size were analyzed, and phytohormone levels were evaluated via LC-MS/MS. Confocal fluorescence microscopy was used to observe epiphytic colonization of the leaf and co-localization of labeled bacteria with fungal hyphae in the necrotic tissue of Ssc-infected leaves.

Results: When grown under natural conditions, CLLF exhibits a strongly increased leaf bacterial load but no disease symptoms or reduced survival, indicating a rebalance of the microbiome. LC-MS/MS analysis revealed increased levels of plant immunity-related phytohormones. SrG protected against Ssc *in planta* in the wild-type. Protective effects of live SrG *in planta* were not significant in the mutant, while heat-killed SrG significantly reduced Ssc lesions in both genotypes. Reduced JA levels were observed in CLLF in response to SrG before pathogen invasion. Co-localization of SrG with Ssc hyphae was only observed in the mutant, suggesting that altered (plant-)microbe-microbe interactions influence live SrG protection against Ssc.

Conclusion: Plant host factors may select for different microbial interactions in the phyllosphere. The findings of this study suggest that colonization barriers and plant immune responses may not only limit colonization but also influence inter-microbial interactions that shape plant health.

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**IBP016**

**Zinc transporter MptABC is required for *Mycobacterium avium* subspecies *paratuberculosis* fitness and virulence**

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*Mycobacterium avium* ssp. *paratuberculosis* (MAP), the causative agent for a progressive, fatal enteritis in ruminants, is able to survive and replicate in the phagosome of intestinal macrophages, despite macrophage phagosomal defence mechanisms such as zinc deprivation. Hence, MAP zinc importers seem to be of considerable importance for its survival. In mycobacteria, zinc homeostasis is regulated by the zinc sensing antagonists SmtB and the global zinc uptake regulator Zur. They allow expression of zinc exporters and importers, respectively, to maintain appropriate zinc levels. Our previous works showed that MAP is exceptionally well equipped with zinc uptake systems, of which two are MAP specific: the Zur regulated MptABC (*map*3736c-34c) and Zur independent ZnuABC-like (*map*3776c-74c) [1-2]. Among those, expression of *mpt*A and other zinc starvation marker genes were induced in MAP in the absence of zinc and also after infection of macrophages.

Due to the importance of zinc in host-pathogen interaction it is of special interest to further characterize the role of MptA for MAP pathogenicity.

A MAPΔ*mpt*A mutant and the complemented strain, MAPΔ*mpt*A$^{C}$, were constructed. Growth experiments were performed in different media with or without zinc supplement for 4 weeks. To analyse survival of the mutant, mouse macrophages were infected with MAPwt and MAPΔ*mpt*A and were cultivated under either elevated zinc concentrations or standard conditions. Survival was monitored after 2 and 72 h by determination of CFU. The relevance of MptABC for MAP pathogenicity was investigated in a mouse infection model. Mice with normal or elevated zinc serum levels were infected with MAPwt and MAPΔ*mpt*A for 4 weeks. After sacrifice, liver and spleen were disrupted, plated on MB agar for determination of CFU and zinc levels in serum of mice were analysed.

Growth of MAPΔ*mpt*A was not affected in full medium, but a severe growth defect was observed in low zinc Watson Reid medium, which could be rescued upon complementation or zinc supplementation. Survival and virulence of MAPΔ*mpt*A was strongly reduced in macrophages and mice, respectively, even though complementation could not restore MAPwt phenotype in mice. Zinc feeding of mice did not change survival of MAPwt or MAPΔ*mpt*A.

Our analyses strongly indicate that MptABC is required for full biological fitness of MAP in culture, in macrophages and for virulence in mice.

1. doi.org/10.1128/JB.00049-21
2. doi:10.1186/1471-2164-15-1076

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**IBP017**

**Temperature-dependent fis control: Unraveling its influence on motility and virulence in *Yersinia***

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As a pathogenic bacterium, *Yersinia pseudotuberculosis* has to cope with adverse environmental stresses and host defense mechanisms. For a quick adaption to environmental changes, many complex regulatory networks and mechanisms are needed to ensure adequate regulation of gene expression and precise coordination of the infection machinery. Among these mechanisms, changes in the DNA

topology of specific chromosomal regions (e.g., gene promoters) as well as global changes in DNA architecture are mediated by nucleoid-associated proteins (NAPs). These NAPs function as transcription factors and/or chromatin remodeling enzymes and consequently regulate gene expression. Fis, a transcriptional regulator and one of the most abundant proteins in bacteria, regulates gene expression by binding and bending DNA.

To gain a comprehensive insight into the global effects of Fis on *Y. pseudotuberculosis* at ambient and host body temperature, we conducted RNA-seq analyses in the wild-type and a Δ*fis* mutant at 25 and 37 °C. Remarkably, the results uncovered more than 300 regulated genes that were distributed over 14 functional gene categories. Genome-wide analysis of Fis-binding motifs and comparison with phenotypic characterization approaches revealed that the absence of Fis causes significant physiological consequences in *Yersinia*. More precisely, Fis positively regulates chemotaxis- and motility-associated genes and inhibits the expression of many virulence genes located on the virulence plasmid at 25 °C. In summary, our findings demonstrate that Fis plays a crucial role in repressing the expression of virulence factors, such as the main virulence regulator LcrF at 25 °C. Our results suggest that Fis is a critical modulator of pathogenicity in *Yersinia* and shed light on the complex regulatory networks underlying bacterial virulence.

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**IBP018**

**Rifampicin tolerance and growth fitness among isoniazid-resistant clinical *Mycobacterium tuberculosis* isolates: An in-vitro longitudinal study**

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Introduction: Antibiotic tolerance in *Mycobacterium tuberculosis* leads to less effective bacterial killing, poor treatment responses and resistant emergence. There is limited understanding of antibiotic tolerance in *M. tuberculosis*.

Objective: We investigated the rifampicin tolerance of *M. tuberculosis* isolates, with or without pre-existing isoniazid-resistance.

Materials and methods: *In-vitro* rifampicin survival fractions were determined using a minimum duration of killing assay, for isoniazid susceptible (IS, n=119) and resistant (IR, n=84) *M. tuberculosis* isolates. Rifampicin tolerance was correlated with bacterial growth, rifampicin minimum inhibitory concentrations (MIC) and isoniazid-resistant mutations. The longitudinal isoniazid-resistant isolates were analyzed for rifampicin tolerance based on collection time from patients and associated emergence of genetic variants.

Results: The median duration of rifampicin exposure reducing the *M. tuberculosis* surviving fraction by 90% (minimum duration of killing-MDK90) increased from 1·23 (95%CI 1·11; 1·37) and 1·31 (95%CI 1·14; 1·48) to 2·55 (95%CI 2·04; 2·97) and 1·98 (95%CI 1·69; 2·56) days, for IS and IR respectively, during 15 to 60 days of incubation respectively. The increase in MDK90 time indicated the presence of fast and slow growing tolerant sub-populations. A range of 6 log10-fold survival fraction enabled classification of tolerance as low, medium or high and revealed that isoniazid-resistance associates with increased tolerance with faster growth (OR=2·68 for low vs. medium, OR=4·42 for low vs. high, *P*-trend=0·0003). The high tolerance of longitudinal isoniazid-resistant isolates was specific to those collected during rifampicin treatment in patients and was associated with bacterial genetic microvariants.

Conclusions: Our study identifies a range of rifampicin tolerance and reveals that isoniazid resistance is associated with higher tolerance with growth fitness. Furthermore, rifampicin treatment may select isoniazid-resistant isolate microvariants with higher rifampicin tolerance, with survival potential similar to multi-drug resistant isolates. These findings suggest that isoniazid-resistant tuberculosis needs to be evaluated for rifampicin tolerance or needs further improvement in treatment regimen.

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**IBP019**

**The metalloprotease immune inhibitor A – A potential virulence factor for *Paenibacillus larvae*?**

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Worldwide, considerable losses of honey bee colonies are caused by the gram-positive, spore forming bacterium *Paenibacillus larvae,* the causative agent of American Foulbrood (AFB). There are two epidemiologically relevant genotypes of *P. larvae*, ERIC I and ERIC II, which differ in their virulence at both the individual larva and bee colony level. In the last two decades, considerable progress has been made in identifying and functionally characterizing several species- and genotype-specific virulence factors according to which *P. larvae* ERIC I and ERIC II follow different pathogenesis strategies leading to the observed differences in virulence. To further our understanding of the molecular basis of the interaction between *P. larvae* and its host, the honey bee larvae, we continued our quest for putative genotype-specific virulence factors. We here present our data on the identification of an M6-metalloprotease, exclusively expressed by *P. larvae* ERIC II and highly homologous to immune inhibitor A (InhA) originally identified in the *Bacillus cereus* group. Proteases have been suspected to be relevant virulence factors of *P. larvae* for decades and InhA-homologues have been shown to play a role in virulence in other bacterial species. Therefore, the aim of this work was to functionally characterize *P. larvae* ERIC II InhA and to elucidate its role during pathogenesis of *P. larvae* ERIC II infection.

To this end, we generated a gene inactivation mutant for *inh*A in *P. larvae* ERIC II (ERIC II Δ*inh*A) and performed exposure bioassays with wild type *P. larvae* ERIC II and *P. larvae* ERIC II Δ*inh*A. We recombinantly expressed InhA as GST-fusion protein in *E. coli* and HIS-fusion protein in *B.*

*subtilis* which were subsequently purified using GST – and IMAC-Nickel-columns, respectively, for functional testing.

The larval exposure bioassays revealed that InhA is a virulence factor of *P. larvae* ERIC II, since *P. larvae* ERIC II Δ*inh*A showed a significantly reduced virulence compared to the corresponding wild type strain. First functional tests of the recombinant InhA for protease or collagenase activity were performed using substrate gels impregnated with casein or gelatine, respectively. No activity could be demonstrated so far, hence, the substrate of *P. larvae* ERIC II InhA is still elusive and will be identified in further assays.

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**IBP020**

**Preclinical evaluation of phospholipase PlaF and the membrane phospholipid remodeling pathway as an antibiotic target**

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*Pseudomonas aeruginosa* is a Gram-negative pathogen known for its ability to adapt to hostile environments and rapidly develop antibiotic resistance. Therefore, the development of novel antibiotics for the treatment of *P. aeruginosa* infections has become increasingly important. In this study, we focused on PlaF, an intracellular phospholipase A1 that we recently discovered and demonstrated its function in the glycerophospholipid remodeling pathway. Through various virulence and biofilm assays, we established PlaF as a potent determinant of *P. aeruginosa* virulence, which is highly conserved among significant pathogens, making it a potential target for broad-spectrum antibiotics.

We conducted pre-clinical repurposing studies to evaluate the PLA-mediated phospholipid remodeling pathway, with specific focus on PlaF as a drug target. To identify potential PlaF inhibitors, we performed a structure-based search among 25,349 compounds approved for clinical use or under preclinical investigation. Among the 23 target compounds tested, we identified 12 inhibitors of *in vitro* PlaF activity, four of which significantly ($p < 0.05$, n = 9) enhanced *P. aeruginosa* biofilm formation (35 – 51% compared to the non-treated samples), while three inhibited *P. aeruginosa* growth under planktonic conditions (33 – 57%). Notably, a concentration of 200 µM of the human sphingomyelinase inhibitor, GW4869, inhibited PlaF activity and *P. aeruginosa* growth, while enhancing the production of *P. aeruginosa* biofilm.

Furthermore, we assessed the synergistic effects of specific drugs targeting PlaF and/or *P. aeruginosa* on four commonly used antibiotics. The findings demonstrated that the combination of the human lipoprotein-associated phospholipase A2 inhibitor, Rilapladib (100 mg/l), and the last-resort antibiotic, imipenem (2 mg/l), when added to the culture medium, completely eradicated the growth of *P. aeruginosa*. In contrast, when each compound was added separately to the medium, *P. aeruginosa* continued to grow.

Further investigations are required to understand the underlying mechanisms by which these compounds affect *P. aeruginosa* and to explore their potential applications as antibiotics or antibiotic adjuvants.

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**IBP021**

**Sugar uptake of the brassicaceae smut fungus Thecaphora thlaspeos during biotrophic interactions**

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In plants, sugar allocation and transport play a vital role in survival, signaling and immune responses. During pathogen infection, sugar transport changes by defense responses to fight off the invader and through pathogenic sugar uptake. Smut fungi like *Ustilago maydis* or *Thecaphora thlaspeos* are biotrophic pathogens, depending on living plant tissue to spread disease. Smut fungi are of agricultural importance, as they infect crop plants, and they are model organisms in research about plant-pathogen interactions. This study aims at understanding sugar allocation during smut fungal infection.

The sugar transporters of the maize smut *U. maydis* and the Brassicaceae smut *T. thlaspeos* are compared. Different infection styles suggest variation in nutrient allocation, with *U. maydis* producing plant tumors, while *T. thlaspeos* grows asymptomatic and systemically. In *U. maydis*, **S**uc**r**ose-**t**ransporter-**1** (Srt1) and **H**e**x**ose-**t**ransporter-**1** (Hxt1) are responsible for pathogenic sugar uptake and have homologs in *T. thlaspeos*. Complementation of a *U. maydis* deletion strain with *T. thlaspeos* Hxt1 variants supports conserved transporter and sensor function. Hence, sugar uptake and sensing by Hxt1 is conserved, although the different infection styles, indicating higher conservation of sugar sensing in smut fungi.

Annotation of the genome of *T. thlaspeos"* host *Arabis hirsuta* revealed 57 sugar transporters, of which 49 have homologs in *Arabidopsis*. In *Arabidopsis*, downregulation of SWEET12 was observed during colonization with a beneficial fungus *Trichoderma harzianum* to restrict sugar loss from plant to fungus and initial analysis in *T. thlaspeos* infected *Ar. hirsuta* suggests a similar regulation. Further differential gene expression analysis of our published RNASeq data will allow to identify more plant candidate transporters induced or repressed during infection.

To test these transporters experimentally, an infection system for *T. thlaspeos* culture was established, enabling analysis of genetically modified fungal strains. Infection showed culture and spore infection are comparable during early stages in *A. thaliana* and *Ar. hirsuta*. Using this method, *T. thlaspeos* infection of *A. thaliana* sugar-transport-mutants will give more insights about the key-players of pathogenic sugar uptake.

Ultimately, a sugar-transporter-atlas showing the expression of transporters in different plant organs will reveal specificity of *T. thlaspeos* sugar uptake during endophytic growth.

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**IBP022**

***Tet*-controlled gene expression in *Stenotrophomonas maltophilia***

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Introduction: *Stenotrophomonas maltophilia* is increasingly recognized as an underscored nosocomial pathogen among the Gram-negative bacteria (1). Intrinsic resistance to different classes of antibiotics makes treatment of infections challenging. A deeper understanding of *S. maltophilia* physiology and virulence requires molecular genetic tools.

Objective: We here describe the implementation of tetracycline-dependent gene regulation (*tet*-regulation (2)) in *S. maltophilia*.

Materials & Methods: A *tet*-regulatory region from transposon Tn*10* was cloned into a broad host-range plasmid and was tested in *S. maltophilia* with a *gfp* variant as a quantifiable reporter. Also, the *rmlBACD* operon, encoding factors for the synthesis of O-antigen, was cloned into a plasmid to obtain *tet*-control in an *S. maltophilia rml* deletion mutant. Plasmids were introduced into strain K279a by triparental mating using one *E. coli* donor strain and one *E. coli* helper strain. Synthesis of O-antigen was tested by SDS-PAGE of purified lipopolysaccharide (LPS) and silver-staining or Western-Blot.

Results: In case of the GFP reporter, fluorescence intensity was directly correlated to the concentration of the inducer anhydrotetracycline (ATc) and the duration of induction. When regulation of the *rml* operon was investigated, the LPS pattern was similar to that of wild-type *S. maltophilia* in presence of ATc, whereas without inducer, less and apparently shorter O-antigen chains were detected.

Conclusions: Our findings underscore the functionality and usefulness of the *tet*-system for gene regulation, and prospectively, the validation of targets for new anti-*S. maltophilia* drugs.

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**MBEP001**

**Isolation, identification and functional characterization of microorganisms from traditional kefir grains**

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Kefir is a complex fermented dairy product produced through the symbiotic fermentation of milk by lactic acid bacteria and yeasts present in a natural polysaccharide and a protein matrix (kefir grain). Kefir consumption has been associated with a range of health benefits based on its antimicrobial activity, antioxidative, cholesterol metabolism and angiotensin-converting enzyme inhibition. This research project aimed at evaluating the antimicrobial and antioxidant properties of both the milk fermented by- and microorganisms isolated from kefir grains. By combining physiological and genomic approaches, probiotic microorganisms from kefir grains were identified and characterized. The kefir supernatant, as well as bacteria and yeast strains isolated from fermented milk kefir grains were screened for antibacterial activity., High inhibitory activity against Gram-positive (*Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) was observed by the diameter of inhibition zone. The isolates were tested for their antioxidant activity using 1,1-diphenyl-2-picryl-hydrazine (DPPH) and Ferrous chelating activity assays. The results demonstrated that the bacterial isolate *Lentilactobacillus kefiri* and the yeast isolate *Kazachstania unispora* have high antioxidant activity. All the isolates showed good survival rates in simulated gastrointestinal tract conditions, that is, by probing acid and bile salt tolerance, as indicator for their probiotic properties. It can be concluded that bacteria and yeast isolated from our kefir grains serve as promising candidates for probiotics foods and beverage since they exhibit potential probiotic properties and antioxidant activities. Our findings will contribute to studies on the development of kefir starter cultures.

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**MBEP002**

**From subsurface to the termite gut – Novel isolates expand our knowledge on the distribution and diversity of the planctomycetal order *Isosphaerales***

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Microbial community studies of various habitats have reported for many years that bacteria of the phylum *Planctomycetota* are ubiquitous. However, planctomycetal axenic cultures have been scarce for decades. This only changed recently with the valid description of more than 70 novel species belonging to more than 30 novel genera. Within the class *Planctomycetia* the described phylogenetic diversity of three out of four known orders (*Planctomycetales*, *Pirellulales* and *Gemmatales*) has been enormously expanded. However, until today the order *Isosphaerales* comprises only six genera for each of which only one or two representative species have been validly published, yet. Here, we present five additional novel isolates from various environmental samples belonging to the family *Isosphaeraceae,* the currently sole family of the order *Isosphaerales*. The five strains were isolated using our deep cultivation approach and characterized. The genomes were assembled from Oxford nanopore long reads and subsequently polished with Illumina short reads. Phylogenomic analyses revealed that three of the isolates represent novel genera according to thresholds of established phylogenetic markers. The proposed type species of the novel genus *Kueselia (Kueselia aquiterrae*, type strain EP7$^T$) is the first member of the phylum *Planctomycetota* that has ever been isolated from subsurface water. The proposed type strain TA3$^T$ of the type species of a second novel genus *Isopterasphaera* has been isolated from the gut of a fungus-farming termite living in South Africa. The third proposed type strain SH657$^T$ of a species belonging to a third novel genus was isolated from leakage water of a garbage pile in the 1980s by the microbiologist Dr. Heinz Schlesner (Christian-Albrechts University of Kiel) – a pioneer in the isolation and cultivation of several budding and prosthecate bacteria including

members of the phylum *Planctomycetota*. Two more strains of two novel species of the known genus *Paludisphaera* were also revived from Schlesner`s strain collection comprising in total around 400 strains. We performed comparative genomics with at least one representative of all nine genera within the *Isosphaeraceae* and evaluated the biosynthetic repertoire. We also computed the pangenome of the family to identify habitat generalists as well as habitat-specific adaptions. Our findings support the well-known Baas Becking hypothesis that "everything is everywhere, but the environment selects".

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**MBEP003**

**Discovery of coerumycin, a novel cinnamycin analog from an *Actinomadura coerulea* strain isolated from meerkat feces**

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The rapid global spread of antibiotic resistance emphasizes the need for new effective therapeutics. The isolation of strains, e.g. of the actinomycetes, from unexplored habitats could potentially provide access to new or endemic species as the new sources for novel secondary metabolites. Here, we investigate a new antibiotic-producing isolate from a meerkat (*Suricata suricatta*) fecal sample with regard to its antimicrobial activity.

Diluted fecal samples were plated on MYM agar. Potential actinobacteria were isolated based on their typical colony and cell morphology. The isolate was phylogenetically classified based on the 16S rRNA gene sequence. Mature colonies on MYM agar plates were overlayed with soft agar containing Gram-negative, Gram-positive or fungal species as indicator strains. Cell extracts from liquid cultures as well as colonies were further analyzed regarding their antibiotic spectrum by applying a panel of whole cell biosensors, in which an antibiotic-inducible promoter is fused to the luciferase cassette. The potential biosynthetic gene clusters (BGCs) involved in the production of antibiotic compounds were identified by genome mining. During the plate-based biosensor screens, we identified naturally resistant *Bacillus subtilis* colonies growing in the zone of inhibition, which were further analyzed. After repeating rounds of selection, highly resistant spontaneous mutant strains were subjected to whole genome sequencing.

16S rDNA sequencing identified the isolate as *Actinomadura coerulea*. The isolate showed a strong and broad range of antibacterial activity against Gram-positive bacteria, including pathogens, as well as an antifungal effect on the growth of *Penicillium chrysogenum*. The secondary metabolites of the isolate potentially inhibit replication, block translation, and interfere with bacterial cell wall integrity. Amongst others, we identified loss of function mutations in *pssA*, encoding a phosphatidylserine synthase. Based on the function of PssA and genome mining, the BGC for a novel cinnamycin analog was identified. Detailed follow-up studies are currently ongoing.

Our approach demonstrates that combining the exploration of niche habitats for actinomycetes with whole-cell biosensor screening and characterization of natural resistance development provides a promising strategy for identifying novel antimicrobial compounds.

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**MBEP004**

**A microfluidic chip for adaption and selection of whole-cells**

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Introduction: Adaptive laboratory evolution (ALE) is widely used in biotechnology and a key tool to study fundamental evolution processes. Conventional experimental setups for ALE lack some features that are common in nature such as spatial physico-chemical gradients. Microfluidics can overcome these limitations as it can mimic naturally occurring microenvironments creating microbial population heterogeneities ranging from planktonic cells to biofilm states.

Objectives: The ability to generate spatially defined gradients of stressors in microfluidic chip systems is still limited and the screening of adapted mutants in these systems is not well optimized. Therefore, the aim was to develop a miniaturized ALE chip design that employs adjustable stressor profiles creating an inflow-gradient which enables an efficient on chip screening of the complete cell population.

Materials & methods: In this poster presentation a microfluidic chip is presented which contains microcompartments and that can be used to perform long-term ALE experiments to adapt microbial cells to defined profiles of stressors such as antibiotics. [1]

Results: The controlled change in the concentration of the antibiotic in the successive compartments of the chip leads to an adaptation of the bacteria to the presence of the antibiotic and also enables the distinction between persistent and resistant cells. Importantly, previously unknown mutations in *Escherichia coli* conferring resistance to nalidixic acid were discovered via adaptation experiments employing the chip. Recently, using this microfluidic device, the thermophilic organism *Thermus thermophilus* was successfully adapted to kanamycin.

Conclusion: The novel microfluidic chip presented on this poster thus enhances the occurrence of mutations employing cultivation under quasi-static conditions with tunable and spatially stable concentrations. This kind of miniaturized chip-based ALE can be applied to generate data to better understand the mechanism of antibiotic resistance and adapt virtually any microorganism to changes in environmental conditions.

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**MBEP005**

**Comparative genomics reveals early speciation in aquatic bacteria with implications for bacterial systematics**

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Early speciation events during bacterial evolution were elucidated using the limnic freshwater bacterium *Sphingorhabdus planktonica* as a model system. Isolates of the phylotype G1A of this species had identical 16S rRNA

sequences but exhibited distinct substrate utilization patterns and growth rates. In addition, their populations showed different seasonal fluctuation patterns in the lakes they were isolated from, indicating that they occupy different ecological niches. De *novo* complete genome analysis and comparative genomics were used to analyze the evolutionary history of 36 *S. planktonica* isolates in detail. They revealed that these strains belong to eight genomically distinct groups within the same phylotype. Four of these groups were also distinguishable by established ANI and dDDH methods. Phylogenomic network analyses of the core genomes showed that recombination events occurred mostly within the eight designated genomic groups but only rarely between them. A comprehensive analysis of horizontal gene transfer demonstrated that transposases constituted the main mechanism of genomic rearrangement, which occurred mostly near insertion sequences and tRNAs. Analyses of functional gene contents resulted in the identification of several metabolic pathways that were either strain- or group-specific. In particular, we discovered the genetic potential for the degradation of aromatic compounds, which had not been previously reported for members of this genus. The *Sphingorhabdus* model system allowed us to identify early processes in genome evolution which underlie the genetic separation, diversification, and ultimately the speciation of bacteria. These insights need to be considered in bacterial systematics beyond current 16S rRNA gene comparisons and genome-to-genome hybridizations.

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**MBEP006**

**A novel clade of fastidious purple sulfur bacteria exhibits unique capabilities for harvesting near infrared light**

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The conversion of light to biological energy through photosynthesis powers most ecosystems by providing the bulk of organic material to the base of each foodweb. While various photosynthetic prokaryotes have developed specialized photosystems to harvest particular wavelengths of visible light and thereby avoid competition, little is known on photosynthetic bacteria capable of exploiting the near-infrared spectrum ranging between 900-1000 nm.

A purple-sulfur bacterium (PSB) isolated from the shoreline of Baltrum, a North Sea island of Germany, was found to contain bacteriochlorophyll *a* (BChl *a*) as its primary pigment similar to other PSB, but had a light harvesting complex (LH) that absorbed light maximally at 966 nm, which represents the furthest infrared-shift documented to date for Bchl *a*-containing photosystems. Through a polyphasic approach that encompassed complete genome sequencing, as well as biochemical and physiological properties, this strain 970 was found to be a relative of *Thiorhodovibrio winogradskyi* DSM $6702^T$ by 26.5, 81.9 and 98.0% similarity via dDDH, ANI, and 16S rRNA gene comparisons, respectively. The photosynthetic properties of strain 970 were unlike any *Thiorhodovibrio* spp., as they contained typical LH absorbing characteristics of 800-870 nm, as well as a newly discovered absorption band at 908 nm. The photosynthetic operon composition was also strikingly different between strain 970 and the other species of the genus. Upon genomic comparisons of the original *Thiorhodovibrio* strains DSM $6702^T$ and strain 06511, the latter was found to be divergent, with 25.3, 79.1, and 97.5% similarity via dDDH, ANI, and 16S rRNA gene homology to *Trv. winogradskyi*, respectively. Strain 06511 was thereby described as *Thiorhodovibrio litoralis* sp. nov., and the unique strain 970 as *Thiorhodovibrio frisius* sp. nov.

The new species *Trv. litoralis* and *Trv. frisius* represent novel taxons of the same genus that individually show distinct and unusual absorption bands between 900 and 1000 nm. These novel phototrophs aid our understanding of photoautotrophy and the evolution of photosynthesis, and expand upon the known wavelengths of light capable of supporting life.

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**MBEP007**

**Convergent evolution in a novel lineage of *Opitutales* (*Verrucomicrobiota*) and other endosymbionts of termite gut flagellates**

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Cellulolytic flagellates perform a key role in the lignocellulose digestion of lower termites. Each flagellate species is colonized by diverse bacterial ecto- and endosymbionts from different, only distantly related phyla. In a recent metagenomic study of 78 termite species, which recovered more than 2000 metagenome-assembled genomes (MAGs), we identified several previously unknown lineages of endosymbiotic bacteria in the phyla *Bacillota*, *Pseudomonadota* and *Verrucomicrobiota*. The latter are represented by 82 MAGs that form a novel family-level lineage in the order *Opitutales*. Comparative genome analysis with other *Opitutales* revealed a severe genome reduction (average genome size 1.68 vs. 2.98 Mbp) and substantial gene losses in carbon and energy metabolism, which were accompanied by the acquisition of a nucleotide triphosphate transporter and numerous transporters for the uptake of sugar phosphates, amino acids and vitamins. A comparison with the gene functions encoded by the MAGs of other, established or putative endosymbionts revealed strong patterns of evolutionary convergence in different, only distantly related lineages of *Actinomycetota* (*Ancillulaceae*), *Desulfobacterota* (*Adiutricales*), *Elusimicrobiota* (*Endomicrobiaceae*), *Bacillota* (*Acutalibacteriaceae*), and *Pseudomonadota* (*Rickettsiales*, RUG11792, UBA3830, UBA9339). Convergent traits include the acquisition of gene functions for the uptake and subsequent conversion of alternative substrates, such as sugar phosphates and hexuronic acids, and in many cases, an ADP/ATP antiporter, which are driven by lateral gene transfer and compensate for functional losses due to an ongoing genome erosion.

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**MBEP008**

**Natural transformation in non-model cyanobacteria**

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Introduction: Recombinant work in new cyanobacteria strains and species is often restricted by the delivery of DNA into the cell. Conjugation, electroporation, and natural transformation are the most commonly used techniques for this purpose. The ability to take up DNA from the environment via natural transformation, termed natural competence, relies in gram negative bacteria on the type IV pilus and additional natural competence specific proteins. Within the cyanobacterial phylum this complete set of the respective genes for natural competence is present in the majority of species, except members of the picocyanobacteria. However, experimental evidence of natural competence, demonstrating DNA uptake

through natural transformation in cyanobacterial strains, is limited to only a few species.

Objectives: The aim of this study is to test for natural transformation in cyanobacteria that belong to parts of the phylum where natural competence has not been previously reported.

Patients & Methods: For this study, we utilized two strains from the Pasteur Culture collection of Cyanobacteria (PCC): *Spirulina major* PCC 6313 and *Chlorogloeopsis fritschii* PCC 6912. The DNA uptake capability was assessed by introducing plasmids encoding antibiotic resistance genes, followed by antibiotic selection.

Results: Here, we show DNA uptake through natural transformation in two filamentous cyanobacterial species. Transformation into *S. major* was established with a plasmid designed for chromosomal integration via homologous recombination. To our knowledge, this is the first report of natural transformation in the order of Spirulinales. Transformation of *C. fritschii* is the first demonstration of natural competence in subsection V and was established with the replicating plasmid pRL25C. While transformation of *C. fritschii* can be achieved with 200 ng of plasmid or more, the lowest tested amount of plasmid DNA, which resulted in the transformation of *S. major*, is 1 ng.

Conclusion: The presence of the complete set of natural competence genes in numerous cyanobacteria, together with rising number of experimentally validated examples of natural transformation, underline the relevance of this trait in major parts of the phylum. From an application perspective, more cyanobacteria species could be made accessible for recombinant work by natural transformation and we recommend including the screening for natural competence in the criteria for choosing new production strains.

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**MBEP009**

**Insights into the diversity and fitness of the potential nasal probiotic *Corynebacterium accolens***

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Introduction: The nasal microbiome plays a crucial role in safeguarding the respiratory tract from invasive pathogens. One of the most abundant nasal microbiome members is *Corynebacterium accolens*, a Gram-positive rod-like bacterium that was first isolated in 1943 and was found to grow near *S. aureus,* therefore named after the Latin word "*accola*" (neighbour). In 2016, Bomar *et al.,* have shown that *C. accolens* can degrade lipids in the nose, thereby releasing anti-*pneumococcal* oleic acid*.* It has also been suggested the ability of *C. accolens* to protect the mucosal barrier from *S. aureus*-induced damage. The closest relative of *C. accolens* is *Corynebacterium macginleyi,* which is known to be an ocular pathogen, and its presence in the nose is hardly reported. Despite the potential benefits of *C. accolens*, its nasal dominance remains poorly understood.

Objectives: This study aims to investigate the genetic divergence of the two species and to identify putative fitness factors of *C. accolens* by comparing the two closely related species.

Materials & methods: In total, 30 *C. accolens* and 25 *C. macginleyi* genomes were retrieved from public databases and in-house sequencing. From these, 12 *C. accolens* and 13 *C. macginleyi* isolates, collected from different countries and years were available for experimental analyses. After phylogenetic and comparative genomics analysis, wet-lab experiments were performed to confirm metabolic differences between the two species.

Results: Initial results highlighted that *C. accolens* is a more diverse species with a smaller mobilome and a bigger pangenome than C. *macginleyi*. Interestingly, adaptation of *C. accolens* to the nasal habitat seems to be facilitated due to modification of lipid metabolism and iron acquisition systems. The latter was of special interest, as it shows that *C. accolens*, over reductive evolution, lost its siderophore production and depends on other siderophore-producing nasal commensals for their establishment.

Conclusion: *C. accolens* is a diverse nasal bacterial species and a highly effective cheater for siderophores.

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**MBEP010**

***E. coli* colony growth, filamentation and resistance evolution under tetracycline and *acrAB-tolC*multidrug efflux pump overexpression**

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Introduction: Efflux pump mediated resistance is an intrinsic mechanism allowing bacterial cells to survive under antibiotic stress. Efflux pumping can inhibit neighbouring cells and also consumes cellular energy, altering bacterial physiology. These effects may alter the fitness landscapes for further resistance evolution.

Objectives: To investigate colony growth, cellular adaptations and evolution under tetracycline in *E. coli* wildtype and efflux pump lacking/overexpressing strains.

Materials and methods: *E. coli* strains MG1655, and strains with efflux pump gene overexpression LM202 (Δ *marR*), JW0453 (Δ *acrR*) or deletion strains JW0452 (Δ *acrA*), and JW0451 (Δ *acrB*) were used in this study. All strains were cultured in Lurie-Bertani (LB) medium or agar at $37^0$C with (RPM 200) or without shaking. Exponential phase *E. coli* strains cultured in LB medium were treated with different concentrations of tetracycline (Sigma-Aldrich) or plated on LB agar with tetracycline and incubated at $37^0$C. Cellular morphology and colony growth were imaged by microscopy (Nikon Ti).

Results: For the wild-type strain MG1655 the minimum inhibitory concentration (MIC) of tetracycline was 1.25 mg/mL, inhibiting bacterial growth completely, whereas LM202 had a higher MIC for tetracycline at 3.0 mg/mL. LM202 showed delayed growth at tetracycline concentrations 2 and 2.5 mg/mL after 20-22 hrs, and microscopic observations revealed bacterial population with cell length heterogeneity and significant filamentation. LM202 growth on LB agar (2 mg/mL tetracycline) was initially as striped, concentric colonies at 2 days followed by emergence of several secondary resistant colonies within the primary colony post 3 days of incubation. Such colony morphology and resistant secondary colonies were also observed in MG1655 with salicylic acid, which induces *acrA,B-tolC* efflux pump expression. In contrast,*acrA,B* deletion strains did not show this distinct colony morphology, confirming the role of efflux pump in the stripy, concentric colony growth and emergence of resistant secondary-colonies.

Conclusions: *acrAB-tolC* overexpression leads to filamentous cellular morphology, stripy concentric colony growth and emergence of secondary resistant sub colonies in *E. coli* at near- MIC concentrations of tetracycline.

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**MBEP011**

**Geographic and ecological diversity of green sulfur bacteria in microbial hot spring mat communities**

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Introduction: As of today, only three strains of thermophilic green sulfur bacteria (GSB, anaerobic anoxygenic phototrophic *Chlorobiota*), representing a single species, *Chlorobaculum tepidum,* have been described; all were isolated from mats in hot springs in Rotorua, New Zealand (NZ).

Objectives: In this project, we studied the presence and biodiversity of green sulfur bacteria in hot spring microbial mats, analyzing taxonomic identification and biogeographic distribution.

Material and Methods: Several hot spring associated microbial mats were sampled in different geographic locations (Rotorua, New Zealand (NZ), Yellowstone National Park (YNP), USA and The Republic of the Philippines (PHL)). Green sulfur bacteria were detected using GSB-targeted amplification and sequencing analysis of 16S rRNA genes and selective enrichment cultures.

Results: In addition to Rotorua hot springs, (NZ), GSB 16S rRNA sequences were detected in three regions of YNP and a PHL spring. GSB enrichments and isolates from YNP and PHL mats contained small, green, nonmotile rods possessing chlorosomes, chlorobactene, and bacteriochlorophyll c. Partial 16S rRNA gene sequences from YNP mats and enrichments formed phylogenetic clades distinct from those formed by sequences from NZ and PHL springs, suggesting geographic isolation. Clades were associated with samples differing in temperature and pH, suggesting adaptations based on these parameters. Sequence differences and evolutionary simulation suggest several possible new GSB species. Sequences from enrichments sometimes formed clades that were distinct from those of corresponding mat sequences, increasing the amount of diversity detected in samples and demonstrating the impact of enrichment bias.

Conclusion: In this study, we describe diverse populations of moderately thermophilic *Chlorobaculum* spp. (GSB) inhabiting hot springs of Rotorua, NZ, Yellowstone National Park, USA and The Republic of the Philippines, increasing the known diversity of thermophilic GSB from currently one known species by four further putative novel species.

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**MBEP012**

**"*Mesoanaerobacter kivui?*" – Evolution of a thermophile to thrive at lower temperatures**

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Introduction: The origin of mesophile microorganisms is still part of an ongoing discussion. Several studies suggest that the last universal common ancestor (LUCA) was a thermophilic organism and that mesophiles derived from thermophiles. Although thermophilic as well as mesophilic microorganisms are not considered monophyletic, adaptations to different temperatures are often associated with similar features like changes in GC-content in 16S-rRNA and genome size, usage of different amino acids (CvP-Bias, IVYWREL-Value) and changes in fatty acid composition.

Objectives: Our goal is to study adaptations of thermophilic microorganisms to lower growth temperatures. This enables us to draw conclusions on cold-adaptation of thermophiles on Early Earth experimentally and identify important traits of thermophiles.

Materials & methods: In an ongoing adaptive laboratory evolution experiment we aim to evolve the thermophile acetogenic bacterium *Thermoanaerobacter kivui* ($T_{OPT}$ = 66 °C) into a mesophile organism using serial passaging. This process of adaption is supported by growth experiments, fatty acid, genome and transcriptome analysis.

Results: After 67 transfers (approximately 180 generations) at 45 °C in complex medium the adapted strain *T. kivui* Adpt45 shows a significant shift of its $T_{opt}$ from 66 °C to 60 °C. In addition there is a slight increase in growth rate at 55 °C but not at 45 °C. No shift in fermentation products, mainly acetate could be observed. Cultivation of Adpt45 in defined medium shows a significant reduction in growth rate compared to the type strain, but also a shift in $T_{opt}$ from 66 °C to 60 °C. Fatty acid analysis revealed an increased proportion of short fatty acids at 50 °C compared to 66 °C in the type strain and in Adpt45. In addition, the latter showed a significantly increased proportion of plasmalogens. Compared to the type strain genome analysis revealed 67 single nucleotide polymorphisms.

Conclusion: We observed a significant and unexpected adaption of *T. kivui* to temperatures slightly below its $T_{OPT}$. This represents the starting part for the future long-term adaptation of *T. kivui* to low temperature towards understanding of thermophily and towards the evolution of mesophily on Early Earth.

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**MBEP013**

**Time-kill kinetics reveal heterogeneous tolerance to disinfectants**

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Introduction: Disinfection is an important mitigation strategy to control and prevent the spread of infections. Incomplete or incorrect usage of disinfection may promote evolution of resistance against disinfectants and antibiotics. Ideally, disinfection reduces the number of surviving bacteria and the chance for resistance evolution. Resistance describes the ability to grow in previously inhibitory concentrations of an antimicrobial, whereas tolerance is associated with enhanced survival of lethal doses. Individual bacteria from

the same population can display considerable heterogeneity in their ability to survive treatment (i.e. tolerance) with antimicrobials, which can result in unexpected treatment failure.

Objectives: In this study, we investigated six active substances of disinfectants, preservatives, and antiseptics against a population of *E. coli* to identify the presence of a tolerant subpopulation.

Materials & Methods: We performed time-kill experiments and analyzed the data with a mathematical model to statistically infer whether the data is best explained by the presence of a tolerant subpopulation.

Results: The analysis identified bimodal kill kinetics for benzalkonium chloride, didecyldimethylammonium chloride, and isopropanol. In contrast, kill kinetics by chlorhexidine, glutaraldehyde, and hydrogen peroxide were best explained by unimodal kill kinetics. These findings have implications for the risk of disinfection failure. In addition, we are currently performing adaptive laboratory evolution (ALE) experiments with the different disinfectants to investigate the potential consequences of tolerant sub-populations for the evolution of antimicrobial resistance and tolerance.

Conclusion: Application of biocides as disinfection is important to prevent thre threansmission of pathogens, particularly during the ongoing antimicrobial resistance crisis. The presence of phenotypically tolerant persistent subpopulations may exacerbate the crisis by surviving transient antibiotic treatment and further antimicrobial resistance evolving.

Reference:

Time-kill kinetics reveal heterogeneous tolerance to disinfectants
Niclas Nordholt, Dominique Lewerenz, Frank Schreiber
bioRxiv 2022.06.22.497202; doi: https://doi.org/10.1101/2022.06.22.497202

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**MBEP014**

**Robust collateral sensitivity to aminoglycoside antibiotics due to ciprofloxacin resistance evolution in *Pseudomonas aeruginosa***

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Antibiotic resistance is an evolutionary problem at its core. Therefore, antibiotic treatments need to account for evolution to be able to counter resistance and sustainably treat infections. Collateral sensitivity based strategies are a promising example of such treatments. Collateral sensitivity is the evolution of hyper susceptibility to one antibiotic upon resistance evolution to another. The inclusion of this trade-off in sequential and combination treatments has been demonstrated to successfully constrain resistance evolution in vitro. The translation of collateral sensitivity for clinical use, however, has faced some problems. One of the challenges is that collateral sensitivity tradeoffs can vary with the exact resistance mutation and genomic backgrounds. Consequently, the repeated evolution of a given collateral sensitivity tradeoff across species, different strains in a species, or multiple bacteria in a population cannot be guaranteed. In this study, we tested the robustness of collateral sensitivity against four antibiotics observed upon ciprofloxacin resistance evolution in *Pseudomonas aeruginosa* strain PA14, across the genomic diversity found in the species and two different evolutionary trajectories commonly observed in treatments. We found that collateral sensitivity to the tested aminoglycoside antibiotics was conserved across genomic backgrounds representative of the species-wide diversity and different evolutionary trajectories such as long-term evolved populations and spontaneous mutants. Of the remaining antibiotics, collateral sensitivity was either not observed or inconsistently observed. Our results identify collateral sensitivity to the aminoglycosides due to ciprofloxacin resistance evolution as a conserved trade-off valuable for the treatment of *P. aeruginosa* dominated infections.

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**MBEP015**

**High diversity of the emerging pathogen *Acinetobacter baumannii* in livestock and human wastewaters**

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Carbapenem-resistant *Acinetobacter baumannii* are causing tremendous treatment problems in hospitals. There is still a knowledge gap on the abundance and stability of acquired resistances and the diversity of resistant *Acinetobacter* in the environment. In this study the diversity and antimicrobial resistances of *Acinetobacter* spp. released from livestock and human wastewater into the environment was studied. Fifty-two *A. baumannii* isolates were cultured from raw and digestated manure of different biogas plants and most stages of the rural wastewater treatment plants (WWTP) (no hospital wastewater receiving) and the two studied urban WWTPs receiving veterinarian and human hospital wastewater. Multi-locus sequence typing (MLST) identified 23 novel and 12 known sequence types (STs) of *A. baumannii*. Most novel ST were cultured from livestock samples and the rural WWTP. *A. baumannii* isolates from livestock and the rural WWTP were susceptible to carbapenems, colistin, ciprofloxacin, ceftazidime, and piperacillin. In contrast, *A. baumannii* isolates from the two urban WWTP showed a clinical linkage with respect to MLST and were multi-drug resistant (MDR). The presence of viable *A. baumannii* in digested manure and sewage sludge confirmed the survival of the strict aerobic bacteria during anoxic conditions. The study showed the spread of diverse *Acinetobacter* strains into the environment with a strong association of clinically MDR *A. baumannii* strains from the inflow of hospital wastewater to WWTPs. A more frequent detection of *Acinetobacter* in sewage sludge than effluent waters indicated that particle-attachment of *Acinetobacter* cells must be considered by the risk assessment of those bacteria.

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**MCP001**

**Bacterial vaginosis associated bacteria act as pathobionts of the protozoal pathogen *Trichomonas vaginalis***

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Background: Bacterial vaginosis (BV) is an enigmatic polymicrobial condition characterized by a depletion of health-associated *Lactobacillus* and an overgrowth of anaerobes. *Trichomonas vaginalis* (Tv), a parasitic protozoan, is a common infection of the urogenital system. Notably, BV-associated bacteria (BVB) and Tv are linked to adverse gynecologic and obstetric outcomes, including an increased risk of sexually transmitted infections,

preterm birth, and cervical cancer. In this study, our objective was to investigate whether BVB act as pathobionts of Tv infection by altering pathogenic capabilities of the parasite, particularly regarding adhesion to vaginal substrates and host immune responses.

Materials/methods: Our in vitro model recapitulates the human cervical epithelium was infected with BVB, followed by interaction with Tv, as a polymicrobial infection. Assays interrogated the performance of Tv adhesion and cytotoxicity to human ectocervical cells (Ect) in this polymicrobial infection model. Multiplex immunoassays and immunoblotting were used to investigate the molecular mechanisms caused by the host immune responses governing inflammation.

Results: An *in vitro* polymicrobial infection was shown to provide adhesion for *Tv.* The binding of parasites to host cells and was modulated by the vaginal BVB. Parasite cytoadhesion was significantly increased by *Prevotella bivi,* and it was able to promote Tv growth. Tv and BVB affect the survival of Ects, *P. bivia* caused cytotoxicity and upregulated expression of increased interleukin (IL)-6, IL-8/ Chemokine (C-X-C motif) ligand 1(CXCL1) through mitogen-activated protein (MAP) kinases (MAPK) and phosphatidylinositol 3-kinases (PI3K) pathways. In addition, BVB enhanced the pathogenic effects of the parasite to host cells.

Conclusions: Cooperative interactions between protozoan and BVB lead to disruption of the cervicovaginal epithelial barrier, and lead to a robust proinflammatory activation of cervical cells. Together, this study showed that BVB accompanying *Tv* infection in the vagina function as pathobionts as they are capable of enhancing the pathogenic capabilities of this parasite. This study highlights the importance of understanding the contribution of the vaginal microbiome to trichomoniasis. Establishing a foundation for investigating complex host-microbe- parasite interactions in the human cervix.

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**MCP002**

**Microbiome friendly – Testing cosmetic ingredients in vivo on the example of UV filters**

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Introduction: The awareness of the need to apply sun protection is rising as more and more people start considering skin aging and skin cancer. At the same time, consumers concern about the human and environmental safety of UV filters. Accordingly, comprehensive studies on the safety profile of UV filters are needed. Only a few studies investigated potential effects of light/UV on cutaneous microorganisms, which is far from any systematic understanding. Especially, the in vivo situation after application of sun protection has not been investigated as of yet.

Objectives: *Microbiome friendly* is a relatively new cosmetic claim appearing on more and more products today. We showcase in vivo testing for this claim on the example of UV filters and their impact on the microbiome.

Materials & Methods: An in vivo study mimicking a vacation application scenario was designed and the skin microbiome analyzed before and after. Emulsions containing different UV filter combinations as well as a placebo without any UV filter were applied to the forearms of 23 healthy persons. The O/W emulsions (beside the placebo) contained the following UV filters Bemotrizinol (BEMT), Ethylhexyl Triazine (EHT), Ethylhexyl Salicylate (EHS) and Phenylbenzimidazole Sulfonic Acid (PBSA). The difference is in the addition of Octocrylene (OCR) / Homosalate (HMS) and / or Disodium Phenyl Dibenzimidazole Tetrasulfonate (DPDT). The products were applied three times daily for a duration of two weeks. At the beginning before the first application and after two weeks application the microbiome was sampled with swabs on a skin area of 4cm x 4cm. The samples were analyzed with 16S rRNA sequence analysis.

Results: The skin microbiome of the participants was found to be stable in its composition over the duration of the study. While typical representatives of a healthy skin microbiome were identified, their average contribution was not impacted neither by the placebo emulsion nor by any product containing UV filters. We have demonstrated that formulas containing the tested combination of UV filters have no impact on the healthy skin microbiome thus supporting the claim "microbiome friendly".

Conclusion: This outcome fulfils the customer expectation regarding save sun care products. It further confirms that it is possible to develop safe cosmetic ingredients that respect and support the natural ecosystems of the human body with their microbiomes.

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**MCP003**

**The role of secondary metabolites in plant associated microbial communities**

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The progressive climate change is threatening plant growth all over the planet. Since plants represent the most important nutrient source for humans, their health is of great interest for our food supply. The plant microbiome is essential for healthy plants and represents a promising target for plant promotive treatments. [1]

The microorganisms within plant microbiomes not only interact with the plant but furthermore strongly affect each other. Recent studies found that the leaf microbiome of *Arabidopsis thaliana* shows a huge potential to produce secondary metabolites. [2] Since these compounds are fundamental units for microbes to sense and respond to their environment, we hypothesized, that they play a major role in mediating interactions in the leaf microbiome.

To investigate secondary metabolite-based interactions within plant microbiomes, we use a beneficial synthetic community (SynCom) assembled from *Arabidopsis thaliana* leaves. We used genome mining to analyze the potential of each Syncom member to produce secondary metabolites. Additionally, by using single strain non-targeted-metabolomics, we identified secondary metabolites and compared the metabolome of single strains to the metabolome of the whole Syncom.

In addition to metabolomics, we aimed to identify and isolate secondary metabolites produced by Syncom members to investigate their effect on the community. In further experiments, we investigate whether the presence or absence of certain compounds change the community

composition on the plant leaf and therefor influences plant infection prevention.

References:


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**MCP004**

**Investigation of the water kefir grain formation and growth by a new image analysis method**


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Water kefir is a fermented beverage which is produced by adding water kefir grains to a sugar solution with dried fruits. The grains, which have a size of approx. 1-10 mm, consist of a polysaccharide matrix and have a jelly-like consistency. They are colonized mainly by lactic acid bacteria, acetic acid bacteria and yeasts. The grains increase in number and size during fermentation cycles. So far, it has not been possible to clarify how new grains form from existing ones and what dynamics the growth is based on. Here, we aimed to find a method for the documentation of grain growth and new formation during the fermentation to enable a kinetic growth description.

We developed a documentation system that takes high quality pictures that were further processed with the Fiji image-analysis software. Kefir grains were selected by size and incubated in a standardized water kefir medium at room temperature. The number and increase of white pixels in the pictures on a black background represent a measure of the two-dimensional grain size and growth respectively.

Two parallel processes were observed: the growth in size of the compact starter grains and the formation of new grains as a rather loose grain-like conglomerate. Both structures were studied with scanning electron microscopy. The size expansion of the starter grains was observed in all experiments, while the formation of new grains depends on the pre-treatment. Macroscopic grain growth could be displayed as a function of white pixels over fermentation time with critical time points.

We showed that new grains are mainly formed by detachment from existing grains but not from dispersed individual cells and their aggregation in the medium. The growth dynamic strongly depends on the pre-treatment and to a minor extent on the number of starter grains and their vitality.

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**MCP005**

**Microbiology of activated carbon filters used in the fourth stage of wastewater treatment plants**


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Conventional wastewater treatment plants (WWTPs) do not sufficiently remove micropollutants such as pesticides, pharmaceuticals including hormones and antibiotics as well as microplastics and nanoparticles, which can affect aquatic organisms. To remove these trace chemicals from effluent water, improved wastewater treatment techniques are being installed, including adsorbing activated carbon (AC) filters. Previous studies have shown that microbial biodegradation can support the physicochemical purification process in AC filters, but the microbiome and microbiological processes are largely unexplored.

Our research seeks to understand microbial communities and their function in AC filters with the perspective of bioaugmentation by adding micropollutant-degrading bacteria.

Preliminary microbiome analysis of AC particles from an operating filter in a wastewater treatment plant revealed that diverse bacteria groups can colonize AC, and *Sphingomonadaceae*, *Chitinophagaceae*, and *Rubinispaeraceae* are the dominant bacterial families throughout the sampling period. Enrichment of bacteria from a lab-scale AC filter that was operated with artificial wastewater effluent containing salicylic acid, which adsorbs very well to AC, led to the isolation of *Pseudomona*s sp. strain salic. Growth experiments with strain salic revealed that it could grow by desorbing salicylic acid from AC without obviously forming a strong biofilm. A strongly reduced growth rate and growth yield of strain salic revealed that desorption was a relatively slow process and also incomplete, as supported by chemical desorption of residual salicylic acid at the end of growth. Experiments with a member of the *Sphinomonadaceae* revealed strong, sphingane-depedent biofilm formation with succinate and glucose as substrates, which did not adsorb to AC. Further bacteria strains with high affinity for AC particles have been isolated and are currently being characterized regarding properties facilitating growth in such filters.

Our study reveals that bacteria can interact with AC particles by desorbing substrates as well as by attaching them. Based on the microbiome data, we currently are trying to isolate members of the dominant families inhabiting AC filters for further functional analysis.


Keywords: wastewater treatment plants, the fourth stage of wastewater treatment plants, activated carbon filters, microbiome


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**MCP006**

**Metagenomic analysis of an anoxygenic photosynthetic microbial consortium from a pond ecosystem**


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Blooms of purple sulfur bacteria drive the sulfur cycle in some aquatic ecosystems. Usually they turn the water visibly purple only for a short time. In 2017, in a small pond of a restored wetland in the former Willershausen clay pit (Kalefeld, Lower Saxony), we discovered millimeter-sized pink hollow spherical aggregates of bacteria which have been present in large numbers throughout the year since then. In a preliminary 16S amplicon sequencing study we found them to be formed by four taxa: *Desulfocapsaceae*, *Paludibacter*, *Lamprocystis*, and *Rhodocyclaceae*.

Although it is known that *Lamprocystis* cells form reticular or even spherical aggregates, the formation of the millimeter-sized complexes has not yet been described, and the presence of three other taxa in the aggregates in fixed proportions has not been recognized. This raises the question of to what extent the formation of multispecies aggregates is beneficial for the constituent bacteria.

Using genome-resolved metagenomics we reconstructed four metagenome-assembled genomes (MAGs) and studied the potential for nutrient cycling within the aggregates on genome and transcriptome levels. Here we especially focus on carbon and sulfur cycling.

The MAGs are affiliated with the four taxa we had already seen in the amplicon data and have a completeness of >94%. The *Desulfocapsaceae* representative encodes and expresses the genes for dissimilatory sulfate reduction. In contrast, *Lamprocystis* and the *Rhodocyclaceae* representative feature genes for anoxygenic photosynthesis using reduced sulfur compounds as electron sources. *Paludibacter* does not show a strong link to the sulfur cycle but has the potential to degrade a range of complex carbohydrates, producing propionate and acetate as fermentation products, which could serve as electron donors for the *Desulfocapsaceae*.

We conclude that there may be cross-feeding of sulfur compounds and organic acids between the organisms. The experimental proof of this has to be addressed in future work, though.

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**MCP007**

**Spatiotemporal Probing of Microbial Communities from Winogradsky Columns as a Model Ecosystem**

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Introduction: To explore changes in bacterial communities, an important approach is system perturbation under controlled environmental conditions. Winogradsky columns offer a solution, as they provide a self-sufficient system by encapsulating a sediment sample in a closed column. The metabolism of present microorganisms results in nutrient, oxygen, and sulfide gradients along the column height, which in turn result in segregation of microorganisms based on their preferred habitat, so Winogradsky columns can also be used as enrichment cultures for rare taxa.[1]

Objectives:

1. Develop a method for spatiotemporal sampling of Winogradsky Columns
2. Study the reproducibility of microbial communities in Winogradsky columns growing under similar conditions using an elastomeric probe based on MESIF (macroporous elastomeric silicone foam).[2]
3. Study the development in microbial community composition in the columns and determine the system's stability to perturbations.

Methods: MESIF materials were produced as previously described.[2] In order to mount the MESIF"s to fixed heights in the column, steel frames were designed and fabricated by laser cutting. Soil samples were used to prepare Winogradsky columns as described earlier.[1] Three metal frames, each holding three MESIF pieces, were included in each column, the columns were closed and incubated in a home-made chamber containing programmable LED light sources. At defined timepoints individual frames were retrieved, and the microbial community inside the MESIFs was extracted and analyzed by metagenome sequencing.

Results: Relative abundances of microorganisms could be determined for MESIF probes positioned at different locations in the column. Column duplicates allowed determination of the reproducibility of species found in the community by analysis of similarity. Sampling at different time points provided initial insights into the dynamics of growth patterns and microbial community composition.

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**MCP008**

**Characterisation of non-axenic cyanobacteria – PacBio amplicon sequencing of the complete 16S-ITS region of *Coleofasciculus* and associated bacteria**

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Introduction: *Coleofasciculus* is a genus of filamentous marine cyanobacteria worldwide distributed in microbial mats of tidal flats. In the environment hundreds of heterotrophic bacteria live in close contact with *Coleofasciculus*. The DSMZ harbors the largest collection of *Coleofasciculus* strains, which offers the possibility to investigate and compare the composition of the cyanosphere.

Objectives: Generating complete 16S rRNA gene sequences of non-axenic cyanobacteria is challenging. In a proof-of-concept study 34 strains of xenic *Coleofasciculus* strains were investigated with a novel PacBio amplicon sequencing approach targeting the complete 16S rRNA gene and ITS region of the cyanobacterium and associated heterotrophic bacteria. Sequencing was performed with biomaterial from continuously cultured strains from 2019 and 2023 to investigate the composition of the cyanosphere over time.

Materials & Methods: Sequencing of the 16S-ITS rRNA region was performed on the PacBio sequel platform generating circular consensus sequences. Data were quality-checked, filtered, denoised and checked for chimera with an adapted and modified DADA2 pipeline.

Results: High-throughput sequencing confirmed that all 34 investigated strains were unicyanobacterial. 32 of them were correctly classified as *Coleofasciculus* (cyanobacterial Clade B3). Two supposed *Coleofasciculus* strains were misclassified and represent cyanobacteria of the genus *Salileptolyngbya* (Clade C3). The authenticity of sequences was confirmed by identical Amplicon Sequence Variants (ASVs) in at least two different biological samples. The cyanosphere comprises up to 70 different ASVs of associated heterotrophic bacteria, most of them were classified as *Proteobacteria* and *Bacteriodota.* 434 ASVs of heterotrophic bacteria were validated in at least two sequencing runs and represent bacteria of 12 different phyla. Finally, 16S-ITS sequencing allowed to confirm the axenicity of one *Coleofasciculus* strain.

Conclusion: The 16S-ITS PacBio amplicon sequencing approach provided well-resolved insights into the diverse composition of the cyanosphere. Validation of bacterial ASVs is guaranteed with at least two individually performed sequencing runs. With this proof-of-concept study it was possible to classify closely related isolates of non-axenic cyanobacteria; it was even possible to distinguish between ribosomal operon variations from one cyanobacterium.

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**MCP009**

**Modeling phototrophic microbial communities using biochemical resource allocation analysis**

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Introduction: The evolution of oxygenic photosynthesis in the ancestors of modern-day cyanobacteria gave rise to perhaps the most important biological process within our biosphere. While many properties of growth in axenic cultures are reasonably well understood, cyanobacteria and other microorganisms have evolved as parts of interconnected and dynamic ecosystems, and their physiology can only be understood in the context of this evolutionary history.

Objectives: We seek to understand the emergence of interactions within phototrophic microbial communities using computational models based on biochemical resource allocation analysis. What are the prerequisites and energetic trade-offs for cooperation and division of labor? How do metabolic diversity and mutualistic relationships emerge? Our premise is that the perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms in marine environments.

Methods: We can build upon high quality quantitative computational models of microbial growth and resource allocation developed over the past decade. In particular, we make use of algorithmic model reduction to obtain coarse-grained growth models based on genome-scale reconstructions.

Results**:** The contribution will present a novel computational model that describes interactions between photo- and heterotrophic microorganisms. Our starting point are coarse-grained models of cellular metabolism and growth. The model allows us to evaluate the costs and benefits of interactions, and allows us to outline a plausible evolutionary pathway for the emergence of metabolic dependencies.

Conclusions: The perspective of cellular resource allocation offers a unique opportunity to understand the constraints and energetic trade-offs that govern the emergence of dependencies between photo- and heterotrophic microorganisms

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**MCP010**

**An approach to investigate interbacterial interactions in a community of human gut microbes**

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The efficacy of antibiotics against specific bacteria may differ in diverse microbial communities compared to monocultures. This suggests that microbial interactions are essential within a microbiome. Investigating the molecular pathways of cross-species interactions will allow us to precisely modulate the microbiota to benefit the host"s health and to find new possibilities to fight infections. The goal of this project is to investigate how enteropathogenic clostridia can modulate the human gut microbiota and influence the abundance of common commensal strains. In a first step, we identify interaction partners of *Clostridium perfringens* in a 17-member community. Every community strain is cultured alone and in co-culture with *C. perfringens,* and the growth under the two conditions is compared. In a second step, we aim to entangle the mechanistic details of such microbial interactions to elucidate which metabolic pathways are involved. We used qPCR with species-specific primers to monitor the growth of the strains of interest in monocultures and co-cultures. The molecular mechanisms underlying these interactions are investigated through proteome and metabolome analysis and genetic approaches. The observed phenotypes will be further explored in a bioreactor system combining the knowledge gained from the metaproteomic and metametabolomic study. This approach enabled us to identify beneficial and adverse effects on the growth rate of the strains of interest. Most of the identified interactions were found to be inhibitory under these growth conditions. We identified interactions between the pathobiont *C. perfringens* and human gut commensals by comparing their growth in co-cultures and single cultures. Together with the data from the metabolome and proteome analysis, this knowledge will be valuable in modulating the human microbiota for therapeutic purposes.

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**MCP011**

**Investigation of a novel transcriptional regulator of secondary metabolism in *Streptomyces iranensis* required for activation of fungal silent gene clusters**

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Streptomycetes are renowned for their ability to produce an enormous variety of secondary metabolites, depending on the chosen culture conditions. The term "secondary metabolites" refers to compounds produced by an organism, that are not directly required for the survival of the producer but rather provide an advantage under certain ecological conditions. Since their biosyntheses are costly, microbes usually only produce secondary metabolites under specific conditions, where the compound in question actually provides a benefit. As we have previously shown, *Streptomyces iranensis* HM35 (DSM 41954) has the ability to activate the production of orsellinic acid and various derivatives thereof in the fungus *Aspergillus nidulans* [1,2]. Recently, we were able to show that *S. iranensis* can activate silent biosynthetic gene clusters (BGCs) in various fungi through the secondary metabolite azalomycin F3[3]. By conducting deletion experiments, we identified a group of major regulatory genes. One of them, a new LuxR-type transcription factor is required for the interaction between *S. iranensis* and *A. nidulans*. Furthermore, we were able to verify that this regulator is involved in the production of azalomycin F3, which mediates the interaction of *S. iranensis* and *A. nidulans*. Through transcriptomic analysis and protein-DNA interaction studies we aim to identify the target genes and exact binding motif of the regulator as well as its

potential involvement in mechanisms of export or transport of azalomycin F3 to its recipient fungus. Our data provide insight into the molecular regulation of bacterial-fungal interactions.

1 Schroeckh, V. *et al.* Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 14558-14563 (2009).

2 Nützmann, H. W. *et al.* Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 14282-14287, doi:10.1073/pnas.1103523108 (2011).

3 Krespach, M. K. C., Stroe, M. C. *et al.* Streptomyces polyketides mediate bacteria-fungi interactions across soil environments. *Nat Microbiol*, accepted, doi: 10.1038/s41564-023-01382-2 (2023).

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**MCP012**

**More than just bacteria – Metagenomic analysis reveals a diverse, skin-like microbial community on worn spectacles**

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Spectacles are widespread devices aiding human vision. However, they also represent a reservoir for (potentially) pathogenic microbes, which threaten eye health and might promote the spread of infectious diseases. Using cultivation and 16S rRNA gene amplicon sequencing, we previously obtained a comprehensive view of the bacterial community composition on worn spectacles and similar surfaces, such as slit lamps [1, 2]. Here, we report the establishment of metagenomic DNA shotgun sequencing to obtain a more comprehensive, PCR-independent view of the spectacle microbiota and its hygienic relevance. This approach provides a broader resolution of the microbial community as it is possible to detect prokaryotes, eukaryotes, and viruses simultaneously. Furthermore, functional genes (antibiotic resistance genes, virulence factors, etc.) become accessible.

Harvesting sufficient template DNA was difficult, due to the smooth and small sampling areas. Sequencing swab samples obtained from 33 spectacles worn by university staff and students resulted in 737109 raw sequences per sample, of which 249021 non-human reads per sample remained after quality trimming. Read-based analysis assigned ~ 90% of the sequences to skin and environmental bacteria, dominated by cutibacteria, staphylococci and corynebacteria, thereby corroborating our previous studies. In addition, ~9.5% of the reads were assigned to eukaryotes (mainly plants and yeasts, such as *Malassezia* sp.), ~0.5% to viruses and ~0.05% to archaea. Viruses mostly comprised bacteriophages, but also human-associated types, such as HPV. Based on these preliminary analyses, also the non-bacterial community on spectacles appears to resemble the human skin microbiota. Initial functional analyses proved the presence of antibiotic resistance genes, e.g. against macrolide and beta-lactam antibiotics, and other virulence factors. Future work aims at optimizing the metagenomic workflow and a deeper analysis of functional aspects of the spectacle microbiota, particularly for spectacles worn in clinical environments. In addition, RNA extraction will be established to account for RNA-viruses. Finally, since many of the eukaryotic sequences were assigned to plant DNA, the role of spectacles as a reservoir for allergens also seems worth investigating.

[1] Fritz, B. et al. (2020). Sci Rep 10: 5577/doi:10.1038/s41598-020-62186; [2] Fritz, B. et al. (2021). Front Cell Infect Microbiol 11: 745653/doi:10.3389/fcimb.2021.745653

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**MCP013**

**Linking host genetic landscapes to phyllosphere microbiota recruitment and balance**

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Introduction: A balanced leaf microbiome is necessary for the survival and fitness of plants in nature. However, very little is known about what a balanced microbiome is and how host genotypes contribute to this.

Objectives: To understand the mechanisms behind "dysbiosis" and "rebalancing" of the leaf microbiome in a non-model *Arabidopsis thaliana* line using a forward genetics approach

Methods: We generated mutants in a local wild-type *A. thaliana* background (NG2) using chemical mutagenesis. After screening hundreds of mutants for bacterial load, we compared the leaf microbiome and defense hormones in 13 selected mutants in the mostly homozygous M3 generation. In one of the mutants, "CLLF", we analyzed the glucosinolate profile and tested resilience towards abiotic (high humidity) and biotic threats (Fungal pathogen, *Sclerotinia sclerotiorum,* and bacterivorous amoeba). A field experiment was also conducted to test the survival rates of CLLF in a natural habitat. We are currently outcrossing to identify causal mutations in CLLF and analyzing its transcriptome to have further insights into the regulation of balance in the NG2 microverse.

Results: To our surprise, approximately 5% of mutant plants had a high bacterial load compared to the wild-type, indicating frequently altered microbiota recruitment despite no disease symptoms. Detailed microbiome analysis on selected mutants showed that the high bacterial load was generally accompanied by the recruitment of unusual taxa and varying alpha or beta diversity. Defense hormone regulation was disrupted in most mutant lines but to different extents. CLLF exhibited strongly upregulated Salicylic acid and Jasmonic acid levels and altered glucosinolate profiles. Additionally, CLLF showed improved resilience to abiotic and biotic threats and survival rates the same as the wild type. Furthermore, certain bacterial taxa were favored in CLLF when challenged with a fungal pathogen *S. sclerotiorum*. Similarly, higher humidity treatment and bacterivorous amoeba inoculation also exerted a stronger driving force on CLLF's bacteriome than the WT.

Conclusion: Altered microbiota recruitment does not always lead to disease and may be linked to beneficial responses to some stressors. Plant defense hormones and secondary metabolites like glucosinolates are strongly linked to recruitment. Leaf microbiome appears to rebalance due to

both microbe-microbe interactions and a broad range of host immune system regulations.

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**MCP014**

**Enzymatic textile recycling: Microbial upcycling of synthetic fiber monomers from hydrolysates**

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Introduction: Global production of textile fibers amounted to 109 million tons in 2020, with polyester-based fibers accounting for more than half of the total production.[1] However, less than one percent of textile waste has been recycled fiber-to-fiber once.[2] In the context of end-of-life management, the increasing quantities of fossil-based textiles such as polyethylene terephthalate (PET), polyamide (PA), and polyurethane (PU) in textile production, and the amount of accumulating waste textiles, pose a significant threat to environment and society. To address this problem, alternative and innovative recycling routes are indispensable. For biological upcycling, enzymatically hydrolyzed textile waste serves as feedstock for bacterial fermentation, producing biological polyesters that contribute to a circular (bio)economy of plastics.

Objectives: Defining of a mixed-culture, consisting of *Pseudomonas putida* KT2440*, Paracoccus pantotrophus* DSM 2944 and *Cupriavidus necator* H16 DSM 428, which are capable of upcycling different synthetic textile monomers simultaneously. Production of value-added biological compounds such as the polymer polyhydroxybutyrate (PHB).

Materials & Methods: As feedstock for the mixed-culture monomers ethylene glycol (EG), terephthalate (TA), adipic acid (AdA) and 1,2-butanediol (BDO) were used in a fed-batch cultivation. Genetic engineering and adaptive laboratory evolution were used to enable or improve the growth on various textile monomers. A Growth profiler device (Enzyscreen) was used for media optimization.

Results: After adaptation, *P. pantotrophus* and *C. necator* H16 could grow on EG and AdA, respectively. *P. putida* KT2440 DPHA pLO06 was chosen as a candidate for the mixed culture, since the strain was engineered before to establish PHB production. In addition, the *tph*-operon of *Pseudomonas umsongensis* GO16 was introduced to enable the growth on TA. To create a stable mixed culture, single cultivation parameters were determined. The media composition was modified until strains could grow on equimolar concentrations of 30 mM of EG, TA, AdA, and BDO.

Conclusion: A defined mixed culture was characterized for its degradation of synthetic textile monomers and simultaneous upcycling for biopolymer production. High molecular weight PHB synthesis and its improvement are planned as the next steps.

[1] McKinsey & Company (2022): scaling textile recycling in europe turning waste into value.

[2] TextileExchange (2022): Report 2021

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**MCP015**

**Establishment of an interspecies cross-feeding community to identify novel players in vitamin B6 metabolism**

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Intra- and inter-species metabolic cross-feeding is a fundamental driver for the diversity and function of microbial communities. The presence of auxotrophic species in natural microbial communities indeed suggest extensive exchange of metabolites, which impacts the structure and function of the microbial communities [1]. Metabolic cross-feeding was often employed to design and assemble synthetic microbial consortia in the laboratory [2]. The establishment of these synthetic microbial consortia may be a promising approach for developing robust and stable biotechnological processes [3]. An attempt to map all possible cross-feeding interactions among *Escherichia coli* mutants uncovered that vitamins are ideal shared goods for establishing synthetic microbial consortia [4]. Recently, the adaptive laboratory evolution of a community consisting of yeast and lactic acid bacteria led to the identification of bacteria showing enhanced secretion of vitamins [5]. To identify novel players in vitamin B6 metabolism (uptake and export systems as well as proteins involved in vitamin B6 homeostasis) we established a synthetic metabolic cross-feeding community consisting of *Corynebacterium glutamicum* and *Bacillus subtilis* mutants that are auxotrophic for the B6 vitamer PLP and for glutamate, respectively. While the *C. glutamicum pdxST* mutant lacks the PdxST PLP synthase complex and the *B. subtilis* mutant *gltAB* mutant is unable to produce glutamate. PLP as an essential cofactor for amino acid metabolism is required in small amounts. By contrast, Glutamate serves as the major amino group donor and is thus required in higher amounts. Co-cultivation experiments using liquid and solid media revealed that the *C. glutamicum* and *B. subtilis* mutants established a stable metabolic cross-feeding community irrespective of the initial ratio of the cross-feeding strains. Currently, we are performing an adaptive laboratory evolution experiment to enhance the exchange of shared gods. We are also studying the genetic stability of the synthetic consortium. The current status of the project is presented.

[1] Zelezniak *et al.* (2015) Proc Natl Acad Sci USA 112: 6449-6454.

[2] Giri *et al.* (2020) Curr Opin Biotechnol 62: 228-238.

[3] Minty *et al.* (2013) Proc Natl Acad Sci USA 110: 14592.

[4] Guillen *et al.* (2021) Cell Syst 12: 1064-1078.

[5] Konstantinidis *et al.* (2021) Mol Syst Biol 17: e10189.

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**MCP016**

**How do bacteria adapt to life in the community? Answers for a model community using metabolic modelling and proteomic data integration**

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The collective metabolism of bacterial communities is a central mechanism for the numerous functions that microbiomes perform. While single bacteria and crossfeeding relationships between pairs are often characterized (Weiss et al., 2021, Pérez Escriva et al., 2022), the intricate metabolic networks of larger communities are harder to elucidate. Systems biology can be of great value here by combining computational simulations and experimental data.

Here, we present a hybrid approach of metabolic modelling and experimental work to investigate the metabolic network of the OMM12 community. This synthetic community of twelve strains is a model for the murine gut microbiome that is widely used to investigate colonization resistance to pathogens and metabolic and immune-mediated diseases. We use metaproteomic data from different culture conditions to analyse community metabolism and probe the metabolic adaptation of bacteria to the community context. Curated genome-based metabolic models serve as a framework to elucidate metabolite fluxes and exchanges from the proteomic data. We find evidence for multiple functions that are expressed only in the community and undetectable in single culture. We leverage the mathematical possibilities of the computational approach to investigate the signals and constraints that lead to these community adaptations. Thus, we find multiple connections that clarify previously noted but unexplained phenotypes.

Furthermore, we compare metabolism between *in vitro* cultures of different media and *in vivo* samples of the OMM12 community. Previous work (Weiss et al., 2022) has noted that community composition does not necessarily reflect community interactions. To probe how well *in vitro* culturing strategies represent the *in vivo* network in the mouse gut, we use both the aforementioned computational modelling approach as well as direct comparison of proteomic data to give a comprehensive picture of community life *in vivo* and *in vitro*.

Weiss, A. S., A. G. Burrichter, […] and B. Stecher: "Exploring the interaction network of a synthetic gut bacterial community." The ISME Journal, 2021.

Pérez Escriva, P., T. Fuhrer, U. Sauer: "Distinct N and C cross-feeding networks in a synthetic mouse gut consortium." mSystems, 2022.

Weiss, A. S., L. S. Niedermeier, […] and B. Stecher: "Nutritional and host environments determine community ecology and keystone species in a synthetic gut bacterial community." bioRxiv, 2022.

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**MCP017**

**Touching surfaces-testing antimicrobial surfaces in space for life on earth**

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Humans contribute to a majority of the microorganisms in enclosed environments, and microorganisms are impossible to eliminate from inhabited spaces. This is true for spaceflight missions where the crew lives in indoor conditions with harsh outside conditions as well as for clinical settings, where humans are especially vulnerable to their environment. Among these natural and unharmful microorganisms, there are opportunistic pathogens, which might cause a threat to crew members in spaceflight or patients in clinical settings, for example in intensive care units. With the ever-increasing antimicrobial resistance of microorganisms posing one of the top ten global public health threats (WHO), the need for alternative approaches to tackle this problem is essential. One promising approach is the use of innovative antimicrobial surfaces as they have shown the potential to prevent the spread of pathogens.

In the project Touching Surfaces novel copper- based antimicrobial surfaces are tested under real space conditions, in schools, and in clinical settings. In Touching Surfaces, nine different surfaces are implemented in each hardware called "Touch Array". Three different metals are included: stainless steel as an inert metal, copper as an antimicrobial surface and brass (CuZn37) as a moderately antimicrobial surface. Of each metal, three different surface topographies are tested of which one is a smooth reference surface. Using direct laser interference patterning, two different topographies are created in each metal. One pattern roughly matches the size of many bacteria (3 µm) and the second pattern is smaller than the average bacterial cell (700 nm). Touch Arrays were installed in schools in Germany, a hospital, and the ISS, where they were touched frequently over a defined timeframe. Upon return to Earth, the Touch Arrays were swabbed to investigate the microbial community using culture-dependent and culture-independent methods. Additionally, the surfaces were evaluated regarding their robustness after frequent touching using ICP-MS to measure copper ion release as well as electron microscopy to analyse material integrity.

Combining the evaluation of the Touch Arrays from the different settings and testing the surfaces with model organisms will help to further investigate which surface structures and materials are promising antimicrobial surfaces, which could not only be used in space but also on earth in schools, public transportation, or clinical settings.

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**MCP018**

**RNA seq analysis of multispecies biofilms provides information about interaction and competition among lung pathogens in their niche**

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Introduction: Microbial biofilms harboring several pathogens coexisting in close proximity are related to lung infections. The bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Stenotrophomonas maltophilia* and *Candida albicans* are widely known for their substantial role in pathogenicity in infected lungs.

Objectives: By RNA seq analysis and the usage of promotor fusion constructs we want to investigate the interaction and competition among lung pathogens in multispecies biofilms. Furthermore, we want to examine the distribution and organization of each species in these biofilms by CLSM imaging.

Materials and methods: For this study chromosomally labeled fluorescent strains of *P. aeruginosa* PA01, S. *aureus* SH1000, *S. maltophilia* K279a and *C. albicans* SC5314 were used. Multispecies biofilms were formed successfully in flow and static settings and were processed to RNA seq analysis.

Additionally, promotor fusion constructs of selected genes were generated and analyzed with a light sheet fluorescence microscope.

Results: CLSM imaging showed that species interactions affect the structural composition of multispecies biofilms. Layer formation was often observed. In multispecies biofilms, PA01 dominated, while SH1000 was often reduced. The bottom layer was initially colonized by K279a. In a dual species biofilm of K279a with SC5314, K279a cells were attached to the hyphae of SC5314. In coculture with SH1000, lactate metabolism was elevated in K279a. On the other hand, propionate degradation in K279a was upregulated in coculture with of PA01. K279a and PA01 metabolism in multispecies biofilms was primarily fermentative with cytochromes used for anaerobic respiration. The expression of virulence factors, QS signaling and cyclic diGMP was decreased in PA01 in coculture with K279a. Furthermore, promotor fusion constructs of selected genes showed distinct expression patterns inside a biofilm on a single cell level.

Conclusion: We have successfully established artificial multispecies biofilms that serve as model consortia. LSM images show a distinct distribution and layer formation of different microbial species within a multispecies biofilm. Dual and triple species RNA seq analysis of these biofilms and the application of promotor fusion constructs has shown specific and different expression patterns for each species as compared to single species biofilms, suggesting that the different species acknowledge and respond to the presence of one another.

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**MCP019**

**Effects of seed inoculation of wheat and barley in organic farming on plant parameter and the rhizosphere microbiome**

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During the last decades, organic farming has undergone a notable expansion as a common agricultural practice throughout the world. This, together with an increasing demand for fertilizer reduction has led to consider different approaches. Herein, we investigate the effect of *Hartmannibacter diazotrophicus*, a plant growth promoting rhizobacterium, in order to enhance the production of wheat and barley under low energy input at two experimental field stations located in Germany.

For this purpose, wheat and barley seeds were mixed with a resuspension of *H. diazotrophicus* in MgSO4 and gum Arabic at 25%, and finally coated with talc. Randomized block designs were considered for field experiments, evaluating the effect of three factors: organic fertilizer application (with and without), row distance (15 cm and 50 cm), and bacterial inoculation (*H. diazotrophicus* and control without bacteria).

Winter wheat harvesting results obtained during the season 2020-2021 showed a significant effect of bacterial inoculation in the straw yield, as well as, an effect of row distance in the 1000 kernel weight. In the same way, a significant effect of row distance was found in the grain yield and 1000 kernel weight for summer barley. During the season 2021-2022, only a significant effect of row distance in winter wheat was observed. However, harvesting results showed the same trend compared with the previous season. Remarkably, *H. diazotrophicus* has been detected on the roots (5-16 x 105 copies g DW-1) through qPCR 120 days after seeding (DAS), as well as, at the flowering and milk-ripe stages, 237 and 272 DAS respectively. This finding suggests an apparently constant presence of *H. diazotrophicus* through the time, both in wheat and barley rhizosphere. Moreover, β-diversity analysis from the rhizosphere microbiome of the season 2020-2021 showed a shift in the bacterial community composition between treatments grouped by stage and location in winter wheat and summer barley. Future analysis of the rhizosphere microbiome (differential abundance, co-occurrence analysis) will allow us to determine how the different factors modulate the interactions between the bacterial communities.

In conclusion, the field experiment showed that E19 was able to (a) survive on roots, (b) can improve the plant parameter and (c) not affected the bacterial community structure.

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**MCP020**

**Development of a bioelectrochemical system to uncouple and interrogate $H_2$-syntrophic partners in the human gut microbiota**

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Introduction: The fermentation of carbohydrates is one of the primary functions of the gut microbiome, which results in the production of short-chain carboxylic acids and gasses such as hydrogen ($H_2$) and carbon dioxide ($CO_2$). Fermentative $H_2$ production and interspecies H2 transfer predominantly drive colonic $H_2$ metabolism rather than respiration (Waters & Ley, 2019). Accumulating $H_2$ disrupts gut function, harms humans, and needs to be prevented. However, $H_2$ is an important energy source for gut microbes, such as sulfate-reducing bacteria, acetogens, and methanogens. Very little is understood about the $H_2$ economy of the human gut; therefore, interspecies $H_2$ transfer and microbial syntrophy have become increasingly important to fill one gap in understanding the entire human gut microbiome. The family *Christensenellaceae* are heritable members of the human gut and are associated with human health. *Christensenella minuta* is a highly prevalent, heritable, health-associated bacterium that cross-feeds $H_2$ to the methanogen *Methanobrevibacter smithii*. In continuous co-culture, *C. minuta* produces the least butyrate when *M. smithii* is abundant. Due to thermodynamic limitations, $H_2$ accumulation predicts a microbial syntrophy in which carbohydrate degradation can only occur when a microbial partner consumes $H_2$ simultaneously (Ruaud et al., 2020).

Objective: Development of the bioelectrochemical system (BES) and proof of concept of $H_2$ removal with *C. minuta*

Materials & Methods: We developed a BES for this investigation, mimicking a syntrophic microbial partner that takes up $H_2$. The BES includes a Platinum-doped carbon anode (Pt/C) and a close interaction site of microbes with the anode where $H_2$ is actively removed by oxidation. Thus, it provides an environment favored by $H_2$-producing, carbohydrate-degrading bacteria. For a proof-of-concept, *C. minuta* as an $H_2$-producing microbe was used.

Results: We found a shift of the fermentation products of *C. minuta* towards more acetate and less butyrate under conditions when the BES removed $H_2$. Furthermore, we

found that *C. minuta* attached to the Pt/C anode and formed a biofilm by scanning electron microscopy.

Conclusion: The outcomes of this study are essential to developing an isolation approach for gut microbes without requiring a microbial (syntrophic) partner. Culturing *C. minuta* in the BES will help further technical BES development and support our ultimate goal of understanding human gut microbes better.

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**MCP021**

**Interspecies interaction between *P. putida* and *R. capsulatus* based on the fluorescent siderophore pyoverdine**

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Microbial communities exist in almost any habitat worldwide. They play a superior role in essential bio(techno)logical processes like detoxification, food production and pathogenesis. Social intra- and interspecies interaction can strongly affect dynamics and composition of such a microbial community. In these processes, the exchange of beneficial compounds (so-called public goods) plays a vital role. In the presented project, we aim to establish synthetic microbial model consortia to analyze the role of iron-scavenging siderophores as public goods for interspecies interactions. One of these synthetic consortia consists of *Pseudomonas putida* and *Rhodobacter capsulatus*. When confronted with iron limitation, *P. putida* synthesizes the siderophore pyoverdine, which can efficiently bind ferric iron ($Fe^{3+}$). When released to the environment, this public good enables access to $Fe^{3+}$ as a strictly limited resource for all community members that can specifically import pyoverdine. As indicated in preliminary sequence analysis, the genome of *R. capsulatus* harbors copies of putative siderophore receptor genes. However, corresponding siderophore biosynthesis genes could not be identified. Thus, *P. putida* and *R. capsulatus* might be suitable candidates for analyzing interspecies interactions based on a public good.

To determine the role of pyoverdine in the proposed social interaction, we first analyzed growth of *P. putida* and *R. capsulatus* in single and co-cultivation setups. We could demonstrate that the growth of *R. capsulatus* is strongly impaired under iron limitation but could be restored either by adding cell-free pyoverdine-containing supernatant of *P. putida* wild-type cultures or in respective co-cultivation experiments. Our first findings thus indicate that pyoverdine can act as a public good in this microbial model consortium.

To identify further candidates for siderophore-based interaction with the putative xenosiderophore utilizer *R. capsulatus*, we performed plate screenings with pro- and eukaryotic siderophore producers from different taxa. Remarkably, these experiments revealed that *R. capsulatus* might use other microbial siderophores including enterobactin from *Escherichia coli* and ferrichrome from *Ustilago maydis* for Fe3+ uptake under iron depletion. Detailed analysis of spatial and temporal aspects of siderophore-based interactions within these new model consortia will provide new insights into the function of public goods in microbial communities.

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**MCP022**

**Capacity of the swine gut microbiome in phytate dephosphorylation and inositol catabolism**

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Introduction: Phytate, or inositol hexakisphosphate, is not only the major carbon source in soil, but also the main phosphorus storage molecule of plants and therefore part of the diet. Phytases are often added to animal food to raise the amount of phytate-derived phosphate absorbed by the host. Based on an *in silico* survey, we estimated that approximately 27% of all bacterial species are able to utilize *myo*-inositol (MI), which is released from phytate upon complete dephosphorylation.

Objectives: The enzymatic activities of the gut microbiome involved in phytate dephosphorylation and inositol degradation, however, are largely unknown.

Methods: We used fecal and gut section samples of piglets and analyzed their microbiome composition via culturomics by using conventional selective culture techniques in combination with 16S rRNA sequencing. Strains isolated on solid media were screened for their capability to utilize MI as sole carbon and energy source.

Results: 16S rRNA gene sequencing of nearly 300 commensals positive in MI utilization revealed a predominance of *Paenibacillaceae*, *Bacillaceae*, *Enterobacteriaceae* and *Planococcaceae*. Similar compositions were found in feces, colon as well as ileum and cecum of four swine. Under aerobic and anaerobic conditions, the percentage of MI-degrading colonies ranged from 10% to 70% depending on culture conditions. To then investigate how many and which commensal bacteria isolated by culturomics produce phytases, we established a phytase assay to screen our bacterial swine gut library. To corroborate our findings, we performed a systematic bioinformatic survey of all bacterial species producing phytases. Analyses on all taxonomic levels revealed that the main classes of phytases are not ubiquitously present in bacteria. In a follow-up approach, we will derive the metagenomes and metatranscriptomes of feces samples to delineate the influence of increasing amounts of phytate in the diet on the microbiota composition and activity.

Conclusion: This project combines different omics approaches to analyze the swine gut microbiome with a focus on the taxonomic distribution of phytases and MI utilization activities. The results are expected to improve our understanding of the phytate fate following ingestion and to pave the way for probiotic interventions to increase phosphate availability in the gut and to reduce eutrophication

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**MCP023**

**Characterizing the sucrose metabolism of *Ustilago maydis* as a prerequisite to study carbon economics in a microbial cross-kingdom consortium**

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Introduction: Until now, research has mostly concentrated on studying individual microbial species. However, in nature microorganisms usually exist in communities. One example are lichens, where a fungus forms a scaffold in which algal or cyanobacterial cells are anchored. In this intimate community, the alga or cyanobacterium provides carbon

sources through photosynthesis and the fungus returns shelter and micronutrients. We aim to understand such interactions by assembling and studying a synthetic community mimicking lichens, using the cyanobacterium *Synechocystis* the basidiomycete *Ustilago maydis* and the ascomycete *Saccharomyces cerevisiae*. Initially, we will address the carbon economics within this synthetic community focusing on the exchange of sucrose which is released by the cyanobacterium and deals as a public good to sustain the two fungi.

Objectives: We will decipher how distinct sucrose utilization types, namely public and private metabolizers, but also cheaters, influence the composition and stability of the synthetic consortium.

Materials and Methods: Distinct sucrose metabolization strategies will be implemented genetically for both fungi. Co-cultivation experiments combining members with different strategies will be exploited to quantify the composition of the consortium over time.

Results: In *S. cerevisiae* the key players of the sucrose metabolism are known. For *U. maydis*, deletion of the two known invertases did not abolish growth on media containing sucrose as single carbon source. Hence, there are likely additional genes which contribute to the sucrose metabolism in *U. maydis.* Blast analyses identified two candidates, a putative maltase and a putative maltose permease. Since these proteins are known to not only accept maltose but also sucrose as substrate in *S. cerevisiae*, this might also be the case in *U. maydis*. In accordance, the transcripts of both genes are highly abundant during growth on sucrose. Currently, we test this hypothesis by yeast complementation.

Conclusion: Thus, we identified promising candidates that might play a key role for sucrose metabolism in *U. maydis.* Experimental validation will increase our knowledge and take us one step further towards the assembly of a synthetic lichen.

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**MCP024**

**Towards the production of sesquiterpenoids in a light-driven microbial consortium**

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Introduction: Synthetic microbial consortia exhibit extended metabolic capacities compared to monocultures and therefore have a great potential for biotechnological applications. Here, we envision to combine the photoautotrophic features of cyanobacteria with the versatile production potential of the fungal chassis *Ustilago maydis* to produce mushroom-derived sesquiterpenoids from $CO_2$ and light. Mushroom-forming basidiomycetes are a rich source of sesquiterpenoids, but their slow growth and low isolation yields limit the access to those valuable compounds. Moreover, successful expression of the underlying biosynthetic pathways in traditional heterologous expression platforms can be challenging. *U. maydis*, a basidiomycete itself, is a promising alternative production host potentially offering metabolic compatibility and tolerance to sesquiterpenoids that are toxic to other microorganisms.

Objectives: While modified cyanobacteria, provided by cooperation partners, will serve as the "breadwinner" of our consortium by photosynthetically converting $CO_2$ and light into sucrose, we aim to engineer *U. maydis* to efficiently convert the sucrose to high-value sesquiterpenoids, such as antimicrobial Lagopodin B or antiproliferative Antrocin.

Methods: Beside establishing the basic requirements to set up a stable synthetic consortium, our focus lays on the transplantation of heterologous sesquiterpenoid biosynthesis pathways. Metabolic engineering will be exploited to increase sesquiterpenoid yields.

Results: Following the successful establishment of common growth conditions and growth monitoring for our two species community, first co-cultivation experiments show that the sucrose release by the cyanobacteria is too low to support a constant increase in fungal cell count under our current conditions. Nevertheless, we could demonstrate that sucrose-releasing cyanobacteria improve the cell viability indicated by a stable cytoplasmatic Gfp fluorescence over several days. Moreover, independent from the availability of optimized cyanobacteria, we are already able to produce the Lagopodin B precursor sesquiterpene α-cuprenene and concentrate now on yield increase and strain generation for Lagopodin B and Antrocin synthesis.

Conclusion: Promising first steps towards the production of sesquiterpenoids in a highly sustainable and efficient synthetic microbial consortium have been achieved.

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**MCP025**

**Calcium peroxide-induced elevated dissolved oxygen (eDO) for ecological lake restoration**

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Lake restoration is a critical process aiming to enhance water quality in lakes, often highly valued by local communities but vulnerable ecosystems. Unfortunately, nutrient input, e.g., from industry or agricultural runoff, has increased the trophic state, especially in smaller lakes and ponds. Many methods to reduce nutrient availability in eutrophic lakes have been established throughout the last decades, with dredging remaining one of the most common but expensive and environmentally destructive measures. Alternatively, applying calcium peroxide ($CaO_2$) for lake restoration can be less costly and labor-intensive. When applied to lakes, $CaO_2$ dissociation slowly releases oxygen at the sediment surface, increasing the dissolved oxygen concentrations (elevated dissolved oxygen, eDO) and stimulating aerobic microbial organic matter degradation. Additionally, eDO may reduce harmful bacterial and algal blooms via P-elimination. In this newly established ZIM project, collaboration partners from German industry and universities are working together to analyze the factors influencing organic matter degradation and mud reduction via eDO in small lakes (<5 ha). Even though $CaO_2$ has often been used successfully for organic matter and pollutant degradation in aquatic systems, it is still unknown which biotic and abiotic factors influence its success. In this project, a number of lakes have been selected for $CaO_2$ application in Northern Bavaria and Saxonia, accompanied by intensive monitoring of biotic and abiotic parameters. In parallel, microcosm incubations are set up to investigate $CaO_2$-induced effects via microsensor measurements, gas chromatography, and 16S rRNA gene amplicon sequencing. In preliminary experiments, $CaO_2$

addition indeed increased the concentration of (bio-)available oxygen deeper into the sediment, while microbial community composition remained mostly unchanged. Additionally, applying $CaO_2$ may stimulate microbial activity, indicated by increased carbon dioxide production in oxic microcosm incubations, while anoxic incubations should not have increased activity. Furthermore, the emission of greenhouse gases, i.e., methane and nitrous oxide, is tracked in our ongoing work. Ultimately, we aim to elucidate how $CaO_2$ influences biotic and abiotic parameters in freshwater sediments *in vitro* and *in situ*. Results from this project will contribute to a better understanding of lake restoration measures via eDO as a non-destructive alternative to dredging.

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**MCP026**

**Plant growth-promoting (PGP) effects of microbial consortia in a peat-free substrate**

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In horticultural sciences, the development and improvement of peat-free substrates becomes increasingly important. In this study, defined microbial consortia are selected on the one hand to replace the functional properties of peat and on the other hand to improve the balance of plant-available nutrients and microbial nitrogen immobilization in peat-free substrates. For that, it is important to characterize the microbial representatives of a consortium regarding their plant growth-promoting properties and interspecific interactions.

From an established strain collection, bacterial consortia consisting of *Pseudomonas alvandae*, *Paraburkholderia strydomiana*, *Rhizobium gei*, *Paenibacillus cineris*, *Azotobacter chroococcum*, *Brevibacterium frigoritolerans* and *Bacillus* sp. were *in vitro* characterized physiologically including their microbe-microbe interactions. Furthermore, these consortia were used to investigate their plant growth-promoting potential on *Petunia hybrida* cv. "Mitchell", *Petunia exserta* and *Ocimum basilicum* in sterile and non-sterile peat-free substrate. For the improvement of physical and chemical properties, the white rot fungus *Schizophyllum commune* was added to several systems. During the culture period, plants were phenotyped, the physical and chemical properties of substrates were analyzed and the nitrogen content of dried plant biomass investigated.

First results indicated PGP effects on *Petunia hybrida* cv. "Mitchell" in peat-free substrate by one of the tested bacterial consortia, by the *S. commune* biomass on both, basil and petunia plants, and by sterilization independent of the used plant-microbe combination.

Additional studies are intended to investigate the mechanisms of the effects in order to further improve the design of the microbial consortia including the fungal mycelium additions and to evaluate the effect of sterilization.

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**MCP027**

**Preliminary comparison of microbiota composition of odorous and non-odorous domestic washing machines using metagenome shotgun sequencing**

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Washing machines can become significantly contaminated with a variety of microorganisms that benefit from the moist and nutrient-rich environmental conditions inside and form biofilms on surfaces that are rarely cleaned. Proliferation of microorganisms inside washing machines can cause, among others, aesthetic problems such as machine and laundry odour [1-2]. However, the causes that can lead to machine malodour are far from being fully understood.

Here, we wanted to obtain more information about the differences in microbial community composition between washing machines with and without malodour. We also wanted to obtain a more comprehensive overview of the microbial community beyond the well-studied bacterial kingdom.

To achieve this, we swap sampled 20 private household washing machines from the greater area of Villingen-Schwenningen, Germany, at two different locations (detergent drawer, rubber door seal). As indicated by the users, ten machines produced bad odour while no odour was reported for the other ten. Total extracted DNA was analyzed by shotgun sequencing using Illumina MiSeq technology.

On average, sequencing yielded 340,204 raw sequences per sample, of which 304,792 non-human reads per sample remained after quality trimming. Preliminary, read-based analysis using KRAKEN2 revealed that 99.8 % of the sequences in washing machines were of bacterial origin. Archaea, microbial eukaryotes and viral sequences together had a relative abundance of only 0.2 %. The main bacterial representatives were *Acinetobacter*, *Moraxella*, *Pseudomonas* and *Rhizobium*, which well matches previous studies [1-4]. Interestingly, washing machines with odour problems appeared to have a lower alpha diversity, especially in the door seal, than machines without odour problems, suggesting a role of a "distorted" microbiota composition in odour production.

In future steps, the bioinformatics workflow will be adapted to cover not only taxonomic but also functional aspects of the microbial community, such as the presence/absence of genes coding for enzymes involved in the production of malodorous metabolites, for virulence factors or for antibiotic resistance mechanisms.

[1] Jacksch et al. (2020) *Microorganisms*, *8*(1), 30 ; [2] Zinn et al. (2022) *Microorganisms,* 10(7), 1465 ; [3] Jacksch et al. (2021) *Microorganisms*, *9*(5), 905 ; [4] Nix et al. (2015). *Tenside Surfactants Deterg* 52, 432–440

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**MCP028**

**Mining novel quorum quenching biomolecules from *Aplysina aerophoba* for harmful biofilm inhibition**

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Bacteria are becoming increasingly resistant to antibiotics, and thus bacterial infections are becoming more and more challenging to treat. Alternatives to classical antibiotics are urgently needed. Quorum sensing is a process by which bacteria communicate with each other or potentially with a eukaryotic host through the production and detection of small

signalling molecules. Those signal molecules regulate gene expression and behavior in populations in a coordinated manner, including biofilm formation and pathogenicity. Thus, a promising target to prevent and inhibit biofilms and potentially infections is the interference with bacterial communication (quorum quenching, QQ). This study aimed to identify novel QQ compounds from a basal metazoan, the sponge *Aplysina aerophoba,* which interfere with the communication of their colonizing bacteria to control the microbiome establishment and maintenance. An expressed sequence tag (EST) library was constructed from mRNA isolated from *A. aerophoba*, containing > 29,000 EST clones. The library was successively screened for acyl-homoserine lactone (AHL) and autoinducer-2 (AI-2)-interfering activities with the respective reporter strains AI1-QQ.1 and AI2-QQ.1 (Weiland-Bräuer et al., 2015). The first screen identified a total of 51 clones to be QQ-active against the intraspecies signalling molecule acyl homoserine lactone (AHL) and the interspecies signalling molecule autoinducer-2 (AI-2). The identified and confirmed single clones are currently characterized concerning their biotechnological and ecological function. Static and dynamic biofilm assays will further elucidate their potential to prevent and inhibit disease development with opportunistic pathogens. The obtained frequency of QQ compounds identified in the basal metazoans illustrate them as a promising source for novel bioactive molecules.

Ref:

Weiland-Brauer, N., Pinnow, N. & Schmitz, R. A (2015). Novel reporter for identification of interference with acyl homoserine lactone and autoinducer-2 quorum sensing. *Applied and environmental microbiology* 81, 1477– 1489.

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**MTP001**

**Tartrolon resistance mechanisms in *Listeria monocytogenes* depend on the TimAB transporter and the ClpP proteasome**

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Introduction: *Listeria monocytogenes* is a foodborne pathogen belonging to the *bacilli*. It is the cause of listeriosis, a lethal infectious disease in patients with a suppressed immune system. *L. monocytogenes* is ubiquitously present in the environment, where it is constantly exposed to competitors secreting toxic compounds to gain selection advantages. One way of dealing with these compounds is the production of ATP binding cassette (ABC) transporters to export these compounds out of the cell.

Objectives: Aim of this study was the characterization of novel ABC transporters genes regarding their compound specificity and induction mechanisms.

Materials & Methods: Promoters of of putative *L. monocytogenes* ABC transporter genes were fused to *lacZ* and screened with a natural compound library for induction. Expression of the *timAB* transporter genes were induced by tartrolon B, and thus, the *timAB* genes and their impact on tartrolon B resistance were analyzed by construction of deletion mutants, determination of minimal inhibitory concentrations (MICs) and further analyses.

Results: We here have identified the *L. monocytogenes timAB* genes as genes encoding a novel ABC transporter exporting tartrolon B, a macrolide antibiotic synthesized by the myxobacterium *Sorangium cellulosum* to battle competitors. Expression of *timAB* was repressed by TimR, a tartrolon-sensing transcriptional repressor. Tartrolon-resistant suppressors inactivated *clpP* (*lmo2468*), encoding an important protease subunit, anda Δ*clpP* strain was highly tartrolon-resistant. Tartrolon did not inhibit ClpP *in vitro*, but instead acted as a potassium ionophore. Remarkably, a Δ*timAB* Δ*clpP* double deletion strain had a comparable minimal inhibitory concentration (MIC) as the Δ*timAB* single mutant strain, indicating that the high level tartrolon resistance of the Δ*clpP* mutant is *timAB*-dependent. Mutants in the three Clp ATPase genes were also contructed and a Δ*clpCEX* triple mutant phenocopied the high level tartrolon resistance of the Δ*clpP* strain, further suggesting that TimAB could be a ClpP substrate.

Conclusion: With *timAB*, we describe a novel compound exporter that supports the survival of an important human pathogen in its natural reservoir. Interlacing TimR-dependent control of *timAB* transcription with ClpP-dependent control of TimAB stability generated an exceptionally wide dynamic range of tartrolon resistance over four orders of magnitude.

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**MTP002**

**Insights into the molecular mechanism of the $Na^+$ – Translocating NADH: Quinone-oxidoreductase ($Na^+$-NQR) from *Vibrio cholerae***

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The sodium-translocating NADH:quinone oxidoreductase ($Na^+$- NQR) of *Vibrio cholerae* is a membrane bound protein complex consisting of six subunits NqrABCDEF. This respiratory complex possesses four flavins, two iron-sulfur centers and ubiquinone-8 as redox active groups. $Na^+$ -NQR is found in many pathogenic bacteria. The $Na^+$ - NQR couples the oxidation of NADH and reduction of quinone to the translocation of sodium ions from the cytoplasm to the periplasm. The generated sodium gradient, the so-called sodium motive force, is essential for many cellular processes like motility, exchange of cations and multidrug resistance efflux pumps. Moreover, $Na^+$ - NQR activity is associated with production of virulence factors and is therefore considered an important target for the development of novel antibiotics. The X-ray structure of the $Na^+$-NQR revealed large distances between redox cofactors [1] suggesting that the molecular mechanism of coupling sodium translocation to electron transport involves large domain movements to decrease the distance between redox centers, hereby promoting efficient electron transfer. We address this problem by a mutagenesis approach where individual domains are compromised in their function. Understanding the mechanism of the $Na^+$ - NQR, a putative target protein for antibiotics, is a prerequisite to identify novel inhibitors against this bacterial enzyme which is not present in humans.

[1] Steuber J, Vohl G, Casutt MS, Vorburger T, Diederichs K, Fritz G. Structure of the *V. cholerae* $Na^+$-pumping NADH:quinone oxidoreductase. Nature. 2014 Dec 4;516(7529):62-7. doi: 10.1038/nature14003. PMID: 25471880.

**MTP003**

**Towards characterising the putative ABC transporter EslABC in the human pathogen *Listeria monocytogenes***

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Transporters mediate many crucial processes in the cell. One conserved group of transporters are ATP-binding cassette (ABC) transporters, which usually facilitate the translocation of their cognate substrate across the membrane via ATP hydrolysis. Interestingly, recent studies have revealed alternative mechanisms not directly linked to import or export, resulting in a more dynamic use of the term. One candidate for this is the putative ABC transporter EslABC, which was recently identified in the human pathogen *Listeria monocytogenes*. Preliminary experiments demonstrate the involvement of the transporter in creating intrinsic lysozyme resistance and ensuring correct cell elongation. As part of the innate immune system, lysozyme serves as a natural antibiotic, thus, identifying proteins involved in its resistance will aid efforts to counteract pathogenic bacteria. Our findings also reveal that the transporter plays a crucial role in peptidoglycan biosynthesis, cell wall integrity and in the overall surface charge. Even though, the mode of action of EslABC, as well as its exact connection to peptidoglycan biosynthesis and remodeling remains elusive, EslABC sheds light on cognate transporter functions and their versatility in cellular processes.

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**MTP004**

**Identification of heteropolymeric subcomplexes and interaction of subunits of the DNA transporter in *Thermus thermophilus* HB27**

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Introduction: The uptake of free DNA from the environment in *Thermus* cells is performed by a DNA transporter that shares essential components with type IV pili (T4P), consisting of a secretion channel, pilins and motor unit components[1]. However, the precise mechanism of interaction between the secretin channel and the motor unit components is still unknown.

Objectives: We aimed to enlighten the interplay of inner membrane (IM), outer membrane (OM) and motor unit components of the DNA transporter in *T. thermophilus*.

Materials & Methods: Site directed mutagenesis, *in vivo* co-affinity purification, MALDI-TOF MS Analyses, column-based interaction studies, transformation studies, western-blot analyses, twitching motility, biofilm formation, cell-cell adherence test.

Results: Through *in vivo* co-affinity purification of PilW and MALDI-TOF MS analyses of purified protein complexes we identified a DNA transporter complex consisting of PilM, PilN, PilO, PilW and PilQ. Within this heteropolymeric complex, we determined that a PilMNOWQ subcomplex interacting with the IM platform protein PilC. *In vivo* co-affinity purification of PilC led to the detection of different protein complexes. A PilC complex was found to mediate the connection between pilins, OM and IM components of the DNA transporter. Furthermore, column-based interaction studies revealed that PilC interacts with the IM component PilO and the OM anchored PilQ, and the motor ATPases PilF and PilT1.

Conclusion: The *in vivo* co-affinity purification experiments and MALDI-TOF MS analyses of purified DNA transporter complexes lead to the conclusion that PilW, PilM, PilN PilO, PilQ and PilC form a heteropolymeric structure of T4P and the DNA transporter, that spans the cell periphery of *T. thermophilus.* The finding that PilC interacts with both, PilQ and PilO, supports our suggestion that PilC connects the IM and OM subcomplexes of the DNA transporter.

[1] Averhoff, A., Kirchner, L., Pfefferle, K. Yaman, D. (2021). Extremophiles, 25: 425 – 436.

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**MTP005**

**Bidirectional formic acid translocation by FocA: Interplay with the formate hydrogenlyase complex**

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Introduction: Formate is a central intermediate in the enterobacterial mixed-acid fermentation of glucose. During exponential-phase growth, formic acid passes from the cytoplasm to the periplasm via the pentameric formate channel, FocA. Upon entry into the late exponential-phase of growth, or upon a decrease in pH below 6.5, FocA re-imports formate /formic acid into the cytoplasm, where it is disproportionated into CO2 and H2 by the membrane-associated formate hydrogenlyase (FHL) complex. Uptake of formic acid by FocA is dependent on an active FHL complex, suggesting coupling between both systems. Each protomer of the FocA pentamer has a narrow hydrophobic pore through which neutral formic acid can pass. Conserved histidine (H209) and threonine (T91) residues at the center of each pore control bidirectional formate/formic acid translocation; this implies that the pore is not simply a "channel". Objectives: Based on these findings, and other data, we propose that besides passive formic acid efflux, FocA can also switch to a formate-import mode. If this is the case, then FocA, working together with the FHL complex, should either be able to relieve pH stress by taking up formic acid, or allow optimal fermentative growth by importing formate. We tested this hypothesis in the current study.

Methods: We use a formate-responsive reporter system, FocA amino acid-exchange variants, together with *fhl* mutants to assess various physiological parameters, such as determination of internal formate levels, H2 production, to test the impact of FocA and FHL on fermentative growth.

Results: We show that the FocAH209N variant exclusively translocates formate out of the cell and consequently produces only low levels of H2. Such a variant shows poor early exponential-phase growth compared with the parental strain. Mutants that lack an active FHL complex fail to produce H2 and also show poorer early exponential-phase growth than the parental strain.

Conclusion: Our findings indicate that FocA functions together with the FHL complex to maintain relatively constant intracellular formate levels, to improve growth by helping maintain pH homeostasis, and possibly also to contribute to energy conservation during glucose fermentation.

**MTP006**

**Exploring the extracellular domain of the BceAB-type ABC-transporter NsrFP from *S. aglactiae***

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Introduction: The well-known lantibiotic nisin has high potential against human pathogenic Gram-positive bacteria. However, human pathogens like *S. agalactiae* are naturally resistant. This resistance is achieved by expression of a membrane associated protease (NSR), an ABC-transporter (NsrFP) and a two- component system (NsrK and NsrR). The ABC-transporter NsrFP belongs to the BceAB family and confers resistance against multiple antibiotics (George et al., 2022; Gottstein et al., 2022).

Objectives: Here, we aim to characterize the characteristic extracellular domain (ECD) of NsrFP. This hallmark of BceAB-type ABC transporters is located in between membrane helices 7 and 8.

Material & methods: To highlight the importance of this ECD, we employed structural alignments, to determine conservation throughout different species. Furthermore, we performed mutagenesis of two essential lysine residues and investigated their effect on resistance against bacitracin.

Results: Structural alignments revealed that ECDs can be classified in six groups, each characteristic for a conserved arrangement of secondary structure elements. Mutagenesis experiments revealed that the mutations of each lysine resulted in reduced resistance. Further, cells expressing either mutant showed different levels of growth inhibition, if threated with sub-lethal concentrations of bacitracin.

Conclusion: Structural alignments indicate, that the ECD is conserved in bacteria and the classification reveals that members of the individual groups might confer resistance against similar antibiotics. Results from mutagenesis experiments suggest, that both lysine"s are required for substrate recognition and resistance.

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**MTP007**

**The impact of single amino acids on functionality of membrane toxin TisB in *Escherichia coli***

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Introduction: In *Escherichia coli*, the *tisB/istR-1* toxin-antitoxin system plays a crucial role during the response to DNA damage (SOS response). Upon DNA damage, the small hydrophobic TisB protein is produced and integrates into the inner membrane to form pores. This results in membrane depolarization, ATP depletion, and other secondary effects, such as protein aggregation. Consequently, cellular growth is inhibited and dormancy is promoted. The dormant cells exhibit antibiotic tolerance and are referred to as persisters. The "charge-zipper" model suggests that specific charged/ polar amino acids are integral to the formation of TisB tetramers, which underlies pore formation.

Objectives: The aim of this study was to determine the impact of charged/ polar amino acids on the function of TisB.

Material and Methods: First, we established an optimized plasmid-based TisB expression system. The expression plasmid was subsequently mutated to replace charged/ polar amino acids with the hydrophobic amino acid leucine. The resulting strains were analyzed with respect to TisB-dependent growth inhibition, membrane depolarization, ATP concentration, protein aggregation, and persister cell formation. To further corroborate the findings, Lambda Red recombineering was used to construct and analyze chromosomally modified *tisB* alleles.

Results: Our results indicate that the residues lysine 12 (K12; charged) and glutamine 19 (Q19; polar) play a crucial role for TisB functionality. Substitution of these residues resulted in a complete loss of protein function, as evidenced by the absence of growth inhibition, ATP depletion, protein aggregation, and persister cell formation. Other amino acids, such as aspartate 22 (D22) or lysine 26 (K26), contribute to TisB functionality but are not essential.

Conclusions: Our findings suggest that amino acids that are located in the center of TisB play a more crucial role for its functionality than C- or N-terminal amino acids. Further investigation is required to determine whether the loss of functionality is due to impaired integration into the inner membrane, inability to form tetramers, or impermeability of pores.

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**MTP008**

**The ABC transporter MsbA adopts the wide inward-open conformation in *E. coli* cells**

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Membrane proteins are currently investigated after detergent extraction from native cellular membranes and reconstitution into artificial liposomes or nanodiscs, thereby removing them from their physiological environment. However, to truly understand the biophysical properties of membrane proteins in a physiological environment, they must be investigated within living cells. MsbA of *Escherichia coli* is a homodimeric adenosine 5′-triphosphate (ATP)–binding cassette (ABC) exporter that flips core lipid A, the precursor of lipopolysaccharide (LPS), across the cytoplasmic membrane (1,2). Here, we used a spin-labeled nanobody to interrogate the conformational cycle of MsbA by double electron-electron resonance and cryo-electron microscopy (3). Unexpectedly, the wide inward-open conformation of MsbA, commonly considered a nonphysiological state, was found to be prominently populated in *E. coli* cells. Molecular dynamics

simulations revealed that extensive lateral portal opening is essential to provide access of its large natural substrate core lipid A to the binding cavity. Our work paves the way to investigate the conformational landscape of membrane proteins in cells.

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**MTP009**

**Outer membrane vesicle formation of *Pseudomonas aeruginosa***

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The opportunistic pathogen *Pseudomonas aeruginosa* is one of the major causes of morbidity and mortality in respiratory infections of immunocompromised patients. This pathogen causes various types of nosocomial infections by secreting a large array of virulence factors, which contribute to a successful infection strategy. During infection, Gram-negative *P. aeruginosa* produces extracellular membrane vesicles (MVs) that encase and deliver a wide variety of molecules into the eukaryotic host cell regardless of being in direct contact. Therefore, vesiculation plays a key-role in host-pathogen interaction contributing to pathogenesis but it is also crucial in inter- and intra-species cell communication. Despite their important role in virulence, the mechanisms governing vesicle biogenesis remain poorly understood. Thus, elucidating the protein machinery responsible for membrane remodeling and vesicle formation is crucial for targeting *P. aeruginosa* infections. We have discovered a novel family of proteins that appear to be involved in re-structuring events of both the inner and outer membrane. The deletion or overexpression of the genes encoding these proteins strongly impacted vesicle formation. Additionally, recombinant production in *Escherichia coli* and purification of the corresponding proteins are currently being performed for their *in vitro* characterization. Another approach to target infections might be to understand the metabolic adaptation of the microbe during host-pathogen interaction. Therefore, cultivating the bacteria in spent media of vesicle-treated lung cancer cells simulates the vesicle-mediated host-pathogen interaction and can give insights on metabolic changes.

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**MTP010**

**The tat complexes that are detected by BN-PAGE differ in their association with TatA**

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Tat systems translocate folded proteins across energized biological membranes in bacteria, archaea, plastids and plant mitochondria. Based on studies with fluorescent tags and cross-linking, it has been concluded that TatA is recruited to TatBC core complexes in response to substrate-binding. Three distinct *Escherichia coli* Tat complexes (TC1/2/3) could be biochemically differentiated by blue native polyacrylamide gel electrophoresis (Geise et al, 2019), but their TatA content has not been analyzed. By investigating purified Tat complexes with point mutations that specifically enrich certain Tat complexes, we now demonstrate that all Tat complexes contain TatA, but the smallest Tat complex, TC1, contains significantly less TatA than the larger complexes. This agrees with the idea that the larger Tat complexes represent active assemblies that can permeabilize membranes by the aid of multiple associated TatA protomers.

Geise, H., Heidrich, E. S., Nikolin, C. S., Mehner-Breitfeld, D., & Brüser, T. (2019). A potential late stage intermediate of twin-arginine dependent protein translocation in *Escherichia coli*. Frontiers in Microbiology, 10, 1482. doi.org/10.3389/fmicb.2019.01482.

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**MTP011**

**From assembly to function: How inner membrane ring mobility governs the type III secretion system**

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Infectious diseases are a burden to humans and remain a major cause of morbidity and mortality. Among the vast arsenal of virulence factors deployed by infectious agents, the Type III Secretion System (T3SS) is essential for many important Gram-negative bacterial pathogens. The T3SS is a fascinating molecular nanomachine assembled in the bacterial cell wall with a needle-like extracellular appendage giving it a syringe-like structure. Using this molecular syringe, pathogenic bacteria promote their survival and replication by secreting toxins directly into the cytoplasm of eukaryotic host cells. While large parts of the syringe structure are stable, some cytosolic components of the T3SS are mobile and dissociate from the T3SS when the external pH is acidic. Thus, the mobility and dissociation of such components allow the bacteria to better adapt T3SS secretion to changing external conditions. Surprisingly, a central structural component of the T3SS, the inner membrane ring protein SctD, also dissociates under acidic conditions. To better understand the behavior of this core T3SS component under physiological conditions, we monitored the localization and mobility of SctD by fluorescence microscopy and single particle tracking. Interestingly, our results demonstrate that SctD is indeed mobile under physiological conditions, as previously observed for some T3SS components located in the cytoplasm. Next, we investigated whether SctD mobility might play a role in T3SS assembly and secretion, combining different approaches such as targeted cross-linking, functional secretion assays, and fluorescence microscopy. Using a mutant that allows SctD mobility to be influenced by cross-linking neighboring SctD monomers, we found that protein export through the T3SS is impaired in strains with

lower SctD mobility both during and after T3SS assembly. This observation suggests that the mobility of the central structural T3SS component SctD plays an important, albeit yet uncharacterized role in T3SS assembly and secretion. In conclusion, our results provide new insights into the unexpected mobile behavior of a central component of the T3SS and interesting leads on its role that still need to be explored.

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**MTP012**
**The balance between the type III secretion system activity and bacterial replication during *Yersinia* infection**
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The Type III Secretion System (T3SS) is a molecular nano-machine used by a heterogeneous group of Gram-negative bacteria to inject virulence proteins into eukaryotic target cells. T3SS assembly and secretion in the foodborne pathogen *Yersinia enterocolitica* are tightly regulated processes triggered by host temperature and target cell contact (*in vitro* mimicked by Ca2+ depletion), respectively. Activation of secretion induces a severe but reversible bacterial growth arrest, known as secretion-associated growth inhibition (SAGI). Our work aims to better understand how the essential activation of the T3SS is balanced with the SAGI during bacterial infection and colonization. Using single-cell microscopy, we characterized growth, division and T3SS activity in secreting and non-secreting bacteria, discovering a severe down-regulation of the T3SS assembly and secretion at increasing cell density. Using proteome analysis, we confirmed the specificity of the OD-driven T3SS down-regulation and identified the main T3SS transcriptional activator VirF as the target of the regulation. The modulation of the T3SS is reversible and dependent on the growth stage of the bacterial cell. To uncover a possible role of cell-cell communication in the growth-stage dependent down-regulation of secretion, we characterized quorum-sensing mutants. Finally, we used advanced microscopy techniques to better characterize cells in the different conditions tested, allowing us to expand the knowledge on the link between secretion and *Yersinia* cell physiology. Our findings reveal how the function and cost of the essential virulence factor T3SS are balanced, an essential insight to understand the application of the T3SS for *Y. enterocolitica* infection.

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**MTP013**
***In vitro* transport of NisT**
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Introduction: Antibiotic resistance is an increasing problem for the treatment of bacterial infections. One way to circumvent this is the search for new potent antibacterial alternatives such as antimicrobial peptides (AMPs). One group of AMPs, lantibiotics, is defined by intensive post translational modification to form lanthionine containing ring systems. Nisin is one of the best characterized lantibiotics and is used as food preservative in the dairy industry since the 1970s. All lantibiotics are composed of an N-terminal leader peptide and a C-terminal core peptide. After ribosomal expression of the precursor prenisin, residues of the core peptide are posttranslationally modified. NisB dehydrates serines to dehydroalanine (Dha) and threonines to dehydrobutyrine (Dhb). The condensation of these Dha and Dhb residues with cysteins is catalysed by NisC resulting in five lanthionine rings in the case of nisin. This peptide is secreted via the ABC transporter NisT into the extracellular space after which the N-terminal leader sequence of the precursor peptide is cleaved by NisP resulting in active nisin.

Objective: To understand the transport mechanism of NisT, a more thorough characterization via mutagenesis studies of this transporter is imperative for optimization of nisin production.

Methods: To understand the transport mechanism of NisT, sequence alignments were performed as a starting point to generate NisT variants to analyze transport in more detail. *In vitro* expression of nisin in *L. lactis* (NZ9000) was performed in minimal media. To determine the impact of individual point mutations, the secretion of prenisin was determined *in vitro*. The amount of exported prenisin over a course of 5h was determined via HPLC. The NisT expression was confirmed via Western blotting.

Results: We were able to determine the apparent secretion rates of NisT for fully modified prenisin (NisBTC, NisA), dehydrated prenisin (NisBT, NisA) and unmodified prenisin (NisT, NisA). It has been shown that NisT is able to transport all of these nisin states, albeit with decreased efficiency for dehydrated and unmodified prenisin. Via the mutagenesis approach we have narrowed down amino acids that are crucial for the transport mechanism of NisT.

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**MTP014**
**Lipid rafts in *Schizophyllum commune* – insights in localization and composition**
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Lipid rafts are dynamic microdomains within the cell membrane. They are highly enriched in sterols and sphingolipids as well as specific raft proteins. These domains act as platforms for signal transduction, membrane trafficking, protein sorting, and polarized growth (Alvarez *et al*., 2007). Polarized growth of the filamentous basidiomycete *Schizophyllum commune* requires the supply of proteins and lipids to the hyphal tip by vesicle trafficking. Crucial in establishing and maintaining polarity are the actin cytoskeleton and raft domains (Takeshita *et al*., 2008). Rafts contain specific proteins like stomatin and striatin. They play a role in cell signalling and signal transduction, as well as in numerous protein-protein interactions and the assembly of proteins in distinct signalling complexes. The Aim of this work is to investigate the composition of raft domains as well as the localization and role of rafts in *S. commune* using two raft-associated proteins, stomatin and striatin. To analyse the overall membrane composition and the protein-protein interaction of the striatin signalling complex, we performed co-immunoprecipitation followed by LC-MS measurements. Sterol-enriched raft domains were visualized by Filipin staining. Further, stomatin and striatin were labelled with the fluorescence proteins dTomato and eGFP, respectively, and visualized by laser scanning microscopy. To investigate the role of stomatin, we performed a knockout followed by RNA sequencing. Sterol-rich domains were detected at tips and septation sites of the hyphae. Labelling of stomatin showed that it localizes at young branch tips and changes its localization over time to the apex. In the dikaryotic stage of the fungus, stomatin colocalized with eGFP-labeled actin. The deletion of stomatin in *S. commune* showed an impact on the organism related to morphological and genetic changes. The RNAseq results revealed an upregulation of several cell signalling groups like

GPI-anchored proteins, MAPKs, and GPCRs. This study has shown that raft-associated proteins play an important role in hyphal growth and cell signalling in *S. commune*. Enhancing our understanding of the structure and functionality of membrane microdomains in microorganisms could potentially provide valuable insights into mating, the interaction of organisms and the pathogenesis process. Furthermore, these insights might facilitate the way for the development of novel therapeutic approaches utilizing raft-mediated strategies.

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**MTP015**

**Cell type specific KDEL-receptor clustering**

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Retention of endoplasmic reticulum (ER) resident proteins is a crucial factor for proteome homeostasis. A subset of soluble ER chaperones shares a C-terminal KDEL-like amino acid sequence, recognized by highly specialized transmembrane proteins: The KDEL receptors (KDELRs). Once these ER resident proteins leak into the secretory pathway, KDELRs initiate their retrotransport from the Golgi back into the ER lumen. This "core" function is accompanied by the modulation of a broad range of cellular functions via activation of KDELR-associated G-proteins and kinase-associated signal cascades. A dynamic fraction of KDELRs resides at the plasma membrane of yeast and mammalian cells, where they bind extracellular KDEL-like proteins and undergo highly complex internalization and recycling processes. Upon ligand binding, KDELRs form clusters on the plasma membrane (PM) prior to their internalization. Until recently, it has been unknown whether there are species or cell type specific differences in KDELR clustering or how KDELRs are transported to the cell surface. Endocytosis and recycling rates of KDELRs have been compared between several human and murine cell lines via live cell imaging and RT-qPCR was used to detect mRNA levels of KDELRs. Colocalization experiments were used to detect human Rab-proteins (Ras-related in brain), the "master regulators" of transport processes, orchestrating KDELR recycling and transport to the PM. Macrophage cell lines of both species do not develop any clusters, indicating highly specialized roles of surface KDELRs. A low KDELR mRNA level could be excluded for absent receptor clustering in macrophages. We detected preferred docking sites on the PM and how they influence the exponent of the power-law distribution of corresponding cluster sizes. Additionally, a highly diverse correlation between KDELRs and different Rab-proteins could be observed, indicating a precise regulation of transport processes.

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**MTP016**

**New insights into the proton translocation mechanism of the organohalide respiratory complex of *Dehalococcoides mccartyi* strain CBDB1**

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*Dehalococcoides mccartyi* CBDB1 respires with hydrogen as sole electron donor and different organohalides as electron acceptors in an anaerobic process known as organohalide respiration (OHR). The key catalytic step in the OHR is the reductive dehalogenation facilitated by a corrinoid-dependent reductive dehalogenase (RdhA) enzyme. RhdA is part of a membrane-bound OHR complex, which comprises at least seven distinct proteins. The OHR complex lacks quinones, cytochromes and proton pumps, which are commonly found in other respiratory complexes, suggesting an alternative mechanism for the *proton motive force* (*pmf*) formation during the OHR process.

To gain insights into the proton translocation pathway associated with the OHR complex, we developed an enzyme activity assay with deuterium-labeled water ($D_2O$), enabling the precise tracking of proton incorporation into the product. Dehalogenase activity assays were conducted, using CBDB1 cells cultured in $H_2O$- or $D_2O$-containing medium, and activity master mixes with $D_2O$ or $H_2O$, to have either $D_2O$ outside and $H_2O$ inside the cells, or *vice versa*. Then, the deuteration degree of the formed product was determined with GC-MS. To predict the proton-conducting path across the membrane, a multiple sequence alignment of OmeB and NrfD homologs from different bacteria was done and the structure of the OmeAB-HupX submodule and RdhA using AlphaFold2 was calculated.

Our experimental data with $D_2O$ initially outside and $H_2O$ inside the cells, show an increase of the deuteration degree of the product over time, indicating that initially protons are incorporated. The converse experiment shows a decreasing deuteration degree over time. Both experiments therefore suggest that protons are passed through the membrane directly onto the substrate. Structural and sequential analyses revealed that OmeB contains conserved, charged amino acids capable of proton translocation across the membrane. The RdhA subunit was manually docked to the OmeAB-HupX subcomplex, with its distal [4Fe-4S] cluster less than 14 Å distant to the distal [4Fe-4S] cluster of HupX, enabling efficient electron flow. Additionally, in RdhA conserved and charged amino acids were identified that could direct the protons from OmeB to the active site of RdhA.

The dehalogenation reaction directly contributes to the *pmf* by facilitating the transfer of protons from the cytoplasm to the substrate. Thus, RdhA and its substrate exhibit a similar role to that of quinones.

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**MTP017**

**Advances in purification of the organohalide respiratory complex of *Dehalococcoides mccartyi* strain CBDB1**

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Introduction: Strictly anaerobic, organohalide respiring bacteria like *Dehalococcoides mccartyi* strains represent a promising opportunity for the bio-dehalogenation of persistent, halogenated organic compounds, such as chlorinated and brominated benzenes, dioxins, and phenols, utilizing them as terminal electron acceptor in organohalide respiration (OHR). The OHR complex of strain CBDB1 consists of seven protein subunits, including RdhA (a dehalogenation catalyst) and its anchor protein RdhB, hydrogenase subunits HupL and HupS, ferredoxin-like protein HupX with four [4Fe-4S] clusters, as well as OmeA with its membrane anchor OmeB. The overall size of the complex is estimated to be ~340 kDa.

Objectives: To date, the precise structure of the membrane-bound OHR complex of *D. mccartyi* remains elusive, with

only theoretical models and preliminary indications of the function available. Consequently, the objective of this study was to employ different purification techniques to render the OHR complex amendable to rigorous structural analysis *via* cryo-EM.

Results: To maximize the initial biomass, an anaerobic continuous flow reactor was used for cultivation, yielding a cell density of up to 5 x 108 cells mL-1. The cells were harvested by multiple centrifugation, followed by cell disruption, and ultracentrifugation to obtain the membrane fraction, which was solubilized using dodecyl-β-*D*-maltoside. To facilitate mild purification of the OHR complex, size-exclusion chromatography (SEC) alone, or in combination with anion-exchange chromatography (AEX) were used. The quality of the purified protein in the collected fractions was evaluated using a dehalogenase enzyme activity assay, SDS-PAGE, and LC-MS/MS. Collected SEC fractions ranging from 550 to 150 kDa exhibited the main dehalogenation activity in the activity assay, as well as the presence of all proteins associated with the OHR. However, SDS-PAGE analysis revealed that the SEC fractions comprised heterogeneous protein mixtures. Preceding SEC, the implementation of an AEX step led to notable improvements in peak resolution within the obtained SEC fractions. Nonetheless, this enhancement was accompanied by a substantial decrease in enzymatic activity and protein content.

Conclusion: This study successfully demonstrated initial purification of the OHR complex using AEX and SEC. Nevertheless, in order to obtain the necessary degree of purity for structural analyses, further improvements are required.

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**MTP018**

**Enzyme activity screening of hundreds of proteins of unknown Ffnction revealed novel lipolytic hydrolases of *Pseudomonas aeruginosa***

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The discovery of novel enzymes is crucial for a more profound understanding of cellular metabolism and biochemistry, as well as for practical applications in fields such as biotechnology, medicine, and agriculture. Hydrolases, in particular, are of great interest as they are the most abundant enzymes with diverse functions related to the turnover of proteins, polynucleotides, polysaccharides, and lipids. While their protein structures can be accurately predicted using AlfaFold2, their biochemical functions are difficult to anticipate without the use of activity assay-based approaches.

In this study, we aimed to identify novel lipolytic enzymes by screening enzyme activities of hundreds of hydrolases genes with putative or vague annotations from the human pathogenic bacterium *P. aeruginosa*.[1] To achieve this, we cost- and time-effectively constructed 416 corresponding expression plasmids and used them for overproduction of recombinant enzymes in *E. coli* under various conditions. This cell lysate library with an estimated overproduction success rate of 76% was used for unbiased screening of esterase, acyl-CoA thioesterase, lipase, phospholipase A (PLA), and phospholipase C, activities using artificial and natural substrates. Results indicated putative biochemical functions for 91 proteins from all five families. Among them, 21 overproduction strains showed dual or triple functions, as expected for these substrate promiscuous enzymes. Meta-analysis and experimental analysis of transposon mutant phenotypes linked several of those hydrolases with the virulence, antibiotic stress, biofilm formation and phospholipid remodeling. To study the predicted phospholipid remodeling functions, we conducted further analysis on several identified *P. aeruginosa* PLAs that share homology with disease-associated human proteins.

Our functional data about these enzymes of previously unknown function is of general significance for further deciphering their metabolic and virulence functions, not only in P. aeruginosa but also in other bacteria with homologous enzymes.

Babic, N., and F. Kovacic. 2021. "Predicting drug targets by homology modelling of Pseudomonas aeruginosa proteins of unknown function." *PLoS One* 16 (10):e0258385.

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**OTP001**

**Temporal dynamics of BGC regulation in the myxobacterium *Sorangium* sp**

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A better understanding of the regulation of natural compound biosynthesis could accelerate the discovery of new biologically active molecules. To this end, we have investigated the time course of genome-wide transcription in the myxobacterium *Sorangium* sp. So ce836 in relation to its production of natural compounds. Our time-resolved RNA sequencing data revealed that core biosynthetic genes from 92% of biosynthetic gene clusters (BGCs) in So ce836 were actively transcribed in a batch culture. The transcription of most polyketide synthases and non-ribosomal peptide synthetases peaked during exponential bacterial growth. Strikingly, these bursts in BGC transcriptional activity were associated with surges in the net production rates of known natural compounds. Taken together, our time-course data provide unique insights into the dynamics of natural compound biosynthesis and its regulation in a wild-type myxobacterium, challenging the commonly cited notion of preferential BGC expression under nutrient-limited conditions. Currently we explore differential RNA-seq (dRNA-seq) data to gain detailed insights into the temporal dynamics of transcription start site (TSS) usage and small RNA regulatory activities in *Sorangium* sp.

Reference:

Boldt, J., Lukoševičiūtė, L., Fu, C., Steglich, M., Bunk, B., Junker, V., Gollasch, A., Trunkwalter, B., Mohr, K. I.,

Beckstette, M., Wink, J., Overmann, J., Müller, R., & Nübel, U. (2023). Bursts in biosynthetic gene cluster transcription are accompanied by surges of natural compound production in the myxobacterium *Sorangium* sp. *Microbial biotechnology*, *16*(5), 1054–1068. https://doi.org/10.1111/1751-7915.14246

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**OTP002**

**Fragment-based drug discovery targeting the flavin transferase enzyme to combat antimicrobial resistance in gram-negative bacteria**

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The Na+-translocating NADH: ubiquinone oxidoreductase (Na+-NQR) is a unique respiratory chain multi-enzyme complex found in many bacterial species, including gram-negative human pathogens such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [1]. This membrane protein acts as a primary sodium pump, generating an electrochemical gradient of Na+ ions across the cytoplasmic membrane, which is converted to chemical, mechanical, and osmotic energy forms vital for the cell [2]. Notably, the nqrC and nqrB subunits exhibit a unique type of covalent FMN-binding via a phosphoester bond to a Threonine residue. This post-translational modification is widespread in bacteria and catalyzed by the flavin transferase enzyme (ApbE) [3]. Thus, blocking the active site of ApbE would prevent the maturation of Na+-NQR and other enzymes essential for microbial cellular respiration. We are pursuing this target in multidrug-resistant pathogens using fragment-based drug discovery, a high throughput screening technique to identify, develop, and validate chemical scaffolds and lead compounds. Whereby the first promising fragments were identified. This approach potentially serves as a starting point for developing a new class of antibiotics to combat antimicrobial resistance, especially in MDR-gram-negative bacteria.

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**OTP003**

**A ciliate bridging biology and physics – Paramecium bursaria as a model ambassador**

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Like many protozoa, Paramecia exhibit a predominantly predatory lifestyle which is a key factor in controlling microbial biomass in ecosystems. In genetics, epigenetics, cell and molecular biology, *Paramecium* is used as model organism due to its easy cultivation and established genomics. Furthermore, its huge diversity of naturally occurring intracellular symbionts makes it a suitable model in symbiosis' research. In this project, we demonstrate *Paramecium* as model organism obeying a random walk. Random walks are ubiquitously present in all natural sciences and are used as simple models for Brownian motion, e.g., to describe diffusion of pollutants in the air, model motile bacteria, and to describe the travel of data in the world-wide web.
We focus on *Paramecium bursaria*, known for its symbiotic lifestyle with photosynthetically active endosymbiotic algae and photoaccumulative behavior. Assessing its movement in well-defined specific environments, we will test multiple potentially influencing parameters such as nutritional condition, symbiotic status, and different illumination scenarios. We describe our implemented experimental environment, and explain our methods of measuring and analyzing trajectories of individual cells as well as of their mean squared displacement (MSD; squared average distance a particle moves within a certain time). We confirm *Paramecium* as a simple model suitable to verify predictions in statistical physics, active soft matter, and ecosystem modeling. This project will further facilitate predictions of *Paramecium*"s swimming behavior, an aspect highly relevant with respect to its feeding strategy, response to disturbances, and distribution.

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**OTP004**

**The DSMZ collection of cyanobacteria and protists: Characterisation of non-axenic cyanobacterial cultures by metagenomic sequencing**

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Introduction: The Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH is one of the world's largest collections of microorganisms and cell cultures. Recently, the DSMZ expanded its portfolio to include cyanobacteria, oxygenic photosynthetic bacteria of global importance, in the collection of microorganisms. The DSMZ collection of cyanobacteria currently comprises about 1000 strains from different habitats, representing approximately 110 genera. Many of the cyanobacterial strains in the collection so far have been classified based on morphological traits only, while their phylogenetic classification is still pending. Moreover, most cyanobacterial strains are non-axenic, making them difficult to characterise using standard microbiological methods. In addition to the cyanobacteria, the DSMZ has also launched a collection of ecologically relevant protists (diatoms, protozoa), constituting the first depository for protozoa in Germany.

Objective: Our goal is a comprehensive characterisation and genome-based phylogenetic classification of cyanobacterial strains. We further aim to explore the biodiversity of heterotrophs present in non-axenic cyanobacterial cultures.

Methods: We exemplary analysed the microbiomes present in the mixed cultures of DSM 109267 *Oculatella crustaeformantes* and DSM 101393 "*Phormidium* sp." by metagenomic sequencing using the PacBio Sequel IIe Technology.

Results: The complete genome sequence of the corresponding cyanobacterium was obtained in a single run from both cultures. In addition, up to five genomes of accompanying bacteria occurring in the cyanosphere were completely deciphered per cyanobacterial culture. The genome sequences of the cyanobacteria were analysed bioinformatically, resulting in the genome-based taxonomic assessment of the strains. While the taxonomic affiliation of DSM 109267 *O. crustaeformantes* to the genus *Oculatella* was confirmed, DSM 101393 "*Phormidium* sp." was identified as member of the species *Baaleninema simplex*. The genome sequences were further used to determine the toxigenic potential of the cyanobacteria and to elucidate the axenity status of the cultures.

Conclusion: With the further implementation of this approach, cyanobacteria of the DSMZ collection will contribute to an improvement of the cyanobacterial phylogeny and taxonomy. Furthermore, the DSMZ will provide the scientific community and their industrial partners with in-depth characterized cyanobacterial cultures.

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**OTP005**

**Investigating the antibacterial mode of action of gold nanoparticle – peptide deformylase inhibitor conjugates**

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Since the discovery of antibiotics, the evolution of resistance could be observed at the same time. In the so-called "golden age" of antibiotic discovery between 1940s and 1970s during which many new classes of antibiotics were discovered this challenge grew steadily. Since the 1980s only few new antibiotic classes were developed while multi-resistance is spreading, threatening the ability to effectively cure infections (Fernandes and Martens, 2017). In this study we investigated the potential of small gold nanoparticles as a new drug delivery system for antibiotics. Furthermore, we explored the possibility of a dual mode of action lowering the chance of resistance development. To this end, we investigated in a first step new derivatives of the peptide deformylase (PDF) inhibitor actinonin (Chen et al. 2000). In a second step, we examined the mode of action of the coated gold nanoparticles. As core method, gel-based proteomics in the model organisms *Bacillus subtilis* and *Escherichia coli* was utilized. Furthermore, incorporation assays with $^{35}$S-methionine and TEM imaging were used. We observed a broad activity of the free actinonin derivative against different gram-positive and gram-negative bacteria. Furthermore, the gel-based proteomics revealed that the derivative still functions as PDF inhibitor. The actinonin derivative – gold nanoparticle conjugate still exerted this mode of action. $^{35}$S-methionine incorporation assays showed that gold nanoparticles themselves (without antibiotic load) also caused a minor decrease in protein synthesis rates. Overall, we could show that gold nanoparticles and PDF inhibitor derivatives separately affect bacteria and show a promising antibacterial activity as conjugate.

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**OTP006**

**Characterization of the three CobW proteins shed new light on the metal pools of *Cupriavidus metallidurans* CH34**

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Zinc is an essential trace element due to its occurrence in a high number of metalloenzymes and metalloproteins with functions in all cellular pathways. In the beta-proteobacterium *Cupriavidus metallidurans* CH34 a zinc proteome has been described accommodating 120.000 zinc-binding proteins. This number is higher than the amount of 70.000 zinc ions accumulated per cell when the wild type strain is cultivated in Tris Minimal Medium, therefore allowing the cell to buffer incoming zinc ions. Moreover, the correct allocation of this trace element is of uttermost importance, both when found in scarcity or excess. As a consequence, a prerequisite condition is to maintain zinc homeostasis. Central to all these processes is the Zur regulon comprising three members: Zur as transcriptional regulator (Zinc Uptake Regulator), ZupT, a zinc importer of the ZIP family, and three recently characterized members belonging to the COG0523 family of the G3E superfamily of P-loop signal-recognition-associated SIMIBI class GTPases, named $CobW_1$, $CobW_2$ and $CobW_3$.

Our aim is to understand the contribution of the three CobW proteins in buffering and allocation of zinc ions between the loosely- and tightly-bound zinc pool of the cell. Furthermore, the roles they play in the low-specificity pool of other transition and heavy metals is of interest in the high metal resistance model organism *Cupriavidus metallidurans* CH34.

In this endeavour, a multi-branched approach is undertaken by combining physiological characterization with molecular approaches, a state-of-the art proteomic approach and development of an HPLC-ICP-MS analytical system for the separation of metalloprotein pools.

Although all three genes of CobW members are part of the Zur regulon, only *cobW*1 is strictly regulated and repressed by sufficient zinc conditions and strong induced under zinc starvation. On the other hand, *cobW*2 and *cobW*3 show a high constitutively expressed level. The proteomic approach allows a quantification of these gene products under several conditions and separation of metalloprotein pools gives us an insight into the metal protein ratio and allows a qualitative assignment. Moreover, the loss of CobW2 and CobW3 influences these metal pools.

The CobW proteins are part of the zinc repository by acting as metal buffering compartments and are involved in allocating metals efficiently to metal-dependent proteins.

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**OTP007**

**Investigating the production of natural products with antibacterial activity by *Streptomyces* isolates**

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Natural products are hard to beat in their chemical diversity and efficacy as antibiotics. They offer great potential for the development of new antibiotics urgently needed in view of the increasing incidence of multi-resistant microorganisms. Many of the antibiotics used in medicine today are based on structures of natural products produced by *Actinomycetes*, including bacteria of the genus *Streptomyces*. Genomic studies have uncovered that the potential of *Streptomyces* bacteria for secondary metabolite synthesis is many times greater than originally assumed[1].

Here we present an approach to discover and analyse the production of natural products with antibacterial activity against pathogens by *Streptomyces* isolates. A set of *Streptomyces* strains, isolated from various soil samples, was selected, which showed inhibition of *E. coli* EP1581 during cocultivation. Whole genome Nanopore and Illumina sequencing was performed. By using antiSMASH[2] biosynthetic gene clusters (BGC) were predicted and BGC sequence similarity networks constructed by BiG-SCAPE[3]. In addition, secondary metabolites produced by isolates under diverse conditions were investigated for their potential antibacterial activity against model organisms and ESKAPE pathogens. Metabolomic analyses of active extracts were performed by liquid chromatography-mass spectrometry.

In summary, we used genomics and metabolomics to explore specialized metabolite diversity. Among the predicted BGC only a few are described to give rise to known antibiotics. Additionally, the production of natural products under different cultivation conditions and their antibacterial properties were compared. Growth inhibition of ESKAPE pathogens was observed for extracts of several *Streptomyces* strains. Further effort is directed to the detection of bioactive natural products, their purification, structure elucidation and characterization of their mode of action.

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**OTP008**

**Investigation of the unusual PE-III phycobiliprotein of *Prochlorococcus marinus* SS120, using *Synechocystis* sp. PCC 6803 as a biosynthetic platform**

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The marine cyanobacterium *Prochlorococcus* is known to be the smallest photosynthetic organism on this planet, nonetheless, its sheer abundance makes it ecological one of the most significant cyanobacteria in the ocean. Unlike other cyanobacteria, *Prochlorococcus* has abandoned the effective light harvesting complexes, called phycobilisomes. Instead, this organism relies on divinyl-chlorophyll-antenna, harvesting blue light very efficiently. Interestingly, *Prochlorococcus* kept small amounts of a remnant of phycobilisomes (PBS) in form of a single phycobiliprotein, phycoerythrin III (PE-III). This PE-III of low-light adapted *P. marinus* SS120 (CCMP1375) is composed of an α- and β-subunit (SU) and likely carrying covalently attached phycoerythrobilin (PEB) and phycourobilin (PUB) chromophores in a 1:3 ratio. Genes required for the assembly of PE-III are encoded in a ~10 kb gene cluster, encoding the SUs and five putative phycobiliprotein lyases for the proper stereochemical attachment of chromophores to apo-PE-III. Apart from the cluster, genes are found encoding biosynthetic enzymes for PEB and phycocyanobilin (PCB). The function of lyases and their role in the assembly of apo-PE-III were investigated by a heterologous *E. coli* expression system. To date only the function of lyase CpeS was confirmed by this approach, ligating (3*Z*)-PEB to Cys82 on the β-SU CpeB. Although the remaining lyases can be expressed in *E. coli* as verified by Western blot, none of them displayed activity, pointing to a problem related to protein folding in the foreign host, possibly due to a bias in codon usage. With a novel approach, we will aim to circumvent this problem by introducing components of the PE-III gene cluster and different chromophore biosynthesis enzymes into the cyanobacterium *Synechocystis* sp. PCC 6803. The modular cloning system CyanoGate will allow a quick exchange of different combinations of lyases as well as the generation of a synthetic polycistronic operon, resembling the gene cluster of *P. marinus* SS120. Isolated native and recombinant phycobiliproteins from *Synechocystis* and tagged PE-III SUs will be examined by UV-Vis- and fluorescence spectroscopy, Zinc-blot, and HPLC to evaluate their composition. In addition, the possible interaction of introduced proteins with the natural phycobiliproteins will be analyzed. Overall, this project aims at understanding the assembly and function of the unusual phycobiliproteins in low-light adapted *P. marinus* strains.

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**OTP009**

**Tropolone natural product biosynthesis in *Streptomyces sp***

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Tropolones are a group of natural products with potent metal-chelating properties that exhibit antibacterial, antiviral and antitumoral activity.[1] These compounds are hydroxylated derivatives of tropone that consists of a seven-membered, non-benzenoid, aromatic carbon-ring with an additional keto-group.

In bacteria the precursor for these compounds surprisingly originates from primary metabolism, i.e. the CoA-dependent catabolism of phenylacetic acid (paa). However, depending on the producing strain, different sets of enzymes are used to modify this precursor. For the Gram-positive *Streptomyces* sp., the gene cluster encoding the dihydroxytropolone biosynthetic machinery was identified by gene-knockout studies.[2]

We now investigated dihydroxytropolone formation in *Streptomyces* sp. by *in vitro* reconstitution of the biosynthetic pathway using heterologously produced enzymes, which allowed us to gain insight into the individual biosynthetic steps and reaction mechanisms of the partaking enzymes. Conducted assays were analyzed via LC-MS, accumulating products were investigated by NMR and compared to chemically synthesized standards. Additionally, protein X-ray crystallography was performed and a crystal structure of one of the key enzymes could be obtained.

Accordingly, the thioesterase TrlF cleaves the CoA-ester bond from the precursor molecule originating from phenylacetic acid catabolism. The flavoprotein monooxygenase TrlE then mediates the unanticipated series of reactions comprising hydroxylation, decarboxylation and ring oxidation. Finally, the two-component flavoprotein monooxygenase TrlCD catalyzes two consecutive ring-hydroxylations to yield dihydroxytropolone. The role of TrlA,

an enoyl-CoA hydratase homologue encoded in the same gene cluster is still subject to investigation.

Taken together, the discovered enzyme functionalities substantially differ from the previously proposed roles that were based on gene knock out studies. Currently, kinetic and mutagenesis studies of TrlE are carried out in order to gain further insights into the unusual reaction mechanism.

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**OTP010**
**Metabologenomic workflow development for strain prioritization**

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Introduction: Global climatic changes lead to extreme weather events, as droughts and heavy rainfalls and in consequence to a weakening of eco systems. In this tense situation crop pests gain ground. At the same time, the use and misuse of chemical fungicides promotes the rise and spread of resistances, leading to failures in the control of plant diseases.

To counteract these developments, a transformation of agricultural systems towards a bio economical and sustainable food production is inevitable. To support this conversion, functional solutions for the management of diseases and infection events must be provided. Soil living microorganisms are an opportunity to naturally evolved solutions in the battle against fungal crop diseases.

Objectives: The goal of the here introduced project is the characterization of bacterial metabolic profiles and their naturally evolved potential as sustainable control agent for phytopathogenic fungi.

The project is based on the former Sanofi strain collection consisting of >100.000 microbial strains, and therefore a huge variety of spore forming, soil inhabiting bacteria is accessible for testing. Selected strains are rated using a metabologenomic profiling pipeline. The prioritized strains then will be tested *in vitro* and *in vivo* as potential antagonists against *Septoria tritici* and C*olletotrichum coccodes.*

Materials & Methods: Organic extracts of ~300 Bacilliaceae were screened for antifungal activity and rated on account of MS-based metabolomic data. Combined with the genome sequencing of >100 of these strains this forms the basis for our metabologenomic-guided strain prioritization workflow.

The combined consideration of activity and diversity data enabled a strategic strain selection for further studies, as the determination of the production of plant growth promoting compounds.

Results: A prioritized group of 15 out of 300 bacterial strains was selected by taking the extensive metabolomic and genomic datasets into account. Isolation and structure elucidation of unknown compounds with antifungal activities are running.

Conclusion: The project outlines the development of a workflow, on how to process big datasets to identify the most promising candidates for time and cost consuming laboratory experiments in the future.

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**OTP011**
**Selecting an optimal fluorescent marker for single-particle-tracking PALM imaging in bacteria**

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This research focuses on identifying the most suitable fluorescent markers (FM) for single-particle-tracking photoactivated localization microscopy (sptPALM) in bacteria. Our study involves various fluorescent proteins (FPs) and organic dyes to determine proper FM selection, crucial for accurate and quantitative sptPALM results.

Particularly important are high FM labeling specificity to the protein of interest (POI) and stable fluorescence signal for obtaining long sptPALM trajectories by using photostable and non-blinking FMs, both crucial FM characteristics to extract reliable molecular dynamics of POIs and their interaction partners. Additionally, controlled switching capabilities and/or high fluorogenicity of the FMs are essential to ensure measuring individual molecules.

For characterizing the performance of the different FMs, we chose the monomeric inner membrane protein LacY from E. coli as the biological test system [1], employing the inducible rhamnose system as the expression vector [2]. The monomeric and membrane-bound nature of LacY allows for the observation of individual, spatially separated fluorescent spots with slow diffusion characteristics, enabling a detailed and quantitative characterization of the different FMs for sptPALM imaging.

We compare the performance of the FPs PAmCherry, Dendra2-T69A, Dendra2 and mEos4b, along with the protein tag HaloTag7 using covalently bound organic dyes [3]. Furthermore, for extended tracking schemes, we explore the recovery method by 488 nm light for mEos4b imaging [4], and the utility of exchangeable Halo ligands [5].

In conclusion, this study aims to identify the optimal approach for sptPALM for imaging intracellular POIs in bacteria that can be easily adapted for different biological research projects. By systematically comparing current sptPALM imaging strategies and determining the most effective and quantitative approach, this research aims to reduce systematic biases, e.g. currently introduced by limited trajectory lengths or blinking FMs. The findings will have immediate relevance for future sptPALM projects, enabling improved tracking schemes and more accurate data analysis.

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[5] doi:10.1021/jacs.2c11969

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**OTP012**

**Characterizing microbial growth in single–cell microfluidics**

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Single-cell microfluidic systems offer versatile applications in microbial cell biology research, including the comprehensive analysis and monitoring of large populations of bacterial cells and their offspring. The design of the so-called mother machine devices facilitates the tracking of cell generations while providing precise control over environmental parameters, e.g., nutrients, temperature, or active compounds. It is possible to determine growth rates of single-cell lineages under given conditions and to explore the progression of subcellular processes for multiple generations [1, 2].

In this project, we benchmark robust single-cell microfluidic imaging for various microorganisms. This framework does not only enable the characterization of the growth and proliferation of cells under diverse environmental conditions, but also facilitates high-throughput quantitative phase contrast and fluorescence imaging at the single-cell level.

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**OTP013**

**Cultivation of yet unknown microorganisms from extreme aquatic environments**

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Currently less than 1% of microbial taxa are discovered (Locey and Lennon 2016), which shows an enormous knowledge gap. The BMBF funded project MultiKulti was started to tack this, by taking aquatic samples from high-altitude lakes in the Atacama Desert, Chile and the biggest cold-water geyser of the world in Andernach, Germany. Those lakes are of special interest due to their extreme conditions, which are important for astrobiologists and the definition of life limits. Organisms isolated from these habitats are well adapted to their environment, and have developed survival strategies against diverse stress factors. In this study the tolerance of two bacterial isolates and cyanobacteria will be tested upon temperature, pH, salinity and radiation. The pigmentation of these organisms is of interest, due to its wide range of use in biotechnological fields and spaceflight, as radiation protection. Two organisms were chosen, that showed a 16S rRNA similarity to its closest known relative of less than 98.7%, being the threshold for new species according to Kim et al. 2014. One organism was isolated from Laguna Verde in the Atacama Desert, HP23, which is closest related to *Roseovarius tibetensis.*. The second organism, R23, was isolated from the Geyser Andernach, being clostest related to *Arundinibacter roseus*. Both organisms show a different pigmentation compared to the type strains and their ability to tolerate extreme conditions. This will be tested and compared to the closest relative and the results presented. Further experiments include whole genome sequencing, API tests, fatty acid and polar lipid analysis. Experiments upon their pigment analysis are UV-VIS spectrometry, RAMAN, thin-layer-chromatography, and HPLC-MS. For the radiation shielding experiments organisms will be irradiated with UV light and X-Ray, also the pure pigment extracts will be used as a shield for other organisms. This project will lead to a greater knowledge in the cultivation of extreme microorganisms, their ability to adaption and potential usage in space.

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**OTP014**

**The next level: OpenBIS supporting encryption and workflows via a novel user interface**

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Introduction: Today, we generate research data faster than ever before. At the same time, not only the number of data sets is increasing, but also their size. For example in life sciences, sequencing reads that used to be a few hundred base pairs long and were selectively collected, now consist of million reads summing up to many millions of base pairs. Data management systems have been developed to help keep track of such volumes of data, neglecting the challenge of data security that must be considered when processing sensitive data.

Objectives: We extend the open source software openBIS to ensure data security by cryptographic methods. The complete encryption and key management is done client-side, inside the user"s web browser. In addition, workflows can be executed from within openBIS and encrypted results are stored back. Thereby, the protection by encryption must remain.

Materials & Methods: We chose the established Crypt4GH standard for the encryption component. It uses asymmetric encryption protected by keys based on elliptic curves, but can also be extended to meet future requirements. The execution of workflows is controlled by our self-developed workflow registry. This ensures the security of input and output data. For maximum data protection, isolated environments are created in our own cloud infrastructure for the execution of workflows, which are removed instantly after workflow completion. For the web interface, we rely on a modern single-page application written in Vue.js.

Results: Here we present our system which allows to store scientific data securely encrypted, based on the open source data management software openBIS. The decryption information is known only to the user. By encrypting the data for other users, access can be easily granted or revoked. In addition, audited workflows can be run on the user's data

sets without weakening the encryption. The obtained results are also written back to the system in encrypted form. For maximum security, the entire software package can also be installed as open source software within a self-controlled environment.

Conclusion: Our platform allows users to keep control and analysis capability over large amounts of data, while ensuring data protection to meet increased demands in the field of data management and to be ready for future requirements.

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**OTP015**

**A streptolysin S-like hemolysin in *Brachyspira* species**

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Introduction: Hemolysis is considered a major virulence trait in *Brachyspira* species. Various genes have been associated with hemolysis but their role in the development of a strongly or weakly hemolytic phenotype is unclear.

Objectives: We wanted to quantify the variable hemolytic capacity of *Brachyspira* species and search for genomic differences that would explain the variable phenotypes.

Materials and Methods: Strongly and weakly hemolytic isolates of different *Brachyspira* species were investigated for their hemolytic capacity in the presence of the RNase resistant fraction of yeast RNA (RNAcore). Inhibition of the hemolytic activity by trypan blue was assessed. Genomes were mined for a streptolysin S-like haemolysin (SLS) and associated genes (*sag*-gene cluster). The hemolytic activity was quantified when RNAcore was substituted by different DNA oligonucleotides, which were rich in guanine (50-66%) or randomly composed with 25% guanine.

Results: Statistically significant differences in the hemolytic capacity in the presence of RNA core could be demonstrated in the different *Brachyspira* isolates. The hemolytic activity could be inhibited by pre-incubation with trypan blue. A gene for a SLS-like haemolysin, genes for post-translational modification and its export were identified. RNAcore could be substituted by a DNA oligonucleotide with a guanine content of 66% while lower guanine content resulted in significantly less hemolytic activity.

Conclusion: Phenotypic and genomic data suggest that *Brachyspira* species harbour a SLS-like hemolysin and associated genes in addition to previously described hemolysis-related genes. Similar to SLS, hemolytic activity is dependent on the presence of a stabilizer like RNAcore. RNAcore can be substituted by an oligonucleotide predicted to from guanine-quadruplexes (G4). Together this suggests that substrate-ligand interactions between aromatic thiazole and oxazole heterocycles of SLS and G4 molecules confer peptide toxin stabilization necessary for hemolytic activity. Genetic differences in the *sag*-gene cluster of different *Brachyspira* species might explain the strongly or weakly hemolytic phenotype of isolates.

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**OTP016**

**Analysis of Asc1/RACK1s function in hooking cellular signaling to the sensing and processing of stalled and collided ribosomes**

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Ribosomes are the protein biosynthesis factories of cells. Additionally, they are increasingly experienced as versatile hubs for cellular sensing and signaling. In eukaryotes, ribosomes are separated from the gene-encoding chromosomes within the nucleus demanding for communication pathways that coordinate ribosomal mRNA translation and nuclear gene activity. Asc1/RACK1 is a eukaryote-specific Gb-like protein of the head region of the ribosomal 40S subunit (hr40S). It is involved in dealing with ribosomes that unproductively stall and collide during mRNA translation. Sensing of these ribosomes and subsequent ubiquitin-dependent remodeling of the microenvironment at the hr40S appear Asc1-/RACK1-dependent and might directly be associated with signaling pathways that determine nuclear activities. In vivo proximity labeling analysis with biotin (BioID) revealed that (i) the E3 ubiquitin ligase Hel2, (ii) the de-ubiquitination complex Ubp3-Bre5, and (iii) the ubiquitin-binding proteins Def1 and Lsm12 (both regulating stability of nucleic acid polymerases within the nucleus in response to cellular stress) reside within the cytoplasmic hr40S, proximal to Asc1/RACK1. Using (Split-)BioID in combination with mass spectrometry, we characterize stress-induced ubiquitin-dependent protein remodeling at the hr40S that might account for the release of nucleus-targeted factors. Therefore, we express bait proteins of interest like Def1 or Lsm12) as fusion proteins of high-efficiency biotin ligases for subsequent affinity capture of biotinylated proteins and LCMS identification/quantification.

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**OTP017**

**Production of tailored biosurfactants in *Pseudomonas putida***

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Biosurfactants represent a diverse array of amphipathic biomolecules with significant potential for industrial and medical applications. Production in native hosts often presents challenges due to intricate regulatory networks and demanding cultivation conditions. Utilizing recombinant expression strains, such as *Pseudomonas putida* KT2440, provides an advantageous alternative, featuring enhanced laboratory accessibility and optimized production parameters.

We successfully established *P. putida* KT2440 as a versatile platform for the biosynthesis of a broad spectrum of biosurfactants, including rhamnolipids, lipopeptides such as serrawettin W1 and a novel glycine-glucose-lipid conjugate. Rhamnolipids were produced in various compositions, including pure 3-(3-hydroxyalkanoyloxy-) alkanoic acid (100%), mono-rhamnolipid (100%), and mixtures of mono- and di-rhamnolipids (14-94% mRL), each possessing potentially unique properties. Furthermore, we achieved

heterologous production of methylated rhamnolipids, a relatively new class of compounds.

Our findings demonstrate the potential of *P. putida* KT2440 as a robust and efficient platform for the production of an extensive range of biosurfactants, thereby expanding their diversity and paving the way for novel applications in biotechnology.

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**OTP018**

**Sphingolipid inhibitors from fungi: biosynthesis, spatio-temporal organisation and cross-interaction with sphingolipid metabolism**

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Filamentous fungi are responsible for the production of chemically diverse secondary metabolites with the potential for biotechnological applications and drug discovery. Each year, with more genomes becoming available, new secondary metabolites are discovered and so far, their biosynthesis has been mainly investigated on genetical and biochemical level. Little is known about the cellular localization of the enzymes involved in the biosynthesis and only a few have been well studied. Of special interest are sphingofungins, class of sphinganine-analog mycotoxins (SAMs) produced by the soil-dwelling and known human pathogen *Aspergillus fumigatus*. Sphingofungins inhibit serine palmitoyl transferase (SPT), the first and rate-limiting enzyme of the sphingolipid biosynthesis. Previously, we elucidated the biosynthetic cluster and pathway in *A. fumigatus* which enabled us to further investigate the pathway on the cellular level [1]. Localization studies showed that the enzymes involved in the biosynthesis are associated with the cytosol, ER, and ER-derived vesicles and additionally, partially colocalize with its target enzyme – SPT. These results raised the question of how the fungus is protecting itself from its toxin. Unlike the fumonisin biosynthetic cluster, which includes genes encoding additional subunits of the target enzyme, the sphingofungin cluster does not have additional candidate genes, therefore we hypothesized that SphA and SphF, the aminotransferase and 3-ketoreductase of the cluster, can compensate in the sphingolipid pathway. Using *in vitro* and *in vivo* approaches, we could prove that SphA can function as an SPT, and SphF as a 3-ketodihydrosphingosine reductase (3-KDSR) in sphingolipid pathway and provide additional enzymatic activity, revealing their parallel role as enzymes involved in self-resistance.

1. Bissell, A.U., et al., *Biosynthesis of the sphingolipid inhibitors sphingofungins in filamentous fungi requires aminomalonate as a metabolic precursor.* ACS Chem Biol, 2022. **17**(2): p. 386-394.
2. Janevska, S., et al., *Self-protection against the sphingolipid biosynthesis inhibitor fumonisin B1 is conferred by a FUM cluster-encoded ceramide synthase.* mBio, 2020. **11**(3): p. e00455-20.

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**OTP019**

**Innovative Strategies for Enhanced Microalgal Production**

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Microalgae are potential cell factories for producing biomass and complex high-value compounds, which consume only light energy, $CO_2$ and water. However, the relative low cell density (CD) of photoautotrophic cultures has prevented their economic utilization. To overcome this limitation, researchers have focused on the creation and selection of highly productive transgenic strains as well as on novel photobioreactors enabling cultures to reach a strong synthetic activity at high CD. The combination of genetic engineering and high-density cultivation (HDC) resulted in phototrophic diterpene production by *Chlamydomonas reinhardtii* with a product titer above the one reached by conventional mixotrophic cultivation and comparable to those obtained with engineered yeast and *E. coli* (Einhaus et al. 2022). In this study, model organisms were cultivated with CellDEG's HDC system based on membrane-mediated $CO_2$-input. Photosynthetic productivity (g dry mass $L^{-1}$ $d^{-1}$) and final dry biomass concentration are given to illustrate the cultivation performance. Compared with conventional gas bubbling and headspace gassing the membrane-mediated $CO_2$ supply reaches higher mass transfer rates, thus preventing $CO_2$-deficiency at extremely high CD. A further important advantage of the HDC system consists in the almost lossless $CO_2$ supply, which gives a measure of photosynthesis. The studied strains include *Synechocystis sp.* PCC 6803, *Synechococcus sp.* PCC 7002, *Arthrospira maxima* CCALA 27, and *Nannochloropsis salina*. All organisms exhibited a long linear light-limited growth phase with nearly constant productivity >5 g DW $L^{-1}$ $d^{-1}$ and achieved cell concentrations >20 g DW $L^{-1}$. The highest productivity (8 g DW $L^{-1}$ $d^{-1}$) was observed with *Synechocystis*, while the highest CD was achieved with *Arthrospira* reaching 46 g DW $L^{-1}$ in a 1000 mL volume. The HDC technique enabled similar high productivities in all strains, irrespective of their different maximum specific growth rate. Most important parameters for productive HDC were high CD, rapid gas exchange with the liquid phase, high PFD, intensive turbulent mixing in a short light path, and special balanced mineral media with high concentration of all macronutrients. In conclusion, HDC cultivation with membrane mediated $CO_2$-supply significantly enhances the biomass productivity of all studied organisms compared to previously published data.

Keywords: microalgae, high-density cultivation, photobioreactors, productivity, CD, $CO_2$ supply, genetic engineering, biotechnological performance.

References:

Einhaus, A., Steube, J., Freudenberg, R. A., Barczyk, J., Baier, T., & Kruse, O. (2022). Engineering a powerful green cell factory for robust photoautotrophic diterpenoid production. Metabolic engineering, 73, 82–90. 10.1016/j.ymben.2022.06.002

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**OTP020**

**Enrichment and isolation of acetogenic bacteria as biocatalysts for industrial waste gas fermentation**

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Acetogenic bacteria have high potential as biocatalysts that can convert waste gas carbon, such as carbon dioxide ($CO_2$) or carbon monoxide (CO), into biomass or biochemicals. These C1 gases contribute significantly to climate change, making carbon recycling by this specialized group of strictly anaerobic bacteria and their unique Wood-Ljungdahl pathway a promising solution to bridge the gap between increasing demand and environmental concerns. However, currently industrial applications of acetogenic bacteria are limited by growth rate, product spectrum and yield. In this study, novel acetogenic isolates were enriched from diverse environmental samples with the aim of overcoming some of these limitations. Enrichments were performed under anaerobic conditions (N2 (80%)/$CO_2$ (20%) or $H_2$ (67%)/$CO_2$ (33%), total pressure 2 bar) at 35 and 60 °C. Three minimal media with 0.05 g $L^{-1}$ yeast extract, pH values from 6 to 8, 12 different substrates including H2/$CO_2$, 2,3-butanediol, 3,4,5-trimethoxybenzoic acid, acetoine, betaine, ethanol, ferulic acid, formic acid, lactate, methanol, syringic acid, and vanillic acid were tested. A total of 19 environmental samples were used for inoculation, ranging from local composting sites, river sediments, waste water and feces, to extreme environments, such as volcanic hot springs, deep sea or desert. Overall relative abundance and diversity of acetogenic bacteria were highest (>65% relative abundance) in cultures at pH 7 with vanillic acid as substrate and compost as inoculum, based on 16S rRNA gene amplicon analysis. Acetogenic bacteria belonging to the genus *Sporomusa* were most prevalent. In addition, acetogenic bacteria of the genera *Alkalibaculum*, *Clostridium*, *Moorella*, *Terrisporobacter* and *Thermacetogenium* were also enriched. Bacterial isolates from the enrichments capable of acetogenesis will be presented, and genomic similarities and differences to reference strains will be discussed, including an early assessment of their potential as biocatalysts.

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**PBP001**

**Structural and biochemical dissection of the SPbeta arbitrium prophage master repressor MprR (YopR)**

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Prophages can either reside in the genome of their bacterial host or enter the lytic cycle resulting in lysis of the cell. Hence, they must have evolved sophisticated strategies to control their lifestyle. The genome of *Bacillus subtilis* contains a prophage called SPb whose lysogenic state strongly depends on the master repressor MrpR (YopR) (1,2,3). The systematic analysis of an historic *B. subtilis* strain harboring the heat-sensitive SPb c2 mutant, allowed us to analyze MrpR as a key component of the lysis-lysogeny decision system. We demonstrate that the heat-sensitive SPb c2 phenotype is due to a single nucleotide exchange in the *mrpR* gene, rendering the encoded MrpRG136E protein temperature sensitive. The structural characterization of MrpR revealed that the protein is a DNA-binding protein with an overall fold like tyrosine recombinases. Yet, further biochemical, and functional analyses indicate that MrpR has lost the recombinase activity. HDX-MS experiments showed that the G136E exchange impairs its higher order structure and DNA binding activity. Finally, we assume that the MrpR master regulator binds to several regions within the genome of the SPb prophage presumably repressing gene expression and thereby hindering activation of the lytic lifecycle, thus maintaining its lysogeny.

(1) Brady, A., et al. (2021) Curr. Biol. 31: 5037-5045.

(2) Kohm, K., et al. (2022) Environ. Microbiol. 24: 2098-2118.

(3) Bremer, E., et al. (2023) Microb. Biotechnol. 00: 1-29.

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**PBP002**

**Antiphage properties of aminoglycoside antibiotics produced by *Streptomyces spp***

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Bacteria are under the constant threat of predation by bacteriophages (or phages), thus they evolved a diverse arsenal of anti-phage defense systems. Most of the defense systems described so far are RNA or protein mediated and acting at the single cell level[1]. In our studies, we are focusing on the prokaryotic immune system of multicellular bacteria of the genus *Streptomyces* [1,2] .

Environmental bacteria like *Streptomyces spp.* produce a variety of bioactive molecules including a vast majority of all known and pharmaceutically used antibiotics. These molecules play an important ecological role and are multifunctional; besides being anti-bacterial agents, they can also have anti-fungal, anti-cancer and anti-viral properties. Recently, we could demonstrate that aminoglycoside antibiotics, produced by *Streptomyces*, inhibit phage infection in *Streptomyces venezuelae*. This inhibitory effect was observed using purified compounds as well as spent medium from the producer strain[2], emphasizing the physiological relevance of the observed effect.

In this study, we aim to unravel the mechanism of action, how aminoglycoside antibiotics inhibit phage infection by using super-resolution microscopy and specific phage-DNA labelling. Furthermore, we will elucidate the potential of secondary metabolites produced by several *Streptomyces spp.* as a community defense by analysing the anti-phage activities of spent media. Preliminary results showed inhibition of phage infection to varying degrees. These results suggest a broad prevalence of chemical defense against phage infection and highlights its importance for community interactions.

1 Aël Hardy, Larissa Kever, and Julia Frunzke* "Antiphage small molecules produced by bacteria – beyond protein-mediated defences" (2022), doi: 10.1016/j.tim.2022.08.001

2 Kever, L. et al. "Aminoglycoside Antibiotics Inhibit Phage Infection by Blocking an Early Step of the Infection Cycle" (2022), doi: 10.1128/mbio.00783-22

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**PBP003**

**Lysis inhibition (LIN) of phage T4 requires a dimeric T4 holin/antiholin complex**

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Lytic phages control the timepoint of host cell lysis by timing the holin-mediated release of cell wall-degrading endolysins. In phage T4, the antiholin RI inhibits the holin T, thereby preventing early release of the T4 endolysin and lysis. The antiholin is believed to sense phage superinfections that give rise to phage DNA inside the periplasm of infected cells, which is thought to be responsible for a lysis inhibition (LIN) that increases the chance for lysis in an environment with lower phage concentration. The holin T consists of a small N-terminal cytoplasmic domain, followed by a transmembrane-helix and a periplasmic C-terminal domain. The antiholin is targeted to the periplasm by a cleavable N-terminal signal peptide. The soluble domains of T and RI have been reported to crystallize to a tetrameric T2/RI2 complex. To investigate the functional relevance of this complex, we reconstituted LIN in a phage-free system, using only RI, T, and the endolysin, and analysed effects of mutations on lysis and LIN. LIN worked without periplasmic DNA. Inactivation of the RI signal peptide cleavage site did not abolish lysis inhibition, indicating that RI can function in a membrane-bound state, which does not fit to the current tetramer-model [1]. We therefore carried out mutational analyses that indicated that the physiologically relevant complex is a dimer, which is also supported by AlphaFold prediction. The reported tetramer of the soluble domains thus may have been formed from dimers during crystallization. The dimeric complex can explain, how T and RI can interact in a membrane-bound state.

[1] Mehner-Breitfeld D, Schwarzkopf JMF, Young R, Kondabagil K, and Brüser T (2021) The phage T4 antiholin RI has a cleavable signal peptide, not a SAR domain. Front. Microbiol. 12:712460. doi: 10.3389/fmicb.2021.712460

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**PBP004**

**Metabolic footprint of prophage induction**

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Viral lysis of bacterial hosts results in the release of metabolites and cell lysates promoting energy and material fluxes (viral shunt). Viruses can lyse their host immediately after infection (lytic cycle), or replicate their genome alongside the host genome (lysogenic cycle). The integrated viral genome (i.e. prophage) can be induced to enter the lytic cycle under certain conditions (e.g. environmental stress). Less is known about the importance of virus-mediated cell lysis in terrestrial food webs.

In this project we aim to demonstrate that the lysis-driven release of particulate and dissolved compounds in subsurface systems (groundwater) is relevant for the growth of microbial communities. In particular, we hypothesize that, compounds released after lysis are available to subsets of the subsurface microbial community.

As a first step, we determined changes in the endo- and exometabolome upon prophage induction. Bacterial strains from groundwater were induced with mitomycin-c, a common method to study prophage induction. Out of 146 isolates, 54 were inducible and used for further analysis. The inducible strains were sequenced using nanopore sequencing technology to identify prophages in the bacterial genomes. Endometabolomes were analyzed by liquid chromatography coupled to mass spectrometry, and exometabolomes were determined by direct injection mass spectrometry. The results show distinct endo- and exometabolome responses in bacteria treated with mitomycin as compared to media controls. These metabolites are being further analysed and will also be tracked in induced groundwater microbial communities to identify metabolic biomarkers of viral lysis.

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**PBP005**

**Archaeoviral binding and recognition of HCTV-6 & HCTV-8 to their host *Haloarcula californiae***

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Viruses infecting archaea are extraordinary within the global virosphere. They display unique virion morphologies and their genomes are puzzling since they encode proteins with so far unknown functions and low similarity to any other protein species. Thus, archaeal virus-host interactions might show unique and so far undescribed features. Nevertheless, information about archaeal virus-host interactions is scarce. It is our goal to study their interactions with an appropriate model system. The haloarchaeon *Haloarcula californiae (H. californiae)* is an interesting candidate to study virus-host interactions since a broad range of molecular and microscopic methods is available for it. Haloarcula californiae tailed virus 6 (HCTV-6) & Haloarcula californiae tailed virus 8 (HCTV-8) of the *Hafunaviridae* both infect *H. californiae*. Their virus-host interactions were characterized by adsorption assays and one-step growth curve experiments, in order to get insight into the entry mechanisms of these viruses. This work will generate deeper understanding into the host binding mechanisms of tailed haloarchaeal viruses and will support further research on the entry of archaeal viruses in general.

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**PBP006**

**Identification of a novel bacteriophage receptor in *Staphylococcus epidermidis***

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Introduction: Bacteriophages are extremely abundant in the earth"s biosphere and important for bacterial population dynamics and horizontal gene transfer. Phages infecting the genus *Staphylococcus* are most thoroughly investigated for the clinically relevant species *Staphylococcus aureus.* For attachment and infection, bacteriophages need to bind a specific surface structure via their receptor binding proteins (RBPs). In case of *S. aureus*, these receptors are differentially glycosylated cell wall bound alditol polymers called wall teichoic acid (WTA).

Objectives: Up to now, other species of *Staphylococci* have been neglected in this research, but might function as hubs

for antibiotic resistance genes, which can be spread by phages through horizontal gene transfer. We aim to identify how glycosylated WTA in coagulase-negative Staphylococci determines the bacteriophage infection dynamics.

Material & methods: Since most coagulase-negative *Staphylococci* (CoNS) share a similar WTA backbone consisting of poly-glycerol-phosphate (GroP), we used the well-known coagulase-negative species *Staphylococcus epidermidis* for identification of the bacteriophage receptor binding site. We therefore created a transposon mutant library of *S. epidermidis* strain 1457 and challenged it with siphovirus ΦE72.

Results: Transposon insertions were identified in genes with high homology to the phosphoglucomutase PgcA, uridylyltransferase GtaB and glycosyltransferase TagE of *Bacillus subtilis*. All mutants showed decreased siphophage but increased podophage binding capacity, while binding of all tested myophages remained unchanged.

Conclusion: The identified enzymes are involved in the synthesis and transfer of UDP-glucose to GroP-WTA, and deletion of the TagE homologue in *S. epidermidis* resulted in increased attachment of an n-acetylated sugar to the GroP-WTA, suggesting that an additional glycosyltransferase is involved in this process.

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**PBP007**
**Induction of violacein synthesis in *Janthinobacterium lividum* by an unknown signal molecule**
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Bacterial violacein is an antibiotic bisindole, synthesized by several Gram-negative bacteria such as *Janthinobacterium lividum*. Even though the purple pigment is utilized by bacteria as an antibacterial agent, we observed that it is also synthesized in the presence of bacteriophages.

Bacteriophages are viruses that specifically infect bacteria and can coexist or kill their host. Bacteria-phage interactions have a fundamental impact on bacterial evolution and ecology by altering the competition, maintaining diversity, and facilitating horizontal gene transfer among bacteria. Not all bacteria are susceptible to bacteriophages as some have developed defense mechanisms, including CRISPR-Cas and restriction-modification systems, which are activated upon recognition of phage proteins or DNA. Information on an infection can also be communicated by some bacteria to the bacterial population.

However, the role of violacein in bacteria-phage interactions remains elusive. Hence, we were interested to determine how the violacein synthesis is induced upon infection of *Janthinobacterium lividum* with bacteriophages. In our study, we used the bacterial strain *J. lividum* EIF1. In an overlay plaque assay. EIF1 was treated with water from a local sewage plant (Göttingen), which served as viral source material. We isolated the phage vB_JliS-Donnerlittchen a siphovirus with a genome size of 58,220 bp, containing one tRNA and 74 protein-encoding genes. During phage reinfection, we recorded that EIF1 produced violacein. Interestingly, the purified lysate of the infected bacteria was able to induce violacein synthesis. Thus, we assume that it contained an unknown inducer. In conclusion, the results indicated a putative new phage defense mechanism, which includes post-infection signaling to induce protective violacein production in uninduced cells. The nature of this defense cascade and the identification of the signaling molecule remain to be clarified by future studies.

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**PCBP001**
**Elucidating Auranofin's (AF) mechanism of action in bacterial cells**
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Bacterial resistance against antibiotics is one of the main challenges for our public health. Infections with resistant bacteria currently claim at least 700 000 lives per year, projected to increase to 10 million by 2050. Conventional *de novo* drug discovery is a lengthy and expensive process. A promising approach consists of screening libraries of approved compounds to repurpose these molecules to create new viable therapies. One candidate drug is Auranofin (AF). AF is a gold-based compound utilized to treat rheumatoid arthritis, and it is known to inhibit human thioredoxin reductase (TrxR) by binding to thiol and selenol groups in the active site of the enzyme. Studies using AF demonstrated its antibiotic potential against gram-positive bacteria, including multi-drug-resistant pathogens. However, it lacks significant activity against Gram-negative species for unclear reasons. Using *E. coli* as a gram-negative model organism, we aim to identify AF's target(s) in bacteria. Due to the well-established inhibition of human thioredoxin reductase, it stands to reason that AF has similar effects on the bacterial homologs of that protein. We tested this hypothesis with enzymatic assays *in vitro*. In *E. coli,* 1 µM TrxR is fully inhibited using 1 µM of AF, while the level required to exhibit cytotoxicity against this bacterium is around 188 µM. To understand the recovery of the cells during AF treatment, we used mutants lacking parts of the thioredoxin (Trx) and glutaredoxin (Grx) system, known pathways to help cells to overcome oxidative stress. The lack of the first enzyme of Grx does not influence the cells, while the glutathione mutant showed a MIC of 11.7 µM. The lack of Trx-system proteins only decreased the susceptibility in 4-fold. We performed proteomic experiments studying *E. coli's* response to AF using sub-lethal concentrations to determine potential targets. We identified 20 proteins that were upregulated, which are mainly involved in the oxidative stress response. However, *E. coli* mutants lacking these proteins did not show to be more sensitive to AF's treatment, which led us to conclude that AF might not have one specific target. So far, our data suggest that AF globally perturbs the bacterial thiol proteome. AF's mode of action seems to inactivate thiol-containing enzymes, including those involved in the oxidative stress response, with subsequent induction of systemic oxidative stress and detrimental effects on bacterial metabolism.

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**PCBP002**
**Well protected: Three CRISPR-Cas systems in antibiotic producer *Streptoalloteichus tenebrarius***
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CRISPR-Cas systems are known as adaptive prokaryotic immunity systems. They play an important role in the defence against foreign mobile genetic elements like plasmids or phages. Recently, the involvement of CRISPR-Cas in other cellular processes including DNA-repair, stress

tolerance or the regulation of virulence was described. CRISPR-Cas systems are commonly found in prokaryotes. But the presence of more than one CRISPR-Cas system occurs only in 9 % of all sequenced prokaryotic genomes. So far, the knowledge about the function of CRISPR-Cas in *Actinomycetes* is very limited. Many bacteria of the class *Actinomycetes* are important producers of secondary metabolites with potent properties like antimicrobial, anti-tumor or immunosuppressive activity. However, a large portion of the natural products remain unexplored as the biosynthetic gene clusters (BGCs) are in many cases not expressed and require an activation (e.g., by genetic manipulation). Such genetic engineering is often prohibited by mechanisms that are largely uncharacterized in *Actinomycetes*.
The goal of this project is the functional analysis of CRISPR-Cas systems in antibiotic-producing *Actinomycetes*. This includes adaptive immunity as well as functions beyond immunity, such as a potential role of CRISPR-Cas in antibiotic production (e.g., regulation of antibiotic production). For this, we use the industrial aminoglycoside antibiotic producer *Streptoalloteichus tenebrarius* as model strain. It is particularly interesting, as its genome possesses 33 BGCs and three CRISPR-Cas systems (Region 1-3). In a first approach, the adaptive immunity function was investigated using DNA-uptake assays based on transformation, conjugation and phage-infection. As the genetic manipulation of *S. tenebrarius* is very challenging, heterologous expression of the CRISPR-Cas systems in *Escherichia coli* and *Streptomyces coelicolor* was chosen. Expression of the CRISPR-Cas systems from *S. tenebrarius* in heterologous hosts did not lead to adaptive immunity in naive DNA-uptake assays (non-primed system). The next step is the performance of primed assays. Furthermore, CRISPR-Cas deletion mutants in *S. tenebrarius* were generated. The deletion of CRISPR-Cas Region 1 led to a sporulation-deficient phenotype with influence on the aminoglycoside antibiotic production. This suggests an involvement of CRISPR-Cas in cell-differentiation processes in *S. tenebrarius*. The potential non-immunity function must be further investigated.

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**PCBP003**

**A direct link between control of peptidoglycan biosynthesis via MurA degradation and cell division in *Listeria monocytogenes***

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Introduction: *Listeria monocytogenes*, a gram-positive bacterium, is characterised by a thick cell wall consisting mainly of peptidoglycan. This ensures the viability of the cell in the presence of high turgor pressure. Therefore, enzymes that control peptidoglycan biosynthesis are important targets for antibiotics.

To fully understand peptidoglycan biosynthesis and gain further insight into potential targets for antibiotics, we are investigating a recently discovered regulatory pathway, that regulates peptidoglycan biosynthesis[1,2]. This pathway controls the proteolytic degradation of MurA, the enzyme that catalyses the first step of peptidoglycan biosynthesis. As far as we know, MurA can be degraded by the protease ClpCP in the presence of its interaction partner ReoM. However, when ReoM is phosphorylated by the PASTA-domain containing protein serine/threonine kinase PrkA, the interaction with MurA is inhibited and MurA degradation is prevented[1,2].

Objectives: We wanted to clarify at which conditions ReoM is phosphorylated by PrkA and thus peptidoglycan biosynthesis is favoured.

Material & Methods: To analyze the phosphorylation state of ReoM *in vivo*, we generated a ReoM specific antiserum, performed native PAGE to separate the differently phosphorylated ReoM species, followed by western blotting and immunostaining of ReoM using cell lysates from different mutants and strains cultivated at different growth conditions.

Results: Our results indicate that ReoM is fully phosphorylated under most tested laboratory growth conditions. However, deletion of GpsB, a protein that is part of the divisome, prevented the phosphorylation of ReoM.

Conclusion: The regulatory pathway for MurA degradation via ReoM phosphorylation appears to be directly linked to cell division. With further studies, we would like to clarify this link to cell division in more detail and try to identify growth conditions that inhibit ReoM phosphorylation and thus force the cell to shut down peptidoglycan biosynthesis.

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**PCBP004**

**Molecular and mechanistic basis for spatiotemporal organization of polar flagella**

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Motility through flagella is one of the most important modes of locomotion within bacterial populations. Bacteria can exhibit different flagellation arrangements: Flagella can either be located all over the cell body (peritrichous) or at one or both cell poles, where they occur as a single flagellum (monopolar) or a flagella bundle (lophotrichous). Our model organism *Shewanella putrefaciens* is a peritrichously flagellated bacterium with two distinct flagellar systems: the polar and lateral system. With regard to the polar flagella system, it is already known that the SRP-GTPase FlhF assumes the role of a polar marker and is responsible for the arrangement of one polar flagella. Together with the polar landmark protein HubP, FlhF initiates the recruitment of further components like FliG and FliF, building blocks of the flagellar basal body. Our current model suggests that only a few proteins are required to initiate flagella formation and that their recruitment to the cell pole in *S. putrefaciens* follows a specific order, starting with the polar landmark protein HubP, followed by FlhF, FliG and FliF. To investigate whether our model is complete or whether additional proteins are involved in this process, we analyzed the arrangement in a heterologous host, *Escherichia coli*, that is unable to produce its own flagellar proteins. To this end, fluorescently labeled versions of HubP, FlhF, FliG and FliF were introduced into *E. coli* cells using a plasmid-based system and their localization behavior was analyzed via fluorescence

microscopy. In addition, the stability of the fluorescent fusions was verified by western blot experiments and the swimming behavior analyzed using a motile *E. coli* MG1655 strain. We showed that in *E. coli* HubP localizes to both cell poles and the cell division plane, where it can also recruit FlhF. The presence of FlhF also allows FliG to localize to both poles as well as to the cell division plane. Furthermore, we demonstrated that FlhF plays an important role in the stability of FliG and FliF. Taken together, the results confirm our proposed model of flagellar protein recruitment.

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**PCBP005**

**Novel insights into the peptidoglycan remodeling machinery of *Caulobacter crescentus***

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Bacterial morphology is mainly determined by the peptidoglycan (PG) cell wall. This mesh-like structure lies between the outer and inner membrane and surrounds the whole cell, creating the PG sacculus and giving each bacterium the characteristic shape. This rather rigid meshwork has to be constantly remodeled to allow the cells to grow and divide, which critically relies on the incorporation of new material at the right time and place. For PG synthesis two enzymatic activities – transpeptidation and transglycosylation – are required, while PG hydrolysis relies on various autolysins cleaving at specific sites. Control of PG-remodeling enzymes has been intensively studied for organisms with rather simple cell shape, but how more complex cell morphologies arise still remains incompletely understood.

Bacteria of the class Alphaproteobacteria exhibit various elaborate shapes such as arcs, stars or spirals and some are highly asymmetric as they grow cellular extensions such as stalks. Among these complex species is the crescent-shaped freshwater organism *Caulobacter crescentus*, which is characterized by a biphasic life cycle involving two morphologically distinct cell types and asymmetric cell division into a swarmer and a stalked cell.

To achieve coordinated cell growth and division, a complex network of PG synthesizing and degrading enzymes is at work, changing dynamically over the course of the cell cycle. Especially when inserting new PG material, hydrolysis of the sacculus and insertion of new PG monomers has to be tightly regulated to allow the incorporation of the new cell wall material without risking cell lysis.

The class of bifunctional penicillin-binding proteins (bPBPs) – containing both enzymtic domain – is highly involved in the incorporation of new PG and in *C. crescentus* one of these is most important for normal cell wall integrity. By using a combination of genome wide screening approaches such as CoIP and transposon sequencing followed by standard molecular *in vivo* methods, we aimed to identify novel players acting in the PG synthesis pathway of the bPBPs. Furthermore, we have identified and characterized a novel PG-degrading enzyme predominantly acting in the late exponential growth phase using *in vivo* and *in vitro* methods. Together, our results provide new insights into the spatiotemporal control of PG remodeling during cell division in *C. crescentus* and, thus, shed new light on the complex machinery driving bacterial PG biosynthesis.

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**PCBP007**

**A chimeric KaiA-like regulator extends the KaiB3-KaiC3 clock system in bacteria**

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Introduction: Due to earth"s rotation, organisms are subjected to certain environmental changes. Cyanobacteria are able to predict the daily external changes via an intrinsic circadian clock system and prepare the metabolism for the autotrophy-heterotrophy switch. The interplay of the proteins KaiA, KaiB and KaiC forms a chemical oscillator and the phosphorylation state of KaiC determines the cyclic gene expression. In addition to this standard protein oscillator, *Synechocystis* sp. PCC 6803, a model organism for cyanobacterial research, harbors several, diverged clock homologs. One such nonstandard system, KaiB3-KaiC3, has been proposed to influence metabolic changes in response to darkness.

Objective: In this study, we provide evidence for a direct interaction of KaiC3 with Sll0485, a potential new chimeric KaiA homolog, named KaiA3 and show, that KaiC3 exhibits a diurnal phosphorylation pattern dependent on KaiA3.

Methods: We characterize KaiA3 (Sll0485) bioinformatically in the genomic context of Cyanobacteria and other bacteria. Using yeast two-hybrid assays, we prove an interaction with KaiC3. In addition to examine the deletion of *kaiA3* in growth assays, we also investigate the effect of KaiA3 on KaiC3 phosphorylation *in vitro* via phosphorylation assays and *in vivo* using a Phostag™ assay.

Results: The N-terminus of KaiA3 bears resemblance to a response regulator receiver domain of the NarL-type. Whereas the C-terminal domain has similarity to the standard clock protein KaiA. We detect the formation of a high molecular weight complex of KaiA3 together with KaiB3 and KaiC3 and KaiA3 facilitates the phosphorylation of KaiC3 *in vitro*. Phosphorylation of KaiC3 exhibits a rhythmic pattern of 48 hours in the presence of KaiA3 and KaiB3 *in vitro* as well as in cells subjected to light-dark conditions followed by continuous light. The deletion of *kaiA3* leads to KaiC3 dephosphorylation and results in growth defects during mixotrophic growth and in darkness.

Conclusion: In conclusion, we propose that KaiA3 represents a novel nonstandard homolog of KaiA, thereby expanding the understanding of the KaiB3-KaiC3 system in cyanobacteria and potentially other prokaryotes. We suggest that the KaiA3B3C3 system is a free-running oscillator and, in conjunction with the canonical KaiAB1C1 system, plays a role in regulating the transition between auto-/heterotrophic growth in the facultative heterotroph, *Synechocystis*.

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**PCBP008**

**The Incredibles: Insights into the non-canonical cell division of the *Planctomycetota***

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Strains belonging to the phylum *Planctomycetota* are known for peculiar cellular features including their large genomes, their complex lifestyle, and their atypical mode of cell division. The latter sparked many questions since members of the phylum *Planctomycetota* can divide with a symmetrically binary fission-like mode (central division, classes *Phycisphaerae*[1] and *Ca.* Brocadiae), but are more famous for their asymmetrical cell division. Here, a daughter cell originates either from one cell pole of the mother cell (polar division) or from the lateral side (lateral division, "*Kolteria novifilia*")[2]. In contrast to almost all other bacterial species known today, the otherwise canonical cell division proteins (except for the DNA translocase FtsK) appear to be absent in all members of the phylum, which also includes the contractile ring-forming hallmark cell division protein FtsZ[3,4,5]. If all canonical cell division proteins are absent, then one question remains overarching: Which proteins facilitate proper cell division in members of this phylum?

Starting this journey, the presentation will describe hallmark morphological and temporal characteristics of planctomycetal cell division modes by employing time-lapse transmission light and epi-fluorescence light microscopy in combination with image analysis. Species from various planctomycetal families will be compared yielding data sets describing their wild-type cell division process.

Since future research will focus on identifying and describing proteins likely involved in planctomycetal cell division, these data sets can be used to identify genetic changes introduced into the cell division machinery.

This line of research will also benefit from advances in the development of genetic tools that facilitate the construction of genetically defined mutant strains for the inactivation or altered expression of native genes or introduction of heterologous genes. The latter can e.g. be used for complementation experiments or the construction of translational fusion proteins. Therefore, this work lays the foundation for the investigation of the planctomycetal non-canonical cell division apparatus.

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**PCBP009**

**Single molecule dynamics during replication restart**

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Introduction: The replication machinery has to overcome different obstacles during replication. RNA polymerase is one of these obstacles, where transcription and replication collide (Lang and Merrikh 2018). The essential, primosomal protein PriA is located at the replication fork and is required for replication restart. The interaction of PriA with SSB pre-recruitments PriA to the replication forks, but is not essential for viability. It is known that alterations in the 8-C motif between the helicase domain IV and V do not hinder DNA binding of PriA, but interfere with the interaction with SSB. The winged helix domain (WH) of PriA may also be a hub for protein-protein interaction and is critical for the functionality of PriA *in vivo* especially for growth on rich media and to survive DNA damage (Matthews and Simmons 2022). Additional studies focusing on the interaction partners of PriA are necessary to gain insight into the mechanism underlying replication restart.

Objective: We wish to elucidate the single molecule dynamics of PriA *in vivo* and combine this with the introduction of a roadblock into the genome that will lead to defined restart events allowing for studies to identify PriA interaction partners *in vivo*. These interaction partners should accumulate under replication restart conditions.

Materials & methods: To visualise PriA in *B. subtilis* an N-terminal mNG-PriA fusion was generated, tagging PriA from the original locus. Single molecule tracking (SMT) was used to study the dynamics of PriA *in vivo*.

Results: Studying the mNG-PriA fusion using SMT revealed three populations. The populations account for a static fraction, which locates at the replication fork, another fraction that represents PriA with a bound interaction partner, maybe to its WH-domain, and a third fraction which is more distributed over the whole nucleoid but still DNA associated.

Conclusion: The localization of PriA in wildtype and in a *ssb*Δ35 mutant could already be visualized with epifluorescence. PriA single molecule dynamics suggest the existence of three distinct populations having low mobility, i.e. those that are likely bound to replication forks, and medium and high mobility. A medium mobile population indicates that PriA might be interacting with another restart protein in search for blocked forks. This preassembled restart complex could result in a ready-to-act-mode of PriA to intervene once replication restart is necessary and therefore become more static.

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**PCBP010**

**Uptake of environmental DNA in *Bacillus subtilis* occurs all over the cell surface through a dynamic pilus structure**

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At the transition to stationary phase, a subpopulation of *Bacillus subtilis* cells can enter the developmental state of competence, which can result in the incorporation of novel genetic information *via* uptake of environmental DNA. The model organism uses a cell envelope-spanning competence machinery to take up exogenous DNA. This multiprotein complex facilitates the steps necessary for import of DNA into the cytoplasm where it can integrate into the genome through homologous recombination if sufficient homology exists. According to a prevailing model, DNA is transported across the cell wall by a pseudopilus that is formed by polymerization of the major pilin ComGC together with minor pilins. After transport, DNA will be stored and buffered in the periplasm before it is transported into the cytoplasm, which is restricted to the cell pole. In the cytoplasm, possible integration will be facilitated by a set of proteins, some of which are also competence depended. In previous work pili have been visualized *in vivo* by introducing a cysteine substitution in the major pilin and subsequent labelling with maleimide dyes. We applied this staining technique to reveal

that *B. subtilis* indeed has a dynamic pilus. By using high resolution microscopy we observed that cells had one or more labelled filaments exposed at the surface, of a size of roughly 500 nm, suggesting that cells can take up DNA at many places on their surface. In order to follow the motion and dynamics of the major pilin Single-molecule tracking was used. ComGC was found in two distinct populations, one that would correspond to ComGC freely diffusing throughout the cell membrane, and one that is relatively stationary, likely reflecting pilus-incorporated molecules. The ratio of 65% diffusing and 35% stationary ComGC changed towards more stationary molecules upon addition of external DNA, while the number of pili per cell did not strongly increase. These findings suggest that the pilus assembles stochastically, but engages more pilin monomers from the membrane fraction in the presence of transport substrate. Taken together, our data are in favour of a model in which the DNA uptake is a two-tier process in which uptake into the periplasm, in which DNA can freely diffuse bound to ComEA, occurs over the whole cell surface, while cytosolic uptake is limited to the poles, where binding of DNA recombination proteins guides incoming single stranded DNA onto the nucleoid(s) for homology search.

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**PCBP011**

**Novel oxidoreductases from *Pseudomonas aeruginosa***

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*Pseudomonas aeruginosa* is a highly virulent opportunistic pathogen known for its remarkable antibiotic resistance. Oxidoreductases (OXRs) are crucial enzymes in primary metabolism and contribute to infections, making them important targets for antibiotics. Since a significant portion of *P. aeruginosa* genes remains uncharacterized, exploring novel OXRs may lead to the identification of new targets for the development of antivirulence drugs or antibiotics.

In this study, we cloned 149 putative OXR genes from *P. aeruginosa* into the pGUF plasmid for recombinant expression in both *Escherichia coli* and *P. aeruginosa*. Additionally, 30 putative OXR genes were cloned into a modified pET22b vector to enable high-yield protein production. Results from SDS-PAGE and Western blot analyses confirmed the overproduction of more than 80 novel OXRs. These proteins were purified using immobilized metal affinity chromatography (IMAC) in a 96-well plate format. Their functions were experimentally determined by untargeted enzyme activity profiling with 23 substrates, including alcohols, aldehydes, and amino acids. Additionally, the nicotinamide cofactor specificity of the novel OXRs was tested using four different compounds.

We identified putative functions of four novel OXR proteins: PA2575 exhibited NAD(P)H-dependent oxidase activity, while PA4656, PA0565, and PA2822 showed NAD(P)+-dependent alcohol, aldehyde, and amino acid dehydrogenase activities, respectively. Notably, OXR PA2822 demonstrated the strongest methionine reductase activity, indicating its potential role in methionine metabolism.

Our ongoing research aims to investigate the biological and physiological roles of these novel OXRs in *P. aeruginosa*, as well as their contribution to virulence.

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**PCBP012**

**Analysis of the interactions of chromosome segregation and cell division proteins in *Caulobacter crescentus***

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The successful proliferation of all cells depends on maintaining genome integrity. One of the most essential steps is the correct positioning of the division plane, which is coordinated by DNA segregation proteins. Although chromosome segregation and cell division have already been studied extensively, it is interesting to uncover how different lineages of bacteria use distinct machineries to coordinate these tasks.

MipZ is a member of the P-loop ATPases in *Caulobacter crescentus* involved in the spatiotemporal regulation of chromosome segregation and cell division. It plays an essential role in ensuring the positioning of the cytokinetic Z-ring, composed of the tubulin homolog FtsZ, at mid-cell. MipZ has an antagonistic action on FtsZ polymerization by forming a bipolar gradient, which is established by non-specific DNA binding coupled with its innate ATPase activity.

The distribution of MipZ is influenced by different interaction partners, with ParB being one of them. ParB is a member of the ParABS system involved in chromosome segregation. It interacts with MipZ monomers and recruits them to the pole, stimulating dimerization. MipZ dimers diffuse away from ParB, binding DNA non-specifically. Intrinsic ATPase activity of MipZ determines its residence time on the DNA, as ATP hydrolysis triggers dissociation into monomers lacking significant DNA-binding activity. Thus, ParB acts as a sink, capturing monomers and releasing dimeric MipZ to bind DNA at random sites. This constant recycling results in a MipZ gradient, with the highest concentration at the poles and lowest at mid-cell.

The role of ParB in mediating gradient formation is intriguing. However, mechanistic insights into the ParB-assisted dimerization of MipZ are still missing. By using *in vitro* methods such as bio-layer interferometry and hydrogen-deuterium exchange mass spectrometry, we aimed to identify ParB sites mediating interaction with MipZ. In the current study, we determined that the C-terminal region of ParB is essential for binding, providing the basis to further explore the significance of residues in this region for the ParB-MipZ interaction, using *in vitro* and *in vivo* methods. The results lay the foundation to further investigate the molecular mechanism underlying ParB-dependent MipZ dimerization, thereby providing new insights into the coupling of chromosome segregation and cell division in *C. crescentus* and the mechanistic principles driving biomolecular gradient formation.

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**PCBP013**

**Transcription factor YhbD has different effects on motility in different strains of *Bacillus subtilis***

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Many bacteria use flagella to swim in liquids and swarm on surfaces, which provides a trait to survive in an environment that is dominated by nutrient gradients. In *B. subtilis* this capability is important for its mode of life. Flagella consist of basal body, hook and a filament. In *B. subtilis* the assembly of the hook and filament of flagella depend on bactofilins.

The genes of the bactofilins are located in an operon together with the gene *yhbD*, whose function is still unknown. Its orthologous protein Cap0037 acts as a transcription factor for multiple genes involved in metabolism in *Clostridium*. Because *yhbD* is located in a putative operon with the bactofilin genes, we investigated whether YhbD has an influence on the motility of *B. subtilis*. We found that a deletion of *yhbD* leads in PY79 and 168 to faster swarming behavior than in wild type. Suggesting that YhbD has a role in regulation of motility in *B. subtilis* cells. To investigate whether YhbD influences the motility as a transcription factor, we used an electromobility shift assay. We observed that the protein is indeed able to bind DNA through its Helix-turn-Helix motif. Using in vivo labelling of the FlgE (hook) with a maleimide dye, we show an increased quantity of hooks in PY79. To follow the localization and dynamics of the protein, we used single molecule tracking. The fusion protein YhbD-mNG arrested at the membrane and was diffusive in the cytosol. Taken together our data suggests that YhbD functions as a transcriptional repressor of motility, but operates in a highly unusual manner by arresting at sites close to the membrane.

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**PCBP014**

**The D,D-endopeptidase Csd2 within the helical-cell-shape-promoting protein complex of *Helicobacter pylori***

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Approximately half of the world population is infected with the human pathogen *Helicobacter pylori* which can cause inflammation, chronic gastritis or peptide ulceration. The helical shape of *H. pylori* is one important factor promoting colonization of the upper digestive system. Several cell shape-determining proteins have been identified, of which some form the so-called "shapesome", being a protein complex contributing to the formation of the helical shape. This protein complex is considered be comprising peptidoglycan hydrolases (Csd1 and Csd2), an enzyme for the synthesis of peptidoglycan precursors (MurF), nonenzymatic transmembrane proteins (Csd5 and Csd7), and the bactofilin CcmA. Csd1 and Csd2 are both members of the M23B metallopeptidase family and, as D,D-endopeptidases, are capable of cleaving the d-Ala4-mDAP3 peptide bond of cross-linked dimer muropeptides. Although the structure has been studied in biochemical approaches, the in vivo role and interaction of Csd2 with other potential factors is still rather unknown. Therefore, we visualized Csd2 with structured illumination microscopy and analyzed the dynamics of Csd2 in the presence and absence of potential interaction partners (Csd5, Csd7, and CcmA) by single molecule tracking. Thus, our results contribute to a refinement of the current model of for this cell wall-modifying and cell shape-forming complex.

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**PPMP001**

**Elucidating the function of host-derived cysteine in bacterial virulence**

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The amino acid cysteine imported from the extracellular environment has been primarily considered to be the source of sulfur. The presentation will focus on the emerging role of cysteine in bacterial resistance to oxidative stress. It is intriguing that some bacteria have multiple cystine transporters (e.g. three in *Bacillus subtilis* and *Salmonella* Typhimurium), some transporters evolved to import both the reduced and oxidised form of this amino acid (e.g. *Listeria monocytogenes* TcyKLMN), and some bacteria (such as, for example, *Neisseria gonorrhoeae*) have two separate import systems, one for the reduced and one for the oxidised form. This suggests that the significance of the role of cysteine in bacterial survival and virulence has been underappreciated. Given that the first and the last example species, *B. subtilis* and *N. gonorrhoeae*, lack a glutathione/glutaredoxin antioxidant system, and that the free thiol group of cysteine allows it to participate in thiol-disulfide exchange reactions, it is thought that bacteria can utilises cysteine as a component of an alternative thiol-based antioxidant system to withstand oxidative stress. The presented research focuses on *Helicobacter pylori* - a significant human pathogen causing gastric and duodenal ulcers which, if left untreated, can lead to cancers. Our immune system responds to stomach infection with *H. pylori* by producing high levels of defence molecules called reactive oxygen species (ROS). However, *H. pylori* can withstand ROS, and they fail to clear the infection, but in the long term, ROS damage the stomach lining resulting in disease. *H. pylori* relies on the uptake of host-provided nutrients for its proliferation and pathogenicity. The presented research test the hypothesis that *H. pylori* uses amino acids cysteine and cystine available in the stomach lining to combat oxidative stress brought on by ROS - a trait that can be used by many other pathogens that lack the glutathione/glutaredoxin system.

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**PPMP002**

**Beyond peptides: A RiPP pathway produces an unusual protein complex with post-translational modifications**

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Introduction: Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a diverse class of secondary metabolites with a wide range of bioactivities. They all have a ribosomal origin, as the peptide sequence is encoded in a precursor gene. The precursor peptide usually consists of a leader followed by a core sequence. Biosynthetic enzymes recognize the leader and install post-translational modifications on the core peptide, which is released as the mature natural product after proteolytic cleavage. Often, precursor peptides are short and lack clear structural features. An exception is the "nitrile hydratase leader peptide" (NHLP) family, which is characterized by unusually long leader sequences that show similarity to the eponymous enzyme. Moreover, in species of the order *Burkholderiales*, these NHLP precursors occur as tandem genes, i.e. two copies of the precursor are present in a row or even fused into a single protein. The nature of the resulting RiPPs and the function of the two NHLP domains are currently unclear.

Objectives: We aimed to study these previously uncharacterized RiPP clusters and identify the resulting natural products, characterize the biosynthetic enzymes, and explore the role of the two leader domains in the precursor.

Materials & methods: We heterologously expressed the RiPP gene clusters and analyzed the precursor protein by LC-MS for the appearance of modifications. In addition, the precursor from *Acidovorax oryzae* was characterized after proteomics-guided native purification. Finally, we determined crystal structures of the modified precursor proteins.

Results: Co-expression of the precursor with maturases from the gene cluster, a cyclodehydratase and a methyltransferase, resulted in dehydration of a C-terminal cysteine (leading to a thiazoline) and methylation of a threonine. The same modifications were observed in the precursor protein purified directly form *A. oryzae*. Unexpectedly, we discovered that the modified precursor protein assembled into a hexameric complex of ~180 kDa, and determined its crystal structure.

Conclusion: Such a high degree of structural organization, leading to multimeric complexes, is unexpected for a RiPP precursor. The lack of a peptidase in the cluster, as well as the absence of any cleavage products, suggests that the modified precursor may be the end product of the RiPP pathway. We are currently investigating this hypothesis to determine the function and bioactivity of the precursor complexes.

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**PPMP003**

**Regulation of the carbon flux in *Synechocystis* using the PGAM-PirC switch**

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Cyanobacteria are autotrophic organisms, which perform oxidative photosynthesis. Their ability to oxidize water and use the electrons for reducing $CO_2$ to form organic matter by using solar energy makes them an interesting organism for biotechnological applications. For a sustainable bioeconomy, $CO_2$-neutralyity is becoming pivotal. Therefore, using cyanobacteria as "green cell factories" is a promising way to produce valuable bioproducts, such as chemicals and fuels. The unicellular freshwater model strain *Synechocystis* sp. PCC 6803 is well studied, yet further research has to be done concerning the metabolic flux to broaden the bioengineering platform. The signal transduction protein PII acts as a signal integrator of the carbon and nitrogen status by sensing the levels of ADP, ATP and 2-oxoglutarate. An important control point of the carbon flux is the 2,3-bisphosphoglycerate-independent phosphoglycerate-mutase (PGAM), which converts the first $CO_2$ fixation product 3-phosphoglycerate (3-PGA) to 2-phosphoglycerate (2-PGA). Hence, carbon is withdrawn from the Calvin-Benson cycle and directed towards lower glycolysis for the production of amino acids, fatty acids, polyhydroxybutyrate (PHB) and more. Pervious work in our lab has shown that a small protein, PirC, is inhibiting PGAM activity through binding, while PirC itself is repressed by the PII sensor protein in presence of ATP or ADP [1]. Furthermore, it has been shown that a PirC-deficient mutant accumulates PHB up to 80 % per cell dry mass under nitrogen starvation [2]. Additional, preliminary work studying the PGAM production showed its downregulation in response to N-starvation after 2 days. Our work aims to use this key hub to direct the metabolic flux towards lower glycolysis and the production of PHB and other feedstock chemicals. Therefore, the pSOMA plasmid series [3], a molecular toolset for extensive metabolic engineering of cyanobacteria, is employed to adjust both PGAM expression and repression by PirC. Inducible and tunable promoters, such as $P_{petE}$ and $P_{petJ}$, will be tested to deviate the carbon flux towards lower glycolysis. Hence, by tuning the PGAM-PirC switch, a platform will be established for the redirection of carbon flow for enhanced valuable chemical production.

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**PPMP005**

**Studies on specificity and distribution of the $\Delta^{4,6}$-variant of bacterial bile acid degradation**

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Bile acids are steroids produced in the digestive tract of vertebrates. After their excretion, these compounds are used as substrate by environmental bacteria that employ different degradation pathways. Two main variants have been reported during which intermediates with $\Delta^{1,4}$ or $\Delta^{4,6}$-structures of the steroid skeleton are formed [1]. While the $\Delta^{1,4}$-variant is common among pseudomonads and actinobacteria, the $\Delta^{4,6}$-variant appears to be specific for the *Sphingomonadaceae* family [2], where 7α/β-hydroxysteroid dehydratases (Hsh2/3) are key enzymes in this variant. In this study, the specificity and distribution of the $\Delta^{4,6}$-variant of bacterial bile acid degradation is further explored.

Physiological analysis of a novel freshwater isolate, *Novosphingobium* sp. strain Chol12, indicated that this strain degrades the bile salt cholate with a C12-hydroxyl group via the $\Delta^{1,4}$-variant during the early steps, leading to an $\Delta^{1,4}$-androstadiendione as intermediate, which is then dehydrated to the $\Delta^{4,6}$-compound 12β-hydroxy-androsta-1,4,6-triene-3,17-dione (HATD). In contrast, degradation of chenodeoxycholate, without a C12-hydroxyl group, was degraded via the $\Delta^{4,6}$-variant in the early steps. This instance suggests that Hsh2 of strain Chol12 has different specificities for bile acids with or without a C12-hydroxyl groups which would be different from other bile acid-degrading *Sphingomonadaceae*. This hypothesis is currently being explored on the biochemical level.

So far, no bile acid-degrading bacteria using the $\Delta^{4,6}$-variant have been isolated from marine environments. For systematically searching for such bacteria. Different mesophilic heterotrophic bacterial strains were enriched and isolated from salt marshes from Juist island (Germany) using different bile acids as sole carbon sources. The metabolic diversity of bile acid degradation occurring in these environments is currently being analyzed.

This study on the diversity of bile acid degradation will add new insights of their environmental fate and its potential biotechnological applications.

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**PPMP006**

**Same same but different – The global response of *Escherichia coli* to five different LpxC inhibitors**

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A promising antibiotic target in difficult-to-treat Gram-negative bacteria is LpxC, the key enzyme in the biosynthesis of lipopolysaccharides (LPS), which are the major constituents of the outer membrane. To gain insights into the mode of action of five different LpxC inhibitors, we conducted a comparative phenotypic and proteomic analysis. All five compounds bound to purified LpxC from *Escherichia coli*. Treatment of *E. coli* with these compounds changed the cell shape and stabilized LpxC indicating that the inhibitor-bound enzyme is not degraded by the FtsH protease. LpxC inhibition sensitized *E. coli* to the cell wall antibiotic vancomycin, which typically does not cross the outer membrane. Four of the five compounds led to an accumulation of lyso-PE, a cleavage product of phosphatidylethanolamine (PE), generated by the phospholipase PldA. The combined results suggested an imbalance in phospholipid (PL) and LPS biosynthesis, which was corroborated by the global proteome response to treatment with the LpxC inhibitors. Apart from LpxC itself, FabA and FabB responsible for the biosynthesis of unsaturated fatty acids were consistently upregulated. Our work also shows that antibiotics targeting the same enzyme do not necessarily elicit identical cellular responses. Compound-specific marker proteins belong to different functional categories, like stress responses, nucleotide or amino acid metabolism, and quorum sensing. These findings provide new insights into common and distinct cellular defense mechanisms against LpxC inhibition. Moreover, they support a delicate balance between LPS and PL biosynthesis with great potential as a point of attack for antimicrobial intervention.

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**PPMP007**

**Protein on a double mission: The (p)ppGpp-binding RNase RnhB influences lipopolysaccharide biosynthesis in *Escherichia coli***

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Lipopolysaccharides (LPS) serve as a vital defense mechanism in Gram-negative bacteria, protecting against harmful chemicals and antibiotics. The integrity of the outer membrane relies on a delicate balance between LPS and phospholipids (PL), making their biosynthesis tightly regulated. This study aimed to explore the impact of the signaling nucleotide (p)ppGpp, known for inhibiting PL and peptidoglycan biosynthesis, on the initial step of LPS biosynthesis. We investigated the role of RNase H RnhB, which is encoded in the same operon as LpxA and elicited a similar phenotype when overproduced, in this process.

To assess the role of RnhB, we evaluated cellular LPS levels and quantified outer membrane vesicles (OMVs) to determine LPS content. Additionally, we employed thermal shift assays to detect (p)ppGpp protein interactions and microscale thermophoresis (MST) to analyze the LpxA-RnhB interaction. To evaluate the effect of (p)ppGpp and RnhB on LpxA, we employed a fluorescence-based LpxA activity assay, and for RnhB, a fluorescence-based RNase H assay.

Our findings demonstrate that (p)ppGpp reduces LpxA activity, potentially through its interaction with the fatty acid chain substrate donor, acyl carrier protein (ACP). We also discovered that RnhB binds (p)ppGpp, leading to inhibition of its RNase H activity. Furthermore, RnhB interacts with LpxA and enhances its activity. Consequently, a Δ*rnhB* mutant exhibited slightly reduced cellular LPS levels, while overproduction of RnhB resulted in increased OMV formation and LPS content within OMVs.

This study highlights the interaction between (p)ppGpp-binding RnhB RNase and LpxA, influencing LPS biosynthesis and leading to physiological effects. The extent to which (p)ppGpp modulates the effect of RnhB on LpxA warrants further investigation and serves as the focal point for our ongoing research. Further studies in this area could provide valuable insights into the modulation of bacterial virulence and pave the way for potential therapeutic interventions targeting Gram-negative bacteria.

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**PPMP008**

**The role of MftG in respiration and mycofactocin regeneration in *Mycolicibacterium smegmatis* under ethanol metabolism**

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Introduction: Alcohol consumption is one of the highest risk factors for death and disease progression in tuberculosis patients. Despite this importance, the ethanol metabolism of Mycobacteria, including *M. tuberculosis*, the causative agent of tuberculosis, is poorly characterized. Mycofactocin (MFT) is a redox cofactor produced by *M. tuberculosis* and *Mycolicibacterium smegmatis* and is essential for the growth on ethanol as a carbon source (1,2,3).

Objectives: We hypothesized that the glucose-methanol-choline oxidoreductase enzyme MftG encoded in the MFT cluster (*mftA-G*) might be involved in MFT re-oxidation and electron transfer to the respiratory chain.

Materials & methods: To test this hypothesis, we generated a Δ*mftG* mutant and complement strain of *M. smegmatis*. Phenotypic characterization using growth curves and flow cytometry were implemented. The impact on the metabolite production was determined using LC-MS or quantification kits. Transcriptomic analysis was used to assess the impact of *mftG* in ethanol grown bacteria and complemented with respiration assays using a respirometer. Results

The growth of the Δ*mftG* mutant was impaired on ethanol as the sole carbon source and growth was restored when genetically complemented. Propidium iodide staining revealed reduced survival of Δ*mftG* during ethanol exposure. Comparison of metabolite extracts of wild type (wt) and Δ*mftG* showed the depletion of oxidized MFTs and the accumulation of reduced MFTs in the Δ*mftG* strain. Decreased ratios of NADH/ NAD+ and ATP/ADP were observed in the Δ*mftG* strain on ethanol. Transcriptomics analysis suggested that the absence of *mftG* affected the cell division, respiration, and redox homeostasis during ethanol metabolism. Respiration assays recorded a boost of oxygen consumption when reduced mycofactocin was added to wt

membrane fractions and revealed a decreased respiration rate of the Δ*mftG* mutant compared to wt. Conclusion

This work exposed the transfer of electrons from MFT to the mycobacterial respiratory chain and the effect of the *mftG* gene on MFT regeneration and redox homeostasis of mycobacteria, that can potentially lead to novel targets for antimycobacterial treatments.


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**PPMP009**

**A novel protein lipoylation pathway demonstrated in sulfur oxidizing bacteria**


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Introduction: Many sulfur-oxidizing bacteria and archaea use a sulfur oxidation pathway involving a heterodisulfide-reductase like enzyme system with lipoylated LbpA proteins as essential components [1]. LbpAs from sulfur oxidizers do not serve as as substrates established lipoate-binding protein biosynthetic machineries [1]. Instead, novel lipoylating enzymes are encoded in close proximity to the *shdr-lbpA* clusters, indicating a specialized way of lipoate synthesis. Among these enzymes is a lipoate ligase acting specifically on LbpA but not on closely related GcvH proteins [1]. The other proteins include two unusual radical SAM proteins resembling LipS1 and LipS2 from the archaeon *Thermococcus kodakarensis*, which have recently been shown to act together as lipoyl synthase *in vitro* [2,3]. In addition, the lipoylation gene cluster in sulfur oxidizers usually contains a gene for a geranylgeranyl reductase-like FAD-binding protein.

Objectives: Our work aims to provide conclusive evidence for the existence of a novel sLpl(AB)-LipS1/S2-based lipoate assembly pathway not only in archaea but also in bacteria and to elucidate of the mechanism of lipoate maturation in sulfur oxidizing bacteria.

Materials & methods: We performed gene inactivation studies in the Alphaproteobacterium *Hyphomicrobium denitrificans* and characterized the resulting phenotype. We also heterologously produced LbpA from various sulfur-oxidizing bacteria in the presence of biosynthetic enzymes and verified the products by native PAGE and mass spectrometry.

Results: A specific ATP-dependent ligase, sLpl(AB), attaches either free lipoate or octanoate to apo-LbpA and then two radical S-adenosyl-L-methionine domain proteins with an unusual Fe/S cluster ligation, LipS1 and LipS2, successively add two sulfur atoms. Mercaptooctanyol-LbpA is formed as an intermediate on the way to holo-LbpA. The FAD-containing oxidoreductase (LipT) is a likely candidate to provide electrons, probably derived from NAD(P)H, for the reductive sulfur insertion catalyzed by LipS1/S2.

Conclusion: Our work provides evidence for a novel lipoylation pathway not only in archaea but also in bacteria. A specific lipoate protein ligase sLpl(AB) and a novel lipoate synthase LipS1/S2 work together in the assembly of lipoate on LpbA.


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**PPMP010**

**Investigation of the biological function of small DUF1127 proteins in *Agrobacterium tumefaciens***


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In any given organism, the function of one-third of all proteins is unknown. A short, arginine-rich domain of unknown function is DUF1127. Proteins belonging to this domain are highly positively charged. Proximately 20,000 proteins with the respective DUF1127 domain have been identified in about 5000 bacteria. In the plant pathogen *A. tumefaciens,* we identified seven proteins containing the DUF1127 domain: three small proteins (47 and 48 amino acids) and four long proteins (72 to 101 amino acids) [1]. The respective genes are differently regulated by the LysR-type transcriptional regulator LsrB and expressed under different stress conditions. Our current study focusses on deciphering the biological role of the small DUF1127 proteins and the finding of interaction partners.

In a triple mutant having all three small DUF1127 genes deleted, we found a variety of phenotypes, including a growth defect in stationary phase, increased cell aggregation, upregulated siderophores and altered phosphate uptake. Complementation with one of the small DUF1127 proteins restores the wildtype phenotypes. In addition, DUF1127 proteins from different bacteria like *E. coli*, *Rhodobacter spaerhoides*, *Rhizobium rubi*, *Sinorhizobium meliloti* and *Neorhizobium galegae* are able to complement the phenotypes of the triple deletion mutant. Our results demonstrate that the function of the small DUF1127 proteins is conserved across different species. RNA-Seq of the triple mutant revealed a large number of differentially regulated genes in late exponential and stationary growth phase. One major group of upregulated genes in the mutant is involved in phosphate uptake and belongs to the *pst* operon. Additional deletion of *pstS*, the gene coding for the phosphate binding protein, in the triple deletion mutant complements all defects and restores wildtype phenoytypes. Our results suggest that the small proteins have a high impact on the phosphate metabolism in *A. tumefaciens*.


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**PPMP011**

**A novel silver-containing antimicrobial potentiates aminoglycoside activity against gram-negative pathogens**

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Background & Questions: The rapid dissemination of antibiotic resistance combined with the decline in the discovery of novel antibiotics represents a major challenge for infectious disease control that can only be mitigated by investments into novel treatment strategies. Alternative antimicrobials including silver have regained interest due to their diverse mechanisms of inhibiting microbial growth. One such example is AGXX, a broad-spectrum antimicrobial that produces highly cytotoxic reactive oxygen species (ROS) to inflict extensive macromolecular damage. Due to connections identified between ROS production and antibiotic lethality, *we asked whether AGXX could potentially increase the activity of conventional antibiotics.*

Methods & Results**:** Using time killing and growth curve-based assays, we screened possible synergistic effects of AGXX on several antibiotic classes in a number of Gram-negative patogens. We found that the combination of AGXX and aminoglycosides tested at sublethal concentrations led to a rapid exponential decrease in bacterial survival and restored sensitivity of a aminoglycoside-resistant *P. aeruginosa* strains. Using spectroscopic and flowcytometric approaches combined with fluorescence microscopy, we deciphered elevated ROS production to be a significant contributor to the synergy between AGXX and aminoglycosides and demonstrated that the addition of ROS scavengers resulted in reduced endogenous ROS levels and increased bacterial survival. Consistently, *P. aeruginosa* strains deficient in ROS detoxifying/repair genes were more susceptible to AGXX/aminoglycoside treatment. We further demonstrate that this synergistic interaction was associated with a significant increase in outer and inner membrane permeability, resulting in increased antibiotic influx. Our study also revealed that AGXX/aminoglycoside-mediated killing requires an active proton motive force across the bacterial membrane.

Conclusions & Outlook: Overall, our study highlights the effectiveness of the silver containing antimicrobial AGXX in potentiating aminoglycoside activities a number of Gram-negative pathogens and provide an understanding of cellular targets that could be inhibited to increase the activity of conventional antimicrobials. These findings emphasize AGXX's potential as a route of antibiotic adjuvant development and shed light into potential targets to enhance aminoglycoside activity.

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**PPMP012**

**Utilization of the key regulatory protein CP12 to re-direct energy flux towards products in cyanobacteria**

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Cyanobacteria as phototrophic microorganisms bear great potential to produce chemicals and fuels, including biotechnological hydrogen from sustainable resources such as light, $H_2O$, and $CO_2$. The recently gained knowledge on metabolic electron and carbon fluxes and their regulation brings about opportunities to channel the resources towards product formation. For instance, intrinsic regulators might pose interesting engineering targets to direct metabolic fluxes towards products. With recent research opening the window towards a more comprehensive view on fundamental principles of metabolic regulation, small regulatory proteins that are often unique to cyanobacteria got into focus. For instance, the Calvin-Benson-Bassham (CBB) cycle and thereby the assimilation of inorganic carbon is mainly controlled by a redox-controlled small protein named CP12 that interacts with and inhibits glyceraldehyde-3-phosphate dehydrogenase and phosphoribulokinase, when light availability is not sufficient[1,2].

Some cyanobacteria, including model strain *Synechocystis* sp. PCC 6803, are capable of hydrogen fromation via intrinsic, bidirectional [NiFe] hydrogenases that function as valve for surplus electrons. However, hydrogen formation is in direct competition with the CBB cycle as it is the major electron sink. Here we made use of phage-based CP12 variants to downregulate the CBB cycle minimizing the electron drain in *Synechocystis*. Unlike the native CP12 of *Synechocystis*, which only inhibits the CBB cycle in the dark and gets deactivated by a reduced cell status, CP12 homologs from cyanophages appear to be active when the infected host cell is exposed to light. This, together with other metabolic interventions, lead to an enhanced electron availability for phage reproduction[3]. To simulate this circumstance, we constructed *Synechocystis* strains that encode those CP12 homologs under the control of the $Ni^{2+}$-inducible *nrsB* promoter. Indeed, an inhibitory effect on growth upon induction of heterologous gene expression was observed. Moreover, the generated strains showed enhanced production and a reduced re-uptake of hydrogen. With this, we demonstrate that CP12 homologs from phages can be used to engineer the metabolism of *Synechocystis* to boost processes that are directly fueled by electrons from the photosynthetic machinery.

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**PPMP013**

**Phosphoglucomutases – Overlooked key enzymes in bacterial glycogen metabolism**

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In bacteria, glycogen turnover is not only crucial for survival in fluctuating environmental conditions but can also influence bacterial transmission and pathogenicity [1]. Within glycogen metabolism, the reactions of phosphoglucomutases (PGM) play a vital role in glycogen accumulation and mobilization. For the cyanobacterium *Synechocystis* sp. PCC 6803 glycogen metabolism and regulation of PGM have been extensively studied in our lab. Recent studies describe an elaborate phosphoregulation of the *Synechocystis* PGM (*Syn*PGM) and confirm the effect of bisphosphorylated sugars on the PGM reaction [2, 3]. While glucose-1,6-bisphosphate acts as an activator, fructose-1,6-bisphosphate inhibits the activity of the *Syn*PGM. Furthermore, a cryptic secondary *Syn*PGM was described as the first bacterial glucose-1,6-bisphosphate synthase. By converting fructose-1,6-bisphosphate and glucose-1-phosphate/glucose-6-phosphate to glucose-1,6-bisphosphate, this enzyme

provides cells with this essential activator of both *Syn*PGM and other α-D-phosphohexomutases [3]. While these studies established PGMs as central regulatory key points of glycogen catabolism within photoautotrophic bacteria, many aspects of the glycogen metabolism, PGM regulation, and the origin of glucose-1,6-bisphosphate in heterotrophic bacteria remain unknown. Here we aim to analyze if this modulation of PGM activity by bisphosphorylated sugars is also prevalent in heterotrophic bacteria. For this heterologously expressed PGM enzymes from different human-associated bacteria were characterized. Additionally, these PGMs were tested for potential secondary enzymatic activity as glucose-1,6-bisphosphate synthases. Our results confirm that the activity of all tested PGMs from *Escherichia coli*, *Yersinia enterocolitica,* and *Enterococcus faecium* is regulated by bisphosphorylated sugars. Additionally, we discovered an *Enterococcus faecium* PGM producing glucose-1,6-bisphosphate via the same reaction as the mammalian glucose-1,6-bisphosphate synthase.

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**PPMP014**

**Exploring the targetome of IsrR, a Fur-regulated sRNA controlling the synthesis of iron-containing proteins in *Staphylococcus aureus***

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Introduction: As integral parts of gene regulatory networks, small regulatory RNAs (sRNAs) play important roles in the adaptation of bacteria to environmental conditions. To adapt to iron limitation, many bacteria exploit an "iron-sparing" response mediated by Fur-regulated sRNAs such as RyhB of *E. coli*, allowing them to reduce the synthesis of iron-utilizing proteins not essential for growth.

Objectives: In this study, we aimed to confirm the regulatory role of the sRNA IsrR (Iron-sparing response Regulator) of *S. aureus* [1,2] and to characterize the IsrR targetome in order to better understand the pathogens adaptation to iron-limited conditions often encountered in the host.

Methods: To identify potential IsrR targets, two different experimental approaches were used. In the first, proteome profiles of *S. aureus* HG001 were compared to those of an isogenic Δ*isrR* mutant under iron-limited conditions. In the second approach, the effects of IsrR on the proteome under iron-rich conditions were analyzed using a strain in which IsrR was constitutively expressed from a plasmid. *In silico* target prediction was performed using the CopraRNA2 tool [3].

Results: Presence of IsrR caused a large number of consistent changes in protein levels both under iron-limited or iron-rich conditions. Among the proteins exhibiting significantly different abundance, 63 were in common between both experiments. Since sRNAs can have direct and indirect effects on global gene expression, the experimental approach was combined with *in silico* target prediction. In this way, 21 very likely direct IsrR targets were identified, which include catalase (KatA) and three tricarboxylic acid (TCA) cycle enzymes (CitB, SdhA, SucA). Follow-up analyses confirmed that IsrR negatively affects KatA and CitB enzymatic activities. In contrast to most identified IsrR targets, the predicted interaction region of IsrR with the *katA* mRNA does not overlap the ribosome binding site, suggesting that IsrR cannot only inhibit translation, but also directly affect mRNA stability.

Conclusion: A putative IsrR targetome was identified. In agreement with Fur-regulated sRNAs of other organisms, IsrR negatively affects the expression of TCA cycle and heme biosynthesis enzymes. We are currently analyzing the effects of IsrR on the secretome to gain further insights into IsrR`s role in the regulation of virulence genes in *S. aureus.* [1] Mäder et al. PLoS Genet. 2016 [2] Coronel-Tellez et al. Nucleic Acids Res. 2022 [3] Wright et al. Proc Natl Acad Sci USA. 2013

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**PPMP015**

**Biosynthesis of the unusual redox cofactor mycofactocin and the impact of glycosylation on its physiology**

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Introduction: Mycofactocin (MFT) is a recently discovered redox cofactor involved in dehydrogenation reactions [1] which belongs to the class of ribosomally synthesized and post-translationally modified peptides (RiPPs) present in a variety of actinobacteria [2][3]. Its redox-active core, formed through modification of the precursor peptide MftA by enzymes encoded in the MFT biosynthetic gene cluster (BGC) *mftABCDEF*, can be decorated with up to ten glucose moieties by the glycosyltransferase MftF [1]. Glycosylation is not essential for the redox function of MFT *in vitro* [4]. However, *mftF* is an integral part of the MFT BGC [2] and knock-out of the gene results in intracellular absence not only of glycosylated MFT but also the redox-active aglycone [1], indicating high importance of the MFT glycosylation *in vivo*.

Objectives: While all biosynthetic enzymes of MFT are known, the order of biosynthetic steps leading to the final glycosylated cofactor, its subcellular location, and the role of glycosylation remained unclear. Thus, we sought to shed light on these open questions of the biosynthesis of MFT.

Material & Methods: The objectives of this study were approached via genome editing in mycobacteria, *in-vivo* biosynthesis complementation, synthetic biology for the production of MFT precursors, and heterologous expression of proteins in conjunction with *in-silico* protein structure prediction.

Results: We could show that glycosylation of MFT prevents intracellular loss of aglycones, localize MftF in cell membranes, and get further insights into the order of mycofactocin biosynthesis. Moreover, it was shown that it is possible to produce different MFT precursors in a heterologous host.

Conclusion: MFT with its extensive modifications and redox functionality presents an intriguing molecule for current microbiology. With our recent work, we could gain further insights into the biosynthetic route of MFT, provide evidence that MftF is located in cell membranes, and that glycosylation of MFT serves as a cell anchor for the redox cofactor.

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**PPMP016**

**Unravelling the function of YejL – Another potential regulator of the LPS biosynthesis**

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The outer membrane of Gram-negative bacteria confers protection against hydrophobic molecules, for example antibiotics, or bile salts. Since an imbalance of the two major membrane compounds, lipopolysaccharides (LPS) and phospholipids (PL), disturbs the membrane integrity and leads to cell death, their synthesis pathways are co-regulated. The functional interplay of several essential proteins ensure the optimal ratio of LPS/PL. The regulation of this complex protein network remains poorly understood. Recent studies showed that LapB is a central hub that co-localizes proteins involved in membrane biogenesis at the inner membrane (Möller *et al.,* 2023). Its antagonist, YejM, recognizes periplasmic surplus of LPS, which subsequently triggers LapB-mediated LpxC turnover (Guest *et al*., 2020; Clairfeuille *et al*., 2020; Shu & Mi, 2022). The *yejM* gene is located in an operon with a so far completely uncharacterized gene *yejL*. Unlike other LPS-related proteins, YejL is not essential and rather small with a protein size of only 8 kDa. It is conserved among gammaproteobacteria, forms dimers and contains the domain of unknown function 1414 (Finn *et al.,* 2016).

To investigate the importance of YejL, a Δ*yejL* mutant strain was phenotypically characterized by its bacterial fitness, e.g., growth curves in different media and sensitivity assays. Loss of YejL confers sensitivity towards vancomycin and seems to impair the outer membrane. The bacterial two-hybrid assay showed that YejL interacts with FabZ and possibly LpxA, suggesting YejL involvement in the node of PL and LPS synthesis. Overall, YejL is likely involved in the regulation of LPS synthesis, but its function remains to be elucidated.

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**PPMP017**

**A tool box to investigate Clp protease-mediated protein homeostasis in *Staphylococcus aureus* and its function in pathophysiology**

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Introduction: Protein homeostasis, including protein synthesis and degradation, is essential for all organisms. In the pathogen *S. aureus*, degradation is mainly mediated by the Clp system consisting of the ClpP peptidase and the unfoldases ClpX and ClpC. The Clp protease system is indispensable for general proteolysis but as well for targeted proteolysis, which depend on unfoldases interacting with specific targets. Targeted proteolysis allows for rapid regulation of protein levels and thereby plays an important role in cellular physiology. In particular, ClpXP is crucial for virulence in *S. aureus* [1].

Objectives: The majority of ClpX and ClpC targets remain elusive in *S. aureus* despite the clear role of the unfoldases in virulence and fitness. We aim to construct a set of *S. aureus* strains allowing more detailed studies on ClpX"s and ClpC"s role in *S. aureus* pathophysiology.

Methods: For construction of strains, newly developed plasmid systems are employed - the allele exchange plasmid pSauSE derived from pBASE6-based and the controllable expression plasmid pTripleTrep. Deletion mutants and mutants expressing tagged wild type unfoldase variants from native loci are constructed by applying pSauSE. Further, by introducing pTripleTrep variants into *S. aureus*, plasmid-born transcript and protein levels of unfoldases are inducible and controllable by anhydrotetracycline.

Results: The introduced systems prove their efficacy by the successful construction of *S. aureus* HG001 Δ*clpX* and *clpXTwStr*, where the essential *clpX* gene is manipulated. Initial proteomic profiling *via* mass spectrometry confirmed known ClpX targets such as Spx and Sle1. Additionally, phenotypic screening of the unfoldase mutant underlined its important roles in stress response. In particular, those analyses suggest a so far underestimated role of ClpX during osmotic stress conditions. In ongoing experiments, the developed methods are being used for the construction and analysis of *S. aureus* HG001 Δ*clpC* and *clpCTwStr* mutant strains.

Conclusion: The strain tool box for analyzing ClpX and ClpC function will enable detailed *in vitro* studies under infection mimicking conditions such as iron-limitation and micro-aerobic conditions. By combination with proteomics profiling, a global picture of the role of the unfoldases in *S. aureus* pathophysiology will be revealed.

[1] Jelsbak *et al.* PLoS One. 2010

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**PPMP018**

**The sulfur relay system, which delivers sulfur to a lipoate-binding protein involved in sulfane sulfur oxidation**

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Introduction: In many sulfur-oxidizing prokaryotes, several sulfurtransferases form a relay system that transfers sulfane sulfur towards the cytoplasmic sulfur-oxidizing heterodisulfide reductase-like (sHdr)[1] or Dsr systems[2], with the terminal acceptor protein becoming the direct substrate for the redox reaction. Accordingly, the sHdr system is encoded along with genes for accessory sulfurtransferases

(DsrE, TusA, rhodaneses) and at least one lipoate-binding protein, LbpA[3].

Objectives: Here, we aimed to elucidate the enzymes, the transfer reactions and the possible terminal acceptors involved in sulfur trafficking in sulfur oxidizers. This involved the phylogenetic analysis of the sulfurtransferases present in prokaryotes operating sHdr systems and the elucidation of the sequential sulfur transfer reactions.

Materials & methods: The sulfurtransferases from the sHdr-operating bacteria *Thioalkalivibrio* sp. K90mix, *Thiorhodospira sibirica* and *Hyphomicrobium denitrificans* were analyzed for sulfur transfer *in vitro* by mass spectrometry. The genetically accessible Alphaproteobacterium *H. denitrificans* was used for *in vivo* site directed mutagenesis and sulfurtransferase knockouts.

Results: The proteins TusA, DsrE3B, DsrE3C, a rhodanese and sHdrI were investigated as putative sulfur transferases. Lipoylated holo-LbpA was included as a potential terminal sulfur acceptor. The homotrimeric DsrE3-type proteins efficiently donated sulfur to TusA, which in turn is an efficient donor to LbpA. Deletion of the *dsrE3C* or the *shdrI* gene in *H. denitrificans* resulted in significantly impaired sulfur-oxidation capacity. Exchange of DsrE3C cysteines implemented in the *H. denitrificans* chromosome revealed the catalytically active cysteine. *In vitro*, the importance of this residue was confirmed by mass spectrometric analysis of sulfur transfer reactions. Similarly, mutagenesis of a conserved cysteine in TusA completely abolished *in vitro* sulfur transfer. LbpA serves as the terminal acceptor for the sulfane sulfur supplied by both transferases. The importance and role of the rhodaneses encoded in *shdr-lbpA* gene clusters are being investigated using the same techniques.

Conclusions: In a wide range of sulfur oxidizers, sulfurtransferases form a sulfur relay system, channeling sulfane sulfur to the lipoate-binding protein(s) involved in the sulfur-oxidizing sHdr system.

[1]Koch, Dahl 2018 ISME J 12, 2479

[2]Stockdreher et al 2014 JBC 289, 12390

[3]Cao et al 2018 eLife 7, e37439

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**PPMP019**

**The non-canonical NRPS PA14_11140 of *Pseudomonas aeruginosa* is involved in regulation of virulence factors**

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Introduction: The opportunistic pathogen *Pseudomonas aeruginosa* produces a variety of virulence factors, which enable the bacterium to invade and colonize the host. Virulence factor production may be influenced by metabolites acting as signalling molecules, which are synthesized by non-canonical non-ribosomal peptide synthethases (NRPS). An example for such a non-canonical, monomodular NRPS, consisting of an adenylation-, thiolation- and reductase-domain, is PvfC from *P. entomophila* (1, 2). In *P. aeruginosa*, a yet uncharacterized NRPS encoded by the gene *pa14_11140* exhibits the same domain structure as PvfC. Its metabolites thus might contribute to the regulation of virulence in *P. aeruginosa*.

Objectives: Our aim was to characterize the biological effects of *pa14_11140* with respect to the production of different virulence factors.

Materials & methods: A *pa14_11140* deletion mutant of *P. aeruginosa* PA14 was constructed and complemented with a plasmid containing a copy of *pa14_11140* under the control of an IPTG-inducible pLacUV-promoter. *P. aeruginosa* transformed with the empty vector served as control strain. Different virulence-associated traits of these strains were compared to those of the PA14 wild-type. Levels of the quorum sensing (QS) signalling molecule PQS (*Pseudomonas* quinolone signal) and its precursor were quantified by HPLC. Biofilm formation, pyoverdine, pyocyanin, and rhamnolipid production as well as elastase activity were determined by established spectrophotometric assays.

Results: Overexpression of *pa14_11140* markedly increased the production of pyoverdine, pyocyanin, and rhamnolipids compared with wild-type levels. The levels of PQS and its precursor were also significantly higher upon overexpression of the NRPS.

Conclusion: The increased production of the virulence factors pyoverdine, pyocyanin and rhamnolipids in the *pa14_11140* overexpression mutant can probably be explained by the as well increased levels of PQS, since all these virulence factors are known to be regulated by the PQS-QS system. However, the mechanism of how metabolites produced by the non-canonical NRPS PA14_11140 stimulate the PQS-QS system remains to be elucidated.

References:

(1) Morgan GL, Li K, Crawford DM, Aubé J, Li B (2021). ACS Chem. Biol. 16 (12), 2776–2786.

(2) Kretsch AM, Morgan GL, Acken KA, Barr SA, Li B (2021). ACS Chem. Biol. 16 (3), 501-509.

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**PPMP020**

**Catabolic network of *Phocaeicola vulgatus***

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Introduction: *Phocaeicola vulgatus* is known for the ability to use complex glycans and depolymerization products thereof as growth substrates in the gut microbiome of humans and mammals. The formed fermentations products provide health benefits for the host and have potential industrial relevance as sustainable bulk chemical.

Objectives: The aim of the study was to gain integrated insights into the catabolism of *P. vulgatus* by determining its growth stoichiometry and energetics as well as the architecture of its degradation network for 14 selected substrates.

Material & Methods: *P. vulgatus* ATCC 8482 (DSM 1447) was cultivated in a defined mineral medium with 14 selected substrates. Within the framework of stoichiometric experiments, growth behavior, substrate depletion & product formation (via HPLC), cell dry weight, total cell count and pH shift were determined. The energetics were studied by

integrating balances of reduction equivalents, ATP and formed fermentation products. For proteomics, cells were harvested at ½ $OD_{max}$ and analyzed shotgun, membrane fractions, nanoLC-ES-MS/MS.

Results: The stoichiometric experiments yielded the following range of substrate-specific parameters: growth rate (0.19-0.01 $g_{CDW}/L*h$), biomass formation (0.27-0.01 $g_{CDW}/mM_{substrate}$), fermentation product formation, (5.01-0.72 $mM_{product}/mM_{substrate}$), ATP-yield (28.39-1.95 $mM_{ATP}/mM_{substrate}$). In particular with rhamnose and fucose as provided substrates, propane-1,2-diol was formed amongst other known fermentation products (propanoate, succinate, lactate, formate and acetate). The reconstructed degradation network is comprised of 56 predicted proteins, all could be identified by proteomics. While protein complements of central metabolomics modules such as glycolysis were constitutively formed, those e.g. channeling gluconate into glycolysis were formed with high substrate-specificity. *P. vulgatus* possesses a complex respiratory chain, most constituents of which (33 of 41 predicted) could be identified by proteomics. The strain is amply equipped with paralogs of the Sus-like system required for glycan uptake.

Conclusion: The generated growth stoichiometric and proteogenomics data provide a holistic perspective on the catabolism of *P. vulgatus*, which could be of applied interest and form the basis for a future systems biology level understanding of this model organism.

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**PPMP021**

**5-Keto-D-Fructose, a natural diketone and potential sugar substitute, significantly reduces the viability of prokaryotic and eukaryotic cells**

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5-Keto-D-fructose (5-KF) is a natural diketone occurring in micromolar concentrations in honey, white wine, and vinegar. The oxidation of D-fructose to 5-KF is catalyzed by the membrane-bound fructose dehydrogenase complex found in several acetic acid bacteria. Since 5-KF has a sweetening power comparable to fructose and is presumably calorie-free, there is great interest in making the diketone commercially available as a new sugar substitute. Based on a genetically modified variant of the acetic acid bacterium *Gluconobacter oxydans* 621H, an efficient process for the microbial production of 5-KF was recently developed. However, data on the toxicology of the compound are completely lacking to date. Therefore, this study aimed to investigate the effect of 5-KF on the viability of prokaryotic and eukaryotic cells. It was found that the compound significantly inhibited the growth of the gram-positive and gram-negative model organisms *Bacillus subtilis* and *Escherichia coli* in a concentration-dependent manner. Furthermore, cell viability assays confirmed severe cytotoxicity of 5-KF toward the colon cancer cell line HT-29. Since these effects already occurred at concentrations of 5 mM, the use of 5-KF in the food sector should be avoided. The studies performed revealed that in the presence of amines, 5-KF promoted a strong Maillard reaction. The inherent reactivity of 5-KF as well as the Maillard products formed could be the trigger for the observed inhibition of prokaryotic and eukaryotic cells.

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**PPMP022**

**Non-canonical amino acids in food: Unraveling the metabolism of the advanced glycation end product *Nε*-Carboxymethyllysine in bacteria**

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Proteins are normally composed of twenty natural amino acids. Enzymatic catalysis or spontaneous reactions lead to the formation of post-translational modifications (PTM) and thus extend the genetic code by a large number of non-natural amino acids (NAA) (Lassak *et al*. 2019, 2022). One of the best known PTM is glycation as a result of the Maillard reaction also known as non-enzymatic browning. In particular, cooking or baking favors the reaction of reducing sugars such as glucose with amino acids (Maillard *et al.* 1912). Accordingly, the resulting glycation products are part of our daily diet, among them *N*ε-carboxymethyllysine (CML). However, humans are unable to break down CML but instead rely on the gut microbiota. *Escherichia coli*, as one of the major species in the gut plays an important role in the decomposition of CML: Three major compounds have been identified as metabolites of CML: Carboxymethylcadaverine (CM-CAD), Carboxymethylaminopentanoic acid (CM-APA), and a piperideinium ion (Hellwig *et al.* 2019). However, the molecular "players" mediating the underlying reactions remain unknown.

We have now found the first enzyme in the CML degradation pathway. Specifically, we identified a CML-decarboxylase. A biochemical characterization indicated that CML is decomposed by underground metabolism and we are currently delineating the full pathway.

Understanding CML-metabolism will improve studies on intestinal microbial ecology owing to inclusion of potentially bioactive metabolites. Moreover, it will enable enzyme engineering to remove CML from thermally processed food to eliminate potential unwanted side effects associated with its ingestion.

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**PPMP023**

**Beyond MurA: Investigating how *Escherichia coli* responds to fosfomycin**

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Introduction: A promising strategy to fight the antimicrobial resistance crisis is to better understand the properties of old-fashioned antibiotics, such as fosfomycin (FOS). Mostly used to treat urinary tract infections caused by *Escherichia coli,* it is still effective against multidrug-resistant bacteria and is therefore useful in synergistic therapies. FOS blocks the first step of the peptidoglycan biosynthesis, covalently bonding the MurA enzyme. It remains however unclear how MurA inhibition results in cellular lysis.

Objectives: We aimed to study in *E. coli* the transport of FOS across the outer membrane (OM) and additionally its cellular response to the drug. We affirm that such knowledge is fundamental to optimize FOS usage with other antibiotics.

Material and methods: The translocation through the OM was studied by whole-cells antibiotic susceptibility (E-test, agar dilution method and growth curves) coupled with electrophysiological measurements (single-channel zero current assay).

The phenotype of *E. coli* cells treated with FOS at different growth time-points was explored through continuous measurement of OD 600 nm in liquid medium and sustained by time-lapse microscopy. The intracellular effects of the drug were studied through metabolomics and transcriptomics. The resulting hints were used for further targeted investigations.

Results: We compared a wild type strain with double- and triple-deletion mutants of *ompF, ompC* and *lamB* and showed that these porins are important for FOS translocation across the OM of *E. coli*. The permeability of each channel to FOS was confirmed by electrophysiology.

With time-lapse microscopy we observed that FOS-treated cells not only lyse, but also form blebs, turn to spheroplast-like structures, and finally regrow by reverting to a bacillar shape. Such morphological alterations were comparable to the growth patterns seen in liquid culture. Our omics results of FOS-treated cells (before lysis and after adapted regrowth) showed multifaceted cellular responses, involving the CreAB response and altered metabolism of amino acids and nucleotides.

Conclusions: In addition to the previously shown OmpF, we identified OmpC and LamB as important OM translocators of FOS. We further revealed a complex cellular response of *E. coli* to FOS. Our study is pivotal in providing a deeper understanding of FOS action, which will be fundamental to design more efficient synergistic therapies.

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**PPMP024**

**Identification and characterization of a novel pathway for aldopentose degradation in *Acinetobacter baumannii* ATCC 19606**

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Introduction: *Acinetobacter baumannii* is an emerging opportunistic pathogen that is a significant cause of nosocomial infections. The emerging antibiotic resistances but also its high desiccation resistance have contributed to its success. Another factor contributing to the adaptation to the human host is its broad metabolic versatility, such as different amino acids, aromatic compounds, fatty acids, alicyclic compounds, alcohols, and sugars are used as carbon and energy source. Information concerning sugar metabolism and its link to pathogenicity is very limited.

Objectives: The aim of the study was to characterize the aldopentose degradation pathway in *A. baumannii* ATCC 19606.

Materials & methods: Transcriptomics, mutant studies, growth studies, enzyme activities.

Results: Transcriptome analyses performed with *A. baumannii* ATCC 19606 grown on L-arabinose led to the identification of two significantly upregulated gene clusters. Cluster I contains the genes *araC1*, *araD1,* and *araE1*, a potential regulator and importer gene, and cluster II the isogenes *araC2*, *araD2,* and *araE2*, a potential regulator, importer, and two genes of unknown function. *araC-E* encode proteins with high similarities to enzymes that catalyze the oxidation of a pentonate to α-ketoglutarate which is then funneled into the Krebs cycle. Genes encoding a potential dehydrogenase and lactonase mediating the oxidation of the pentoses to the pentonates could not be identified by transcriptome analyses. However, pentose dehydrogenase activity was observed in cell-free extracts. Deletion of *araC1-E1* led to a loss of growth on L-arabinose, whereas deletion of the isogenes abolished growth on D-xylose and D-ribose. The two genes of unknown function in cluster II are essential for growth on D-ribose, but not D-xylose. The regulation of the two branches differs: regulator I acts as a repressor and regulator II as an activator. Mutational analyses confirmed the role of the putative importers in L-arabinose (cluster I) or D-xylose and D-ribose (cluster II) transport. Furthermore, we could demonstrate that utilization of L-arabinose supports longterm survival and desiccation resistance.

Conclusion: *A. baumannii* oxidizes aldopentoses *via* pentonate to α-ketoglutarate by a novel branched pathway. L-arabonate is degraded to α-ketoglutarate by AraC1, AraD1, and AraE1, and D-xylonate and D-ribonate by AraC2 – AraE2.

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**PPMP025**

**Unraveling the physiological relevance of pyruvate: Quinone oxidoreductase (PQO) from *Corynebacterium glutamicum*: Activity highlighted under acidic pH and low growth rates**

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*Corynebacterium glutamicum* is a Gram-positive bacterium widely used for amino acid production, with the phosphoenolpyruvate-pyruvate node playing a crucial role in carbon flux distribution, hence being target of metabolic engineering. Pyruvate:Quinone Oxidoreductase (PQO) is part of this node, catalyzing quinone-dependent pyruvate oxidation to acetate. Although PQO has been previously identified as a tetrameric flavoprotein and being activated by lipids and detergents, its physiological function remained unclear[1]. This study aims to further characterize PQO and shed light on its potential physiological significance.

Batch fermentations were carried out in a bioreactor at different extracellular pH values to determine the PQO activity in response to different pH levels. This was done, as prior studies indicated increased *pqo* transcript levels during acidic cultivation[2]. Additionally, to examine the impact of the growth rate, a continuous culture of *C. glutamicum* was established and specific PQO activities were determined in relation to the dilution rate, and thus, to culture's growth rate.

In batch fermentations with *C. glutamicum* at different constant extracellular pH values, the specific PQO activity in cell extracts showed a clear correlation with the extracellular pH, a lower pH resulting in higher PQO activity. In the exponential growth phase, the growth rates of *C. glutamicum* were different depending on the extracellular pH, an acidic pH was associated with lower growth rates. In accordance, the specific PQO activity was generally higher during

stationary phase than during exponential growth under various cultivation conditions. Determination of PQO activities in cells cultivated in continuous fermentation processes at different dilution rates showed that lower dilution (growth) rates resulted in higher specific PQO activity.

These findings suggest that PQO may be particularly relevant under conditions of low growth rates and acidic conditions in *C. glutamicum*, highlighting its potential physiological significance.

[1]**Schreiner, M.E. & Eikmanns, B.J**. Pyruvate:Quinone Oxidoreductase from *Corynebacterium glutamicum*: Purification and Biochemical Characterization. *J Bacteriol* **187**, 862–871 (2005)

[2]**Follmann M, et al**. Functional genomics of pH homeostasis in *Corynebacterium glutamicum* revealed novel links between pH response, oxidative stress, iron homeostasis and methionine synthesis. *BMC Genomics* **10**, 621 (2009)

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**PPMP026**

**Unravelling the physiological function of the Entner-Doudoroff pathway in *Synechocystis* sp. PCC 6803 by enzyme characterization**

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Introduction: Although the Entner-Doudoroff (ED) pathway has been demonstrated as one of four glycolytic routes in the cyanobacterial model organism *Synechocystis* sp. PCC 6803 its physiological function remains to be elucidated1. Deletion of the *eda* gene, encoding ED aldolase (EDA), revealed significance of the ED pathway for glycogen utilization in the light, resuscitation from nitrogen starvation and high to low CO2 shift2,3,4. However, 13C flux analysis of photomixotrophically grown cells revealed no flux via the ED pathway under photomixotrophic metabolic steady state conditions. In contrast, fluxes were affected by the deletion of eda under these conditions as especially the flux through the OPP shunt was reduced and glycogen synthesis was enhanced. In addition, an unknown function of EDA was suggested5.

Objective: This study aims to further decipher the role of the ED pathway and its key enzyme EDA.

Materials and Methods: The codon optimized gene encoding a His-tagged version of the *Synechocystis* EDA (*sll0107*) was heterologously expressed in *E. coli*. The enzyme was purified and biochemically characterized with respect to substrate specificity, kinetic and regulatory properties.

Results: EDA catalyzes the cleavage of 2-keto 3-deoxy 6-phosphogluconate with a high catalytic efficiency of 0.026 sec-1mM-1 (Vmax of 9 U/mg, Km of 0.15 mM). In contrast, 4-hydroxy 2-oxoglutarate was only converted with a low catalytic efficiency of 0.0000120 sec-1mM-1 (Vmax of 0.95 U/mg, Km of 2.8 mM). Moreover, effector studies revealed that 6-phosphogluconate (6PG) inhibits EDA activity with a Ki of 1.6 mM.

Conclusion: Our findings confirm that *sll0107* possesses EDA activity. The regulation by 6PG points towards a more sophisticated regulation of the ED pathway in interaction with the complex carbon network that enables adaptation to metabolic and energetic needs in a changing environment.

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2. Makowka A, Nichelmann L, Schulze D *et al*., Mol. Plant 2020; 13(3):471-482
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5. Schulze D, Kohlstedt M, Becher J *et al*., Microb Cell Fact. 2022; 21:69

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**PPMP027**

**_Zymomonas mobilis_ ZM4 ATPase mutants dependent on $O_2$ for growth**

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*Zymomonas mobilis* is a Gram-negative bacterium characterised by high rates of glucose uptake and ethanol production. The metabolism of *Z. mobilis* is fermentative and yields high amounts of ethanol (98% of the theoretical maximum). Despite of its fermentative metabolism *Z. mobilis* is capable of respiring significant amounts of oxygen. However, respiration is not coupled to an increased biomass formation and does not appear to be used for energy production. The mechanisms behind this "uncoupled" growth phenotype remain unknown (1). The *Z. mobilis* genome encodes a typical $F_1F_0$ ATPase. In contrast to many other bacteria, the two subunits are encoded at two separate loci on the chromosome. To analyse the function of the $F_1F_0$ ATPase in *Z. mobilis* ZM4, we deleted both subunits independently and analysed the growth behaviour of the resulting mutants.

Deletion of the subunits was achieved by homologous recombination and gene replacement using the Zymo-Parts toolbox (2). This resulted in heterozygous genotypes with cells harbouring both the WT allele and the replaced allele. After several generations of culturing the cells under aerobic conditions, clean ΔF0 and ΔF1 mutants were obtained. The mutant strains and the wild type ZM4 were cultivated in shake flasks as well as in bioreactors. Both mutants behave similarly. They depend on the presence of oxygen for growth. Decreasing the oxygen supply leads to lower growth rates of the mutants while the growth rates under well aerated conditions are only slightly lower than that of the wild type, as are glucose uptake and ethanol production rates. No differences in biomass yield were observed, further suggesting that the ATPase is not used for the production of ATP.

A notable observation was that the mutants did not completely consume the substrate glucose in shake flasks, whereas this was possible in bioreactors. Whether this difference in behaviour is due to the better aeration or to the pH control in the bioreactor could not be clarified. However, we observed that the ΔF0 and ΔF1 mutants have a lower acid tolerance than the wild type.

In conclusion, we hypothesise that the F1F0 ATPase in *Z. mobilis* has an important function in maintaining or establishing the proton gradient across the membrane. In the

presence of oxygen this role can be taken over by the respiratory chain.

(1) Kalnenieks et al. 2020. Adv Microbial Phys 77: 37-88

(2) Behrendt et al. 2022. ACS Synthetic Biology 11(11), 3855-3864

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**PPMP028**
**Influence of the gut microbiota on the formation of nitrosamines from drug substances**
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Nitrosamines (NA) are potent carcinogens that can pose a significant health risk to humans. There is an increasing interest whether gut bacteria are involved in the nitrosation of active pharmaceutical ingredients (API) containing secondary amines. The aim of this work was to analyze the impact of APIs on the growth behavior of representative strains from the intestinal flora as well as the nitrite consumption by selected gut organisms. This was done with regards to the question whether the gut microbiota can contribute to the arise of API- nitrosamines (API-NA). Based on literature, 10 of 84 core species present in the human colon and representing members of the most prominent phyla Bacteroidota, Bacillota, Proteobacteria, Actinobacteria and Verrucomicrobia were selected. Assuming that the species function as model organism of the respective genus, these genera cover ~ 83 % of all gut bacteria. To mirror gut conditions, the published SHIME® (Simulator of Human Intestinal Microbial Ecosystem) medium was modified for the small and large intestine. The bioavailable amount of nitrite during cultivation was adapted to the physiological concentration of 50 µM present in the colon. The maximum daily dose of the respective API was used in the experiments conducted. For growth experiments, organisms were incubated for 24h at 37°C and optical density was recorded every 20 min. These experiments revealed an inhibitory effect of Propanolol, Amitryptyline, Fluoxetine and Loratadine on the growth of bacteria. However, testing different concentrations of APIs showed that inhibitory concentration varied from one species to another. Additionally, a kinetic of the nitrite consumption was determined. Here, especially *E. coli* was found to reduce nitrite with very high rates, whereas other organisms like e.g. *A. rectalis* did not lead to a significant diminution of the nitrite concentration after 24 h. Analysis of samples by liquid chromatography-mass spectrometry (LC-MS) revealed evidence for API-NA formation by some organisms. This approach provides a deeper understanding of the complex interaction between nitrite, APIs and the gut microbiota and the associated possibility to form API-NAs.

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**PPMP029**
**Investigating the cellular role of CutA proteins: Identification of a putative binder through native mass spectrometry-based metabolomics**
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All living organisms share a pool of common features. Among those commonalities are orthologous proteins with often still undiscovered functions - such as the family of CutA proteins which are found in all domains of life.

While the human CutA protein is specifically expressed in the brain where it is expected to play a substantial role in Alzheimer pathology [1], the bacterial CutA was assumed to confer tolerance against copper in *Escherichia coli* and labeled "copper uptake and transport (cut) gene or protein A" [2]. However, a recent study could not support this assumption for other bacterial strains [3]. Therefore, there is still very little sound information about the cellular role of CutA.

CutA structurally resembles the prominent trimeric signal transduction proteins of the PII superfamily that act as major regulatory hubs to orchestrate key steps of the nitrogen and carbon metabolism [4]. Furthermore, the CutA protein is widely distributed in nature, occurring in archaea, a plethora of bacterial strains and - in contrast to PII - also in eukaryotes, like plants or animals. Hence, we expect this highly conserved protein to play a crucial role in prokaryotic and eukaryotic cells.

To shed light on this protein and its properties, we searched for putative binders of CutA with native mass spectrometry-based metabolomics. Here, the intact trimeric protein was ionized under almost physiological buffer conditions to maintain folding and binding affinities for small molecules [5]. In parallel, crude bacterial cell extract was separated via UHPLC so the molecules in the cell extract are offered to the protein as potential binding partners. With this approach, we could identify a previously unknown putative binder to CutA that was purified and further investigated in this study.

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**RNAP001**
**Characterization of *Aspergillus fumigatus* sRNA biogenesis pathways**
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*Aspergillus fumigatus* is an airborne, spore-forming filamentous fungus and a leading cause of a fungal disease known as Aspergillosis that primarily affects immunocompromised individuals with devastating outcomes. For successful invasion, the pathogen needs to respond and adapt to various stressors with which it is confronted in the human host. Over the past few decades, it has become evident that small RNA (sRNA) molecules are involved in the

regulation of eukaryotic gene expression, including fungi. For example, transfer RNA (tRNA) fragments have been shown to be an emerging class of important regulators under various stress conditions, such as oxidative stress. In order to study the involvement of sRNA-mediated regulation mechanisms, our aims were to: (i) identify candidate enzymes that can bind RNA and potentially modify and cleave tRNAs, (ii) identify novel RNase III enzymes that could contribute to the biogenesis of tRNA fragments independent of known RNase III enzymes like Dicer, and (iii) investigate the environmental conditions that could lead to the biogenesis of tRFs. Northern blot analysis was performed to study tRNA cleavage patterns associated with these enzymes. Moreover, growth assays under various stress conditions were performed to study stress-related phenotypes of the mutants generated. It was found that DclA/B (dicer-like proteins of the RNA interference/RNAi machinery) are not involved in the biogenesis of a particularly abundant tRF derived from *A. fumigatus,* tRNA (tyr). In contrast, RdlP did influence the biogenesis of this particular fragment. Moreover, various environmental conditions were found to influence the fragmentation pattern of tRNAs, and we observed that stressors such as heat, pH, salt, oxidative and hypoxia stress led to increased production of sRNAs in the wild type. Overall, this study hints at the importance of tRNA-derived sRNAs in the response to different stressors in an important human pathogen, and identified components that may be involved in the RNAi-independent biogenesis of these sRNAs in *A. fumigatus.*

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**RNAP002**

**A guard protein mediated quality control mechanism monitors 5-capping of pre-mRNAs**

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Maturation of RNA polymerase II transcripts consists of 5''-capping, splicing, and 3''-polyadenylation. These processes can generate faulty transcripts, which have to be prevented from nuclear export and have to be degraded. An mRNA gets exported to the cytoplasm only after having gone through processing successfully, enabling its translation. Faulty transcripts, on the other hand, get degraded by the nuclear degradation machinery. In *Saccharomyces cerevisiae,* the SR-like guard proteins Gbp2 and Hrb1 control splicing and retain unspliced transcripts in the nucleus. The guard protein Nab2 controls the quality of the 3''-poly(A) tail in a similar way. On correct transcripts, these factors recruit the export receptor Mex67-Mtr2. Only mRNAs which are sufficiently covered with it can pass through the nuclear pore complex (NPC). Passage is controlled by Mlp1, a part of the NPC. For the SR-like protein Npl3, which is highly homologous to Gbp2 and Hrb1, no particular function as a guard protein had been reported so far. By using techniques such as genetical interaction studies, protein and RNA co-immunoprecipitation experiments, nucleo-cytoplasmic fractionation experiments, GFP microscopy, fluorescence *in situ* hybridization experiments as well as an enzymatic assay, we show that Npl3 acts as a guard protein in 5''-capping. Npl3 retains uncapped transcripts in the nucleus and recruits the 5''-3''-end degradation factors Rai1 and Rat1 to them. Most importantly, in absence of Npl3 faulty mRNAs are not retained in the nucleus for degradation but escape retention and leak into the cytoplasm. On correct pre-mRNAs Npl3 interacts with the cap binding complex (CBC), the indicator of a correct 5''-cap. Npl3 binds independently of it and their interaction is lost on uncapped transcripts, suggesting Npl3 detects its presence. Similar to the other guards, Npl3 recruits Mex67-Mtr2 to correct, quality-controlled transcripts while also interacting with the CBC. The binding of Npl3 to the CBC and Mex67 and its binding to Rai1, which supports the association of Rat1, is mutually exclusive, indicating that it might act as a switch between degradation and export. The fate of the transcript is most probably decided through the contact between Npl3 and the CBC.

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**RNAP003**

**Surveillance of 3' mRNA cleavage during transcription termination requires CF IB/Hrp1**

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CF IB/Hrp1 is part of the cleavage and polyadenylation factor complex (CPF-CF), which is responsible for 3' cleavage and maturation of pre-mRNAs. Although Hrp1 supports this process, its presence is not essential for the cleavage event. Here, we show that the main function of Hrp1 in the CPF-CF complex is the nuclear mRNA quality control of proper 3' cleavage. As such, Hrp1 acts as a nuclear mRNA retention factor that hinders transcripts from leaving the nucleus until processing is completed. Only after proper 3' cleavage, which is sensed through contacting Rna14, Hrp1 recruits the export receptor Mex67, allowing nuclear export. Consequently, its absence results in the leakage of elongated mRNAs into the cytoplasm. If cleavage is defective, the presence of Hrp1 on the mRNA retains these elongated transcripts until they are eliminated by the nuclear exosome. Together, we identify Hrp1 as the key quality control factor for 3' cleavage.

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**RNAP004**

**Multiple functions of the SAM-II riboswitch in *Sinorhizobium meliloti***

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Introduction: For the SAM-II riboswitch, present in many Alphaproteobacteria (1), the SAM binding mechanism is well understood, but the effects on gene expression were not studied. In *Sinorhizobium meliloti*, the 5''-UTRs of the *metZ* and *metA* genes harbour SAM-II riboswitches predicted to regulate transcription (1). However, our prediction suggested that the *metA* riboswitch regulates translation.

Objectives: To study the physiological role of the *metA* SAM-II riboswitch in *S. meliloti*.

Materials & methods: Northern blot, RT-qPCR, *egfp* reporter fusions and suitable mutants were used.

Results: Upon SAM binding, structural change in the *metA* riboswitch was predicted to regulate translation from an AUG1 codon. We validated a Rho-independent terminator between AUG1 and a downstream, in-frame AUG2 codon. Transcriptional termination between AUG1 and AUG2 strongly limits *metA* expression and generates an sRNA RA1 that is processed to RA2. Under high SAM conditions, the activity of the *metA* promoter and the steady-state level of the read-through *metA* mRNA were decreased, while the level of the RA2 sRNA was increased. Under these conditions, the RA1 and RA2 sRNAs and *metA* mRNA were stabilized. Reporter fusion experiments revealed that the

Shine-Dalgarno (SD) sequence in the *metA* riboswitch is required for translation, which however starts 74 nucleotides downstream at AUG2, suggesting a novel translation initiation mechanism. Further, the reporter fusion data supported the following model of RNA-based regulation: Upon SAM binding by the riboswitch, the SD sequence is sequestered to downregulate *metA* translation, while the mRNA is stabilized (2). Our data also suggest that the *metA* riboswitch could have additional functions: the originally proposed role in *metA* transcription regulation and roles in *trans* as a part of the sRNAs RA1 and RA2.

Conclusion: In *S. meliloti*, the SAM-II riboswitch upstream of *metA* has at least two contrary functions (downregulating translation while stabilizing the mRNA upon SAM binding), which can serve to ensure basal *metA* and *metZ* mRNA levels under high SAM conditions (2). This probably helps to adapt to changing conditions and maintain SAM homeostasis. This riboswitch might has additional functions, which are under investigation.

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**RNAP005**

**Determination of 5' and 3' RNA-ends in *Aspergillus fumigatus***


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*Aspergillus fumigatus* is a filamentous fungus and an opportunistic human pathogen. In the environment the fungus thrives in compost piles and on other decaying organic materials. Upon nutrient starvation, *A. fumigatus* forms conidia (asexual spores), which are easily aerosolized and dispersed. Every human inhales roughly several hundred of these spores daily. The immune system of a healthy person clears these spores from the lungs. However, in immune-compromised patients, the fungus can cause several forms of disease, like severe aspergillosis, with mortality rates up to 40-90% in susceptible populations. Fungal pathogens are predicted to become a bigger healthcare threat in the coming years, and the world health organization released a human fungal pathogen priority list for the first time in 2022, with *A. fumigatus* categorized in the highest threat group. Although a lot has been done to improve our understanding *A. fumigatus*, we still have a limited understanding of the transcriptome and gene expression mechanisms of this deadly pathogen.

In this study we aimed to determine the 5" and 3" ends of the mRNAs of *A. fumigatus* to get a more complete picture of the transcriptome. To do so, I enriched for poly(A)-tailed RNA and performed a specialized RNA-seq library-preparation to enrich for either the 5" or the 3" RNA-ends. After initial quality control of the sequenced reads, like removing adaptors, the sequences were aligned to the *A. fumigatus* genome using HISAT2. Manual screening of the aligned reads revealed that there are some genes with alternative untranslated regions (UTR) at their 5" or 3" ends. UTRs have various potential roles in regulating gene expression. For example, an alternative 5" UTR could harbor a mitochondrial targeting sequence, or a longer 3" UTR could lead to enhanced RNA stability. Several of the manually identified alternative UTRs were then validated by qRT-PCR, and further bioinformatic analysis will include peak calling of enriched end sites to predict all starts and stops of transcription. Our preliminary analysis suggests that individual peaks can be assigned to approximately 60% and 70% of all genes of *A. fumigatus* using the 5"RNA- and 3"RNA-END-seq approaches, respectively. In addition to improving our understanding of fungal gene expression, we are also likely to obtain a better idea of how the fungus reacts during stress and infection, and hopefully identify new therapeutic targets for treatment of these terrible infections.

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**SSMP001**

**Resource allocation in engineered *Pseudomonas putida*: Synthetic amino acid sinks through recombinant derivatization**


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Microbial production of heterologous proteins is an essential asset in modern day biotechnology. This process drains the depletable pool of resources that are available to the host cell, which results in slow growth and low productivity. One of the key cellular resources are the 20 proteinogenic amino acids, which are the building blocks of proteins. If the intracellular level of amino acids drops below a certain threshold, the stringent response is triggered, which results in a redistribution of resources towards amino acid biosynthesis. Although this phenomenon is generally understood, not much is known about the role of specific amino acids and how their availability influences cell performance. Therefore, we investigated the effect of depletion of specific amino acids in the biotechnology relevant host *Pseudomonas putida*. To this end, we designed six different *P. putida* strains each producing another recombinant enzyme, which derivatizes a specific amino acid. The products of these enzymatic reactions, the amino acid derivatives, serve as a resource sink. To battle the effects of the diminishing levels of amino acids, the cells are expected to channel more resources towards the synthesis of the respective amino acid, at the expense of cell growth. We could show that in order to see a quantitative effect on the growth rate of *P. putida*, the amino acid derivative needs to be a dead-end reaction, meaning that the derivative cannot enter the central carbon metabolism again. Therefore, we blocked the degradation of the amino acid derivatives in several of the engineered strains by genetic engineering. Another important aspect was to discern between growth reduction caused by heterologous enzyme production or by amino acid depletion. We could show that resupply of amino acids in the medium did not fully restore growth in the engineered strains, thus growth reduction not seems to be solely caused by the lack of available amino acids. In two cases, the derivative concentration even increased upon amino acid supplementation, whereas growth remained unaffected, showing that the enzymes were not saturated with substrate. In summary, this works reveals new aspects of resource allocation in *P. putida* and highlights the importance to differentiate between effects brought about by the production of the recombinant enzymes on one hand and by the depletion of a specific amino acid through derivatisation on the other hand.

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**SSMP002**

**Engineering methane-converting platform organisms for a future bioeconomy**


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A desired bioeconomy is a prerequisite for a carbon-neutral or even slightly carbon-negative society. While the advanced production processes of industrial biotechnology are based on glucose from starch and sucrose, the challenge of land use and competition with the food industry are coming into focus. This is especially true for the economic production of low-cost bulk chemicals or biofuels. As the most reduced form of carbon, methane is not only a potent climate gas, but can also serve as an excellent energy and carbon source for methane-based fermentations. However, in recent decades, various research groups have failed to produce the crucial enzymes for methane conversion in industrial relevant platform organisms [1].

We demonstrated for the first time the heterologous production of catalytically active soluble methane monooxygenase (sMMO) from the marine *Methylomonas methanica* MC09 in *Escherichia coli* [2,3]. Key to this was the co-synthesis of the chaperonin GroES/EL, which bears great similarity to one of the proteins within the sMMO operon. For comprehensive characterization by biochemical and spectroscopic techniques, we purified the sMMO by affinity chromatography. Iron determination, electron paramagnetic resonance spectroscopy, photometric assays, and immunoblotting in native gel revealed the incorporation of the non-heme di-iron center and the formation of homodimers of the active sMMO [2,3].

Future development of methane-converting platform organisms and their biotechnological applications will be discussed.

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**SSMP003**
**SporoBeads: Using the inner and outer coat of *Bacillus subtilis* endospores as a protein displaying platform**
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Many bacterial species differentiate into dormant cell types to survive adverse conditions. Upon starvation, the Gram-positive model organism *Bacillus subtilis* initiates the formation of highly resistant and dormant endospores. The core of these spores contain packed DNA, which is protected by the cell wall cortex and three different protein layers named inner coat, outer coat and crust.

Earlier experiments have demonstrated that the crust can be utilized for displaying proteins [1]. Here, we further exploit the potential of the spore envelope as a protein displaying platform by diving deeper into the spore surface and using proteins of the inner and outer coat as anchors. Previous work indicated the endospore proteins OxdD (inner coat) and CotB (outer coat) of *B. subtilis* as promising anchor protein candidates [2], which were therefore analyzed in this study as future targets for protein auto-immobilization inside the endospore coat.

Fluorescence microscopy was chosen to be the first detection method to determine putative anchor proteins on the isolated spores. All constructs were put under the transcriptional control of the crust gene promoter P*cotYZ* , which is active during sporulation, and ensures a proper timing of fusion protein production. The reporter gene *sfgfp* was fused N- and C-terminal to selected anchor proteins via Gibson Assembly and transformed into *B. subtilis.* Initial results reveal the suitability of both CotB and OxdD to act as anchor proteins for sfGFP display, with the former giving rise to a stronger fluorescence signal compared to the latter. In line with previous studies, N-terminal fusions generally resulted in a higher fluorescence intensity, which would make this the best promising fusion side for the putative anchor proteins. Currently we are working on additional anchor proteins from the inner coat (CotF, CotP, CotD) and outer coat (CotM, CotO, CotH), using the experience from CotB- and OxdD-fusions as design rules for the subsequent constructs. First experiments reveal promising new anchor proteins in deeper layers.

The results from this ongoing study will lead to a better understanding of the potential of using the endospore envelope as a protein auto-immobilization platform for biotechnological applications.

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**SSMP004**
**Targeting of antibiotic resistance and biofilm genes with synthetic small RNAs in *Escherichia coli***
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Introduction: Synthetic biology is a rapidly advancing field that involves the design and construction of novel biological systems with specific functions. One of the emerging areas of synthetic biology is the use of synthetic small RNAs, which are short RNA molecules with a regulatory function. Regulatory RNAs contribute significantly to regulatory networks in all aspects of life. In particular, bacteria use small regulatory RNAs (sRNAs) to regulate mRNA translation. Typical sRNAs consist of a seed and a scaffold region, which are distinct modules that can be designed and recombined to create synthetic sRNAs. The resulting synthetic sRNAs have shown potential in various applications.

Objectives: The aim of this study was to test different synthetic seed regions and scaffolds for efficient repression of *csgD* and *ampC* translation.

Material & methods: Golden Gate cloning was used for assembly of seed regions and scaffolds to construct synthetic sRNA expression plasmids. Synthetic seed regions to target *ampC* (β-lactamase) mRNA were predicted by the computational tool SEEDling. The functionality of these synthetic sRNAs was demonstrated using the chromogenic cephalosporin substrate nitrocefin. As a second target, *csgD* was chosen. CsgD is a master regulator of Curli proteins, which are important for bacterial biofilm development. To test *csgD* repression by synthetic sRNAs, a phenotypic screening based on Congo red was performed. Congo red is a red dye that binds efficiently to amyloid fibers such as curli.

Results: It is assumed that the constructed synthetic sRNAs exhibit different regulatory activities depending on the chosen seed and scaffold regions. The respective target also plays a decisive role. Certain scaffolds can specifically and more effectively regulate specific targets, while other scaffolds are less effective. In addition, the seed region of the sRNAs is a significant factor in the regulation of gene expression. The length and binding site of the sRNAs also have an influence on the regulatory activity and thus on the regulation of the respective target.

Conclusion: It may be necessary to test various seed regions and sRNA scaffolds for different mRNA targets to achieve the desired regulatory effect.

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**SSMP005**

**Metabolic network reconstruction and analysis of the cyanobacterium *Synechocystis* sp. PCC 6803**

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Introduction: Phototrophic microorganisms, particularly cyanobacteria, play a vital role in sustaining life on Earth by utilizing light energy to convert atmospheric CO2 into organic compounds. They have also garnered attention as a sustainable source of bioplastics, renewable products, and pharmaceuticals. Computational modeling of phototrophic metabolism and growth is a valuable tool for understanding the principles of light-limited growth in cyanobacteria.

Objectives: In this study, we present an updated reconstruction of the metabolic network of *Synechocystis* sp. PCC 6803 and analyze it using flux balance analysis (FBA). The reconstructed model enables the calculation of the metabolic costs of cellular compounds and serves as a starting point for investigating autotroph-heterotroph interactions.

Materials & Methods: We used literature-based data to update the metabolic reconstruction of *Synechocystis* sp. PCC 6803. We then analyzed the resulting model using FBA to predict quantitative properties of phototrophic growth in a light-limited turbidostat. Additionally, we developed a novel approach to describe light absorption and compared the results with quantitative experimental analyses.

Results: Our reconstructed metabolic model accurately predicts experimentally observed growth behavior of *Synechocystis* sp. PCC 6803 under different light intensities, including light-induced growth inhibition. The model enables the calculation of the metabolic costs of cellular compounds and is suitable for simulating biotechnological production pathways and engineered strains. Furthermore, the model predicts several physiological properties such as NADPH/ATP ratio and O2 evolution.

Conclusion: Our updated metabolic reconstruction of *Synechocystis* sp. PCC 6803 and its analysis using flux balance analysis represents a significant advancement towards improving the biotechnological applications of phototrophic microorganisms. The model serves as a valuable tool for predicting and analyzing phenotypic properties of the bacterial metabolic process and can facilitate investigating autotroph-heterotroph interactions.

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**SSMP006**

**Establishing *Bacillus subtilis* as production platform for mersacidin bioproduction**

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The effective production of bioactive metabolites with antimicrobial properties is necessary as the development of new antibiotics is limited. For screening novel substances, *Bacillus subtilis* appears to be a promising production organism that has already established a reputation as a workhorse for industrially and pharmaceutically useful proteins and fine chemicals.

In order to develop strategies for the production of antimicrobials such as lantibiotics, the biosynthesis of mersacidin as a representative of peptide antibiotics was investigated in several promising *B. subtilis* production strains by integrating the corresponding gene cluster for mersacidin biosynthesis. Here, the genome-reduced *B. subtilis* strain PG10 showed higher sensitivity to mersacidin compared to the other production strains. Using an adapted minimal medium, promising bioproduction of mersacidin was demonstrated for the laboratory strain *B. subtilis* 168. Interestingly, neither coexpression of mersacidin-specific immunity genes nor activating secretory protease were required for production. In contrast, a close relative of strain 168, *B. subtilis* 3NA, which enables high cell density fermentation processes due to mutations in the *spo0A* (regulator of sporulation initiation) and *abrB* (regulator of adaptation in the transient growth phase) locus, showed significantly reduced production performance, suggesting the influence of master regulators in cell differentiation on mersacidin production. In further steps, inducible and externally controllable molecular systems for the bioproduction of mersacidin are to be established and more detailed insights into the mechanisms of gene regulation in *B. subtilis* as a promising microbial host for the production of bioactive metabolites are to be elucidated.

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**SSMP007**

**Reinventing natures No. 1 $CO_2$-fixing enzyme: Using synthetic (micro)biology to build RubisCO *de novo***

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Introduction**:** The most abundant enzyme Ribulose 1,5-bisphosphate Carboxylase/Oxygenase (RubisCO) is the key enzyme in the global carbon cycle. However it is rather slow and shows a high side reaction with oxygen. Improving RubisCO"s kinetics by engineering itself was proved to be difficult. This is mainly due to the natural tradeoff between its

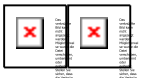
rate and selectivity, which is determined by the complex multi-step reaction the enzyme has to catalyze.

Objectives**:** To overcome the constraints of the RubisCO reaction, we sought to break down the complex, multi-step Rubisco reaction onto individual enzymes that catalyze enolization, carboxylation and hydrolytic cleavage steps of RubisCO individually.

Materials & Methods**:** RubisCO-like proteins (RLPs) from various sources heterologously produced and used in *in vitro* assays. Reaction products were analyzed by LC-MS. $^{13}CO_2$ was used to confirm the carboxylation activity. An *E. coli* SIJ488 strain was engineered to be a *in vivo* testing platform.

Results**:** We demonstrated that two RLP enzymes could be coupled to convert a four-carbon sugar phosphate and $CO_2$ into 3-phosphoglycerate and glycolate. All 3-phosphoglycerate was labeled when $^{13}CO_2$ used as substrate. For the further engineering the reactivity on five-carbon sugar, ribulose 5-phosphate, we constructed a growth coupled selection strain for the anticipated reaction product erythronate 4-phosphate. The strain is pyridoxine auxotroph and able to sense nanomolar range of pyridoxine.

Conclusion: Two RLPs in combination were confirmed to react as carboxylase and hydrolase. Our constructed erythronate 4-phosphate selection strain could be used for engineering their activity on ribulose 5-phosphate. The study will provide a solution of improving carbon fixation using the dominated CBB cycle.



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**SSMP008**

***In vivo* control of siderophore-mediated interspecies communication by light**

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Microbial communities are found in almost all habitats worldwide and play an important role in countless biological processes. Social interactions, including intra- and interspecies communication, can strongly influence the composition and behavior of these communities. In this context, the exchange of various secondary metabolites plays an important role. One class of those secondary metabolites are siderophores. Siderophores are molecules secreted by bacteria under iron depletion. They can bind to Fe3+ with high affinity and are subsequently taken up by the cells via specific receptors. One member of the class of siderophores that is involved in several types of social interactions is pyoverdine (PVD), a fluorescent siderophore produced by various *Pseudomonas* species.To investigate PVD-mediated interactions in bacterial communities, we aimed to generate a *Pseudomonas putida* KT2440 strain in which PVD biosynthesis can be dynamically controlled independently of the iron availability. To achieve that goal, the photocaged inducer NP-cIPTG[1] in combination with a LacI/Ptac-based system was used, allowing light-dependent transcriptional activation of gene expression in various bacteria including *P. putida*[2,3]. This light-responsive switch was applied to control expression of the *pfrI* gene encoding the alternative sigma factor that activates transcription of all PVD biosynthesis genes in *P. putida*[4]. The *lacI*/Ptac *pfrI* gene cassette was genomically integrated via yTREX [5] and gradual light induction of PVD biosynthesis was demonstrated in batch cultures by increasing the exposure time. This *P. putida* strain will next be used to dynamically manipulate and analyze siderophore-mediated social interactions in microbial communities at the single-cell level.

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**SSMP009**

**A synthetic genomics approach to decipher the moonlighting function of the yeast SEN complex**

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Introduction: tRNA splicing is catalyzed by the SEN complex, a heterotetrameric endonuclease complex, conserved and essential in all eukaryotic cells. This complex consists of two catalytic (SEN2 and SEN34) and two structural (SEN15 and SEN54) subunits. Interestingly, it has been found that the SEN complex possesses an unknown essential function that is not related to tRNA splicing but remains elusive. Budding yeast is a model organism for cell biology and many cutting-edge tools are available to conduct pioneering research. In particular, the emergence of synthetic biology developed various novel tools, technologies and approaches that can now be employed to study biological functions such as the SEN complex in an isolated and detailed manner *in vivo*.

Objectives: The SEN complex has a moonlighting function which is aimed to be identified and characterized by (i) analyzing the SEN complex associated RNAs on a global level and (ii) isolating the SEN complex on a neochromosome for genetic functional screens using laboratory automation.

Materials & Methods: Genetic engineering strategies were used to tag the individual SEN complex genes at their native loci. The strains were subsequently used to identify associated RNAs by UV crosslinking and analysis of cDNA (CRAC). Further, we performed strain engineering for easier analysis of the SEN complex based on synthetic biology approaches coupled to laboratory automation and established a long-read sequencing pipeline for strain characterization.

Results & Conclusion: Initial CRAC experiments validate the association of the SEN complex with tRNA introns. Clear and distinct sequencing signals are obtained. The dataset further provides information of other associated RNAs and may

allow to uncover the moonlighting function of the SEN complex in budding yeast. We have established the CRAC workflow to analyze RNAs associated with the SEN complex. The initial results will undergo further analysis to identify potential non-tRNA targets of SEN complex. Additional conditions, where canonical substrates of SEN complex are repressed, will give a more detailed picture of the RNAs associated with the SEN complex.

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**SSMP010**

**A novel promoter-repressor system for tunable gene expression in *Magnetospirillum gryphiswaldense***

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The alphaproteobacterium *Magnetospirillum gryphiswaldense* biosynthesizes magnetosomes, which consist of membrane-enveloped magnetite crystals aligned in linear chains. As one of only few tractable magnetic bacteria, it has emerged as a widely used model for the study of prokaryotic organelle formation and the bioproduction of magnetic nanoparticles. However, analysis and engineering of magnetosome biogenesis has been hampered by the limited toolset for controlled gene expression in this bacterium.

Here, we investigated novel promoters for the strong and tunable expression of endogenous and foreign genes. A set of putative and known promoters from *M. gryphiswaldense* and other bacteria was evaluated in in our host using the bacterial luminescence reporter. The resulting signal was measured in the absence and presence of the cognate repressors. Among all tested promoters, P*cym* and *cymR*, a promoter-repressor system derived from *Pseudomonas putida,* caused the highest level of expression, which exceeded the activity of currently used inducible promoters for *M. gryphiswaldense* such as P*lac* and P*tet*. Furthermore, P*cym* was tightly repressed in the absence of cumic acid, but rapidly induced and tunable by the addition of the inducer at different concentrations.

To explore the P*cym* promoter and *cymR* repressor for the induced expression of magnetosome genes, we placed this system in front of a suboperon harboring five genes, namely *mamRBSTU,* equivalent to one of the five transcriptional units that were recently identified within the large (18 kb) *mamAB* operon known to be essential for magnetosome biosynthesis. Magnetosome formation could be initiated in a non-magnetic mutant by controlled magnetosome gene expression in the presence of the inducer. To gain more information, the remaining four suboperons of the *mamAB* operon will also be placed under control of P*cym* and *cymR*. By varying the inducer concentration, it will be possible to tune the stoichiometry of various magnetosome proteins to reveal and engineer their relative contribution to the control of size, shape, and number of magnetosomes.

In conclusion, the novel genetic tools can be used for functional analysis and engineering of prokaryotic organelle formation, specifically to identify the "adjusting screws" for controlled magnetosome biogenesis in *M. gryphiswaldense.*

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**SSMP011**

**Establishment of a CRISPR-Cas9-based mutagenesis procedure in the roseoflavin producer *Streptomyces davaonensis***

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Introduction: Roseoflavin is structurally similar to riboflavin (Vitamin B2) and exhibits antimicrobial activity against Gram-positive bacteria. *Streptomyces davaonensis* is one of the very few known roseoflavin producers. *S. davaonensis* has an unusual high number of riboflavin biosynthetic genes and thus has a good potential to be developed into a roseoflavin and/or riboflavin producer. The lack of efficient genetic manipulation tools for this microorganism hampers further investigations of this unique bacterial species. CRISPR technology has shown its great power in genome editing and replicative CRISPR-Cas9 mediated genome editing has been reported for some *Streptomyces* species. Unfortunately, *S. davaonensis* is neither compatible with the pSG5 replicons of the employed replicative plasmids nor with pIJ101 (another widely used replicon in *Streptomyces* species). Therefore, we set up to develop an integrative CRISPR-Cas9 system based on pSET152. The integrative CRISPR-Cas9 plasmid showed an ability to generate deletions or insertions as a response to repairing the Cas9-generated double strands break in the genome of *S. davaonensis*. We show that these deletions introduced frame shifts and thus led to targeted inactivation of genes.

Objectives: The objective of this poster is to present an integrative Cas9 plasmid, pSET152_CRISPR-Cas9, which successfully silences genes by introducing indels near the cleavage site of the Cas9.

Materials & methods: The integrative pSET152 was used as a backbone. The pCRISPR-Cas9 plasmid was used as a template to PCR amplify the Cas9 cassette. The cassette was inserted into pSET152 to genernate pSET152_CRISPR-Cas9. The *E.coli* ET12567 (pUZ8002) was transformed with the resulting plasmid and the corresponding strain was conjugated with *Streptomyces* spores. Exconjugants were selected by apramycin and nalidixic acid on MS agar plates. The genome modifications were validated by DNA sequencing.

Results:

- (1)Deletion of *rosA*: seven strains were picked and in 4 strains a nucleotide deletion or insertion.
- (2)Deletion of ribM: 18 colonies were picked, while 6 colonies were

Conclusion: Indels were introduced the genome using the inintegrative CRISPR-Cas9. As a result, a frame shift generated in within the targeting gene. Different targeting genes could have different frequency of introducing indels. In summary, integrative CRISPR-Cas9 could be efficient approach to quickly disrupt genes in *Streptomyces daovaonensis.*

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**SSMP012**

**Analyzing phenotype-less SCRaMbLEd synthetic yeast strains in high-throughput**

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Introduction: The international synthetic yeast project (Sc2.0) is on its path to create the first fully synthetic designer eukaryote. All chromosomes are generated in individual

strains by distinct teams. The synthesis of the synthetic chromosomes underlies common design principles. One of them is the integration of symmetrical loxP sites downstream of every non-essential genes. Once Cre-recombinase is expressed in these cells the genome undergoes drastic recombination events which are caused by Cre mediated deletions, inversions, translocations and duplications. This process is called Synthetic Chromosome Rearrangement and Modification by LoxP-mediated Evolution (SCRaMbLE). SCRaMbLE has been shown to be an effective method to create novel genotypes which lead to higher stress resistance or the ability to utilize novel carbon sources. However, if no selectable phenotype can be observed, for example when attempting to create minimized genomes, the identification of suitable strains can become very cumbersome.

Objectives: SCRaMbLE is an effective tool to create a large variable genotype pool from a single genotype. For future applications, we aim to develop a strategy to select potential candidates in a high-throughput manner for subsequent whole genome analysis. This may be required due to a lack of directly measurable phenotype or to limit redundancies in the process of whole genome sequencing.

Materials & Methods: Within this study various molecular and synthetic biology methods are employed for the creation and characterization of synthetic yeast strains. In particular, qPCR, long-read sequencing and flow cytometry are utilized for strain characterization.

Results & Conclusion: We developed a strategy to efficiently screen SCRaMbLEd yeast strains based on a miniaturized qPCR-based endpoint genotyping procedure. The strategy allows to quickly perform an initial genotype assessment prior to genome sequencing. We further established a long-read based workflow to solve the complex rearranged synthetic yeast chromosomes. Finally, our results indicate a best practice workflow must be established to be able to transfer results from SCRaMbLEd synthetic yeasts towards other strains.

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**SSMP013**

**Engineering cyanobacteria as the basis for a synthetic microbial community**

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In nature, different microorganisms form highly complex communities in which every species has a distinct role. One example are lichens, symbiotic associations of phototrophic cyanobacteria (or algae) with heterotrophic fungi. To increase our understanding of the complex structures and interactions within microbial communities and the role of each microbial partner, the design and analysis of simplified microbial model communities is necessary.

In this collaborative project, our goal is the *de novo* design of a synthetic microbial cross-kingdom community based on the well characterized and genetically amenable model organisms representing cyanobacteria (*Synechocystis* sp. PCC 6803, or *Synechococcus elongatus* PCC 7942), ascomycete (*Saccharomyces cerevisiae*) and basidiomycete fungi (*Ustilago maydis*).

Co-cultivation is based on the carbon source sucrose, which is produced by the phototrophic cyanobacterium using light and carbon dioxide. Sucrose secretion into the culture medium is achieved by inducible, heterologous expression of a sucrose permease gene in the cyanobacteria. This approach is often combined with salt stress and some other metabolic modifications such as overexpression of sucrose pathway genes, or the deletion of competing pathways or carbon sinks to increase sucrose production.

An important aspect for creating a synthetic community is to design and establish tools for the formation of stable co-cultures and the analysis/quantification of the microbial partners of the community. Thus, in this part of the project, we test different cultivation devices and photobioreactor setups for online monitoring of co-cultures as well as single-cell flow cytometry analysis for quantification of individual populations within the co-culture. Another important aspect for the formation of a synthetic microbial community is the characterization of the optimal cultivation conditions for cyanobacterial sucrose production and simultaneous growth of all co-culture partners. In order to track the carbon source sucrose and other important metabolites within the co-culture, we also intend to establish biosensors in cyanobacteria.

Exploiting these established tools, we will create a stable synthetic microbial community which will then be further used to characterize the nutrient exchange in microbial consortia with a special focus on carbon economics and logistics.

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**SSMP014**

**Enhanced carbon fixation in *Synechocystis* sp. PCC 6803 by implementation of a new-to-nature β-keto-acid-cleavage enzyme**

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Photosynthetic carbon assimilation in cyanobacteria, as well as in plants, relay on the activity of RubisCO, an enzyme with relatively low activity and affinity toward the substrate $CO_2$. *In its side reaction, the oxidation of ribulose-1,5-bisphosphate, 2-phosphoglycolate is produces and has to be detoxified during photorespiration in which afore fixed $CO_2$ is released.*

*In our collaborative project we aim to turn photorespiration into a carbon neutral process by implementing a synthetic carbon fixation pathway. Key enzyme is the new-to nature* β-keto-acid-cleavage enzyme (BKACE). Together with designed and adopted enzymes a cycle was created to produce formate from bicarbonate and acetyl-CoA. *Formate is than subsequently incorporated into photorespiration via the C1-pool and thus compensates for the natural loss of $CO_2$ during the detoxification of 2-phosphoglycolate.*

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**STRGP001**

**A novel locally c-di-GMP-controlled exopolysaccharide synthase required for bacteriophage N4 infection of *E. col***

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A major target of c-di-GMP signaling is the production of biofilm-associated extracellular polymeric substances (EPS), which in *Escherichia coli* K-12 include amyloid curli fibers, phosphoethanolamine-modified (pEtN-)cellulose and poly-N-acetyl-glucosamine (PGA). However, the characterized c-di-GMP-binding effector systems are largely outnumbered by the 12 diguanylate cyclases (DGCs) and 13 phosphodiesterases (PDEs), which synthetize and degrade c-di-GMP, respectively. *E. coli* possesses a single protein with a potentially c-di-GMP-binding MshEN domain, NfrB, which – together with the outer membrane protein NfrA – is known to serve as a receptor system for bacteriophage N4.

Here, we show that NfrB not only binds c-di-GMP with high affinity, but as a novel c-di-GMP-controlled glycosyltransferase, synthesizes a secreted EPS, which can impede motility and is required for N4 infection. In addition, a systematic screening of the 12 DGCs of *E. coli* K-12 revealed that specifically DgcJ is required for the infection with phage N4 and interacts directly with NfrB. This is in line with local signaling models, where specific DGCs and/or PDEs form protein complexes with particular c-di-GMP effector/target systems.

Our present study highlights a novel example of how specificity in c-di-GMP signaling can be achieved by showing NfrB as a novel c-di-GMP binding effector in *E. coli*, which is controlled in a local manner specifically by DgcJ. We further show that NfrB is involved in the production of a novel exopolysaccharide. Finally, our data shine new light on host interaction of phage N4, which uses this exopolysaccharide as an initial receptor for adsorption.

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**STRGP002**

**The MerR-family regulator NmlR is involved in the oxidative stress defense in *Streptococcus pneumoniae***

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Introduction: As a leading cause of community-acquired pneumonia worldwide, *Streptococcus pneumoniae* has to cope with different thiol-reactive species, including the strong oxidant hypochlorous acid (HOCl), during host-pathogen interactions.

Objectives: In this work, we analyzed the HOCl stress response of *S. pneumoniae* D39 and focussed hereby on the strongly induced NmlR regulon (1).

Materials & methods: We performed RNA-seq analyses to analyze global gene expression changes upon HOCl treatment. Further, we studied the function and regulation of the redox-sensing MerR-family regulator NmlR under oxidative stress using genetic, biochemical, qRT-PCR, and phenotype analyses and the broccoli-FLAP *in vitro* transcription assay.

Results: In the RNA-seq transcriptome, the NmlR, SifR, CtsR, HrcA, SczA, and CopY regulons and the *etrx1-ccdA1-msrAB2 operon* were most strongly induced under HOCl stress, which participate in the oxidative, electrophile and metal stress response in *S. pneumoniae*. NmlR controls the *adhC* gene, encoding a class III alcohol dehydrogenase. In this study, we demonstrated that NmlR senses not only aldehydes (2) but also HOCl stress to activate transcription of the *nmlR-adhC* operon. Hereby, the conserved Cys52 of NmlR was essential for redox-sensing of HOCl stress *in vivo.* NmlR was shown to be oxidized to intersubunit disulfides or *S*-glutathionylated under oxidative stress *in vitro*. A broccoli-FLAP-based assay further revealed that both thiol modifications significantly increase transcription initiation at the *nmlR* promoter by the RNAP *in vitro*, which depends on Cys52. Phenotype analyses demonstrated that NmlR functions in the oxidative stress defense and promotes survival of *S. pneumoniae* inside human macrophages.

Conclusion: Altogether, our studies identified NmlR as an important HOCl-sensing transcriptional regulator, which activates transcription of *adhC* under oxidative stress by different thiol switches in *S. pneumoniae*.

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**STRGP003**

**The c-di-AMP-binding protein DarA of *Bacillus subtilis* plays a role in osmoadaptation**

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The nucleotide c-di-AMP is the only known essential second messenger. Several functions have been linked to this nucleotide, including osmoregulation, potassium homeostasis, cell wall biosynthesis and DNA integrity. The conserved c-di-AMP receptor DarA of *Bacillus subtilis* is a PII-like protein with a yet unknown function (1). PII and PII-like proteins are the largest family of signal transduction proteins and interact with a plethora of targets such as transporters, enzymes, and transcription factors (2). Despite extensive research, the function of DarA has so far not been described.

In order to gain insight into the role of DarA, phenotypic profiling and suppressor screenings under conditions when DarA is crucial for the growth of *B. subtilis* were performed. The results revealed that DarA is indispensable for growth under osmotically challenging conditions and extreme potassium limitation where the level of c-di-AMP is critically low. Interestingly, suppressor mutations that enhance potassium ion influx can overcome the essentiality of *darA* under these conditions. To explore the role of DarA in osmotic adaptation, a transcriptome analysis was performed to compare transcript levels of a c-di-AMP free strain in the presence or absence of DarA. The analysis showed that the presence of *darA* affects the expression of numerous genes, including many involved in osmotic adaptation. This supports the hypothesis that DarA plays a role in regulating potassium homeostasis under osmotically challenging conditions. It seems plausible to assume that in a situation of extreme potassium limitation DarA integrates the signal of the low c-di-AMP level and counteracts the osmotic conditions.

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**STRGP004**

**Understanding the role of transcriptional and post-transcriptional regulation for the production of the antimicrobial epipeptide EpeX**

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Bacteria must respond to their environment in dynamic ways that ensure they establish and maintain themselves against their competitors. Cannibalism is a bacterial form of programmed cell death during the early stages of sporulation. This survival strategy enhances multicellular differentiation by prolonging or potentially even preventing commitment to endospore formation under starvation conditions. Cannibalism toxins are postulated to kill susceptible cells that have not yet initiated sporulation – and hence lack autoimmunity. The lysed cells then release nutrients that can be used by the toxin producer. In the model Gram-positive *Bacillus subtilis* the cannibalism toxins SKF (sporulation killing factor) and SDP (sporulation delaying protein) are already well known. Biosynthesis for a third cannibalism toxin, the epipeptide EPE, is encoded by the *epeXEPAB* operon. The pre-pro-peptide EpeX is post-translationally processed by the radical SAM epimerase EpeE and the membrane-anchored peptidase EpeP in order to export the 17 aa linear mature peptide EPE. Production of such weaponry is often very tightly regulated at different levels, including transcription but also post-transcriptionally and at the level of production of the active molecule. This project aims at unraveling the role of post-transcriptional regulation of the *epe* locus. Both luciferase and fluorescent reporter strains were applied to analyze the activities of the different promoters driving *epeXEPAB* expression and compare them between different mutants. Furthermore, a collection of mutants was analyzed by Northern blotting and multicellular differentiation assays, to determine transcripts and phenotypes, respectively. We identified a regulatory connection between ComK, the master regulator of competence, and the production of EPE. Moreover, several regulatory RNAs and a 5' UTR were shown to influence the *epe* operon, linking it to the regulation of iron homeostasis, surfactin production, and repression of *comK*. Our investigation leads to a better understanding of the processes of bacterial programmed cell death by expanding our knowledge on the complex network of differentiation decision making in *B. subtilis*.

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**STRGP005**

**Activation of cryptic genes by antagonizing xenogeneic silencing**

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Introduction: Xenogeneic silencing (XS) proteins facilitate the acquisition of novel genetic material into the host genome by silencing foreign gene expression (1). In a previous study, we identified the Lsr2-like XS protein CgpS of *Corynebacterium glutamicum*, which was found to be crucial for maintaining the lysogenic state of the large CGP3 prophage (2). Genome-wide profiling of CgpS DNA binding and in vivo reporter studies revealed a clear preference of CgpS towards AT-rich regions containing multiple TpA steps (3).

Objectives: We investigated how transcription factor (TF) and sgRNA/dCas9 binding can counteract CgpS at silenced prophage promoters leading to promoter reactivation.

Methods: Activities of CgpS target promoters in response to modulated binding of specific TFs or sgRNA/dCas9 complexes were measured by promoter-reporter gene (e*yfp*) fusions via fluorescence-based methods.

Results: Binding of specific TFs to silenced promoter regions may oppose XS activity leading to counter-silencing, thereby providing access to horizontally acquired DNA. In a synthetic counter-silencing approach, CgpS target promoters with synthetically inserted TF operator sites were fused to a reporter gene (*eyfp*) to systematically assess the interference between the XS protein and the specific TF. Various CgpS target promoters showed increased promoter activities upon binding of the effector-responsive TF acting as counter-silencer. Here, the operator sequence as well as its position showed a significant impact on the dynamic range and maximal promoter output (3, 4). Interestingly, we also achieved TF-independent counter-silencing by using sgRNAs guiding dCas9 to silenced phage promoter regions. This sgRNA/dCas9-mediated counter-silencing does not

require the modification of target promoter sequences, but also allowed modulated activation of CgpS target promoters.

Conclusion: Overall, this study demonstrates the capability of both counter-silencing strategies to reactivate cryptic genes in a precise and targeted manner. These findings highlight the relevance of counter-silencing as a bacterial strategy allowing evolutionary network expansion and its biotechnological potential for the production of valuable, but cryptic metabolites.

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**STRGP006**

**SoxR, a sulfane sulfur-responsive repressor of sulfur oxidation**

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Introduction: In organisms that use reduced sulfur compounds as alternative or additional electron donors to organic compounds, transcriptional regulation of sulfur oxidation is needed to adjust metabolic flux to environmental conditions**.** However, little is known about the sensing and response to inorganic sulfur compounds such as thiosulfate in sulfur-oxidizing bacteria.

Objectives: Here, we aim to close this knowledge gap and studied the ArsR-SmtB-type transcriptional repressor SoxR from the Alphaproteobacterium *Hyphomicrobium denitrificans.* The organism is obligately heterotrophic and oxidizes thiosulfate as an additional electron donor during growth on C1 compounds [1].

Materials & methods: The DNA binding properties of SoxR and the role of its two conserved cysteine residues were characterized via site directed mutagenesis, mass spectrometry, gel permeation chromatography, MalPEG assays and electrophoretic mobility shift assays.

Results: We show that SoxR acts as a homodimer and senses sulfane sulfur. The repressor is crucial for the expression not only of *sox* genes encoding the components of a truncated periplasmic thiosulfate-oxidizing Sox-system but also of several other sets of genes for enzymes of sulfur oxidation. DNA binding and transcriptional regulatory activity of SoxR are controlled by polysulfide-dependent cysteine modification. The repressor uses the formation of a sulfur bridge with one, two or three sulfur atoms between two conserved cysteines as a trigger to bind and release DNA and can also form a vicinal disulfide bond to orchestrate a response to oxidizing conditions.

Conclusion: Our study shows, that SoxR allows *H.denitrificans* to adapt to changes in thiosulfate availability via thiol persulfidation chemistry. The whole process may involve transporters and sulfurtransferases encoded in the same genetic island.

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**STRGP007**

**PrrA/B and MSMEG_0243 potentially regulate phosphatidylinositol mannoside acetylation in mycobacteria**

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Two-component systems (TCSs) are a common signal transduction mechanism in many bacteria. In this system, a membrane-bound histidine kinase is autophosphorylated upon binding a signal molecule with ATP consumption at a histidine residue. This kinase can then phosphorylate a response receiver at an aspartate residue, which triggers the cellular response.[1] Bacterial TCSs play important roles in virulence or drug resistance, and are potential drug targets themselves.[2] In *Mycobacterium smegmatis*, the histidine kinase MSMEG_0246 (PrrB) and the response receiver MSMEG_0244 (PrrA) form a TCS. In the same operon as *msmeg_0244* and *msmeg_0246*, there are two periplasmic genes, *msmeg_0242* and *msmeg_0243*.[3] Both corresponding proteins are annotated heme-binding proteins. Knockout mutants showed that biofilm formation was abrogated when *msmeg_0243*, *msmeg_0244*, and *msmeg_0246* were knocked out, compared to the wildtype *M. smegmatis*. The lipid analysis of this knockout mutant showed a defect in processing acetylated phosphatidylinositol mannosides. [3] The aim of this study was to test the heme binding properties of MSMEG_0242 and MSMEG_0243, investigate potential interaction partners of MSMEG_0246, and examine the cellular effects of MSMEG_0243, MSMEG_0244, and MSMEG_0246. UV/Vis spectrometry and biolayer interferometry were performed to test the heme binding properties of MSMEG_0242 and MSMEG_0243. The latter was also used for KD determination and interaction assays with MSMEG_0246, which was additionally tested by co-purification assays. While MSMEG_0242 showed no binding to heme, MSMEG_0243 bound heme with a KD value of ~300 nM. Neither MSMEG_0243 nor MSMEG_0242 showed direct binding to MSMEG_0246, in contrast to the extracellular protein Ag85B (involved in cell wall construction in mycobacteria), which showed binding to MSMEG_0246. Several open questions remain, especially regarding the signal transduction mechanisms and downstream gene regulation, which are the subject of further studies.

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**STRGP008**

**Characteristics of the GlnH and GlnX signal transduction proteins controlling PknG-mediated phosphorylation of the 2-oxoglutarate dehydrogenase inhibitor OdhI in *Corynebacterium glutamicum***

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Introduction: The 2-oxoglutarate dehydrogenase (ODH) of *Corynebacterium glutamicum* is regulated by a signal transduction cascade involving phosphorylation of the small regulatory protein OdhI by the serine/threonine protein kinase PknG. Non-phosphorylated OdhI inhibits ODH activity, whereas phosphorylated OdhI does not. Previous studies indicated that the kinase activity of PknG is controlled by the secreted putative glutamine-binding protein GlnH and the integral membrane protein GlnX.

Objectives: Our aim was to biochemically and structurally characterize the proteins GlnH and GlnX in order to obtained a detailed molecular understanding of the signal transduction cascade.

Materials and methods: The ligand binding properties of purified GlnH were determined by isothermal titration calorimetry (ITC) and tryptophan fluorescence quenching. The cellular localization of GlnX was analyzed by fluorescence microscopy of an mVenus-GlnX fusion protein. The GlnX topology was determined by PhoA and LacZ fusions. Structural models of GlnH and GlnX were generated using the Colabfold2 pipeline by AlphaFold2.

Results: GlnH was shown by ITC to bind L-aspartate and L-glutamate with moderate to low affinity, but not L-glutamine, L-asparagine, or 2-oxoglutarate. Based on a structural comparison with GlnH of *Mycobacterium tuberculosis*, two residues critical for the binding affinity were identified and verified. An mVenus-GlnX fusion protein was found to be localized in the membrane. The predicted topology of GlnX with four transmembrane helices and two large periplasmic domains was confirmed by LacZ and PhoA fusions. *Ab initio* structure prediction of GlnX by AlphaFold2 revealed a novel architecture named as Helical Tandem Module (HTM) composed of three four-helix bundles (4HBs) – with one 4HB located in the membrane and two 4HBs in the periplasm.

Conclusion: Our results provided novel information on the GlnH-GlnX-PknG-OdhI-ODH signal transduction cascade, which serves to adapt the flux of 2-oxoglutarate between ammonium assimilation via glutamate dehydrogenase and energy generation via the tricarboxylic acid (TCA) cycle to the availability of the amino group donors L-glutamate and L-aspartate in the environment.

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**STRGP009**

**First insights into the role of second messengers for regulation of symbiosis and pathogenicity in *Photorhabdus luminescens***

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The role of second messengers for regulation of various phenotypes and host adaptation in bacteria came more and more into focus in the last years. The entomopathogenic bacterium *Photorhabdus luminescens* is a symbiont of nematodes and plants, and a pathogen against insects. The bacteria are characterized by two phenotypic different cell forms: the primary (1°) and secondary (2°) cells, whereas 2° cells cannot interact with the nematodes and in the soil and undergo a symbiotic interaction with plants. Both cell forms are pathogenic against insects. Since *P. luminescens* colonizes different eukaryotic hosts, the cells need to precisely adapt to the different interaction partners. To investigate whether second messengers are involved in this regulation, we analyzed the intracellular concentrations of various second messengers in the presence and absence of host-specific signals. When treated with signal molecules from nematodes, insects or plants, the concentration of 2",3"- and 3",5"-cyclic nucleotide monophosphates (cNMPs) differed from the non-treated cells. Moreover, the concentration of these second messengers differed between 1° and 2° cells, while the general amount of 2",3"-cNMPs was higher compared to the others under all tested conditions. This indicates a central role of the different second messengers in phenotypic switching of *P. luminescens* as well as host adaptation. Even though *P. luminescens* forms biofilms, cyclic di-GMP was not found to be involved in switching from motility to sessility as described for various other bacteria. This is in accordance with the uncommon finding that *P. luminescens* does not have any proteins containing a PilZ- and GGDEF-domain responsible for cyclic di-GMP synthesis or binding. Especially, *P. luminescens* harbors a LuxR-type receptor with a putative N-terminal GAF domain that is supposed to bind second messenger(s) like cAMP as signal(s). The deletion of the gene encoding GAF-LuxR led to differences in *P. luminescens* characteristic phenotypes like luminescence, interbacterial killing and support of nematode development. This indicated a central role of GAF-LuxR in sensing second messengers in *P. luminescens*. Overall, our studies suggest a central role of several cNMPs in the switch between symbiosis and pathogenicity of this biotechnological relevant bacterium.

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**STRGP010**

**Genome-wide analysis of iron- and heme-dependent regulatory networks in *Corynebacterium glutamicum***

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Iron is an essential mineral required by nearly all cellular organisms as it plays a crucial role in several fundamental biological processes, including electron transport, the TCA cycle, and peroxide reduction. In iron-scarce environments, heme, an iron-bound protoprophyrin IX, represents an important alternative source of iron. Heme also serves as prosthetic group of several critical proteins like cytochromes, hydroxylases or catalases. Although iron and heme are essential for survival, a certain toxicity is associated with elevated levels caused inter alia by the generation of reactive oxygen species via Fenton reaction [1,2]. To maintain proper iron and heme homeostasis, *Corynebacterium glutamicum* relies on a complex transcriptional network comprised of the master regulator of iron DtxR and the heme-responsive regulator HrrA of the two-component system HrrSA [3].

Previous studies gave valuable insights into the genetic targets of DtxR and HrrA [4,5], while combining in vitro data with in vivo studies provides an unprecedented opportunity to gain a view on promoter occupancies in the cellular context. Within this work, genome-wide in vivo profiling of DtxR binding sites using chromatin-affinity purification and sequencing (ChAP-Seq) did significantly expand the repertoire of DtxR targets by genes involved in DNA recombination, methionine synthesis and oxidative stress as well as several prophage genes. Comparing DtxR binding patterns in the presence of either $FeSO_4$ or heme revealed a remarkable difference dependent on the iron source and the same observation was made when further deciphering conditional binding patterns of HrrA. The discovery of

multiple common targets of both DtxR and HrrA highlights the interconnectedness of these two global regulatory networks. Ultimately, our research underscores the significance of in vivo genome-wide binding analyses in unravelling global regulatory networks and the intricate relationships that exist between them.


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**STRGP011**

**The epipeptide toxin EPE and its role in multicellular differentiation processes of *Bacillus subtilis***


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The ability to escape unexpected environmental fluctuations is essential for the survival of *Bacillus subtilis* in its natural habitat, the soil. Facing starvation, a subpopulation of the heterogeneous biofilm population commits to the formation of endospores. However, this differentiation process is complex and energy-demanding and only serves as a last resort to ensure survival of the population. Cannibalism, a bacterial form of programmed cell death, is one strategy of this final decision-making process. It prolongs or even prevents full commitment to sporulation by sacrificing part of the population for further nutrient supply, thereby also enhancing biofilm formation. This is achieved through the production of the cannibalism toxins SDP, SKF, and the recently described epipeptide EPE[1].
EPE is genetically determined by the *epeXEPAB* operon and causes severe membrane perturbations, thereby inducing the LiaRS-dependent cell envelope stress[2]. The antimicrobial peptide EPE is encoded by *epeX* as a pre-pro-peptide, which is then processed via post-translational modifications into its active, 17 amino acids long linear peptide form[1].
We recently investigated EPE"s role within multicellular differentiation processes and biofilm formation, using the undomesticated strain *B. subtilis* NCIB3610. Mutants lacking *epeX* or *epeAB* show severely altered colony morphologies, indicating that EPE is not only acting as a toxin, but is also involved in structuring and functionalizing the biofilm. In differentiating colonies, expression of the *epe* locus co-localizes with the LiaRS-dependent stress response, indicating a spatiotemporally correlation between *epeX* expression and EPE production. Taken together, the results suggest that the cannibalism toxin EPE provides a link between colony morphology, cell death and sporulation. Unravelling EPE's function will provide a better understanding of how multicellular bacterial tissue differentiates and how signalling between cells in a biofilm leads to complex structures and patterns.

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**STRGP012**

**Characterisation of the Cpx system of *Pseudomonas aeruginosa***


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Two-component systems (TCSs) are essential for bacteria to sense and respond to environmental changes, including exposure to antibiotics. They consist of a sensor histidine kinase and a response regulator. A well-studied example of TCSs in *Escherichia coli* is the CpxS/CpxR system, which regulates the bacterial envelope stress response, a crucial mechanism for maintaining the integrity of the bacterial cell envelope. In contrast to E. *coli*, the two-component systems of *Pseudomonas aeruginosa* are poorly understood. Our previous work has shown that mutations in the sensor histidine kinase CpxS protect against negative cellular hysteresis, which describes the phenomenon that short exposure to one antibiotic (e.g. beta-lactams) sensitises bacterial cells towards other antibiotics (e.g. aminoglycosides) [1]. To date, the exact molecular processes underlying the protective effect are not well understood.

Our study aims at a detailed understanding of the phenotypic and molecular consequences of mutations in the Cpx system, especially CpxS and the putative CpxP homologue PA14_22740.

We are analysing resistance profiles, growth behavior and *in vitro* biofilm formation of *P. aeruginosa* PA14 strains carrying mutations in the Cpx system. Functional effects of mutations in CpxS are investigated by *in vitro* analysis of protein function (e.g. kinase activity) and the interaction of CpxS with the response regulator CpxR and the CpxP candidate. These assays are being complemented by *in vivo* analysis of protein localization and protein-protein interactions using pull-downs.

Our results indicate that the analysed mutations in CpxS can influence the susceptibility towards beta-lactams and aminoglycosides. Growth behavior and biofilm formation can also be affected by the tested CpxS mutations. We have utilised homology models of CpxS to map the identified mutations to obtain structural insights into their potential effects. We further investigate how mutations in CpxS affect its function and interaction with other Cpx system components.

Our study yields novel insights into the function of the Cpx system of *P. aeruginosa*. The Cpx system is known to regulate a variety of virulence-associated traits in other Gram-negative bacteria. A better understanding of this cell envelope stress response system in *P. aeruginosa* can potentially contribute to a more targeted treatment approach for *P. aeruginosa* infections.


[1] R. Römhild *et al.*, PNAS (2018)


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**STRGP013**

**A regulatory duet: sHdrR and SoxR team up for transcriptional repression of sulfur oxidation in *Hyphomicrobium denitrificans***

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Introduction: Organisms have different strategies for sensing and responding to reduced sulfur compounds such as sulfide or thiosulfate. In the methylotrophic Alphaproteobacterium *Hyphomicrobium denitrificans,* which uses thiosulfate as an accessory electron donor, one such strategy is the use of two distinct but related ArsR-type transcriptional repressors, sHdrR [1] and SoxR, which share two conserved cysteine residues.

Objectives: We aimed at identification of sHdrR target genes, the signal controlling DNA binding, mapping of sHdrR binding sites and collecting evidence for crosstalk with SoxR.

Materials & methods: The following experimental strategies were applied: individual deletion of both regulator genes, electrophoretic gel mobility shift assays (EMSA), qRT-PCR and comparative RNAseq analyses of wild type and mutant strains.

Results: sHdrR affects the expression of numerous genes for encoding enzymes of the cytoplasmic sulfur-oxidizing heterodisulfide reductase-like (sHdr)-LbpA system. While SoxR also affects the expression of these genes, it additional acts as a strong repressor of the *sox* genes, which encode the periplasmic thiosulfate oxidizing Sox complex. The combined action of both repressors was demonstrated with EMSA experiments. For sHdrR, the importance of its two conserved cysteine residues was shown *in vitro* by studying variants with cysteine to serine exchanges and *in vivo* by phenotypic characterization of mutant strains carrying chromosomal substitutions of either one or both cysteines to serine. Two potential transporters, SoxT1A and SoxT1B, are encoded in the *H. denitrificans* sulfur oxidation island, and strains lacking either of these genes have a thiosulfate-oxidation negative phenotype. SoxT1B appears to be a likely signal transducing unit for SoxR, since a Δ*soxT1B* Δ*soxR* double mutant regains the ability to oxidize thiosulfate.

Conclusion: Thiosulfate oxidation in *H. denitrificans* is controlled by concerted action of two repressors, sHdrR and SoxR. Whether they interact directly or indirectly is an important research question for the future.

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**STRGP014**

**pH-dependent activation of the transcriptional regulator AdiY of *E. coli***

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Introduction: Adaptation to acid stress is an important factor in the transmission of intestinal microbes, such as the enterobacterium *Escherichia coli*. To counteract acidic stress and maintain pH homeostasis, *E. coli* activates, amongst others, three enzyme-based proton-consuming acid resistance (AR) systems: the glutamate decarboxylase (Gad), the arginine decarboxylase (Adi), and the lysine decarboxylase (Cad) system to elevate the intra- and extracellular pH[1,2]. These three systems are activated at different levels of acid stress. Briefly, the Cad and Adi systems are heterogeneously activated in an *E. coli* population at extracellular pH ($pH_e$) of 5.8 and 4.4, respectively. The Gad system is heterogeneously expressed mainly in the stationary phase. Whereas activation of the Adi system correlates with the Gad system, the Adi and Cad systems are mutually exclusive in individual cells[3].

Objectives: Because the degree of heterogeneity of the Adi system was influenced not only by the copy number of the main regulator AdiY[3] but also by the intracellular pH, we now characterize AdiY as a potential soluble pH sensor.

Materials & methods: The mechanism of activation and regulation of AdiY was characterized via promoter studies, alanine scanning mutagenesis of histidine residues, and, DNA binding affinity studies both in *vivo* and *in vitro*.

Results: The promoter activities of *adiA* and *adiC* are activated under acidic conditions ($pH_e$ 4.8-4.4) in an AdiY-dependent manner. The ability of AdiY to sense decreasing intracellular pH is mediated by two histidine residues within the N-terminal ligand-binding domain that are crucial for activation of the target promoters. Complementary *in vitro* analysis of the DNA-binding ability of AdiY revealed increased affinity to the target promoter at a pH ≤ 6.5. This is achieved via pH-dependent conformational changes with constant native copies of AdiY in acidic environments.

Conclusion: AdiY undergoes pH-dependent conformational changes and is an example of the poorly known cytoplasmic pH sensors.

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**STRGP015**

**A novel regulatory module important for cell cycle regulation in the stalked budding bacterium *Hyphomonas neptunium***

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Unlike many bacterial model organisms, the alphaproteobacterium Hyphomonas neptunium does not undergo binary fission but generates offspring through the formation of buds at the end of a stalk-like cellular extension. The control of this intricate biphasic life cycle requires a precise regulation of cell cycle progression, morphogenesis and cell division. Here, we report the identification of a two-component signaling protein that is critical for normal growth and development in H. neptunium. A deletion mutant shows pleotropic defects,

including severely swollen mother cell bodies and growth defects. This new regulator is part of a regulatory module which components mediate essential cellular functions and show cell cycle-dependent localization patterns. Together, our results reveal the existence of a novel and conserved regulatory pathway that contributes to the control of cellular development in H. neptunium and, potentially, also other members of the alphaproteobacterial lineage.

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**STRGP016**

**Role of Asp23 family proteins in fatty acid acquisition in *Bacillus subtilis***

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Fatty acids are essential precursors for the synthesis of phospholipids that serve as the main components in biological membranes of virtually all organisms. The acquisition of fatty acids in *B. subtilis* is based on either essential *de novo* biosynthesis or the uptake of exogenous fatty acids. The highly specific acetyl-CoA-carboxylase (ACCase) complex catalyzes the conversion of acetyl-CoA to malonyl-CoA to initiate the biosynthesis of fatty acids. Exogenous fatty acids are phosphorylated by a two-component fatty acid kinase (Fak) prior to incorporation as acyl-phosphates and further processing. The regulation of both committed steps in fatty acid acquisition, however, remains unknown.

We have identified the unknown and highly expressed proteins YqhY and YloU of the conserved Asp23 protein family as promising candidates to regulate fatty acid acquisition. The genes *yqhY* and *yloU* encoding the paralogous Asp23 family proteins share conserved operons with *accBC* and *fakA*, respectively. Based on the genomic co-localization, protein-protein interaction experiments revealed physical interactions between YqhY and AccBC, and YloU and FakA, respectively. Additional suppressor analyses and fatty acid profiling in *yqhY* deletion strains supported the initial hypothesis. This work aims at characterizing the putative regulatory effects of the Asp23 family proteins on fatty acid acquisition in *B. subtilis.*

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**STRGP017**

**Dshi_1135 protein of *Dinoroseobacter shibae* is activating expression of the photosynthetic gene cluster by acting as a light-dependent antirepressor of PpsR**

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Introduction: The marine bacterium *Dinoroseobacter shibae* possesses a photosynthetic gene cluster (PGC), encoding all components for aerobic anoxygenic photosynthesis. Screening of the transposon mutant library of *D. shibae* for loss of pigmentation and reduced Bchl *a* absorbance identified the gene locus Dshi_1135, encoding a potential blue-light dependent LOV (light, oxygen, voltage) histidine kinase. Transcriptome analysis revealed an essential role of Dshi_1135 for expression of the PGC. The protein Dshi_1135 is capable of a reversible blue light induced photocycle. Co-purification analysis, subsequent mass spectrometry and specific immunodetection indicated a light-dependent interaction of Dshi_1135 with PpsR presumably acting as anti-repressor.

Objectives: Verification of the light-dependent antirepressor function of Dshi_1135 via interaction with the PpsR repressor.

Materials & methods: A heterologous *in vivo* interaction system was established by expressing the regulators Dshi_1135 and PpsR alone or in combination while transcriptional regulation was monitored by *bchF-lacZ* reportergene expression after growth under different light conditions.

Results: The expression of the *bchF-lacZ* reportergene in the *E. coli* NovaBlue DE3 blue test strain showed a level of 5000 Miller Units while a strain harbouring the empty vector showed no ß-galactosidase activity. Expression of the PpsR repressor protein reduced the ß-galactosidase activity to 1200 Miller units under light, blue light and dark growth conditions indicating repressor function. Simultaneous expression of PpsR and Dshi_1135 led to increased *bchF-lacZ* expression compared to the strain expressing PpsR alone indicating derepression by Dshi_1135. Since this derepression only occurred under dark conditions a light-dependent antirepressor function of Dshi_1135 was suggested. In addition, the Dshi_1135C61A mutant protein, unable to perform the blue light induced photocycle, lost the ability to function as antirepressor.

Conclusion: In *D. shibae* the newly identified regulator protein Dshi_1135 is interacting with the transcriptional repressor PpsR and acting as an antirepressor under dark growth conditions. Thus, we suggest to rename the Dshi_1135 protein by LdaP (light-dependent antirepressor of PpsR). Moreover, the blue light induced photocycle is essential for the antirepressor activity of Dshi_1135. A regulatory model of light-dependent regulation of the PGC is proposed.

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**STRGP018**

**Lost in Translation? – The role of elongation factor P paralog EfpL in efficient protein synthesis in bacteria**

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Translation is a non-uniform process, and its speed depends on the amino acid to be incorporated [1]. The insertion of proline is particularly slow, and polypeptide synthesis comes to a halt when consecutive proline coding sequences are translated [2, 3]. To alleviate this stalling, almost all living organisms utilize a specialized elongation factor known as EF-P in bacteria [4, 5]. Interestingly, around 12% of all Eubacteria encode a paralogous protein that we have now renamed elongation factor P-like (EfpL). EfpL can be found in various γ-Proteobacteria including *Escherichia coli*, δ-Proteobacteria, Planctomycetes, and Acidobacteria. To gain a deeper understanding of EfpL"s function, we conducted a ribosome profiling. Our studies now reveal that the sequence motif spectrum targeted by EF-P and EfpL exceeds Polyproline-motifs. We identified motifs that, in combination with certain other amino acids, only require one proline to cause an elongation factor-dependent arrest. Combined with mutant-phenotypic characterization and in vivo reporter analyses [6], we were able to unravel the physiological role of EfpL. Overall, we found that EfpL appears to have an accessory role in *E. coli*, whereas EfpL can fully compensate for the absence of EF-P in *Vibrio* species. Taken together,

these findings provide new insights into translation regulation in bacteria.


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**STRGP019**

**Under control – Combat *Listeria monocytogenes* infections**


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PrfA is the master regulator of virulence gene expression in the human pathogen *Listeria monocytogenes* that plays an important role during host infection. Many regulatory factors have already been discussed to up- or downregulate the activity of PrfA such as branched-chain amino acids, different carbon sources, glutathione and stress response factors (1). Especially different carbon sources like glucose-1-phosphate and cellobiose are discussed to be of high importance in the sugar-dependent regulation of PrfA (2). Our research project aims to identify the required factors in this process. For easier handling, the experiments will be performed in *Bacillus subtilis* first, where artificially introduced PrfA was shown to be active. Thus, all factors necessary for PrfA activity seem to be present in *B. subtilis.* This is remarkable considering that it produces bacillithiol instead of glutathione, the essential cofactor of PrfA (3, 4). Therefore, we will also investigate whether bacillithiol can replace glutathione to stimulate PrfA activity. Our goal is to generally characterize the regulatory factors that influence the PrfA activation and repression in more detail. This could help with the development of novel strategies to suppress the virulence potential of *L. monocytogenes* within the host.


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**STRGP020**

**LsrB- A conserved LysR-type transcription factor in pathogenic and symbiotic Rhizobiales**


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The order of Rhizobiales contains species that live in close association with hosts, ranging from symbionts to pathogens. The host-associated lifestyle requires rapid adaptation and tightly regulated response systems to adapt to quickly changing environments upon host encounter. A key role in this adaption is assigned to small regulatory RNAs (sRNAs). A global regulator of numerous sRNAs, in rhizobial species, such as *Agrobacterium tumefaciens, Sinorhizobium meliloti* and *Brucella abortus*, is the LysR-type transcriptional regulator LsrB. LsrB is encoded in a conserved bicistronic operon with TrxB (thioredoxin reductase). Deletion of *lsrB* has been associated with reduced virulence/symbiosis and generally low fitness, compared to the respective wildtypes. Hence, we wondered if the shared regulatory function of LsrB proteins originates in recognition of a conserved environmental signal and activation mechanism. Furthermore, we elucidated the regulation of the *trxB/lsrB* operon itself.

Through phenotypic and transcriptional analysis, we found that complementation of an *A. tumefaciens lsrB* deletion mutant with homologous LsrB proteins from *S. meliloti* and *B. abortus*, restores wild-type phenotypes. Through structural and biophysical analysis, we found that all three LsrB proteins bind a small phenolic ligand in a conserved binding pocket. Via EMSA assays we showed that LsrB binds the *trxB*/*lsrB* promotor, suggesting autoregulation. To gain further insights into the regulation of the operon, we developed a reporter assay (promotor-eGFP-fusion) to investigate differential promoter activity. The obtained results will be the basis of DNA-pulldown assays to identify putative superior regulatory factors of the *trxB/lsrB* operon. In conclusion, the results strongly suggest that LsrB proteins share a conserved activation and regulation mechanism among Rhizobiales.

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**STRGP021**

**The role of pulcherriminic acid in multicellular differentiation of *Bacillus subtilis b*iofilms**


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*Bacillus subtilis* has established various survival strategies to adjust to changing conditions in its natural habitat, the soil. One possibility is the production of secondary metabolites, such as antimicrobial compounds, to suppress the growth of competitors, or siderophores that provide access to limiting iron concentrations in the environment. Pulcherriminic acid is a cyclodipeptide produced by the Gram-positive model organism *B. subtilis*, which leads to depletion of iron by forming unsoluble pulcherrimin, a red-brownish Fe(III)-pucherriminic acid complex. The pulcherriminic acid, encoded by the *yvmC-cypX* operon, chelates Fe3+, and has a high affinity to ferric iron, thereby resulting in a bacteriostatic effect on competitive microorganisms [1]. Since it also controls growth arrest of *B. subtilis* biofilms [2], the metabolite production must be tightly controlled during the multicellular differentiation processes. Here, we investigated the importance of the pigment in patterning, differentiation, and coloring of *B. subtilis* biofilms and analyzed the underlying regulation. Differential gene expression of the pulcherriminic acid locus was analyzed by luminescence reporter strains, consisting of P*yvmC-luxABCDE* fusions, and correlated with the abscence or presence of various competence-related genes. Both,

spatial and temporal gene expression patterns were examined by either performing plate reader assays with liquid cultures, or colony differentiation assays on solid MSgg media. Furthermore, we focused on the characterization of multicellular phenotypes as a function of different iron concentrations.
We observed a regulatory relationship between early competence genes (including *comA*) as well as the surfactin biosynthesis cluster and pulcheriminic acid production. Furthermore, significant differences in colony morphology and pulcherrimin release into the agar were observed. The study improves our understanding of the regulatory network in multicellular biofilms, and the role of iron availability in the differentiation process.

[1] Yuan S, Yong X, Zhao T, Li Y, Liu J. Research Progress of the Biosynthesis of Natural Bio-Antibacterial Agent Pulcherriminic Acid in Bacillus. Molecules. 2020 Nov 28;25(23):5611.
[2] Arnaouteli S, Matoz-Fernandez DA, Porter M, et al. Pulcherrimin formation controls growth arrest of the Bacillus subtilis biofilm. Proc Natl Acad Sci U S A. 2019;116(27):13553-13562.

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**STRGP022**
**Characterisation of LysR-type transcription regulator YdcI in *Escherichia coli***
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YdcI belongs to the family of LysR-type transcription regulators (LTTRs) which constitutes the largest family of transcription regulators in bacteria involved in a broad spectrum of biological processes. *Escherichia coli* K12 codes for 46 LTTRs out of which YdcI is one of the conserved LTTRs that has not yet been well characterized.

The main objective of the study is to gain insight about YdcI's regulatory and functional role in *E. coli*. In order to identify the target genes of YdcI, an RNA-seq analysis was performed using wild-type and *ΔydcI* strains. Potential target genes of YdcI were identified by a comparative analysis of the RNA-seq data with genomic ChIP-exo data[1] and with RNA-seq data of other LTTRs (LrhA, YafC and YhaJ). EMSA DNA-binding analysis and expression analysis were used to further validate the identified potential target genes. Our data confirm DNA-binding sites for YdcI upstream *of iraP* (phosphate-starvation stress response), *gltA* (citrate synthase), *mqo* (malate:quinone oxidoreductase), *yobA-yebZY* (copper-binding required for NADH dehydrogenase II), *yhdVWXY* (putative ABC transporter system) as primary targets. Further, expression analyses using *iraP* and *ydcI* promoter *lacZ* reporter fusions show that the *ydcI* promoter is induced by phosphate stress independently of the PhoBR two-component phosphate stress system. Our data further show that the phosphate stress induction of the *iraP* promoter is mediated by YdcI in addition to and independent of PhoBR, suggesting a role of YdcI in phosphate stress induction of *iraP*. Taken together, our data indicate that YdcI has a function in regulation of TCA cycle genes and the phosphate stress response and this will help to decipher YdcI's functional role.

[1] Gao et al. (2018) Nucl. Acids Res. *46*, 10682-10696.

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**STRGP023**
**Spatio-temporal regulation of type IV pilus assembly in *Synechocystis* sp. PCC 6803**
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Introduction**:** To exert directional responses towards environmental stimuli, unicellular prokaryotes need to establish cell polarity and dynamically localize proteins to different cell poles. One such behavior is cyanobacterial phototaxis in which polar activity of type IV pilus machinery leads to directional movement towards or away from a light source. In the model organism *Synechocystis* sp. PCC 6803 phototaxis is governed by multiple chemosensory systems and other signal transduction pathways that employ CheY and PatA-like response regulators. In particular, the PatA-like regulators have been shown to interact with the pilus extension ATPase PilB1 which is proposed to act as a central regulatory hub during phototactic decision making.

Objectives**:** We hypothesize that polar interactions of pilus motor components and response regulators mediate directional motility. To elucidate the signaling pathway, we will investigate the subcellular localization of PilB1 and the phototaxis response regulators and their interactions during directional movement in *Synechocystis*. Furthermore, we aim to identify the function of response regulator phosphorylation. Specifically, we will study the role of phosphorylation in the localization of these response regulators and their ability to modulate polar pilus activity. Lastly, we plan to identify novel interactors of the pilus machinery by employing an adenylate cyclase-based two-hybrid (BACTH) system for library screens.

Methods and Materials: Using confocal microscopy, we profile radial intensity of fluorescently-labeled response regulators and the motor ATPase PilB1 to analyze protein localization during phototaxis. Furthermore, we aim to establish a FLIM-FRET microscopy system to study protein-protein interactions in live *Synechocystis* cells subcellularly.

Results: *Synechocystis* strains expressing fluorescent protein-tagged PilB1 and response regulators were established. PilB1 and PixG localize polarly correlating with the movement direction. We have successfully established a genomic BACTH library identifying both known and novel interactors of PilB1. Preliminary results demonstrate that visualization of a PilB1-mTurquoise2 fusion protein by FLIM is possible and localized to the inner membrane.

Conclusion: Pilus proteins and response regulators show differential distributions correlating with movement direction during phototactic movement. Fluorescent fusion proteins in *Synechocystis* can be visualized using FLIM.

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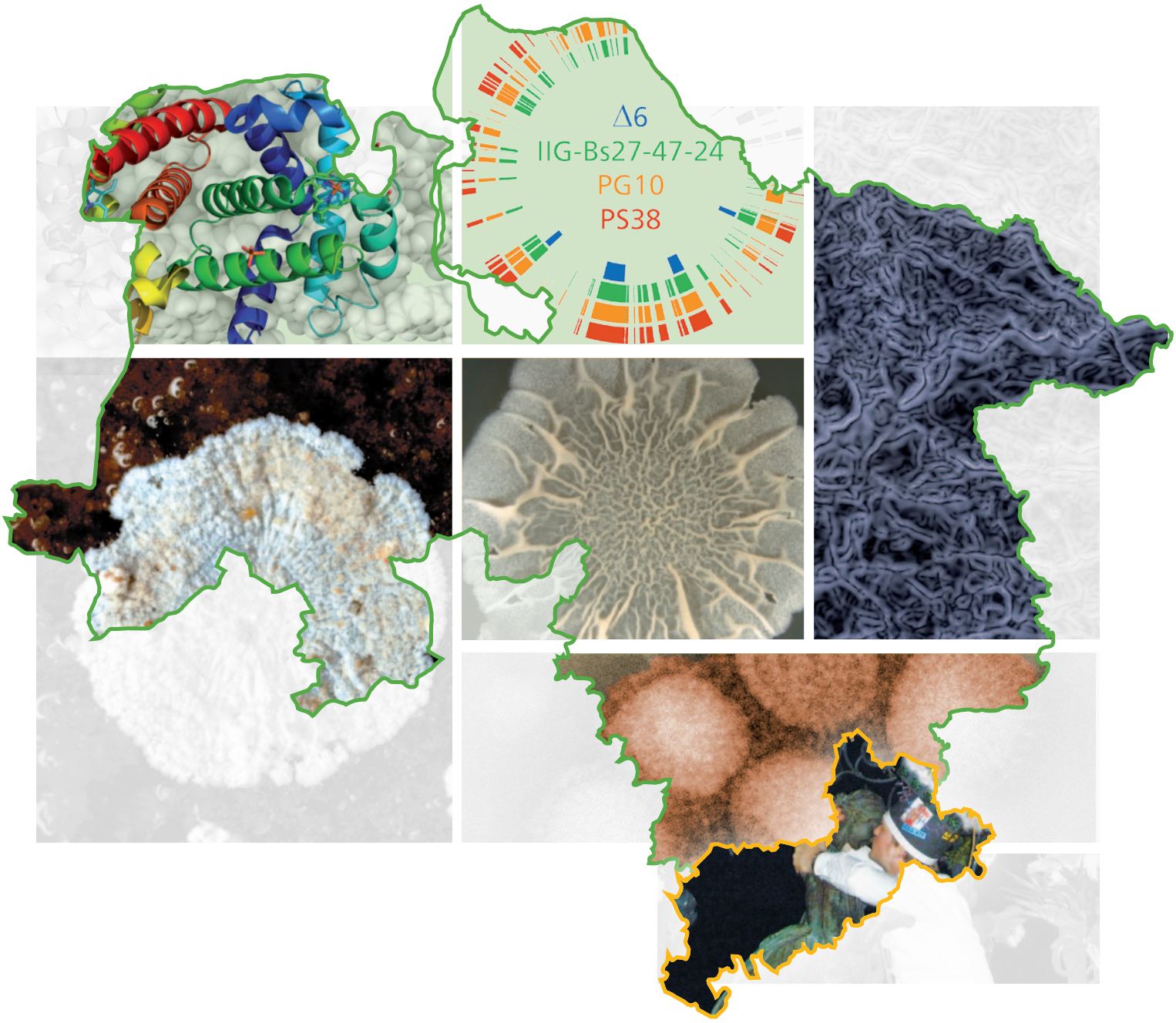


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