Abstract book

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[1] G-quadruplex DNA - hemin interactions induce Staphylococcal extracellular electron transfer

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Bacterial biofilms possess various biochemical structures such as polysaccharides, proteins, and nucleic acids which confer rigidity, elasticity and protection to the biofilm cells. Recent findings suggest that biofilms can possess canonical and non-canonical forms of nucleic acids structures such as G-quadruplex DNA in their extracellular matrix. Physiological roles of these G-quadruplexes in biofilms are still being explored. G-quadruplex DNA has been observed to interact with hemin forming complexes in Staphylococcal biofilms. Here, we investigated the possibilities for Staphylococci to interact with G-quadruplex DNA/hemin complex and engage in extracellular electron transfer (EET) due to electroactive properties of G-quadruplex/hemin complexes. Using Staphylococcus epidermidis models, voltammetric analyses showed that extracellular DNA and exopolysaccharides interacting with hemin were important for EET in S. epidermidis. We validated for the first time that G-quadruplex DNA and hemin interacting with cell surface exopolysaccharides mediate electric charge from the cells by the flow of electrons through the cell-bound G-quadruplex DNA/hemin complex. Microscopy images of biofilms formed under poised electrode potentials showed DNA-cell interactions which increased with time as more G-quadruplexes were incorporated into the biofilms. The G-quadruplex DNA on the cells led to slightly increased cellular tolerance of hemin, and G-quadruplex DNA/hemin complex on the cell surface degraded low doses of hydrogen peroxide under oxygen limited conditions, while using hydrogen peroxide as an external electron acceptor. These properties show that G-quadruplex/hemin complexes could be utilized by bacteria in gaining energy and surviving in low-oxygen stress possibly occurring in infections.

[2] Bringing the heat: Using isothermal microcalorimetry for rapid diagnostics and antimicrobial susceptibility testing

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Osteomyelitis and orthopedic implant infections are a significant healthcare burden, with severe complications for patients. Given the rising prevalence of diabetes and the aging population, a dramatic increase in bone infections is anticipated. Diagnostics, reliant on culture-based methods, are laborious, slow, and plagued by high false-negative rates. As a result, patients are often administered broad-spectrum antibiotics and risk a worsening of their condition if targeted antibiotics cannot be initiated. This study aims to refine diagnostics by leveraging the capabilities of isothermal microcalorimetry (IMC) to reduce time to detection (TTD) and the time to an antimicrobial susceptibility test (TTAST) result. IMC is highly sensitive and measures heat flow in real time at a scale of µW, providing hidden insights into the bacterial metabolism without the need for any labeling. Here we used clinical osteomyelitis isolates in a unique antibiotic panel designed for IMC to combine detection and antimicrobial susceptibility testing in one assay. In this study we found that IMC can successfully lower the TTD and detect all clinical isolates tested in less than 4 hours (median 1.87, range 1.01-3.31). Furthermore, susceptibility profiles for all clinical isolates were obtained within 2-22 hours (median 4.85, range 1.28-21.78). The susceptibility results are highly accurate when compared to the susceptibility results obtained by disk diffusion at the Department of Clinical Microbiology, Rigshospitalet, scoring 100% accuracy, sensitivity, specificity, positive predictive value, and negative predictive value. This method makes early targeted antibiotics a possibility in osteomyelitis treatment and can potentially improve patient outcomes, lower the duration of hospitalization, and the economic burden associated with these infections. IMC diagnostics paves the way for faster and personalized treatments, ultimately improving patient safety while advancing precision medicine.

[3] From single strain bioinoculants to consortia

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Microbes engage in vibrant and complex social interactions. The success and stability of bioinoculants is also shaped by interaction with other microbes. Transitioning from single-strain bioinoculants to multi-strain consortia can thus offer significant advantages. Typically, the design of a bioinoculant consortia, is either a top-down approach driven by -omics, or a bottom-up approach driven by synthetic biology, both of which pose challenges.

Here we present two novel methods for the design of bioinoculant consortia that are based on colocalization instead. We define consortia whose members synergistically reinforce each other, as 'collaboromes'. These collaboromes show synergy within the community and can stably colonize and function in the presence of a competitive microbiome.

Our innovations enabled the high-throughput isolation of collaboromes directly from environmental niches, using novel bead and bait approaches. More than 100 promising collaboromes were cultivated and characterized, leading to a catalogue of microbial strains and genes. Our lead collaboromes outperformed single strains on plant protection and growth promotion in greenhouse trials with potato and tomato.

In conclusion, harnessing social interactions within the plant microbiome for the design of bioinoculants holds the potential to revolutionize microbe-assisted crop production. Further, these methods offer opportunities for consortia design in diverse fields beyond agriculture.

[4] Back to Basics – The Fundamentals of the Black Soldier Fly (Hermetia illucens) Microbiome.

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Black Soldier Fly larvae (BSFL) are excellent at converting low-quality industrial waste products to high-value proteins such as aquaculture feed. Unlike other domesticated animals, their production requires minimal resources, rendering BSFL a sustainable and eco-friendly alternative to conventional agriculture. The BSFL farming industry is on the rise, and consequently, there is an increasing interest in optimizing larval production and bioconversion capacities. The BSFL gut microbiome plays a crucial role in digestion and bioconversion, yet the precise mechanisms governing the microbiome establishment remain unknown.

Therefore, this study aimed to elucidate how (via vertical, horizontal, or mixed transmission) and when (at which life stage) BSFL acquire their gut microbiome.

To address this, we designed a transmission study to map the gut microbiome throughout the entire life cycle. We collected samples from the guts of mated females, their eggs, and subsequent emerging larvae, prepupae, pupae, and fully matured females using 16S rRNA gene amplicon sequencing. Nine mated females were selected and transferred to individual sterile containers for egg-laying, with the eggs and neonates maintained on sterile containers to track the transmission route of potentially vertically transmitted symbionts. Meanwhile the emerging larval stages were reared on chicken feed substrate and was routinely sampled for gut and substrate material to monitor the microbiome transmission from substrate.

Preliminary results suggest minimal vertical transmission with subsequent selective acquisition of the BSFL microbiome from the environment, which would allow some degree of microbiome manipulation for optimized larval production and bioconversion.

[5] Streamlined cells display more efficient metabolic and biomanufacturing performance

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A series of Escherichia coli streamlined strains was developed by removing extracellular structures and expression of unessential metabolic enzymes. The streamlined strains exhibited improved metabolic performance, including lower overflow metabolism and higher growth rate, compared to their parental strain. The intracellular levels of ATP were monitored using a genetic sensor, showing the improved resources stewardship of the streamlined cells. The streamlined strains were tested as cell factories to produce plasmid DNA (pDNA) in batch cultures, exhibiting 23 % increase of the specific rate of pDNA production, compared to the parental strain. Recombinant protein expression was tested in the streamlined cells in microbioreactors in batch and fed-batch mode. In batch mode, recombinant protein yield from biomass was up to 70 % higher in the streamlined strains, compared to the parental strain. Furthermore, in fed-batch mode, the recombinant protein yield was 75 % greater in the streamlined cells than in the parental strain. Our results show the benefits of reducing cellular complexity on the biomanufacturing of pDNA and recombinant proteins in culture schemes typical of industrial settings.

[6] Structural insights into Biofilms and Biofilm Forming Functional Amyloids

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Aggregated proteins in the form of amyloid fibrils play a key role/function (functional amyloids) in maintaining the structural integrity of bacterial biofilms and when these proteins are mutated, the biofilms are disrupted and bacteria become accessible to, e.g., antibiotic treatments. Such functional amyloids strengthen biofilms and are a major threat to human health, since the (chronic) infections they cause are difficult to treat due to the biofilm structural integrity and insufficient penetration of drugs, thus promoting antibiotic resistance (antimicrobial resistance, AMR). Antimicrobial resistance (AMR) is an urgent global public health threat and declared as one of the top ten global health problems facing humanity, which results in million casualties per year and is estimated to cause more death than cancer by 2050. Moreover, 80% of chronic infections are related to bacterial biofilms.

Targeting biofilms and their amyloid components could be a novel approach to fight AMR. However, very little structural information exists about biofilms and their fibrillar components and how these components interact with other proteins/parts. Determining unknown structures and unraveling the structure–activity relationship of such fibrils will help to develop better therapies. Here I will show a new structure-based approach to fight AMR by targeting bacterial biofilms at their specific components. The atomic-resolution structural information will be determined by solid-state NMR and Cryo-EM, as well as by other structural and biophysical techniques.

[7] A molecular model for transcriptional regulation of a type II toxin-antitoxin system

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Type II toxin-antitoxin (TA) systems are important cellular regulators in bacteria, and fine-tuning of expression represents a key feature of TA homeostasis. Transcriptional repression of a type II TA system is often achieved by antitoxin binding to the TA promoter region.

We previously determined the structure of a type II TA complex, Xre-RES, from Pseudomonas putida, revealing an unusual 4:2 stoichiometry in which each of the RES toxins of a central dimer interacts with a dimer of Xre antitoxins. Interestingly, each Xre dimer shows structural similarity to the Cro repressor from the lambda phage, suggesting involvement in transcriptional autoregulation. But, how this unusual complex composition might work in transcriptional regulation of promoter activity is unknown.

Here we show that the native promoter of the xre-res operon contains four repeat segments, and that expression of xre-res represses transcription from the promoter in vivo. Supportive of this, in vitro studies show that the purified Xre-RES complex specifically binds an imperfect palindromic sequence within the promoter region. The imperfection of the palindrome appears optimal for binding, as the binding affinity for a perfect palindromic sequence is reduced. We determined the structure of the heterohexameric Xre-RES complex bound to the imperfect palindromic DNA element at 2.6 Å, revealing that major deformation of the DNA takes place during binding. This suggests that the complex recognizes the imperfect palindrome via indirect readout. Lastly, we show that isolated Xre antitoxin is a monomer in vitro and only weakly represses the promoter in vivo. Moreover, we identified a non-binding tetrameric 2:2 complex in vitro.

Together, our data support a model for transcriptional autoregulation in which the Xre antitoxin of the Xre-RES complex exist in an equilibrium between a non-binding 2:2 and a DNA-binding 4:2 form. In this way, transcription is regulated without the risk of releasing the RES toxin.

[8] Identification of genes involved in aggregate associated antimicrobial tolerance of Pseudomonas aeruginosa using Tn-Seq guided discovery

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It is estimated that about 60-70% of hospital acquired infec0ons are caused by biofilms. Since current treatment regimens in many cases fail to cure biofilm-based infections, it is critical to identify the mechanisms which underlie biofilm-based antimicrobial tolerance. In biofilms, the bacteria grow in densely packed aggregates concealed in a self-produced matrix of exopolymers and can tolerate high doses of antibiotics. We use a super-saturated P. aeruginosa PA14 transposon (Tn) mutant library consisting of more than 800.000 unique mutants, to identify mutants with increased susceptibility towards a panel of antibiotics. The transposon mutants are grown in agar plates under conditions where each mutant forms an agar-embedded aggregate. Antimicrobial tolerance increases with aggregate age but even relatively young aggregates display tolerance up to 10-fold higher than their planktonic counterparts. Furthermore, the level of c-di-GMP – as measured through a c-di-GMP-monitor - is significantly higher in the aggregates compared to planktonic growth, confirming that the aggregates represent a biofilm-like growth physiology. For a single experiment, we use large agar plates that combined harbor over 150.000 aggregates. The aggregates are treated with antibiotics, washed, and then disintegrated. The identity and relative number of the mutants present in the pool of antibiotic-treated versus the non-treated bacteria are then determined by Tn-Seq analysis. Under the right selection conditions, Tn-mutants with higher susceptibility will be selectively depleted from the antibiotic-treated pool. Subsequently, all identified genes are ordered according to their potential importance for aggregate-associated antibiotic tolerance thus serving as the starting point for exploring the underlying mechanisms.

[9] Seasonal dynamics and metagenomics of ammonia-oxidizing archaea in a seasonal anoxic and sulfidic basin in Mariager Fjord (Denmark)

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Different ammonia-oxidizing archaea have recently been shown to produce oxygen, when exposed to oxygen depletion. A potential explanation for the frequent occurrence of ammonia-oxidizing archaea in oxygen-depleted environments would be, that they can perform this proposed pathway of oxygen production, NO-dismutation.

We studied the ecology of ammonia-oxidizing archaea in the stratified water column of Mariager Fjord, a sulfidic basin in Denmark over a two-year period. Their abundance varied greatly between seasons. Surprisingly, at time points with the highest abundance of ammonia-oxidizing archaea, amoA gene copy numbers peaked within and in the sulfidic depths adjacent to the chemocline. 16S rRNA gene amplicon sequencing showed that one sequence variant affiliated with Nitrosopumilus dominated the microbial community (up to 12%) in and below the chemocline, together with sulfide and sulfur-oxidizing bacteria. Metagenomic analysis showed that a Nitrosopumilus population could be the main nitrite consumer in anoxic depths during some summer months, therefore, potentially being capable of performing NO-dismutation. Phylogenomic and pangenomic analysis showed that the Nitrosopumilus population is closely related to Candidatus Nitrosopumilus limneticus. Anoxic incubations using copper click chemistry with the nucleoside analog 5-ethynyl uridine showed Nitrosopumilus could be active under anoxia.

All in all, we show that ammonia-oxidizing archaea are not limited to the chemocline of anoxic basins but can remain abundant and active at low sulfide concentrations.

[10] Redefining paradigms in the archaeal virus-host arms race

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Understanding the pan-immune landscape of archaea and the mechanisms by which viruses evade host defenses is key to understanding cellular immunity evolution. Earlier studies on archaeal immunity focused on few systems, namely restriction-modification, CRISPR-Cas, argonautes and toxin-antitoxin modules. Recently studies, however, expanded the prokaryotic immune landscape, revealing its diversity and complexity. Despite this, the distribution of these defense systems in Archaea remains underexplored.

In this study, we analyze 7750 archaeal genomes to comprehensively assess the abundance, distribution and evolution of known defense systems. We also evaluate the effectiveness of current prediction tools. Our results show a skewed distribution of defense systems in Archaea and imply the existence of yet-to-be-discovered defense systems in this domain. However, restriction-modification, CRISPR-Cas, SoFic, AbiE and DNA phosphorothioation systems are common across both domains, implying their universality.

Our data challenge the paradigm that CRISPR-Cas is highly abundant in archaea, showing a decrease in prevalence from 80% to approximately 30%, aligning with bacterial prevalence. Hyperthermophiles and Altiarchaea show higher CRISPR-Cas prevalence, while DPANN archaea exhibit lower levels.

We also developed a bioinformatic pipeline to predict viral inhibitors of archaeal defenses through the analysis of their regulatory sequences, identifying 354 putative anti-defense genes in crenarchaeal viruses. Additionally, we provide experimental validation of Aca8 as an anti-crispr associated (aca) regulator of anti-defense genes, and describe potential new regulators Aca14 and Aca15, which employ novel DNA-binding domains.

Our work emphasizes the importance of considering archaeal defense systems in microbial immunity and lays the groundwork for future research into archaeal virus-host interactions.

[11] Soil microbiome stability to agricultural practices and nitrification inhibitors

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Due to common agricultural practices, microorganisms in Danish agricultural soil experience massive changes in biogeochemistry during an annual crop cycle. At the same time, soil health and crop production depend on microbiome functions such as recycling organic matter, nutrient availability, and soil structure. The pressures on the soil microbiome are increasing with the intensifying climate change causing rising temperatures, drought, and waterlogging. At the same time, climate mitigation tools often involve soil microorganisms. Before the widespread implementation of these tools, any environmental side effects must be determined.

We have investigated the stability of soil microbiomes during four years of alternating spring barley and winter wheat with different fertilization and tillage regimes at two locations with differing soil texture and climate. Furthermore, the side effects of three nitrification inhibitors, reducing the ammonium oxidation and hence emissions of the greenhouse gas nitrous oxide, were monitored. The microbiomes (prokaryotes, fungi, and protists) were studied by amplicon sequencing of DNA and extracellular enzyme activities using MUF substrates.

The combination of location, soil texture, precipitation, and crops were the main drivers of microbiome structure and function, but the tillage and fertilization regimes also significantly affected the microbiomes. The effects of nitrification inhibitors were detectable and slightly increased during the four years. Studies on active microbiomes by amplicon sequencing of 16S rRNA and nitrification gene expressions are ongoing.

Due to the dramatic effects of agricultural practices, soil microbiomes show high annual fluctuations. The environmental effects of nitrification inhibitors were minor compared to agricultural practices; however, accumulating effects seem to occur.

[12] Cross-continental soil prokaryotic phenotypic traits driven by precipitation regime and land cover

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Trade-offs among microbial traits determine community dynamics and affect carbon-cycling feedback to climate change. Using cross-continental temperature and aridity gradients, we determined soil prokaryotic phenotypic traits based on a novel marker gene-based workflow. Genome streamlining and high 16S rRNA gene copy numbers per genome (RRN) conferred high potential maximum growth rates, possibly by allowing for smaller cells with higher surface to volume ratio. Small genomes and high potential growth rates were found under high precipitation seasonality and in barren soils. Large genomes and slow growth rates were found in forests, characterized by high water availability and by abundant and complex organic resources. Our findings suggest that large genomes confer versatility to cope with resource fluctuations and moderate climatic fluctuations while extreme climatic fluctuations and scarcity of resources promote genome streamlining. Seasonal fluctuations in water availability were associated with the ability to form spores and with rapid resuscitation, promoted by high RRN. Moreover, Prokaryotes were less dispersal limited compared to Fungi, presumably due to their smaller size, but within Prokaryotes, smaller taxa were not more ubiquitous. Our trait-based framework highlights that particularly changes in precipitation patterns and vegetation type will cause changes in microbial processes under future climate.

[P1] Exposing Archaeal-Bacterial syntrophic mechanisms through microencapsulation and droplet cultivation

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The discovery and independent evolutionary status of Archaea have sparked interest in their diversity, and ecological and evolutionary importance. High-throughput sequencing has revealed Archaea in a variety of environments, from extreme niches such as hydrothermal vents to more broadly accommodating surroundings like soil and marine sediments. Despite their wide distribution, only a few taxa are cultivable through conventional approaches and thus, to fully understand the functional roles of Archaea in ecosystems and their interactions with other microorganisms, we need to establish alternative cultivation approaches.

In this study, we aim to utilize a droplet-based microfluidic platform to improve the cultivability of Archaea and gain insights into the preference and level of promiscuity of Archaea in relation to their bacterial partners. Specifically, we aim to elucidate cross-domain interactions in hydrogen-mediated syntrophic relationships, such as those giving rise to the last eukaryotic common ancestor.

By collecting sediment along the coast of Zealand, we map the spatial distribution of Archaea along the salinity gradient between Kattegat and the Baltic Sea. This may enable identification of environmental drivers of the community composition and co-occurrence patterns with potential bacterial partners. Employing parallelized droplet co-cultivation of cell assemblages extracted from sediments rich in Archaeal taxa, will allow us to generate millions of individual consortia containing Archaea and a wide selection of possible partners. By manipulating culture conditions to select for hydrogen-mediated syntrophic relationships, we aim to stimulate such interactions and subsequently determine the composition and metabolic profile of successful consortia. Ultimately, this will allow us to tap into the underexplored metabolism of Archaea and unravel the cross-domain interaction mechanisms involved in these syntrophic relationships and in eukaryogenesis.

[P2] The interconnection between plant food bacteria and horizontal gene transfer

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Horizontal gene transfer (HGT) – a mechanism by which bacteria can acquire exogenous plasmids from the environment – plays a crucial role in the evolution of microbial communities and the dissemination of antibiotic resistance genes. Moreover, interspecies interactions could potentially increase the spread of detrimental genetically based traits like resistance genes, further contributing to the antibiotic resistance crisis. Therefore, it is vital to investigate the ability of vegetable-associated microbial communities to receive and transfer a broad range of resistance-carrying plasmids as fresh produce comes into direct contact with humans and animals.

To examine the native vegetable-associated microbiome, we conducted a metagenomic analysis of these communities using Oxford Nanopore Technologies (Rapid Sequencing Kit) to obtain long-reads DNA sequences and enhance ubiquitous plasmid detection. Next, we investigated the transfer of the broad host IncP1 plasmid pB10 to native microbial communities present in several fresh vegetables. The presence of transconjugants was confirmed by flow cytometry using a dual fluorescence gene reporter system in all the tested vegetable microbial communities.

This study provides a foundation for exploring the dynamics of HGT in vegetable-associated microbiomes. The versatility and potential for horizontal gene transfer observed in plasmids can influence the spread of antibiotic resistance and other virulence factors in food products having direct contact with humans, underscoring the importance of monitoring and effective prevention strategies in this area. Our metagenome analysis reveals the diversity found within vegetables and several of these species were found to have the capacity to take up pB10 plasmids. The plasmidome analysis of these bacterial communities could support the evaluation of plasmids with potentially detrimental effects on public health and food safety.

[P3] Adaptation of a methanogen to Fe0 corrosion via direct contact

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Iron (Fe0) corrosion in anoxic environments can be caused by methanogenic archaea, with certain Methanococcus maripaludis strains known for their role in global infrastructure corrosion. Previous studies hinted that free extracellular hydrogenases, encoded on a MIC-island (Microbially Influenced Corrosion-Island), are key to this process. However, free enzymes can be exploited by 'cheater' populations, making them too costly for the producers. We hypothesized that highly corrosive M. maripaludis anchor these key enzymes to their surface, requiring direct contact with Fe0 for effective corrosion.

Our findings challenge the traditional view of free enzymes driving severe corrosion. We show that M. maripaludis strain Mic1c10 only shows strong corrosive activity when in direct contact with Fe0. We obtained the complete genome of this highly corrosive strain and compared it to three other corrosive and three non-corrosive strains. By comparative genomics, we identified five syntenic gene clusters (islands) specific to corrosive strains including [NiFe]-hydrogenases, cohesins, transporters, and toxin-antitoxin systems crucial for inter-strain competition. On the other hand, Mic1c10 has unique glycosyl transferases and highly glycosylated [NiFe]-hydrogenases, which could explain how hydrogenases remain anchored to the cell surface and do not act as free enzymes in the cell filtrate. The acquisition of corrosive traits via horizontal gene transfer (the MIC island), coupled with the retention of ancestral genes in all known M. maripaludis corrosive strains, highlights their adaptability to the constructed environment.

[P4] Interaction of Aspergillus oryzae and Bacillus subtilis var. natto: Effects on Flavor Profile and Consumer Preferences in Koji Fermentation

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Bacterial-fungal interactions are common in fermented foods, where they contribute to the complexity and richness of flavors. This study investigates the interaction between the ancient koji mold Aspergillus oryzae and Bacillus subtilis var. natto to understand their combined influence on flavor development and consumer perception. Through a series of experiments where we characterized the nature of interaction between these two organsims, we identified an ammensalic relationship in which Aspergillus oryzae consistently inhibited the growth of Bacillus subtilis var. natto, driven primarily by nutrient competition rather than volatile compounds or secondary metabolites. Notably, co-culturing these microorganisms produced a koji with a unique flavor profile that was distinct from their respective monocultures. This co-cultured product closely resembled the Aspergillus oryzae monoculture in flavor but exhibited a slight enhancement in overall preference by the taste panel. Additionally, it scored lowest in “strange aromas and flavors,” suggesting that the interaction between Aspergillus oryzae and Bacillus subtilis var. natto may enhance the sensory appeal of fermented foods.

[P5] The chemical language of Archaea and Bacteria

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Archaea and bacteria interact with each other in complex microbiomes. However, the mechanisms of cross-domain communication have not been elucidated. There is evidence of mutualistic archaea and bacteria interactions, from tight symbioses to more loose relationships of sharing metabolites. Interference competition among prokaryotic domains could also occur, affecting microbial community composition and function, yet evidence of such interactions remains uncovered. We investigate two possible ways of archaea bacteria interactions involving (i) acyl-homoserine lactone (AHL) lactonases, and (ii) ribosomally synthesized and post-translationally modified peptides (RiPPs) encoded in archaeal genomes. The occurrence of AHL lactonases across the archaeal domain is unknown, but AHL lactonases have previously been described for four (hyper-)thermophilic archaea and could potentially interfere with bacterial AHL quorum sensing signals. Whereas RiPPs could function as signaling molecules or antimicrobials, which is supported by anti-archaeal activities detected for RiPPs from halophilic archaea. To address the role these compounds play within a microbial community, we screened archaeal reference genomes for a custom AHL lactonase hmm model (hmmer) and for biosynthetic gene clusters for RiPPs (antiSMASH and GECCO). Preliminary data suggest the occurrence of AHL lactonases in novel taxa of the Thermoproteota phylum, with potential differences in substrate specificity. The genome screening indicates the presence of putative RiPPs and RiPP recognition elements across archaeal genomes from multiple phyla including Nanoarchaeota, Thermoplasmatota and Thermoproteota. Overall, our analysis provides a first glimpse into the chemical language of archaea and their potential antagonistic interactions with bacteria or other archaea. This will guide future in-depth investigations on the role of AHL lactonases and RiPPs within co-cultures and microbial mock communities.

[P6] Screening, selection, and characterization of autochthonous potential probiotic bacteria against the rainbow trout pathogen Flavobacterium psychrophilum

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Flavobacterium psychrophilum, causing Bacterial Cold Water Disease and Rainbow Trout Fry Syndrome, threatens salmonid aquaculture globally. With rising antimicrobial resistance and limited vaccine efficacy, sustainable biocontrol measures are needed. This study aims to identify and characterize bacteria with probiotic potential against F. psychrophilum from rainbow trout aquaculture environments, focusing on recirculating systems. A screening assay using F. psychrophilum 950106-1/1 embedded in TYES agar was developed. Bacterial colonies from aquaculture biofilter units were screened for inhibitory activity. Candidate strains showing clear inhibition zones were identified using MALDI-ToF MS and analyzed by Whole Genome Sequencing. AntiSMASH analysis identified biosynthetic gene clusters (BGCs) potentially responsible for antimicrobial activity. Screening of approximately 600 colonies via replica-plating yielded three probiotic candidates with consistent inhibitory activity. WGS analysis revealed an unknown Pseudomonas strain, a Pseudomonas yamanorum strain, and a Janthinobacterium tructae strain. AntiSMASH analysis showed multiple BGCs in all strains. In the Pseudomonas strains, high-similarity clusters (>40%) included NRPS clusters similar to pyoverdine SMX-1 (80%) and viscosinamide A/pseudodesmin A (62%). The J. tructae strain showed RiPP-like, terpene, and thioamide-NRP clusters. These BGCs suggest potential production of various antimicrobial compounds that may contribute to the observed inhibitory effects against F. psychrophilum. Ongoing metabolomic studies aim to elucidate the specific compounds responsible for the antimicrobial activity. This study combines traditional microbiological screening with genomic approaches to identify and characterize autochthonous probiotic candidates. Future work will expand the microbial sample pool to include more aquaculture units and healthy rainbow trout individuals. The elucidation of potential mechanisms of action could lead to the development of sustainable biocontrol strategies for rainbow trout aquaculture.

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[P7] Morphological diversity of Lokiarchaeia from Aarhus Bay sediments

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Asgard archaea are the closest extant prokaryotic relatives of eukaryotes and encode proteins that are homologous to several eukaryotic signature proteins, which indicates a genetic potential for subcellular complexity. So far cultivation efforts yielded only the isolation of two closely related strains from the Asgard archaeal class Lokiarchaeia. Microscopic examinations of these cells revealed complex surface structures but no eukaryote-like subcellular compartments. On the other hand, we previously demonstrated spatial separation of DNA and ribosomes in distantly related yet-uncultivated Lokiarchaeal cells, suggesting a potential for subcellular compartmentalization. Therefore, Lokiarcheal cell structure appears to be diverse but poorly characterized. Here, we visualized diverse Lokiarchaeia inhabiting marine sediments (Aarhus Bay, Denmark). We detected three distinct Lokiarchaeia clades using phylogenetic analysis and designed oligonucleotide probes specifically targeting their ribosomal RNA. We then performed catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) and visualized the labeled cells by epifluorescence microscopy. Our results indicate filamentous and coccoid cells with varying DNA localization, suggesting diverse morphotypes and cell structures in yet-uncultivated Lokiarchaeia. Future studies should address enriching Asgard archaea from marine sediments and imaging their ultrastructure. This would shed light on the diverse cellular architecture in the prokaryote-eukaryote interface and contribute to our understanding of the evolution of the eukaryotic subcellular complexity.

[P8] Dietary glycerol impacts the potential of intestinal microbiota to contribute to endogenous acrolein formation

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Acrolein is a highly reactive toxicant for eukaryotic and prokaryotic cells and has been associated with diabetes, cardiovascular, respiratory, and neurodegenerative diseases. Fecal metagenome data and in vitro studies suggest that gut microbiota possesses the potential for acrolein production, but due to exogenous (environmental, diet) and non-microbial endogenous acrolein background it has been difficult to estimate the contribution of gut microbiota to acrolein production.

We investigated the acrolein producing potential of gut microbiota in 19 volunteers under defined housing and diet conditions to minimise confounding acrolein sources. Glycerol intake was estimated (dietary fat composition) to be on average 6.5 g/day with higher levels at days 4 and 8 (12 g/day). Fecal and urine samples were collected and a combination of analytical and molecular techniques were used to characterize the fecal microbial community and glycerol transformation activity.

Quantitative microbiota profiling indicated an overall decrease of bacterial cell counts during the 11 day study, and a shift in microbial community beta-diversity. Fermentation profiles changed with a trend for lower acetate and higher propionate production. Based on the presence of the pduC gene all microbiota showed the potential for glycerol transformation; Anaerobutyricum hallii was the major contributor across donors and time, with stable abundance. 1,3-propanediol, which is derived from glycerol metabolism, was detected in 62% of fecal samples, while the urinal biomarkers of acrolein HPMA and CEMA were detected in all donors at all time points. Levels of fecal and urinal glycerol/acrolein biomarkers were highest at days 4/5 and 8-11.

Taken together, our data shows that high abundance of glycerol transforming taxa allows for a timely response to dietary glycerol highlighting that the intake of certain fats might temporarily increase the contribution of intestinal microbiota to endogenous acrolein formation.

[P9] Decoding Evolution: The Distinctive Disaggregatase enzyme

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The morphology of Methanosarcina mazei undergoes a significant transition from multicellular aggregates to single cells due to the activity of a unique hydrolyzing enzyme, disaggregatase (DAG). Disaggregatase specifically targets and cleaves a distinctive bond within methanochondroitin, an exopolysaccharide that plays a key role in clustering Methanosarcina cells into multicellular aggregates. This exopolysaccharide is integral to the formation of the extracellular matrix and facilitates cell-cell interactions that promote aggregation. DAG contains a conserved DNRLRE domain and has been characterized as a soluble protein in Methanosarcina mazei. Despite its observed role in disaggregation, the complete structure and precise function of DAG, particularly across different species of Methanosarcina remain poorly understood. These morphological changes, driven by DAG-mediated disaggregation, allow Methanosarcina to adapt to a wide range of environmental conditions. The ability to alternate between aggregated and single-cell forms provides these microorganisms with flexibility in responding to external stresses, such as nutrient availability or changes in ionic concentration.

[P10] Acetic acid bacteria: an evolutionary story of the quest for sugar

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Acetic acid bacteria (AAB) are generally considered beneficial microbes for humans and used to produce several fermented food and beverages, like vinegar, kombucha or kefir. Humans have historically domesticated these bacteria to obtain some of the ferments that we consume, but the question remains on how these microbes got there in the first place. Our work shows that some of these bacteria are found in social insect guts and environment, in the food storages of their nests, and in the flowers and fruits social insects visit. They can also be isolated from other plant parts. These bacteria are adapted to somewhat harsh environments (low pH, high osmolarity) where simple sugars are readily available, and so, colonized by many different types of microbes. Has life within insect microbiomes made these bacteria ideal to colonize host-controlled environments, like our nutritious ferments? This project focuses the adaptions of acetic acid bacteria to these dynamic communities, using their presence in fermented food, plants and insect microbiomes to follow their evolution within microbial communities. Genomic adaptions have been observed in acetic acid bacteria domesticated by insects, namely, genome reduction and base compositional bias towards an AT-rich genome. Both social insect associated bacteria and ferment associated acetic acid bacteria are transmitted vertically and undergo bottlenecks with each new host generation. Could similar patterns be observed in acetic acid bacteria in our ferments? We find that ferment associated AAB only arise from clades associated with plants, and this transmission seems to be mediated by fruit flies. Specifically, it seems that by producing ferments, humans have created an environment that recreates the sugary bits of plants, and makes AAB present in those already prepare to invade these new environments. Social insect associated AAB reduce their genome size to an extent they cannot invade any other environment.

[P11] Diversity and structural of archaea communities in wheat rhizocompartments under effect of genotype and development stages

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Archaea have been detected in various habitats with development of sequencing technologies. Recent studies suggest that archaea play important roles in maintaining plant health. However, there is little information regarding the archaea communities around wheat roots. In this study, we conducted a greenhouse experiment and used 16S rRNA amplicon sequencing to study how the archaea community respond to niche, genotype and development stages of the wheat plant. Our results showed that niche was the most significant factor driving the archaea community, compared to development stages and genotype. The α-diversity of archaea in the rhizoplane was either higher or similar to that of the rhizosphere, depending on the genotype. Additionally, the α-diversity of archaea in the rhizosphere remained relatively stable throughout plant development, whereas fluctuations in α-diversity was observed for bulk soil and the rhizoplane. The ammonia oxidizing Class Nitrososphaeria was the dominant Archaeal Class accounting for almost 98% in all samples, with NS-Delta and NS-Zeta being the two dominant ammonia oxidizing archaea clades. Across eight varieties sampled at the flag leaf stage, NS-Delta was enriched in the rhizosphere while NS-Alpha was enriched in the rhizoplane of specific varieties. Analyzing on a temporal scale, NS-Alpha was enriched in the rhizoplane and increased with developmental stage in the Heerup variety. In contrast, the relative abundance of NS-Delta and NS-Gamma increased in the rhizoplane of the Hilliard variety during developmental until flowering. We also found that the ratio of ammonia-oxidizing bacteria (AOB) to ammonia-oxidizing archaea (AOA) increased in the rhizoplane compared to the bulk soil and rhizosphere, with an increase observed as the plants developed. This study expands our understanding of archaeal communities in the wheat rhizocompartments and underscores the importance of niche-driven recruitment of archaea, particularly in the rhizoplane.

[P12] Enzymatic exposure impacts microbial diversity and reduces biovolume of reverse osmosis membrane-associated biofilms

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Background: Reverse osmosis (RO) filtration is a separation technology employed by industrial plants for water recovery to reduce effluents and water consumption. However, RO membranes face challenges from biofouling caused by microbial biofilm formation, which reduces efficiency and increases energy consumption. A promising, more sustainable alternative to physical and chemical cleaning is an enzyme-based treatment for biofouling removal. This study evaluated the effects of an enzyme mixture (α-amylase, alginate lyase, β-mannosidase, proteinase K, and DNase) on the structure, diversity, and composition of biofilm communities on RO membranes.

Methods: Biofilms were formed by circulating dairy industry wastewater through a lab-scale RO membrane fouling monitor to simulate RO plant dynamics. Microbial abundance and diversity of membranes with and without enzymatic treatment were assessed using confocal laser scanning microscopy, amplicon sequencing, and qPCR to normalize gene copy numbers.

Results: Analyses showed a reduction of microbial biovolume after 4 and 24 h of enzyme treatment, whereas significant decreases of the bacterial (62.39%) and fungal (79.73%) copy numbers were only observed after 24 h. Contrary to the uniform fungal communities, bacterial communities exhibited high diversity and minor shifts in the relative abundance of specific genera. Despite these variations, the treatment generally reduced the gene copy numbers of bacterial genera and reduced bacterial alpha diversity, primarily attributed to decreased richness. Nonetheless, Raoultella and Lactococcus showed greater resistance to the treatment. For fungal diversity, no significant changes were observed.

Conclusions: The enzyme mixture with different targets has the potential to reduce multispecies biofilm formation on RO membranes significantly, with longer treatment durations proving more effective.

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[P13] Impact of Fucosidase Dietary Supplementation on Pig Gut Microbiota and Enteric Methane Emissions

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Enteric methane production is a challenge for livestock (ruminants and monogastrics) production. We hypothesized that dietary supplementation of fucosidase releases fucose from gut mucins or feed ingredients, affecting gut microbiota by favoring propionate-producing, hydrogen-consuming bacteria (e.g., Prevotella spp.), thus outcompeting hydrogen-dependent enteric methanogens. We studied a Bifidobacterium bifidum fucosidase, coated to withstand stomach passage (low pH, protease activity). Weaned piglets (n=240) were divided into four groups: a control and three fucosidase-treatments (Fuc2, Fuc7, Fuc16), receiving fucosidase (100 mg/kg feed) for 2, 7, and 16 weeks, respectively. Gut digesta was collected (day 10, 43 and 114 post-weaning) for in vitro gas production and microbiota analysis (qPCR, 16S rRNA amplicon sequencing), and selected pigs (n=80) were placed in respiration chambers to measure in vivo methane emission (day 43, 78 and 106 post-weaning). A reduction in in vivo methane emission on day 106 in all fucosidase-fed groups compared to the control ($p<0.05$), and a numerical reduction in in vitro methane production in the Fuc16 group on day 114 ($p=0.13$) was observed. Methanogen counts were not different between groups ($p>0.05$), but a decrease in Prevotella and total bacteria was observed in the Fuc2 group on day 43. Overall, the microbial composition did not differ significantly between treatment groups; microbiota richness in proximal colon was higher in the Fuc16 group on day 114 than in the Control group. Individual taxa showed significant abundance differences. Our findings show that fucosidase supplementation may mitigate enteric methane emissions. However, the microbial mechanisms involved need further elucidation.

[P14] Structural properties of short-chain carboxylic acids and alcohols impact the antimicrobial mechanisms against Salmonella in an acidic environment

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Short-chain carboxylic acids (SCCA) and alcohols (SCALC) that are naturally produced by microorganisms are antimicrobial compounds to reduce food waste by preventing microbial spoilage. This study aimed to investigate the structure-function relationships of SCCA/SCALC with 3-carbon chain (propionic, PA; 3-phenylpropionic, 3PP; 3-phenylpropanol, 3PPol), and 3-carbon chain with an additional hydroxyl group (lactic, LA; 3-phenyllactic, 3PL; 1-phenylpropanol, 1PPol) on the fitness, metabolic activity and gene expression of S. enterica.

SCCA showed better inhibition than SCALC towards Salmonella by reducing final density to 50% (MIC50) at 1.5–4.6 mM and 8.2–11.5 mM respectively. The only exception was LA (MIC50 = 28.8 mM). SCCA increased the lag phase of S. enterica up to 6 h. Acetic acid formation was detected in SCCA/SCALC treatments when the relative growth rate was < 80%. Salmonella consumed 67% of 20 mM LA, and produced PA (43–140%) in the presence of PA (1.25–2.5 mM).

Principal component analysis and hierarchical clustering of gene transcripts indicated distinct clusters between SCCA and SCALC. For SCCA, LA and 3PL with an additional hydroxyl group clustered separately from the PA, 3PP and the control. PA and 3PP had higher expression of eut and pdu genes that relate to microcompartments formation during propionic and acetic acid production. LA induced the expression of lldPRD operon encoding proteins relevant to lactate uptake. Both LA and 3PL were linked to induction of cys genes for sulfite metabolism, which is a source of energy for Salmonella, and downregulation of fli genes for flagellar assembly. fep genes that contribute to enterobactin biosynthesis were expressed in S. enterica treated with the SCALC 1PPol and 3PPol.

In conclusion, gene expression analysis and metabolic activity of Salmonella revealed that the antimicrobial mechanism of SCCA/SCALC was affected by compound structure particularly with major impact of the carboxyl group and the presence of a hydroxyl side group.

[P15] Extracellular Nucleic Acids Drive Extracellular Electron Transfer in Methanosarcina barkeri

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Traditionally, methanogenic Archaea have been thought to convert various small molecules (e.g., H2, formate, methylated compounds) to methane, but in recent studies some species of methanogens belonging to Methanosarcina were shown to reduce CO2 to methane on the account of electrons from solid electron donors. Of the Methanosarcina species only a few poses multiheme c-type cytochromes (MHCs) a characteristic conduit for extracellular electron transfer (EET), others lacking MHCs also exhibit EET capabilities by a completely unknown mechanism.

In this study, we investigate the role of extracellular nucleic acids (eNA) in EET by Methanosarcina barkeri. We observed that M. barkeri 800 releases high concentrations of eNA during early growth stages, with levels decreasing in the later growth stages. This pattern contrasts with bacterial biofilms, where eNA typically accumulates during stationary phase due to cell lysis. In M. barkeri, we observed that eNAs, including both extracellular DNA (eDNA) and RNA (eRNA), surround the cell surface and form thread- and nanowire-like structures, studded by quadruplexes which link cellular aggregates. DNA quadruplexes have been implicated in the past in metal coordination, potentially facilitating electron tunnelling.
To confirm that released extracellular nucleic acids have a role in EET we disrupted the eNA network with nucleases. We observed that M. barkeri with a disrupted eNA network exhibits ineffective electron uptake from an electrode although it can still grow effectively on soluble substrates. These findings provide new insights into the mechanism of EET in methanogenic archaea without MHCs, revealing an unprecedented role of extracellular nucleic acids in facilitating electron uptake.

[P16] Harnessing the Power of Legumes: Plant-Based Antimicrobial Compounds for a Sustainable Future

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The shelf life of food is closely linked to the composition of microorganisms. Various preservation methods have been developed to ensure microbial safety, as well as to maintain the nutritional value and sensory qualities of food. However, due to growing consumer concerns about health and higher demands on quality, the demand for natural antimicrobial agents is increasing. Plant-based antimicrobial substances offer promising alternatives to conventional antibiotics and preservatives. A deeper understanding of the interactions between microbiota and food products can therefore significantly aid food producers in product development and quality assurance.

As the global population continues to rise, the food industry faces the challenge of increasing production and distribution speeds. Currently, food production accounts for 25-30% of global greenhouse gas emissions, leading to heightened scrutiny of our dietary choices. The shift towards plant-based and plant-rich diets, seen as an alternative to traditional diets heavy in animal-based foods, is gaining momentum within the food sector. Several legumes, rich in protein, are particularly well-suited to replace a portion of the meat in our diets.

The “Grønholdbar” project, funded by GUDP, aims to further investigate an antimicrobial effect observed in legume ingredients. We will explore these effects across various bacterial species and different legume types. We seek to develop industrially relevant processes for producing both an ingredient with antimicrobial activity and a natural extract of antimicrobial compounds, applicable in various food products. This will help improving the quality and shelf life of many food products in a natural and sustainable manner.

[P17] Microflora Danica: the atlas of Danish environmental microbiomes

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Over the past two decades, remarkable progress has been made towards uncovering the microbiomes integral to vital functions in both natural and human associated environments. Contemporary and extensive metagenome studies have catalogued the diversity of microbial existence in various habitats, including oceans, wastewater, the human digestive system, and earth.

Extensive environmental research has involved the aggregation of thousands of public datasets. However, a smaller number of studies have focused on systematic and uniform sampling and analysis, like the Earth Microbiome Project. While large-scale microbiome surveys or compilations have been extensive, they often lack either functional details (as seen with amplicon sequencing) or uniformity in sample processing (as observed with public data catalogues). Despite covering thousands of locations, these studies sometimes suffer from issues like low resolution, geographical sparsity, or inadequate metadata details.

In this work, we introduce Microflora Danica, our extensive collection comprising 10,874 environmental samples from Denmark, associated with 10,686 metagenomes and 449 complete datasets of prokaryotic and eukaryotic rRNA operons. The sample sites reflect the primary habitat types of the country and is accompanied by a comprehensive 5-level environmental ontology, serving as a detailed habitat classification system.

Utilising this granular data, we explore the unique microbial entities within these habitats as compared to existing databases and analyse the microbial diversity within individual samples as well as across the entire nation. We particularly examine the distribution and diversity of nitrifiers, given their significance as functional groups in Denmark, where agriculture constitutes 60% of the land use. The Microflora Danica database lays a solid groundwork for addressing key questions in microbial ecology regarding the factors that influence microbial diversity, distribution, and functionality.

[P18] Implementation of Terahertz Technology for expedited identification of Microorganisms

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The present study demonstrates the use of metamaterials within the terahertz frequency spectrum to fabricate microbial sensors that demonstrate remarkable sensitivity and selectivity. These sensors can quickly identify microorganisms in both normal outdoor and watery environments. Because their dimensions were similar to the micro-gaps of terahertz metamaterials, the detection of minute amounts of microorganisms became possible. The current study used microbial concentration and dielectric constant analysis to look into the observed resonant frequency shift of metamaterials. The observed shift was found to be precisely explained by a switch in the operative dielectric constant inside a particular gap area. This technique is applicable to many viable microorganisms that inhabit aquatic mediums and ambient conditions, as it is very specific to chemicals presented near the surface. High-frequency metamaterial sensing (THz) is a flexible technique that uses dielectric revelation to provide specific detection by altering surfaces.

[P19] Microsensor measurements reveal microbial activity in coral gastric cavity microenvironment

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Symbiotic microorganisms of corals play an important role in nutrient cycling, defense and stress response in the coral holobiont. The coral gastric cavity (CGC) serves various essential functions such as coral feeding and circulation. Previous study has shown the CGC contains high nutrients concentration and high bacteria counts, suggesting it may be a potential hotspot for microbial activity. However, limited research has been conducted on the CGC due to the technical challenges in measuring the um-mm scale microenvironment. In this study, we utilized electrochemical microsensors (O2, H2, NO, N2O and H2S) to investigate the chemical dynamics and microbial processes within the gastric cavity of Caulestrea corals, with a specific focus on anaerobic respiration processes. In darkness, the O2 concentration decreased and CGC transitioned to hypoxia/ anoxia. Within this anoxic microenvironment, we detected NO and N2O, which are the intermediate products of denitrification. Additionally, we found H2 production in the dark as a byproduct of fermentation, and the highest recorded concentration of H2 reached 0.8 umol L-1. However, H2S was not detected. Overall, this study demonstrates the potential of using microsensor to reveal the denitrification and fermentation processes in CGC, which sheds light on understanding the microbial activity in CGC and coral holobiont.

[P20] Optimizing Screening Methods for Streptococcus thermophilus Strains in Dairy Applications

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The industry is increasingly focused on developing new food products that meet criteria such as sustainability, clean labeling, and health benefits. At Novonesis, a large strain collection is screened to identify those suitable for specific food matrixes and applications. One key area of interest is Streptococcus thermophilus for dairy applications including yoghurt and mozzarella-type cheeses made from bovine or plant-based milks. A critical trait for dairy application is the ability of strains to acidify milk, which is assessed using a high-throughput screening method. This method often does not correlate well with results from larger scale applications, leading to potential false positives and negatives in strain selection. To enable rapid screening, the strains are initially tested for their acidification capabilities using pH indicators in a polypropylene 96 deep-well plate with 2 mL volumes. Following this initial screening, selected strains are further assessed using pH probes in 200 mL glass or plastic bottles with a silicone plug.

A total of 301 Streptococcus thermophilus strains have been characterized in skim milk. Approximately one-third of the strains acidified more rapidly in the well-plate assay, likely due to sedimentation effects. Another third displayed nearly identical acidification profiles across both assays, while one-sixth acidified faster in the 200 mL bottles. The remaining strains exhibited varying differences in acidification profiles between the two assays. A few of the strains have indications of being affected by the presence of oxygen. Oxygen availability can impact the acidification rate in milk for well-characterized Streptococcus thermophilus strains. All strains are genome sequenced and future studies aim to shed light on the roles of genes related to oxygen utilization, sedimentation, and container interaction in influencing the acidification phenotype.

[P21] Antibiotic Resistance in Bacterial Isolates from the Strait of Magellan: A Genotypic and Phenotypic Study in Seno Otway and Charles Islands Bays

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The Strait of Magellan remains a crucial maritime passage with significant marine traffic. This study examines antibiotic resistance in 68 bacterial isolates (35 from Seno Otway and 33 from Charles Islands) recovered using Chromocult agar. Resistance genes, including qnrA, qnrB, qnrS, intI1, intI2, sul1, blaTEM, blaSHV, chromosomal blaAMP-C, aac(6')-Ib, floR, and blaCTX-M (groups 1 and 2), were identified via PCR, and resistance profiles were determined through antibiograms for antibiotics like chloramphenicol, cefuroxime, and ampicillin.

Bacterial identification was performed using 16S rRNA sequencing with the Sanger technique, analyzed via the Silva v.138 database. The predominant genera were Aeromonas spp., Escherichia spp., Pseudomonas spp., Yersinia spp., Lelliottia spp., and Hafnia spp.

One Pseudomonas spp. isolate from Seno Otway harbored the intI1 gene and showed resistance to chloramphenicol, ampicillin, and cefuroxime. Three Pseudomonas spp. isolates from Seno Otway carried chromosomal blaAMP-C, resistant to chloramphenicol, cefuroxime, ampicillin, and ceftriaxone. Three isolates had the aac(6')-Ib gene: two Pseudomonas spp. (from Seno Otway and Charles Islands), resistant to chloramphenicol, cefuroxime, imipenem, ampicillin, and ceftriaxone, and one Escherichia spp. that amplified the gene but showed no phenotypic resistance.

These findings suggest that marine currents and human activity contribute to the spread of multidrug-resistant bacteria, particularly Pseudomonas spp. This is the first report of antibiotic resistance in bacterial isolates from the Strait of Magellan, underscoring the need for continued surveillance to address resistance in marine ecosystems.

[P22] Gut Microbial Profiling in piglets: A comparative between short and long a16sRNA gene sequencing

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The gut microbiome of piglets plays an essential role in their health and development. This microbial composition shifts over time until it reaches a "mature" state (1). This process is influenced by different factors, such as diet changes, in-feed antibiotics, and stress (1). Weaning is a critical period that significantly impacts the gut microbial community, making piglets susceptible to post-weaning diarrhea due to an immature gut and a developing immune system. Understanding the microbiome changes in this period is crucial for improving piglet well-being and developing better diets or alternatives to antibiotics for post-weaning diarrhea, a major issue in the pig industry (2).

To study these dynamic changes, 16S rRNA gene short amplicon sequencing has been widely used (3). However, with the development of long-read sequencing of 16S rRNA gene, we can obtain more detailed resolution at the species level (4). This could strengthen the study of gut microbial shifts by differentiating closely related bacteria that may have distinct roles in the gut. Building on this idea, we compared the taxonomic profiles of the gut microbiome in piglets using both short and long-read sequencing. Preliminary results, on both datasets, showed a similar distribution of core bacteria (3) including, Bacteroidaceae, Lactobacillaceae, Lachinospirae and Clostridiaceae. Interestingly, long-read sequencing could detect subtle changes in the taxonomic composition at species-level, during the weaner period, as in Lactobacillus genera. Therefore, this method can help in refining the description of microbial communities in complex samples without the need for full genome sequencing.

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[P23] Unveiling soil microbial diversity through ultra-deep metagenomic sequencing and co-assembly

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Soil hosts the most diverse microbial community on the planet which is crucial for biogeochemical cycles and provides resources for pharmaceuticals and industry. Many soil microorganisms remain uncharacterized due to limitations of culture-dependent methods, and as shotgun metagenomic sequencing is challenged by the extensive diversity of the soil community making sequencing coverage very low and preventing de-novo assembly.

This study utilized ultra-deep metagenomic sequencing to explore the impact of sequencing depth on characterization of soil microbiomes. We collected 600 soil samples from different agricultural fields in Denmark, resulting in more than 73 terabases of clean metagenomic reads, with an average of 107 gigabases per sample. Despite this extensive sequencing effort, metagenomic coverage was relatively low (47-73%) varying depending on field diversity, and it would require 1-4 terabases of clean forward reads to capture 95% of the community. Co-assembly improved metagenomic coverage, assembly quality, gene recovery, and recovery of metagenome-assembled genes (MAGs), and enabled recovery of bacterial phyla that could not be recovered from single assemblies. Our study highlights the importance of high sequencing depth and co-assembly to capture diversity and improve the characterization of the vastly diverse microorganisms in soil.

[P24] Non-target effects of the nitrification inhibitors nitrapyrin and DMPP on soil microbial communities: perspectives utilizing total RNA sequencing.

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Fertilization of agricultural fields is crucial for achieving crop yields that meet the growing global demand for food production. However, intensified fertilizer applications also pose major environmental challenges, which are caused by the transformation of excess ammonium (NH4+) via microbial nitrification. This process forms nitrate (NO3-) which can be leached into groundwater or further transformed into potent greenhouse gases (GHGs) such as ammonia (NH3) and nitrous oxide (N_2O) contributing to climate change.

The use of various nitrification inhibitors (NIs) could help mitigating these effects by hampering the ammonia monooxygenase (AMO) enzyme, which catalyzes the first step of nitrification (the oxidation of NH4+ to NO2-), and therefore reduces subsequent NO3- leaching and N2O emissions. Despite increasing knowledge about the effect of these inhibitors on nitrifying and nitrogen cycling microorganisms, there is still limited understanding of their potential non-target effects on the broader microbial community and its ecological functions.

By applying total RNA sequencing to field trials treated with two commonly used nitrification inhibitors, nitrapyrin and DMPP, the amoA project aims to assess potentially overlooked side effects of NIs on microbial soil dynamics. The simultaneous analysis of rRNA and mRNA extracted directly from NI-treated soil provides a holistic approach that enables tracking both taxonomic and functional changes within the active microbial community, including prokaryotes and eukaryotes. This approach provides an opportunity to investigate biodiversity and ecological changes in NI-treated soils subjected to conventional fertilization practices, potentially identifying previously overlooked genes and ecological functions affected by NIs. This comprehensive ecological assessment helps to better characterize the environmental risks associated with NI applications and thus contributes to the development of more sustainable fertilization practices.

[P25] Impacts of the mycovirus Fusarium culmorum phenuivirus 1: laboratory and in planta investigations

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Fusarium culmorum phenuivirus 1 (FcPV1) is a mycovirus (fungal virus) that was detected in F. culmorum isolated from wheat (Triticum aestivum) infected with Fusarium Head Blight (FHB). FcPV1 is a negative-sense single-stranded RNA mycovirus from the Phenuiviridae family. Given the increasing interest in using mycoviruses for biological control of FHB, we performed laboratory and in-planta experiments investigating how FcPV1 affects F. culmorum and its pathogenicity. Laboratory experiments revealed no observable phenotypic changes. However, in greenhouse pot experiments with wheat, FcPV1 infection resulted in increased fungal pathogenicity (hypervirulence), whereas in semifield conditions, it led to reduced pathogenicity (hypovirulence). The study highlights that mycovirus impacts, often assumed to be cryptic, may only emerge under specific environmental conditions and assessment criteria. We therefore underscore the complexity of mycovirus-host interactions and the critical role of environmental factors in evaluating their suitability for biocontrol. To our knowledge, this is the first study that compares the effect of mycovirus on fungal diseases in crops grown in both greenhouse and semifield environments.

[P26] Insights into the metabolic profiles of B. cereus and B. subtilis in diverse food matrices

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The genus Bacillus contains pathogens (Bacillus cereus clade) and microorganisms used for food fermentation (Bacillus subtilis clade). Monitoring Bacillus spp. in food matrices is essential for food quality and safety, which requires a deeper understanding of the metabolic responses to different food products. Isothermal microcalorimetry is an effective way to measure metabolic heat production in real-time.

This study provides a novel approach to understanding clade-specific and matrix-dependent microbial activity, by combining phylogeny, microcalorimetry, and metabolomics. Sixteen B. cereus and eight B. subtilis strains were cultivated at 30°C in different matrices: Brain Heart Infusion (BHI) medium, oat drink, whole milk, 5% pea protein hydrolysate (PPH) in water solution, and oat drink supplemented with 5% PPH. A maximum-likelihood phylogenetic tree was constructed based on whole-genome sequences. Metabolic heat curves were analyzed using Gompertz's model to derive calorimetric lag time, maximum calorimetric growth rate, and total accumulated heat. Sugars and organic acids were quantified at selected time points to compare metabolomic traits across phylogenetic clades and food matrices.

Phylogenetic analysis classified the strains into 3 clades within B. cereus and 2 clades within B. subtilis. Cultivation in BHI media resulted in clade-specific metabolism, where B. cereus had a shorter calorimetric lag time, while B. subtilis released significantly higher amounts of total heat. Oat drink with PPH led to a notable increase in maximum calorimetric growth rate for both B. cereus and B. subtilis, indicating a matrix-dependent synergetic response. These findings highlight the value of integrating phylogeny and calorimetry to describe phylogenetic- and matrix-dependent microbial metabolic profiles. Analysis of organic acid and sugar composition will be performed to further comprehend the distinct metabolic profiles of B. cereus and B. subtilis in different matrices.

[P27] Unfolding the collective functional potential of a synergistic multispecies community through genotypic and phenotypic analyses

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The strains Paenibacillus amylolyticus, Microbacterium oxydans, Stenotrophomonas rhizophila and previously, Xanthomonas retroflexus, were co-isolated from the soil residuesphere and possess synergistic biofilm formation capabilities in vitro along with other intrinsic properties, some of which could lead to potential industrial and agricultural applications. Here, we conducted a bioinformatic analysis of the genomes to serve as a guide to identify relevant phenotypic assays. A hybrid genome was assembled for each strain, using both long and short reads. Secondary metabolite gene clusters, cooperative genes, antimicrobial resistance genes, and metabolic pathways were identified. The subsequent phenotypic assays conducted were carbon utilization assays, enzymatic activity tests, and antibiotic clearing zones. After assembling the circularized genomes, we found that the previously regarded X. retroflexus was instead Stenotrophomonas maltophilia. P. amylolyticus had 16 secondary metabolite gene clusters identified in its genome, while the other 3 strains all had 2 clusters. When identifying social genes, P. amylolyticus again came out to have the greatest number of cooperative genes, followed by S. maltophilia, S. rhizophila, and finally, M. oxydans. We also demonstrated enhanced growth as a four-species community on several carbon sources, as well as enhanced enzymatic degradation abilities. Overall, analysis of the complete genomes of this model community uncovered major gene functions which could play a role in the observed community intrinsic properties, as well as provided insight to the positive social interactions observed in vitro.

[P28] Unlocking the Power of Gut Microbes: L-Fucose as a Key to Propionic Acid Production

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L-fucose, which is metabolized by specific gut bacteria and is a component of mucus and human milk oligosaccharides and is present in some plant and algae polysaccharides. L-fucose is converted into 1,2-propanediol (1,2-PD), which can be metabolized into propionic acid, propanol and possibly lactic acid by microbial specialists. The role of complex intestinal microbiota in mediating L-fucose and 1,2-PD conversion remains poorly understood.

To capture dynamic metabolic changes especially during the initial stages of fermentation, a longitudinal in vitro study was conducted to assess the metabolism of L-fucose by complex fecal microbiota. Fecal samples were collected from nine healthy donors, and fermentations were performed in Macfarlane media (control) and with 10 mM L-fucose and the pathway intermediate 1,2-PD at 37 °C.

During fermentation, 1,2-PD was metabolized within 8 h with maximum rates of 1.5 mM/h, while L-fucose was used at 24 h (0.4-1.1 mM/h). Propionic acid production commenced within the first 4 h and levels were higher in the presence of L-fucose and 1,2-PD compared to controls. Propanol was produced from both substrates but decreased between 24 and 48 h. The metabolism of L-fucose predominantly yielded propionic acid, with a recovery of 38 ± 18% (Cmol/Cmol) and the use of 1,2-PD resulted in propionic acid and propanol (combined recovery of 39 ± 13% (Cmol/Cmol)). In the presence of L-fucose, butyric acid production was higher in four samples than in the control; 1,2-PD did not influence butyric acid formation. Low levels (0.2-5 mM) of lactic acid and succinic acid were observed at the beginning of fermentation in the presence of both substrates but were consumed after 12 h.

Our research shows that fecal microbiota prefers 1,2-PD over L-fucose. L-fucose is fermented by gut microbes to mainly propionic acid likely via lactic acid cross-feeding. L-fucose metabolism, therefore, contributes to the formation of two important short-chain fatty acids in the gut.

[P29] SemiBin3: Advancing binning of metagenome assembled genomes with methylation patterns

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Metagenomic binning is essential for reconstructing individual genomes from complex microbial communities, yet it remains challenged by the presence of closely related species and strain-level diversity. Traditional binning approaches primarily rely on sequence composition and abundance profiles, which can be insufficient for resolving ambiguities in highly complex or similar microbial populations, often resulting in fragmented or contaminated bins.

We introduce SemiBin3, a novel metagenomic binning tool that directly incorporates methylation motifs identified by Nanomotif from Oxford Nanopore sequencing reads into the binning process. SemiBin3 adds an orthogonal layer of epigenetic information that can be species- or strain-specific. This integration allows for more precise differentiation between closely related microbial genomes, enhancing binning accuracy and reducing contamination.

Our results demonstrate that methylation patterns are a valuable, yet underutilized, source of information in metagenomics. SemiBin3 capitalizes on the capabilities of Nanopore sequencing to detect native DNA modifications, to create another dimension to metagenomic binning. By integrating sequence, abundance, and methylation data, SemiBin3 offers a unique framework for metagenomic binning.

[P30] Metagenomic analysis of 130 years old Danish starter culture material including sequence analysis of the genome of a Lactococcus cremoris starter

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We recently discovered a collection of old, unopened Danish starter culture products in the veterinary collection at the Department of Veterinary and Animal Sciences, University of Copenhagen. The starter cultures were produced by the defunct company Blauenfeldt & Tvede and date back to the 1890s. These historical samples offer a glimpse into the composition and evolution of starter cultures from the1890s onwards, thereby providing insights into the challenges and possibilities of dairy production by the end of the 1800s. In the summer of 2023, we opened two sealed bottles with starter cultures from 1893 and 1899 with the aim to explore the microbiome and genetic characteristics, using culture-independent methods, as no viable cells remained. DNA was extracted via the phenol-chloroform method and sequenced using whole-genome amplification and Nanopore sequencing technology. The microbial composition was analyzed through metataxonomic profiling and genome mapping against Lactococcus spp. reference genomes. The results revealed a high prevalence of Cutibacterium acnes in both samples, suggesting potential contamination during the traditional production process and different degradation of bacterial taxa over time. Despite the heavy degradation of Lactococcus DNA, the 1893 sample, opposed to the 1899 sample, still contained a notable presence of L. cremoris DNA, with 76% genome coverage against the reference strain L. cremoris MG1363, but with less coverage against reference genomes of L. lactis. Bioinformatic analysis of the L. cremoris genome from the 1893 sample showed several functional genes associated with dairy adaptation, including those involved in casein degradation, lactose metabolism, amino acid catabolism, and acetoin and diacetyl production, the latter compounds potentially contributing to characteristic buttery flavors. Additionally, a lactococcin encoding gene (lcn) was detected both in the 1893 sample and in a more recent dairy-associated isolate, L. cremoris TOM.10.15, indicating a conservation of lactococcin gene over time. This study provided an overview of the microbiome and functional potentials of up to 130 years old starter culture material and highlighted hygienic challenges in 19th-century dairy production.

[P31] Climate change impacts the structure and nitrogen-fixing activities of subarctic feather moss microbiomes along a precipitation gradient

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Associations between feather mosses and cyanobacteria are crucial sources of new nitrogen in arctic and subarctic ecosystems. The physiology of both mosses and cyanobacteria is strongly influenced by environmental factors such as temperature and moisture, which directly affect nitrogen fixation rates. However, these associations may be threatened by climate change, since it is leading to warmer and drier conditions in polar regions. In this study, we investigated the nitrogen-fixing microbial communities associated with feather mosses across a precipitation gradient in three subarctic tundra sites in northern Sweden. Using acetylene reduction assays, nifH gene sequencing and qPCR, we evaluated how climate change-driven shifts in temperature and moisture influence nitrogenase activity and nitrogen-fixing community structure. Our results showed that nitrogen fixation was highest in sites with greater precipitation and increased with both temperature and moisture. Cyanobacteria dominated nitrogen-fixing communities, but currently unclassified bacteria also seemed to play a significant role, particularly at higher temperatures. However, the number of cyanobacterial nifH copies decreased with temperature, while the relative abundance of unclassified bacteria increased. These findings suggest that the diversity of cyanobacteria associated with feather mosses in the subarctic will decline at elevated temperatures and lower moistures, potentially leading to a shift in the composition of feather moss-associated microbial communities in a warmer Arctic.

[P32] Precision Phage Detection & Isolation: A new tool for rapid detection and isolation of specific phages at low concentrations in water samples

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Flavobacterium psychrophilum is a bacterial pathogen that causes rainbow trout fry syndrome and cold water disease in salmonids. Especially in rainbow trout fry F. psychrophilum can lead to losses of 70% in severe cases, causing substantial economic consequences. The use of antibiotics to control the disease is becoming less effective due to the emergence of antibiotic-resistant bacterial strains. Phage therapy represents a promising alternative to antibiotics for the treatment, and prevention, of F. psychrophilum infections, in aquaculture.

Phage libraries are vital to deepen our understanding of phage-host interactions and increase the efficiency of phage-based pathogen control. Typically, the isolation of specific phages from environmental samples is time-consuming and requires large sample volumes and/or phage concentration efforts. High throughput screening methods are therefore needed to boost phage isolation and identification.

By combining phage enrichment of host bacterial cultures with optical density kinetics, we are developing a highly sensitive method for the isolation of F. psychrophilum phages from aquaculture facilities, using a multi-well plate assay.
15 F. psychrophilum strains obtained from rainbow trout aquaculture farms were used as target hosts for phage isolation and the multiwell assay method were compared with a direct plating approach. Of 37 phage isolates obtained, 2 were isolated only by direct plating, 21 phages were only identified using the 96-well enrichment assay and the remaining 15 phages were isolated by both methods.

This method is currently in use for the isolation of F. psychrophilum phages, from fish farm samples. So far 9 samples have been screened for a performance comparison with direct plating.

[P33] Optical diffraction tomography reveals moderate levels of dissolved iron stimulate cellular growth and increase lipid storage in Symbiodinium sp.

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Symbiodiniaceae dinoflagellates are fundamental in coral reef ecosystems. They facilitate essential processes such as photosynthesis, nutrient cycling, and calcium carbonate production. Iron (Fe) is an essential element for the physiological processes of Symbiodiniaceae, yet its role remains poorly understood in the context of cellular development and metabolic health. Recently, optical diffraction tomography, known as holotomography, techniques have emerged, providing quantitative morphological and biochemical information about individual cells and tissues without the need for exogenous labeling agents. This study aims to investigate the effect of iron enrichment on Symbiodinium sp. cultures and quantify their cellular content using advanced techniques such as flow cytometry and holotomography. Our results indicate that moderate levels of dissolved Fe (50 nM) enhance growth rates and cellular content development in Symbiodinium sp., with 57% higher lipids and 10% higher proteins content as measured with holotomography. Our findings contribute to a deeper understanding of the relationship between iron availability and Symbiodinium sp. growth and cellular development, with implications for coral health and reef resilience in the face of environmental stressors.

[P34] From 2D plates to 3D structures: Does termite-farmed Termitomyces need three dimensions to grow?

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The World needs more sustainable food sources as an alternative to meat production to help curb climate change while ensuring nutrition for a growing human population. The cultivation of mushrooms shows potential in this quest, as mushrooms are up to 34 times less energy-intensive to produce than their animal counterparts and they possess excellent amino acid compositions for human consumption. Termitomyces, a genus of fungi that is cultivated by farming termite hosts in Africa and Asia, produce edible mushrooms; however, currently, we can only grow Termitomyces on agar plates and growth is slow and biomass production limited. In termite colonies, Termitomyces grows on complex 3D structures, so called fungus combs, which led us to hypothesise that 2D agar plates are limiting growth. To test this hypothesis, we mimicked the natural physical structure of a fungus comb by inoculating test tubes containing two types of 3D structures covered in growth medium and then quantified growth. Spheres were selected to replicate surface structure and cylinders were chosen to mimic termite passageways in fungus combs. Preliminary evidence suggests statistically significant improved growth in 3D structures as compared to 2D plates. Expanding on this work, we acquired a high-resolution 3D model of a fungus comb with MicroCT imaging, which we are using to replicate the fungus comb structure in biodegradable materials. Ultimately, we hope to provide a technology that allows industrial scale production of mycelial biomass for human consumption, as an alternative to mushrooms collected from termite mounds.

[P35] A Surprising Link in the Gut: Could Lactose and Megasphaera Be Driving Valerate Production?

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The human gut hosts an ecosystem of microbes contributing to the digestion of complex molecules and the production of short-chain carboxylic acids (SCCA) through fermentation and chain elongation, a process that can be modulated through diet. The aim was to investigate the impact of fermented dairy consumption, which is a regular dietary item due to high nutritional value and palatability, on fecal microbiota composition and fermentation activity with a focus on lactose utilization potential. This research combined investigation of a one-meal dietary intervention cohort study with in vitro fecal slurry fermentations and co-culture studies.

Fecal samples were collected (0, 6 and 12 weeks) from a cohort of 49 overweight/obese women consuming skyr combined with oats (n= 21, high protein, HP) or a low protein (n = 28, LP) breakfast. While dietary intervention increased ($p<0.05$) fecal microbial diversity and relative abundance of Streptococcaceae, levels of lactate and valerate were higher ($p<0.05$) in feces of HP at 6 weeks. Higher valerate levels were observed in fecal samples that harbored >7 log cells/mL Megasphera, a known valerate producer. In vitro more valerate was produced by fecal microbiota when lactose was if donors harboured Megasphaera. In comparison, fecal microbiota of donors without detectable Megasphera produced less valerate with lactose; valerate production could be restored if Megasphaera elsdenii DSM 20460 was added. In single culture, M. elsdenii was not able to use lactose, but in co-culture with Streptococcus thermophilus LMG 18311, valerate was formed (6.3 ± 1.3 mM).

These findings provide new knowledge on the metabolic interactions that lead to valerate formation, a frequently observed but little studied SCCA. Lactose availability influenced valerate production when Megasphaera was present through cross-feeding activity highlighting the complex interplay between diet, gut microbiota, and fermentation processes.

[P36] Actinomycete Genome Sequencing as the Foundation for Discovery

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DTU Biosustain

We have generated high-quality genome sequences of 1865 filamentous actinobacterial strains from Pacbio, Nanopore and Illumina data, gaining new insights into the genome composition and organization of this important clade of bacteria. Using the antiSMASH predictions of 48561 Biosynthetic Gene Clusters (BGC) in these genome sequences, we have analyzed the general and specialized metabolism. We have identified rare, new, and interesting BGCs, which are responsible for production of specialized compounds with potential function in agricultural contexts. By cloning these BGCs and engineering the native hosts, we are unlocking the biosynthetic potential of the strains. Complementing genome mining and molecular biology efforts, metabolomics is being used to leverage the genomic information to aid the discovery of natural products.

[P37] epsSMASH: A Platform for Automated Identification of Biosynthetic Gene Clusters Associated with Extracellular Polysaccharides

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Polysaccharides perform a myriad of essential functions in the extracellular space surrounding most bacteria. Capsular polysaccharides envelop bacterial cells, aiding in aggregation, adhesion, and protection against harmful substances. Similarly, lipopolysaccharides, which constitute the outer layer of the Gram-negative outer membrane, perform many of the same functions while also enabling evasion from host immune systems. Polysaccharides secreted into the extracellular space, without attachment to the cell surface, provide the producer with vital functions, including structural integrity, toxin adsorption, and water retention. The biosynthesis of these extracellular polysaccharides (exoPS) is often complex and highly diverse, with significant differences even between bacterial strains. Nevertheless, exoPS biosynthesis can generally be grouped into four major pathways, each typically organized within biosynthetic gene clusters (BGCs).

Despite the ubiquity of exoPS in microbes, attempts to assess their genomic potential for production have been limited. Here, we introduce epsSMASH, a bioinformatic tool designed to predict both known and novel exoPS BGCs in bacterial genomes. epsSMASH identifies 23 distinct exoPS BGCs using strict rules derived from the antiSMASH framework. In addition, it employs less stringent criteria to predict putative novel exoPS BGCs. A user-friendly web interface, as well as a command-line tool for more experienced bioinformaticians, will be publicly available to enable researchers to explore the exoPS BGCs in their microbes of interest. Given its comprehensive analysis capabilities and ease of use, we believe epsSMASH will serve as an essential tool for microbiologists investigating exoPS production in various microbial species.

[P39] New insights on the phylogeny and function of the agricultural soil microbiome

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Soil is a habitat that hosts an abundant and diverse microbial community. At the same time, the soil microbiome plays a crucial role in ecosystem processes, such as biogeochemical cycles, and has been a main source for novel compound discovery, e.g. antimicrobials. In agricultural fields, the soil microbiome impacts soil fertility and plant health. Despite its significance, the soil microbiome remains poorly characterized, with few members being cultured and a small fraction of soil communities being represented in public genome collections even through culture-independent characterization. Nevertheless, comprehensive understanding of the agricultural soil microbiome is key for providing sustainable solutions for crop production.

In this study, we aimed to better characterize the agricultural soil microbiome through deep metagenomic sequencing and genome resolved metagenomics. We sequenced 455 soil samples collected from two different agricultural fields in Denmark, resulting in ~70 TB of raw sequencing data. This data resulted in 58,831 medium to high quality metagenome-assembled genomes (MAGs), which could be further clustered in 9,090 species level genomes (SGBs) spanning 46 different bacterial and 3 archaeal phyla. Our genome catalogue covers unexplored diversity as 96.2% of the SGBs have not been previously reported. Characterization of the functional potential of the genomes revealed their role in a range of different pathways, such a nitrogen and sulfur cycling. In addition, we illustrated the broad and diverse biosynthetic potential of soil microbiome with the identification of > 46k biosynthetic gene clusters (BGCs), across 8,182 SGBs. In general, our catalogue greatly expands the known soil prokaryotic diversity and provides a valuable genetic and genomic resource.

[P41] Microbiology in tiny guts: lessons from tropical corals

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Coral reefs are highly biodiverse, yet vulnerable ecosystems in tropical oceans. Reef-building corals, simple colonial animals related to jellyfish, host diverse microbial communities in their tissue, skeleton, external mucus layer, and gastrovascular cavity. Some of these communities are involved in key physiological processes and may thus be important for coral health. However, coral microbiomes are often treated as "environmental samples" and characterised in bulk from whole coral fragments. As a result, our knowledge of these communities lacks the spatial, compartment-specific resolution that is necessary for functional studies of animal microbiomes.

The coral gastric cavity is a small, semi-closed sac, surrounded by skeleton. It is the fulcrum of fundamental physiological processes such as feeding, reproduction, and nutrient exchange. Yet, due to the technical challenges of working in such a small and inaccessible space, we still know very little about this vital compartment and the microbial community that inhabits it. We have used microsensors to characterise the physico-chemical environment in the coral gastric cavity both in the field and during experimental manipulation, revealing a low oxygen environment suitable for supporting specialised microbial communities, as observed in the gut of higher invertebrates and vertebrates. Additionally, we have developed a method to sample and characterise the gastric cavity microbial community of individual coral polyps, using microscale sampling techniques and a low-input DNA extraction protocol. Our results suggest that a core "gut microbiome", enriched in potential anaerobic and microaerophilic taxa, may exist at least for some coral species. Like the gut microbiome of higher organisms, these communities may play critical roles in the resilience of corals to climate change. Our methods are applicable to the study of other aquatic microbial communities in small, inaccessible environments.

[P42] Neonatal gut Bifidobacterium associates with indole-3-lactic acid levels in blood risk of ADHD at age 10

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The gut microbiome has been associated with brain health. The neuromodulatory effects of microbial-derived metabolites is supported by experimental evidence, and alterations of gut microbiota have been associated with the pathophysiology of certain neuropsychiatric disorders. Research on the gut microbiome's role in attention-deficit/hyperactivity disorder (ADHD) has been predominantly cross-sectional and seldomly within the neonatal period, a critical period, where long-term neurodevelopment is shaped. This study addresses how initial colonization and timing of specific gut bacteria, and their metabolic byproducts, may influence the risk of future ADHD. Using the deeply-phenotyped COPSAC2010 prospective mother-child cohort, we show that a higher level of Bifidobacterium in the child's one-week gut microbiome, after extensively adjusting for genetic and early-life factors, is associated with ADHD at age 10. Our analyses further reveal that the tryptophan-derived metabolite indole-3-lactic acid (ILA) in the neonates' blood associates with ADHD and mediates the relationship between Bifidobacterium and ADHD risk. The association between neonatal blood ILA and ADHD was replicated in two independent cohorts. Bifidobacterium is known to affect human health; however, these findings suggest that the initial temporal colonization pattern of Bifidobacterium may be particularly important for neurodevelopment. In particular, elevated Bifidobacterium-derived metabolite ILA levels may have adverse consequences on the child's neurodevelopment during the first week of life, potentially on neural networks associated with ADHD. This suggests that by promoting a suitable temporal colonization pattern for Bifidobacterium and its production of ILA in the newborn may represent a potential strategy for clinical intervention for supporting adequate neurodevelopment and mitigating the risk of future ADHD.

[P43] Staphylococcus aureus coagulases rescue Pseudomonas aeruginosa during coinfection

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Most infections harbor more than one microbe. Within such polymicrobial infections, microbes often produce extracellular factors that facilitate bacterial growth and virulence. While one member may produce these factors, their production can potentially benefit all constituents within a local group or population. Staphylococcus aureus is a prominent member of polymicrobial infections where it secretes two clotting factors, staphylocoagulase (Coa) and von Willebrand factor binding protein (vWbp), that contribute towards staphylococcal survival and persistence during infections. As is the dilemma with many extracellular factors, we find that coagulases are also exploitable by co-inhabiting bacteria that do not take part in producing them. In this study, we find that Pseudomonas aeruginosa, a Gram-negative bacteria, is able to benefit from the coagulases produced by S. aureus. P. aeruginosa displays enhanced survival in an in vitro clinical model and ex vivo samples of human blood where coagulases are available. Using a murine model of infection, we demonstrate that coagulases also contribute towards the hematogenous spread and persistence of P. aeruginosa in organ tissues. Our results provide a possible explanation as to why S. aureus and P. aeruginosa coinfections are recalcitrant and result in worse clinical outcomes.

[P44] Development of the Early-Life Airway Microbiota and Mucosal Immune Profiles in Persistent and Transient Asthma Phenotypes

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The airway microbiome plays a crucial role in respiratory health, with compositions in infancy linked to early wheezing and pre-school asthma. The immune system interacts dynamically with the airway microbiota, associating with distinct asthma phenotypes.

Previous studies on the early-life airway microbiome and asthma focus on cross-sectional data and early phenotypes, neglecting the rapid development of the early-life microbiome and differing immune responses between transient and persistent asthma.

This study characterizes the development of the nasopharyngeal microbiota in infancy (at ages one week, one month, and three months), its interactions with mucosal airway immune profiles measured at age one month, and associations with distinct asthma phenotypes up to age ten years, in the COPSAC2010 prospective birth cohort of 700 children with over 90% follow-up.

Our findings reveal an altered development of the early-life airway microbiota in children with persistent asthma compared to those with transient or no asthma, with distinct microbial patterns associated with changes in mucosal immune responses. Notably, Staphylococcus aureus was associated with persistent rather than transient asthma phenotypes, displaying a negative association in early infancy and a positive association later in infancy.

These results underscore the importance of temporal dynamics in the infant airway microbiota and its interactions with the immune system concerning disease outcomes. By identifying early-life microbial trajectories associated with asthma and microbe-specific immune response patterns, this study advances our understanding of childhood asthma pathogenesis, highlighting potential windows of opportunity for intervention. Understanding these dynamic relationships could inform targeted prevention strategies, potentially reducing the long-term burden of asthma.

[P45] Biofilms colonizing stone heritage: the case of Assistens kirkegård in Copenhagen.

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Biofilms colonize any kind of surface and environment, including all those artefacts and monuments with an artistic, aesthetic, ethnological or anthropological value that constitute our cultural heritage. Biofilms can be particularly problematic when the heritage piece is outdoors, because of limited or no possibility of controlling climate, and thus the biofilms' growth. Because of biofilms' tolerance to chemical and physical stress, biocides efficacy is low, forcing conservators to repeat treatments with increasingly aggressive and toxic compounds. Innovative approaches for biofilm removal based on a detailed knowledge of microorganisms' interaction with our heritage material are thus needed.
Here, we introduce a longitudinal study (September 2023 to August 2024) to describe and monitor the evolution of bacterial and fungal biofilms colonizing the stone surface of six tombstones at the Assistens Kirkegård in Copenhagen. Biofilms colonizing stones are known for being complex communities of both phototrophic and heterotrophic microorganisms, supporting each other metabolism and resilience to stress. With a conservation treatment in October 2023, the biofilm was removed from the six tombstones, but it is expected to grow again. To monitor the biofilm development and identify the pioneer species, we collected samples for microbiome analysis just before the treatment and for a year after. In addition, pre-conservation samples have been processed for isolation of phototropic and heterotrophic microorganisms to then reproduce similar communities in the laboratory and investigate strategies for biofilm and fungal removal. The project is expected to shed light on the interactions among microorganisms responsible for degradation of our heritage, on the evolution of these communities and response to conservation treatments.

[P46] Potential occupational health hazard to museum and library employees from exposure to xerophilic Aspergillus spores

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Inhalation of fungi in an indoor environment is a known respiratory irritant that is linked to the development of chronic diseases. Xerophilic/xerotolerant (following called xerophilic) fungal growth in heritage sites, such as museums and libraries, is a documented phenomenon, but the potential health impact of fungal spore exposure to employees remains unclear. To determine the reactive oxygen species (ROS) inducing potential of xerophilic fungi, 18 different xerophilic Aspergillus species were grown in pure culture and put into a standardized, single spore suspension. Neutrophil-like HL-60 cells were exposed to the spore suspensions alongside spores of A. fumigatus as a positive reference. ROS production from HL-60 cells exposed to spore suspensions was monitored for 3 hours continuously. Cytotoxicity and inflammatory cytokine expression in HL-60 cells was determined after 5 hours of exposure to select fungal species. In vitro exposure showed a diverse response, with some xerophilic species having a 3.5x higher ROS production than A. fumigatus and others having no increase above baseline. All but one tested species was cytotoxic after 5 hours of exposure, and viability correlates to ROS production levels. All xerophilic species increased expression of TNFα, IL-8, IL-1β, and CCL2 in HL-60 cells. Additionally, expression of TNFα and IL-8 also correlates to levels of ROS production. To see if this in vitro phenomena is applicable to occupational settings, ROS production assays were conducted on whole air samples collected using personal and stationary samplers from a historical library and a museum. The concentration of fungi in these samples was determined, and samples with higher fungal concentrations correlated with samples that induced higher ROS production. The results of this study suggest that spores of xerophilic fungi have potential to initiate a diverse inflammatory response in human lymphocytes.

This project was funded by the Augustinus Foundation.

[P47] Couples therapy in the microbial world: unravelling how marine bacteria cope with the antimicrobial secondary metabolite tropodithetic acid (TDA)

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The secondary metabolism of marine bacteria is a research field met with increasing interest, particularly because many secondary metabolites have antimicrobial activities. For example, several species of the obligate marine genus Phaeobacter are promising biocontrol agents, capable of eliminating pathogenic Vibrio spp. from aquacultures by production and release of the antimicrobial secondary metabolite tropodithietic acid (TDA).

In a previous project, a synthetic microbial community (SynCom) was established consisting of the aquaculture algae Tetraselmis suecica, alongside six phylogenetically representative bacterial isolates from its microbiome. Intriguingly, the SynCom was stable over the course of eight days, regardless of whether the potent TDA-producing strain Phaeobacter piscinae S26 was included or not. A minimal inhibitory concentration (MIC) test revealed that five members of the SynCom are tolerant to TDA, most prominently Roseovarius mucosus and Marinobacter excellens. Additionally, a fluorescent gene reporter assay showed that sterile-filtered supernatants from these two SynCom members caused an increase in expression of tdaC, one of the core biosynthetic genes required for TDA production in P. piscinae S26.

These findings raise the question of whether a dedicated mechanism exists in these SynCom members that allows them to sense and deflect TDA, thereby explaining their tolerance. Currently, we are using targeted metabolomics to monitor if the SynCom members can degrade or otherwise modify TDA into a less toxic derivative, a concept known as biotransformation. In parallel, the potential inducing effect that the SynCom supernatants have on tdaC expression in P. piscinae S26 will be investigated further using RT-qPCR analysis. By showing how TDA influences a synthetic microbial community, and vice versa, the outcome of this PhD project will help increase our understanding of the full ecological impact of marine microbial secondary metabolites.

[P48] Strain-link: Linking strain-level functional inference and development of microbial communities

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The human gut microbiome plays a crucial role in host health, which is directly linked to the specific composition and functional capacities of the microbes therein. This is especially true in the early life microbial communities, which show long-term effects on immune maturation and disease risk. In this study, we leverage longitudinal metagenomic sequencing data from the COPSAC2010 birth cohort, comprising faecal samples from children at 1 month, 1 year, and 10 years of age.

Using the genetic signatures we are developing a method for imputing the missing genetic information and functional traits even for low-abundant strains and how they influence the host.

A particular focus was placed on Bifidobacterium, a genus closely associated with infant health, to investigate species- or strain-specific functions that may influence the metabolic status within individual children. Using our in silico tool MAGinator, we have produced SNV-level phylogenetic trees for all metagenome-assembled genomes (MAGs) in the COPSAC2010 cohort. Our analysis has revealed differences among the MAG clusters, where some cluster according to time point and others according to individual. Even within the Bifidobacterium genus we see differences, indicating that some strains persist over time, whereas others act as transient colonisers.

By linking the presence and functions of Bifidobacterium to environmental exposures–such as antibiotic use and sibling presence–and health outcomes, including infections and asthma, we aim to uncover novel microbial mechanisms that contribute to disease development.

[P49] Loperamide increases mouse gut transit time in a dose-dependent manner with treatment duration-dependent effects on distinct gut microbial taxa

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Background: Intestinal transit time has been recognized as an important factor in shaping the gut microbiota community. Still, the understanding of causal links between transit time and the gut microbiota is incomplete.

Aim: The study aimed to evaluate the effect of different loperamide doses on the mouse intestinal transit time, and to investigate the effects of increasing the transit time on the mouse gut microbiota.

Methods: 4 groups of conventional C57Bl/6 mice were included in the study (saline, 5, 7.5, and 10 mg loperamide per kg bodyweight). Loperamide/saline was administered orally to the animals for one week, and transit time was measured at 4 different time points. DNA for 16S rRNA ion torrent gene amplicon sequencing was extracted from fecal samples collected before and during treatment, and SCFA levels were quantified in fecal and cecal samples.

Results: Loperamide significantly increased the mouse intestinal transit time in a dose-dependent manner. We observed a significant difference between the control group and the loperamide-treated groups in the abundance of the bacterial families Bacteroidaceae, Erysipelotrichaceae, Porphyromonadaceae, and Akkermansiaceae after 7 days of loperamide treatment, with the bacterial families responding to the increased transit time at different rates. We found higher cecal propionate levels, but not acetate and butyrate levels, in the high-dose group than in the control and low-dose groups.

Conclusion: The findings of this study emphasize the need for models such as the loperamide-induced increase in transit time to improve our understanding of the links between transit time and the gut microbiota.

[P50] The Spx stress regulator confers high-level β-lactam resistance and decreases susceptibility to last-line antibiotics in MRSA

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Infections caused by methicillin-resistant Staphylococcus aureus (MRSA) are a leading cause of mortality worldwide. MRSA has acquired resistance to next-generation β-lactam antibiotics through the horizontal acquisition of the mecA resistance gene. Development of high resistance is, however, often associated with additional mutations in a set of chromosomal core genes, known as potentiators, which, through poorly described mechanisms, enhance resistance. The yjbH gene was recently identified as a hot spot for adaptive mutations during severe infections. Here, we show that inactivation of yjbH increased β-lactam MICs up to 16-fold and transformed MRSA cells with low levels of resistance to being homogenously highly resistant to β-lactams. The yjbH gene encodes an adaptor protein that targets the transcriptional stress regulator Spx for degradation by the ClpXP protease. Using CRISPR interference (CRISPRi) to knock down spx transcription, we unambiguously linked hyper-resistance to the accumulation of Spx. Spx was previously proposed to be essential; however, our data suggest that Spx is dispensable for growth at 37°C but becomes essential in the presence of antibiotics with various targets. On the other hand, high Spx levels bypassed the role of PBP4 in β-lactam resistance and broadly decreased MRSA susceptibility to compounds targeting the cell wall or the cell membrane, including vancomycin, daptomycin, and nisin. Strikingly, Spx potentiated resistance independently of its redox-sensing switch. Collectively, our study identifies a general stress pathway that, in addition to promoting the development of high-level, broad-spectrum β-lactam resistance, also decreases MRSA susceptibility to critical antibiotics of last resort.

[P51] Interactions in Marine Microbial Synthetic Communities

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Microbial secondary metabolites play significant roles in shaping microbial communities and confer important physiological and ecological functions for the producing organisms. The most renowned secondary metabolites are famous for their antimicrobial activities, while their actual ecological roles in nature are often not understood. Furthermore, some secondary metabolites are only expressed as part of inter-species interactions in complex ecological communities and can therefore be challenging to study.
In the marine environment, such complex communities can often be found in association with macro-organisms or on inert surfaces. However, in situ study of bacterial interactions in these communities remain challenging. A way to bridge the gap between in situ studies and studying bacteria as monocultures is to establish synthetic communities that can be controlled and manipulated in the laboratory.
The purpose of the present study is to assemble a synthetic marine bacterial community to study cross-chemical interactions between community members facilitated by secondary metabolites. The community is assembled from bacteria isolated from marine bryozoans (moss animals) samples (Jyllinge harbor, Denmark), since we have discovered that the bryozoans are a hotspot for potent secondary metabolite producing bacteria. The genomes of the bacteria have been mined for their potential as secondary metabolite producers. Mutants devoid of secondary metabolite production as well as reporter fusions to key biosynthetic gene has been constructed and introduced to the community to explore the impact of secondary metabolites on interactions within the synthetic community, and microbial community assembly and function as well. Finally, a broad range of omics analyses (including metabolomics) is ongoing to unravel the functional role(s) of bacterial natural products within this community and hopefully more complex microbial systems.

[P52] First detection of florfenicol resistance in clinal isolates of Flavobacterium psychrophilum in a Danish rainbow trout farm

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The EU One Health Action plan against AMR emphasizes the need to strengthen surveillance and research to support evidence-based decisions in the food production system. In Denmark, antimicrobial resistance in fish bacterial pathogens is evaluated in relation to commercial diagnostics cases and research projects focused on this topic.

Here, we focused on the bacterium Flavobacterium psychrophilum [etiological agent of Rainbow Trout Fry Syndrome (RTFS) and Bacterial Coldwater Disease (BCWD)] and assessed the antibiotic resistance patterns of eight recent isolates (2019-2023) from Danish rainbow trout freshwater farms (classical microbiology AST (Antimicrobial Susceptibility Testing) methods and whole genome sequencing (Oxford Nanopore R10.4.1 pore chemistry)).

The analyses showed changes in the resistance patterns towards antibiotics used in aquaculture facilities (e.g. oxolinic acid (OA)) and the emergence of florfenicol (FFC) resistance, drug of choice for the treatment of F. psychrophilum infections in Denmark since 1996. The emergence of FFC resistance was observed in three isolates collected in 2021, 2022 and 2023 in a rainbow trout RAS (Recirculating Aquaculture System) farm. The phenotypic resistance patterns were confirmed by the analysis of the genomes from 2021 and 2022 that identified acquired resistance (MFS transporter) towards florfenicol. OA resistance was also detected in this farm as well as in the other isolates included in the study. The sequenced isolates presenting OA resistance on phenotypic testing harboured missense mutations (nucleic acid mutations causing amino acid substitutions) in the Quinolone Resistance Determining Region (QRDR) of the DNA gyrase subunit A (gyrA).

Despite the small sample size of this study, we would like to bring attention to the topic of antimicrobial resistance in aquaculture pathogens and to underline the importance of efficient AST to support effective control measures. The isolates presenting the newly observed AMR patterns belong to a clonal complex (CC-01) that has not been detected previously in Denmark and other Nordic countries thus suggesting the possibility of linking the emergence of these new patterns of antibiotic resistance to the import of eyed eggs, underlining the need for a surveillance program.

[P53] Biofilm formation of Phaeobacter piscinae on pro-bacterial polymer surfaces

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Fish production in aquaculture has increased dramatically to provide food for the growing world population. To control bacterial infections under intensive rearing conditions, antibiotics have often been used leading to fish pathogens becoming resistant to antibiotics. Therefore, new sustainable ways to prevent fish diseases must be found and one option is the use of probiotic bacteria. Bacteria of the Roseobacter group have been tested as probiotics in marine larval rearing, and their production of tropodithietic acid (TDA) enables pathogen control. This secondary metabolite, produced by several Phaeobacter species, has antimicrobial properties, and its production appears enhanced when the bacteria grow in biofilms compared to planktonic cultures. The purpose of this project is to develop a method that allows comparison of biofilm formation and TDA production, measured by proxy using gene expression of one of the TDA biosynthetic genes.

Phaeobacter piscinae were allowed to form biofilm on polypropylene coupons placed in microtiter plates containing 3% Instant Ocean, 0.3% CasAminoAcids and 0.2% Glucose. The surfaces were transferred daily to fresh substrate. When analyzed for biofilm formation, the surfaces were rinsed and stained with Syto62 to monitor the biofilm biomass, while a GFP attached to the promoter of tdaC, a key gene in the TDA biosynthesis, was used to track the TDA gene expression. Images of the biofilm were acquired with a Confocal Laser Scanning Microscope, and the biovolume and gene expression were then processed and quantified using the software BiofilmQ with the Otsu method as a threshold.

Biovolume increased from 1.49*106 µm3 at 48 h to 2.74*106 µm3 at 72 h ($p=0.0009$). Also, tdaC gene expression increased, albeit not significantly ($p=0.2251$). No correlation was detected between tdaC gene expression and biovolume ($p=0.0502$) as tdaC gene expression per biovolume was higher at 48 than at 72 h.

Our ongoing experiments are investigating how different surface topographies and characteristics affect biofilm formation and tdaC expression to design surfaces allowing efficient probiotic activity.

[P54] Early life factors shaping infant gut anellovirus composition and associations with childhood atopic disease

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The Anelloviridae family is highly prevalent and genetically diverse, but factors shaping the composition and the relationship with disease remain elusive.

Here, we characterised faecal viromes of 647 one-year-old children from the deeply phenotyped Copenhagen Prospective Studies on Asthma in Childhood2010 (COPSAC2010) mother-child cohort. In this study, the anelloviruses showed immense prevalence and individual variability. Early-life exposures such as being in day care and having older siblings were associated with a reduced anellovirus abundance and diversity, and influenced the anellovirus composition. Additionally, genetic secretors of the FUT2 enzyme had a higher anellovirus abundance compared to non-secretors. We observed an association between anelloviruses and allergic rhinitis, but no associations with infections or the development of other atopic traits. Our findings indicate that anelloviruses are a commensal component of all infants' faecal virome, and further investigations into how immune-training factors influence their composition longitudinally are pertinent.

[P55] Investigation of transcriptional repression of an antibiotic secondary metabolite produced by a marine bacterium by other antibiotic producing marine bacteria

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Microbial secondary metabolites are famous for their use as clinical antibiotics; however, we know little about their ecological roles in nature, in natural microbial systems. Especially, it remains a mystery how potent antibiotic producing bacteria co-exists and what influence these compounds have on other nearby microorganisms.

Our group recently showed that Pseudoalteraomonas piscicida which produces potent antibiotics can reduce the production of the antimicrobial secondary metabolite tropodithietic acid (TDA) in candidatus Phaeobacter bryozoensis. These two bacteria co-exist in marine biofilms, and the reduction in TDA was seen both during co-cultivation on solid media and in liquid media and shown to be on a transcriptional level. It therefore begs the question if this ability, to reduce TDA-biosynthesis gene expression, is a common trait for the Pseudoalteromonas genus along with what mechanism is responsible for the reduction.

This study will investigate the ability of different Pseudoalteraomonas spp. from around the world to reduce TDA production in Phaeobacter bryozoensis and to uncover the mechanism by which P. piscicida can reduce TDA transcription in Phaeobacter bryozoensis. A screening of 56 Pseudoalteraomonas spp. will be performed using a co-cultivation study on solid media, to verify which Pseudoalteraomonas spp. has the ability. Furthermore, a minimum inhibitory concentration assay (sub-MIC) will be performed using Pseudoalteraomonas supernatant against a P. bryozoensis tdaC GFP reporter strain to further substantiate that it is indeed at gene expression level the inhibitory activity of Pseudoalteromonas spp. takes place. Finally, bioassay guided fractionation of Pseudoalteraomonas piscicida supernatant will be used to pinpoint the molecular mechanism behind TDA repression.

[P56] Evolution of AMR in in vivo experimental evolution biofilm model

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Background: Treatment of biofilm infections is challenging due to their tolerance and resistance to the immune system and antibiotics. We aimed to examine the evolution of antimicrobial resistance (AMR) in a chronic P. aeruginosa lung infection model.

Materials/methods: Experimental evolution using a monitor strain for mutations in nfxB, the regulator of the MexCD OprJ efflux pump, was conducted in mice during 4 passages with a subinhibitory concentration of ciprofloxacin (CIP), 0.25 mg CIP twice/ day for one or two days. Bacteria embedded in alginate beads were inoculated into the lung of BALB/c mice and treatment was started 24h after inoculation. The biofilm populations from the lungs were investigated by population analysis profiles and CIP-resistant colonies from lung homogenates were passed to the next passage, cytokine levels in the lungs were measured, and selected isolates were sequenced.

Results: Comparison between the development of AMR using one or two-day treatment revealed that several CIP treatments increased bacterial resistance. After two days of CIP treatment, 22.8% of the bacterial population survived on 8 mg/L CIP plates, compared to 16.5% surviving on 2 mg/L CIP after one day of treatment. Mutations in nfxB, efflux pumps (mexZ), and two-component systems (parS) contributed to CIP resistance. In the initial two passages, the CIP-treated group exhibited an elevated inflammatory response compared to the control group, which might contribute to the release of mutagenic reactive oxygen species and the development of AMR.
Conclusions: This study illustrates the complex relationship between infection, antibiotic treatment, and immune response.

[P57] Uncovering the methane sink potential and habitat preferences of methanotrophs across 10,000 Danish metagenomes

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Methane emissions need to be reduced if we are to keep global surface temperatures below the critical threshold of 1.5-2 °C. To address this issue, it is crucial to understand the diversity and biogeography of methanotrophs, the only biological sink of methane. Recent studies in functional gene phylogeny and diversity have shown that many putative methanotrophs remain to be discovered. This is especially the case for atmospheric methane oxidisers. Historically, methanotrophs have been identified by the marker genes pmoA and mmoX, encoding two isoforms of methane monooxygenase. However, pmoA and mmoX share sequence similarity to monooxygenases oxidising non-methane compounds. This complicates identification based solely on marker genes and requires detailed information on gene phylogeny and downstream metabolic potential. Here, we screened 10,000 Danish metagenomes for methanotrophy marker genes to identify appropriate samples for long-read sequencing and methanotroph MAG recovery. We successfully expanded poorly described groups, such as Rhodomicrobium (recovered 9 MQ and 4 HQ MAGs) and the potential atmospheric methane oxidizer USCγ (recovered 7 MQ and 3 HQ MAGs), both previously characterised by only a single MQ MAG. Acquiring genomes for groups formerly linked to methane consumption, such as the pmoA-like TUSC, revealed metabolic and phylogenetic affiliation to non-methane oxidisers. The resolution of gene-based identification was improved by incorporating the obtained marker genes, effectively separating clades of plausible candidates from ambiguous sequences. On a national scale, most methanotrophs were unique to a few Danish habitats, while specific groups of potential atmospheric methane oxidisers were widespread across Denmark.

[P58] Oral delivery of a cholera toxin-binding protein protects against diarrhoea in experimental cholera

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Background: Vibrio cholerae can cause severe diarrheal disease mediated by cholera toxin (CTX) binding to the intestinal cell receptor GM1. Oral cholera vaccines are crucial preventive measures against V. cholerae infection. However, their annual distribution of 23 million doses is insufficient since there are one billion at-risk individuals in developing countries.

We developed an orally delivered bivalent single-domain antibody construct BL3.2 to block CTX in the gastrointestinal tract. BL3.2 significantly reduced CTX-associated diarrhea in infant mice challenged with V. cholerae and lowered the bacterial burden in the small intestine.

Methods: In vivo CTX-neutralizing capacity of BL3.2 was determined using a 5-day old infant mouse (CD-1) model. Mice were given two oral administrations of either BL3.2 or bovine serum albumin (BSA) as a negative control, before and after oral delivery of CTX or challenge with a clinical isolate of V. cholerae. Weight loss was measured as a gauge of diarrhea, and the small intestine excised to determine intestinal fluid accumulation. Furthermore, the number of V. cholerae in the small intestine was determined.

Results: Nine hours after CTX delivery, none of the mice given BL3.2 (n = 5) had diarrhea whereas all mice (n = 5) in the control group displayed severe diarrhea. Consequently, mice that received BL3.2 demonstrated a significantly (*) lower weight loss compared to the control. These mice also had significantly (**) less FA in their small intestine.

In mice challenged with V. cholerae, BL3.2 (n = 5) significantly (**) reduced weight loss as well as intestinal fluid accumulation compared to the control (n = 5). Furthermore, the burden of V. cholerae colonizing the small intestine of mice given BL3.2 was significantly less (**) than in the control group.

Conclusion: The functional food protein BL3.2 could serve to protect those at risk of cholera infection, as a part of future cholera management programs along with current practices.

[P59] Exploring the Biosynthetic Potential of Marine Sediments Microbiomes: A Multi-omics Approach

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Marine sediments are habitats for microbial communities that impact global nutrient and carbon cycling. Their diverse biosynthetic potential is a potent source of exploration for novel natural products and likely plays a major role in the sediment microbiomes. Despite this, we still lack a comprehensive map of the Biosynthetic Gene Clusters (BGCs) within this microbiome. This study aims to describe the BGC potential and expression. We will investigate the biosynthetic potential of microbiomes from three environmental locations across Denmark: Nivå, Jyllinge, and Rømø, using a multi-omics approach integrating advanced Nanopore metagenomics and metatranscriptomics sequencing in combination with metabolomics. Improved DNA extraction for long DNA fragments and long-read sequencing will enable the assembly of complete BGCs from the sediment microbiome. Metatranscriptomics will be used to find active BGCs and reveal their expression patterns. Additionally, metabolomic analysis will identify potential secondary metabolites produced by these microbiomes. The omics-results will be compared to environmental data to model any influence of ecological parameters on abundance and expression of BGCs. Preliminary results indicate that deep Nanopore sequencing can assemble hundreds of complete BGCs, most of which are matched with any sequences in the databases. Through the integration of these multi-omics data with ecological modelling, we will explore the ecological roles of secondary metabolites within the marine sediments and their influence on marine microbiomes.

Poster Abstract

[P60] Metabolic activity of vaginal microbiome

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According to WHO, adult infertility affects 17.5% of the global population. Recent studies have shown that both vaginal and semen microbiomes influence infertility for both genders and are a possible treatment target. In this study, the symbiosis of the different bacterial species and viruses will be tested based on the community state types classification. Differences in the vaginal microbiota of healthy and infertile individuals will be investigated. Anonymous vaginal samples are collected from students of Roskilde University which will be characterised in the 4 community state types. The classification of the samples will be conducted by microcalorimetry to identify the different bacterial species and viruses of the microbiome and by amplicon metagenomic sequence to investigate the composition of the microbiome. A fermentation model will be used to investigate the symbiosis/dysbiosis of the bacterial species for the 4 community state types as well as the effect of viruses on the microbiota. Finally, for this model, proteomics and metabolomic analyses will be conducted to detect any changes in the protein and metabolic activity in the microbiomes. By integrating microbiota analysis into clinical infertility diagnostics, both the vaginal and semen microbiomes can be selected as possible treatment targets to solve any dysbiosis-related infertility issues.

[P61] Advancing cultivability of elusive soil microorganisms via Live-Fluorescence in situ Hybridization

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Advances in next-generation sequencing and metagenomics have revealed a vast diversity of uncultivated microorganisms within environmental microbiomes. To overcome the challenge of cultivating these elusive taxa, Live-Fluorescence in situ Hybridization (Live-FISH) coupled with Fluorescence-Activated Cell Sorting (FACS) has emerged as a promising approach, enabling selective enrichment of specific microbial taxa for downstream cultivation. Here, we evaluated the applicability of Live-FISH on soil microbiomes, focusing on its potential to unlock untapped biosynthetic capabilities of previously uncultured soil bacteria. Live-FISH treatment had a significant negative impact on the viability of soil microorganisms as indicated by propidium monoazide viability qPCR followed by sequencing. The results showed a reduction in 16S rRNA gene copies from viable cells, along with an overall decrease in cell viability. We also observed significant shifts in the structure of viable microbial communities following Live-FISH treatment, compared to untreated samples, revealing its impact on the viability of specific community members. Interestingly, certain dominant phyla responded differently to the Live-FISH treatment. Particularly, Planctomycetota and Firmicutes showed resilience, indicating their potential for subsequent cultivation. In contrast, Proteobacteria, Actinobacteriota, and Acidobacteriota showed high sensitivity, suggesting that Live-FISH may have limited utility for these taxa. To validate the potential of Live-FISH, we are applying it to model strains and a soil community using Planctomycetota- and Firmicutes-targeting probes to validate the post-treatment survival of members of these taxa. Collectively, coupling Live-FISH with FACS may facilitate the isolation of promising strains of e.g. the elusive Planctomycetota and unlock the biosynthetic potential of previously unculturable microorganisms.

[P62] Origin and development of two vertically transferred Escherichia coli clones causing colibacillosis in poultry farm

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Whole genomic sequencing including hybrid assemblies of short and long reads to analyse clonal persistence and evolution of avian pathogenic Escherichia coli (APEC). Two vertically transferred E. coli clones, represented by ten isolates from PFGE type 65-ST95 and eight isolates from PFGE type 47-ST131, were selected to identify genomic variations. The isolates had been sampled in broiler production during a period of nine months in a previous study. The most recent common ancestors were estimated back to 2009 for PFGE65-ST95 and to 2011 for PFGE47-ST131, with further divergence occurring in years until sampling in 2014-2015. The genome-wide mutation rate was equivalent to 1.48 mutations per genome per year for PFGE65-ST95 and 2.86 for PFGE47-ST131.The main differences between strains within each clone were related to plasmids, transposases and an incomplete phage which by far exceeded the genetic variation related to point mutations. The methodology introduced in the investigation is able to trace the temporal origin APEC clones in future studies. The investigation challenges the current approach to WGS based tracing of APEC clones since differences in plasmid content, bacteriophage elements, transposons and divergence in coding gene sequences indicate a much higher diversity than SNPs of core genomic elements.

[P63] High throughput detection and sorting of substrate labelled microbial clusters

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Traditional microbiology relies heavily on isolation of pure cultures; however, this is possible only for a small fraction of bacteria. Many keystone microbes in environmental processes are difficult or impossible to culture. They are relegated to studies in mixed cultures, often with tens to hundreds of other microbial types, which complicates the exploration of their individual physiology. To address this, we aim to enable detection and investigation of a microbe of interest together with a small set of accompanying bacteria, forming a tight cooperative community.

This study presents a high-throughput platform for sorting microbial clusters using Raman spectroscopy in a viscoelastically focused stream of microbial clusters within a capillary. This method allows labelling of active cells of the target microbe using deuterium, together with a Raman fingerprint of the cluster it is part of. Based on researcher defined criteria, clusters can then be captured and sent for further analysis or cultivation. We are showing benchmarked results of limit of detection of deuterium labelled bacteria using defined synthetic microbial clusters as well as identification of substrate labelled environmental samples from environmental and engineered communities. Study of simple cooperative communities takes another step beyond traditional pure-culture based microbiology, which does not reflect the typical lifestyle of microbes, and enables broader exploration and application of microbial assemblies.

[P64] Nanopore metagenomic sequencing for rapid bloodstream infection diagnostics evaluated in a prospective observational study

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The gold standard of clinical microbiology diagnostics is to culture the pathogen, which takes 1-3 days and has poor sensitivity. Due to the long turnaround time, critically ill patients, such as those suffering from a bloodstream infection, are administered empiric broad-spectrum antibiotics prior to pathogen identification and an estimated ~20% of patients receive ineffective treatment causing excess mortality.

We present the results from a prospective observational clinical study, where blood samples were collected from patients admitted upon suspicion of bloodstream infection to the Emergency Department at Aalborg University Hospital in Denmark (N=186). Study samples were taken in parallel with samples for routine microbiological diagnostics for comparison. A subcohort (N=40) was analysed consisting of all patients with a positive blood culture (BSI-confirmed: N=11), a negative blood culture with high suspicion of a bloodstream infection (BSI-suspected: N=25), or a negative blood culture with no suspicion of an infection (BSI-absent: N=4). Plasma cell-free DNA was isolated and subjected to Nanopore metagenomic DNA sequencing. In short, all pathogens found in patients from BSI-confirmed were found with plasma metagenomic sequencing. Further, in 12 patients from the BSI-suspected group metagenomic sequencing identified one or more clinically relevant pathogens. Pathogen DNA load was quantified using DNA spike-ins and varied from 0.5-320 genome equivalents per µL, which is in accordance with previously reported DNA titers. No pathogens were identified in the BSI-absent samples with plasma metagenomic sequencing.

These results suggest that the application of Nanopore metagenomic sequencing to bloodstream infection diagnostics is feasible, and the method holds promise as a rapid and sensitive approach that enables aetiology determination within hours of blood draw.

[P65] Microbial bioremediation to tackle PFAS contamination

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Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic compounds produced for their unique properties. The extensive use of PFAS, combined with their inherent resistance to degradation, results in their persistence in both the environment and the human body, where their prolonged biological half-life contributes to bioaccumulation and potential toxicological risks. Firefighting foam used at training facilities is a significant source of environmental PFAS pollution, often resulting in extreme concentrations. Although challenging, microbial bioremediation for PFAS has been an attractive approach. This study focused on screening for bacteria capable of co-metabolizing PFAS. Twenty-two soil and water samples were collected from PFAS-contaminated firefighter training sites in Denmark (Korsør and Værløse). Bacterial communities from these environmental samples were diluted and screened for potential perfluorooctanoic acid (PFOA) degradation. A colorimetric high-throughput defluorination assay was then used to detect fluoride release (F-), indicating PFOA degradation. Samples that exhibited defluorination were selected for further analysis through genome sequencing of the microbial communities. This analysis identified several notable bacterial genera previously linked to the degradation of recalcitrant pollutants, including Sphingomonas, Pseudomonas, and Stenotrophomonas. Preliminary metabolomic analyses reveal that shorter chain PFAS accumulates in defluorinating samples, indicating microbial defluorination of PFAS. Cultivating defluorinating strains in the presence of PFOA was not yet possible, likely due to cellular stress from released fluoride. Future work in this project will focus on isolating pure defluorinating bacterial strains.

[P66] Fit-for-purpose binding proteins for in situ blocking of C. difficile toxins

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Background and Aims: Clostridioides difficile infection (CDI) is a severe gastrointestinal infection affecting individuals with a dysbiotic gut microbiota, often following antibiotic treatment. Bacterial spores germinate and secrete cytotoxin A (TcdA) and B (TcdB), causing severe tissue damage. Since C. difficile colonizes the distal colon, oral delivery of antibody-based toxin inhibitors could be advantageous for early toxin neutralization, before disease onset, protecting gut barrier integrity until the healthy microbiota is restored.

Through a developability selection process we have engineered BL5-6.2, a specific binding protein comprising two monomeric single-domain antibodies (VHHs). BL5-6.2 demonstrates high functional stability in simulated gut conditions. Furthermore, we investigate the neutralizing capacity of BL5-6.2 across various cell models to understand its capacity to protect cell barrier integrity and to study it's effects on TcdB induced epithelial inflammatory activation. Additionally, we evaluated the protective effect a newly developed TcdA blocking VHHs, BL7.1, BL8.1, and BL8.2 in various cell lines.

Methods: The functional stability of BL5-6.2 was assessed by incubating the binding protein in simulated gastric conditions and measuring its binding capacity via ELISA. Since mammalian cells vary in susceptibility to TcdA and TcdB cytotoxicity, multiple cell models were used to evaluate the blocking ability of the binding proteins. TcdB neutralization assays were performed on two human colon adenocarcinoma cell lines. In HCA7 cells, BL5-6.2's effect on toxin-induced loss of viability was measured using a luminescent assay (CellTiter-Glo), while in differentiated Caco-2 cells, the WST-1 assay was used. Concentration of TcdB used were 100 ng/ml and 10 ng/ml for HCA7 and Caco-2 cells respectively. Additionally, BL5-6.2's impact on toxin-induced inflammation was assessed by measuring IL-8 levels via quantitative ELISA. The performance of BL5-6.2 was compared to Zinplava and a VHH Benchmark, all tested at the same molecular:toxin ratio. TcdA neutralization assays for BL7.1, BL8.1, and BL9.1 were conducted on a highly TcdA-susceptible non-human cell line (Vero) and human colon Caco-2 cells at toxin doses of 40 ng/ml and 50 ng/ml, respectively, using WST-1 assay. For statistical analyses, One-way Anova, selected pairs was used with Bonferroni post hoc test or the Mann-Whitney U test was used.

Results: The heterodivalent BL5-6.2 binds two non-overlapping epitopes and retains its binding capacity after exposure to simulated gastrointestinal conditions, with binding ranging from 64% (±5) at pH 1.2 with pepsin to 98% (±8) without pepsin. It also maintains 50% (±4) to 88% (±3) of its binding capacity in the presence of various bile salts. Three VHHs were selected for their nanomolar affinity to TcdA. Both TcdA- and TcdB-blocking binding proteins appeared to neutralize cytotoxicity in vitro in colonic cell lines. TcdB reduced Caco-2 cell viability to 63% (±8) compared to 100% for medium controls ($p<0.05$). Cell viability was maintained at controls levels by BL5-6.2, Zinplava, and the VHH Benchmark. TcdB induced an inflammatory response in Caco-2 cells, measured by IL-8, with the VHH Benchmark showing an increasing trend. However, BL5-6.2, like Zinplava, did not alter TcdB-induced IL-8 release, keeping inflammation significantly lower than with the VHH Benchmark

Conclusions: We show that employing specific binding proteins, comprised of VHH constructs, we can effectively block TcdA and TcdB cytotoxicity in vitro across several colonic cell lines and models emulating epithelial barrier function. BL5-6.2 largely prevented TcdB induced viability loss without affecting TcdB induced IL-8 release, the latter is required for a proper viral defense response. The binding proteins are suitable for oral use and may show applicability for in situ toxin blocking to protect the colon in dysbiotic individuals exposed to C. difficile.

[P67] Microbial degradation of glucose and dodecane and their impact on the microbial cryoconite community on the Greenland Ice Sheet

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Cryoconite holes are water-filled depressions on the surface of glaciers or ice sheets inhabited by an active and diverse microbial community. There is an input of carbon compounds from natural and anthropogenic sources to the Greenland ice sheet (GrIS), and the ability of cryoconite microbes to degrade these compounds is probably an important factor determining their fate on the ice and in downstream ecosystems.

We tested the ability of the GrIS cryoconite microbial community to degrade glucose and dodecane under simulated environmental conditions. The samples were incubated with the individual 14C labeled compounds at two concentrations and the mineralization to 14CO2 was measured. We demonstrate that microorganisms present in cryoconite holes, under the tested conditions, can mineralize glucose and dodecane. Differences in the shape of the mineralization curves indicated a response of the microbial community related to the compound and its concentration. qPCR coupled to high-resolution melt curve analysis showed an increase in bacterial and fungal cell numbers. A shift in the 16S rRNA gene profile was observed in the incubation with a high glucose concentration, while the fungal community shifted during incubation with dodecane. TotalRNA sequencing showed that the active microbial community was dominated by bacteria belonging mainly to the phyla Proteobacteria, Actinobacteria and Acidobacteria, but also contained Chlorophyta and low relative abundances of Cercozoa and Chytridiomycota. The samples incubated with glucose showed a shift in the microbial community over time that is hypothesized to follow a series of growth and predation events involving green algae, parasitic fungi, bacteria and microeukaryotes. In contrast, incubation with dodecane led to only small changes in the microbial community. Metatranscriptomic analysis revealed an influence of the compounds on expression of genes, for example involved in photosynthesis, transcription and transport.

[P68] Modulation of infant fecal microbiota resistant starch utilization by Ruminococcus bromii

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Gut microbial activities play an important role in the development of the immune system. Early colonization by certain bacteria taxa such as Ruminococcus bromii has been suggested to prevent infant atopic dermatitis (AD). R. bromii is known as a key degrader of starch and its appearance in colon may be during the switch from liquid to solid diet during the first year of life. However, the interdependencies of the diet, gut microbial activity and interaction, and ultimately the protection against AD development at infancy have not been fully understood. In this study, we compared the utilization of resistant starch (RS) by the primary degrader R. bromii or Bifidobacterium spp., RS crossfeeding with butyrate producer Anaerobutyricum hallii and investigated the impact of nutritional and microbial interventions on infant fecal microbiota.

When supplied with RS, R. bromii accumulated reducing carbohydrates from RS while Bifidobacterium spp. produced a lot of lactate, formic acid, acetate and ethanol instead of reducing carbohydrate. In co-culture fermentations, A. hallii was able to cross-feed on glucose, maltose, and oligosaccharides generated by R. bromii, or lactate produced by Bifidobacterium spp.; the R. bromii/A. hallii co-culture yielded higher levels of butyrate than the B. adolescentis/A. hallii. Firsts result from in vitro infant fecal batches fermentation showed the addition of starch but not of R. bromii or Bifidobacterium spp. harvested the higher SCFA concentrations, particularly acetate, as well as affected the proportion of gut microbiota community. We are currently determining microbiota profiles using 16S rRNA gene long read by nanopore sequencing technology.

Together, our data suggested the presence of R. bromii can extend the utilization of RS or other non-digestible carbohydrates in gut and hereby enhance gut microbial cross-feeding activity leading to formation of SCFAs, particularly butyrate. Indeed, the primary degraders initiate different microbial cross-feeding activities, which might be crucial for the formation of butyrate in the gut.

[P69] Investigating biotic and abiotic factors influencing the expression of non-ribosomal peptide synthetases in marine sediments.

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Microorganisms produce a plethora of secondary metabolites involved in e.g. intercellular signalling, interference competition, and mutual sustenance. Targeted sequencing of conserved genes involved in the biosynthesis of one of the major secondary metabolite classes, non-ribosomal peptides (NRPs), have uncovered their extensive distribution in the unculturable majority of microorganisms in nature. Marine sediments have shown to be particularly rich in novel NRP-encoding genes. To what extent this NRP diversity is expressed and what role these metabolites serve in this niche remain unknown. Hence, the purpose of the present study is to uncover how large a fraction of the NRP biosynthetic potential is actively expressed, and upon which cues. Specifically, we aim to investigate the influence of both biotic and abiotic factors on the transcription of conserved biosynthetic adenylation (AD) domains of NRP synthetases (NRPSs) in a sediment microcosm experiment. Through addition of biotic proxy compounds (bacterial signaling molecules and antibiotics produced by marine microorganisms), we will determine to what extent NRP expression is affected by biotic factors. For abiotic factors, we will include treatments with increased nutrient content and reduced oxygen tension to infer if NRP expression may aid in competition for organic matter or serve specific roles under transiently anerobic conditions in the sediments. By comparing the transcriptional activity of NRPSs through amplicon sequencing of cDNA of AD domains in different treatments at different time points, we may be able to infer the ecological roles of NRPs in marine sediments microbiomes.

[P70] Isolation and Characterization of Lytic Pseudomonas aeruginosa Bacteriophages PaCCP1 and PaCCP2

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Antimicrobial resistance (AMR) is a major public health threat, exacerbated by the lack of new antibiotics and the emergence of superbugs characterized by resisting multiple treatments. Comprehensive efforts and alternative strategies to combat AMR are urgently needed to prevent significant social, medical, and economic consequences. Pseudomonas aeruginosa is a pathogen responsible for a wide range of infections, from soft tissue infections to life-threatening conditions such as bacteremia and pneumonia. Bacteriophages have been considered a potential therapeutic option for treating bacterial infections. In this study, we aimed to isolate phages capable of infecting P. aeruginosa. We successfully isolated two lytic phages using the conventional double-layer agar (DLA) technique from samples obtained from the influent of a wastewater treatment plant in Concepción, Chile. The isolates, designated PaCCP1 and PaCCP2, were observed via transmission electron microscopy (TEM), and their host range was determined against multiple P. aeruginosa strains using the DLA method. Additionally, their genomes were fully sequenced and analyzed. Phage PaCCP1 belongs to the genus Septimatrevirus and is capable of infecting at least 4 strains of P. aeruginosa, while phage PaCCP2, a member of the genus Pbunavirus, can infect at least 2 strains. Electron microscopy revealed that both phages are tailed and possess an icosahedral head. The genome of PaCCP1 comprised 43,253 bp with a GC content of 54.4%, encoding 60 ORFs, including one tRNA gene. In contrast, PaCCP2 has a genome size of 66,333 bp, with a GC content of 55.6%, encoding 102 non-tRNA ORFs. PaCCP2 contains a putative Anti-CRISPR gene. Neither phage harbors virulence or antibiotic resistance genes, suggesting its potential to control P. aeruginosa, a clinically significant pathogen in both human and animal health.

[P71] Structural Insights and Bioprospecting of the Deep-Sea Sponge Microbiome

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The deep ocean is the largest biome on Earth, accounting for >90% of the planet's marine environment. Sponges (phylum Porifera) harbour unique microbial communities which are a rich source of bioactive compounds for antimicrobial discovery. Much of the work to date has focused on sponges from warm and shallow coastal waters, while sponges from the deep ocean remain less well-studied. Our research initially focused on a metataxonomic assessment of the structure and diversity of the microbial community inhabiting these underexplored deep-sea sponges, revealing intricate, species-specific microbial communities. We uncovered communities dominated by ammonia-oxidizing archaea and reveal a strong influence of sponge phylogeny on microbial composition (1).

Secondly, we bioprospected the culturable members of the microbiome including the isolation of novel deep-sea species from genera including Streptomyces, Micromonospora, Bacillus and Stappia. Two of the novel species - Micromonospora robiginosa sp. nov. and Streptomyces ortus sp. nov. - demonstrated impressive bioactivity against MDR Gram-positive pathogens (2). We performed molecular networking analysis to identify two large clusters of anthracycline like compounds, NMR analysis identified two of the compounds as the known antibiotics kosinostatin and rudolphomycin. This highlights the deep-sea sponge microbiome as an untapped reservoir for novel microbial life with significant biosynthetic capabilities.

In conclusion, our study underscores the underexplored deep-sea sponge microbiome as a fertile source of antimicrobial agents and contributes to enriching our understanding of unexplored deep-sea microbial ecology.

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2 - Williams SE, Back CR, Best E, Mantell J, Stach JEM, Williams TA, et al. Discovery and biosynthetic assessment of "Streptomyces ortus" sp. nov. isolated from a deep-sea sponge. Microb Genom. 2023 May 1;9(5):000996.

[P72] Epidemiology of Nontuberculous Mycobacteria in Denmark: Seven Decades of Clinical Occurrence

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The incidence and prevalence of nontuberculous mycobacteria (NTM) disease and colonisation continue to rise and remain a challenge worldwide. Taking advantage of the International Reference Laboratory of Mycobacteriology (IRLM)´s unique historical collection of freeze-dried mycobacterial isolates at Statens Serum Institut (SSI) in Copenhagen, the changes in NTM epidemiology, clinical manifestation and species distribution in humans over the past seven decades were investigated. This study compared historical NTM-designated isolates (n = 666) from human samples between 1948 and 1978 to contemporary NTM isolates (n = 2485) identified in the IRLM routine diagnostics between 2013 and 2022. All species determinations were performed using the Hain GenoType CM assay, which differentiates between 27 of the most common mycobacteria in clinical settings today. CM of historical isolates resulted in 16 different identifications, with the majority classified as M. species (32.13%). In contrast, recent isolates were dominated by M. avium (40.97%), and M. species accounted for only 7.0%. Among the nine most frequent characterizations, only M. avium and M. abscessus showed significant changes over time, with an increasing prevalence in recent years, consistent with globally reported data. Further species determination by whole genome sequencing was performed for many historical isolates characterised as M. species. This led to the uncovering of several species that are not usually found in diagnostic settings today.

In conclusion, these findings underscore a significant shift in the species responsible for mycobacterial infections in Denmark over the past seven decades. This shift, likely to have clinical implications, reflects the changing epidemiology of NTM. However, it is important to note that societal and environmental changes over the past 70 years should also be considered when interpreting these findings.

[P73] Exploring the microbiome of Danish marine bryozoans: Identification of Phaeobacter spp. isolates

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Due to the rising antibiotic resistance crisis, efforts have been directed towards exploring alternative microbial antibiotic sources such as marine bacteria. One example is Phaeobacter spp., and its antibacterial metabolite tropodithietic acid (TDA). Previous studies suggests that marine bryozoans could potentially be a niche for Phaeobacter spp. The knowledge of ecological niches of antibiotic producing microorganisms is an essential part of the discovery of natural products used for antibiotics. The purpose of this project is to determine if marine bryozoans are indeed a natural niche of potential antibiotic producing Phaeobacter.

Bacteria isolated from bryozoans sampled from three geographical locations: Trykkerdammen, Lillebælt, and Hunderevet were examined. Isolates were derived from two marine bryozoans, Electra pilosa and Membranipora membranacea. Nanopore sequencing of bacterial genomes and subsequent analysis revealed that all isolates were Phaeobacter spp. and were proposed to belong to a new species, as they had ANI lower than 95% with known Phaeobacter spp. Furthermore, all isolates had the genetic potential to produce TDA based on the detection of the TDA operon and TDA genes. Antagonistic assays displayed inhibition of Vibrio anguillarum, and TDA was detected in all pure isolate cultures using HR LC-MS/MS, thus proving that all isolates can produce TDA. No significant difference was found between the production of TDA and the origin of the bacteria isolates (bryozoan species or geographical locations), suggesting that the isolates could be more driven by their ecological niche, the marine bryozoans, than their geographical location. Thus, indicating that a variety of marine bryozoan species could be hosts for TDA-producing Phaeobacter spp., and that marine bryozoans could be considered an ecological niche.

On-going work includes a metataxonomic analysis of the bryozoan microbiome to determine which bacteria can co-exist with the TDA-producing bacteria.

[P74] Anti-Quorum Sensing Phages Silence the Phage-mediated PQS Stress Response in Pseudomonas aeruginosa

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The alarming spread of antibiotic resistance calls for an urgent need to develop alternative therapies. A promising strategy is bacteriophage (phage) therapy, which uses natural enemies of bacteria, phage viruses, to kill bacteria. However, bacteria fight back by launching virulence programs and anti-phage defenses, greatly limiting the effectiveness of phage therapy. To do so, bacteria rely on cell-cell communication, known as quorum sensing (QS). The human pathogen Pseudomonas aeruginosa has multiple intertwined QS systems, including the Las, Rhl and PQS system. The Pseudomonas quinolone signal (PQS) plays a key role in pyocyanin virulence factor production and serves as a warning signal emitted by phage-infected cells, which causes uninfected cells to physically evade phage-infected areas. Here, we explore the potential of using anti-PQS phages to disrupt PQS signaling, limiting both virulence and anti-phage defense activation in P. aeruginosa. We have engineered anti-PQS phages to effectively degrade PQS, rendering P. aeruginosa less virulent and more vulnerable to phage attack. We show that our anti-PQS phages effectively limit PQS accumulation. Using a Galleria mellonella wax moth larvae infection model we find that our engineered phages safeguard P. aeruginosa-infected G. mellonella larvae. Furthermore, we demonstrate that the anti-PQS phages silence the phage-mediated PQS stress response in P. aeruginosa. Thus, anti-QS phages may be particularly useful for therapy.

[P75] Co-cultivation enhances inhibition of fish pathogen Vibrio anguillarum by algal-associated bacteria

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Secondary metabolites play a key role in mediating interactions within microbial communities and have different biological activities, such as pathogen inhibition. Whole-genome sequencing revealed that many bacteria possess a greater secondary metabolite potential than what is observed under laboratory conditions. Standard laboratory cultivations lack the complex abiotic and biotic factors influencing microbial interactions in the environment and traditional single strain culturing often leads to rediscovery of known compounds. Co-cultivation approaches, on the other hand, offer a promising strategy for activating silent biosynthetic gene clusters (BGCs) and unlocking novel bioactivity.

In this study, bacterial strains isolated from the microbiome of the microalga Isochrysis galbana were investigated in an agar-based assay. The microbiome as a community inhibited the fish pathogen Vibrio anguillarum, however, amongst the isolated pure cultures (Alteromonas, Halomonas, Phaeobacter, Roseovarius and Sulfitobacter spp.), only Phaeobacter sp. inhibited the pathogen in monoculture. However, the co-culture of Sulfitobacter pontiacus D3 and Halomonas campaniensis D2 also inhibited V. anguillarum, which was not observed in their respective monocultures. Whole-genome sequencing and antiSMASH analysis revealed that S. pontiacus D3 harbors four and H. campaniensis D2 seven BGCs, potentially encoding antimicrobial compounds. MALDI-MSI analysis suggested a class of compounds that may be involved in inhibition. LC-MS analysis of agar plug extracts from the inhibition zone detected these compounds, although at low concentrations. Therefore, upscaled liquid co-cultures with different initial inoculation ratios were prepared, and their extracts produced faint inhibition zones, confirming bioactivity. Interestingly, the same inhibitory phenotype was observed when co-culturing H. campaniensis D2 with another algal microbiome member, Ruegeria sp. D4. MALDI-MSI indicated overlapping compound profiles between the two co-cultures. Current efforts focus on concentrating active extracts for further LC-MS analysis, followed by bioassay-guided fractionation in hope to identify the specific metabolites responsible for the observed inhibition.

[P77] Phylogenetic conservation of EPS production - a case study of zooglan

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Biofilms are ubiquitous and crucial in various environments, yet they often remain understudied, particularly in environmental and engineered systems with their microbial producers being poorly described. Among these, wastewater treatment plants (WWTPs) offer an ideal model for studying microbial biofilms and flocs, particularly Zoogloea, a key organism for understanding extracellular polymeric substance (EPS) biosynthesis. In the REThiNk project, we aim to use EPS from WWTPs to create sustainable biopolymers as alternatives to oil-based plastics. However, knowledge gaps in EPS composition, formation, and microbial producers must be filled before commercialization. This study aims to uncover the EPS production in Zoogloea through molecular studies along with mapping its phylogenetic distribution in other bacterial lineages to understand the extent of EPS biosynthesis conservation.

Recent discoveries have highlighted the importance of an extracellular polysaccharide (exoPS) gene cluster, comprising 26 genes, in Zoogloea resiniphila encoding machinery for zooglan production. Besides being conserved in the entire Zoogloea genus, the zooglan gene cluster is widely distributed in the Burkholderiales order, mainly in the Rhodocyclaceae family suggesting its critical role in biofilm/floc formation. However, it is not consistently detected in phylogenetic restricted groups, meaning species-level differences within genera, families and orders exist. To validate the structure and conservation of exoPS production, EPS has been extracted from three isolates (Z. caeni, Z. resiniphila, and Z. oleivorans) which are to be used for chemical and structural analyses to verify genomic findings.

Uncovering the phylogenetic distribution of EPS biosynthesis systems is crucial for predicting the production of EPS in microbial systems. This is especially significant when aiming to recover these components.

[P78] Spatially resolved multi-omic landscape of the animal gut microbiome

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Spatial organization is a pervasive feature of natural ecosystems, and the animal gut is no exception. Hidden within animals is a collection of bacteria associated with the intestinal tract, organized in a complex structure that changes alongside variations in the gut habitat. While much has been done to explore the large-scale diversity of the gut microbiota, the fine-scale spatial organization of this complex ecosystem remains largely unexplored. Current approaches used to study the microbiota from fecal material or intestinal contents require homogenizing the biological material. Consequently, these methods are unable to reveal the gut microbial biogeography and fine-scale spatial structure of microbial communities in situ. Due to this lack of resolution, we are missing relevant information, which could contribute to understanding the mechanistic underpinnings of microbial interactions.

The project 3D'omics aims to generate spatially referenced metagenomics, metatranscriptomics and metabolomics data to understand the bacterial landscape in the chicken gut. This poster focuses on the micro-scale metagenomics technology complemented by highly multiplexed fluorescence in situ hybridization (FISH) to visualize and study the microbiota down to a genus and even strain level resolution. Future aims of the project involve querying the metatranscriptomic and metabolomic landscape in a spatial manner and eventually expanding the 3D'omic techniques to study larger livestock such as swine.

[P79] From import to breakdown: Characterisation of the phosphonate transporter PhnCDE in Escherichia coli

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Phosphate is an essential nutrient for cell growth but is often scarce in natural environments. To tackle this, Escherichia coli contains the 14-cistron phn operon (phnCDEFGHIJKLMNOP) encoding C-P lyase that enables extraction of phosphate from phosphonate compounds, characterised by a stable carbon-phosphorus (C-P) bond.
Structures of dodecameric C-P lyase, PhnGHIJKL, revealed an atypical use of two separate ATP-binding cassette (ABC) dimers (PhnK and L), which has raised interest in the phosphonate importer, PhnCDE, also containing an ABC module, PhnC.
Understanding the mechanism of how phosphonates are imported into bacterial cells and passed on to the C-P lyase machinery is therefore of great interest.
Here, we express PhnCDE recombinantly for structural studies by cryo-electron microscopy. The periplasmic protein PhnD is soluble and can readily be expressed and purified. On the other hand, co-expression PhnC and PhnE tagged for purification is challenging and sequence analysis suggests that PhnE is misannotated in the E. coli genome. Consequently, a large improvement of expression was obtained when including a non-annotated longer N-terminus of PhnE. The longer N-terminus is predicted to be intrinsically disordered, which suggests there may be uncharacterised interaction partners.

To investigate this hypothesis, we use the BioID method to screen for new protein-protein interactions using several Phn proteins as bait, to see if the pathway of phosphonate breakdown expands beyond the phn operon.

Ultimately, further mechanistic knowledge about PhnCDE will provide us with new insights into the breakdown of phosphonate compounds.

[P80] Characterising microbial community interactions among pathogens in an in vitro chronic wound model

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Chronic wounds often harbor polymicrobial biofilms that frequently display high antimicrobial tolerance and persistence, therefore leading to worse patient outcomes relative to monospecies infections. This project set out to determine the best in vitro model to investigate the dynamics between four opportunistic human pathogens, namely Acinetobacter baumannii AB5075, Enterococcus faecalis OG1RF, Pseudomonas aeruginosa PA14 and Staphylococcus aureus USA300, that are frequently co-isolated from chronic wounds and are on the ESKAPE pathogen priority list. Traditional rich-media in vitro experiments often favor a singular pathogen out of a multi-species mixture, with one species dominating within 24 hours. This makes it complicated for multi-omic analyses due to the scarcity of RNA and metabolites produced by low-abundant members in these communities.

We found that in an in vitro wound-like model (WLM), these four pathogens proliferate within 24 hours and can be passaged together over the course of 5 days, making it a suitable model to study community dynamics and co-evolution. Through whole genome sequencing, we identified specific mutations that occur when these bacteria are passaged together. Carrying out combinatorial co-culture experiments, we found that this chronic wound model allows otherwise out-competed gram-positive bacteria to survive likely due to coagulases produced by S. aureus, creating anoxic microniches. This is evidenced by the formation of microaggregates that penetrate deeper into the fibrous clots in the WLM. Metatranscriptomics analysis revealed differential gene expression profiles corresponding to shifts in metabolic states of bacteria when co-cultured in WLM. To further elucidate the complex interactions between these bacteria in chronic wounds, we have additionally applied a combination of fluorescence in-situ hybridization and metabolomics.

[P81] Protective effects of multispecies biofilms against bacteriophage predation; the importance of spatial organization and matrix production

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Bacterial viruses (bacteriophages/phages) threaten bacterial populations by specific infection, exerting a large ecological and evolutionary impact. Most bacteria-phage research has focused on homogenous monospecies planktonic cultures, with limited relevance to bacterial life in nature and disease, where bacteria mainly reside in structured heterogeneous biofilm communities of several intermixed species.

To gain more insight in bacteria-phage dynamics in a multispecies biofilm setting, we challenged a synthetic, three-species community comprising Escherichia coli, Kluyvera cryocresens and Vibrio anguillarum with lytic phages. Utilizing confocal laser scanning microscopy and quantitative image analysis, we found that E. coli was eradicated by the T7 coliphage in a monospecies biofilm but was well protected when mixed with and covered by the two other species in the multispecies biofilm. Furthermore, in dualspecies biofilms, we observed that V. anguillarum provided a high level of phage protection to E. coli, leading to its survival, while K. cryocresens conferred little to no protection.

Next, we methodically deleted biofilm associated genes in V. anguillarum and discovered that the rbmC gene and the vpsMNOP gene cluster encode products important for the V. anguillarum-mediated phage protection of E. coli. A similar trend was also observed when testing the V. anguillarum mutants' ability to protect K. cryocresens from the kluyveraphage Lyn, which we isolated from a wastewater facility. Further investigation revealed that these V. anguillarum mutants formed biofilms with significantly lower cellular density compared to the wildtype. Thus, we suggest that the deletion of these matrix genes in V. anguillarum results in a less dense biofilm, allowing the phage to penetrate the biofilm matrix and reach the E. coli cells residing within. This strongly indicates communal protective effects of the biofilm matrix towards phage infections in a multispecies biofilm setting.

[P82] Identifying the regions of pathogen metabolic activity and dormancy in tissue models in vitro

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Pseudomonas aeruginosa is a key pathogen in chronic lung infections and ventilator-associated pneumonia. Examining in vitro infections in lung tissue cultures provides insights into infection dynamics and crucial host-pathogen interactions. However, the spatial and temporal patterns of pathogen growth versus dormancy within tissue structures remain unclear. In this work, we demonstrate where pathogens exhibit active growth and where dormancy is established, using strains engineered to report growth activity. These strains express an unstable green fluorescent protein and are analysed with confocal laser microscopy. Additionally, we investigate the influence of pathoadaptive mutations on the location of these regions. Dormant subpopulations are clinically significant due to their increased resistance to antimicrobials, contributing to infection persistence. By employing growth-reporter strains for real-time analysis, we aim to explore strategies for targeted treatment of dormant populations using drugs and drug-delivery systems in vitro.

[P83] The microbial biogeography of Denmark

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Microbial populations are not randomly distributed at geographical scale but posses characteristic biogeographies. However, we still do not fully understand what governs those patterns. In the past centuries, macroecologists have distilled some broad rules to explain biological assemblages at scale, but many of those rule do not apply to the microbial world, have not been tested yet or have seemingly impossible mechanics. The aim of this work is to explain and predict the microbial biogeography via the integration of the individual processes shaping the communities at scale. We specifically addressed how environment, spatial distribution, phylogeny and species' traits contributed to biogeography. To achieve this we integrated multiple large-scale datasets covering Denmark at an unparalleled resolution. We quantified over 19,000 dereplicated metagenomic assembled genomes across 10,000+ metagenomic samples covering Danish soils, waters and sediments. The environmental landscape was reconstructed via the aggregation of 250+ 10m-resolution maps of Denmark encoding for parameters such as carbon, clay and silt content and 70+ LiDAR parameters (e.g. canopy openness, solar radiation, etc.). We built community models identifying the realized niches of the microbes, proposing species-specific mechanistic hypotheses for the emergence of a microbial biogeography in Denmark.

[P84] The Spx stress regulator confers high-level β-lactam resistance and decreases susceptibility to last-line antibiotics in MRSA

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Infections caused by methicillin-resistant Staphylococcus aureus (MRSA) are a leading cause of mortality worldwide. MRSA has acquired resistance to next-generation β-lactam antibiotics through the horizontal acquisition of the mecA resistance gene. Development of high resistance is, however, often associated with additional mutations in a set of chromosomal core genes, known as potentiators, which, through poorly described mechanisms, enhance resistance. The yjbH gene was recently identified as a hot spot for adaptive mutations during severe infections. Here, we show that inactivation of yjbH increased β-lactam MICs up to 16-fold and transformed MRSA cells with low levels of resistance to being homogenously highly resistant to β-lactams. The yjbH gene encodes an adaptor protein that targets the transcriptional stress regulator Spx for degradation by the ClpXP protease. Using CRISPR interference (CRISPRi) to knock down spx transcription, we unambiguously linked hyper-resistance to the accumulation of Spx. Spx was previously proposed to be essential; however, our data suggest that Spx is dispensable for growth at 37°C but becomes essential in the presence of antibiotics with various targets. On the other hand, high Spx levels bypassed the role of PBP4 in β-lactam resistance and broadly decreased MRSA susceptibility to compounds targeting the cell wall or the cell membrane, including vancomycin, daptomycin, and nisin. Strikingly, Spx potentiated resistance independently of its redox-sensing switch. Collectively, our study identifies a general stress pathway that, in addition to promoting the development of high-level, broad-spectrum β-lactam resistance, also decreases MRSA susceptibility to critical antibiotics of last resort.

[P85] Niche differentiation of cable bacteria species in the rhizosphere of Spartina alterniflora phenotypes

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Coastal ecosystems are essential for biodiversity, element cycling, and shore protection at the land-sea interface. Much of the USA's Eastern coastline is protected by saltmarshes with the cordgrass Spartina alterniflora that occurs as tall type phenotype (TT) at the water interface, and short type (ST) further inland. TT leaks more oxygen into the sediment, which is also constantly flushed with water and therefore more oxidized, whereas ST sediments are very reduced, with low oxygen and high sulfide concentrations. Sulfide-oxidizing bacteria (SOB) thrive in the rhizosphere of both Spartina types but are more abundant in ST. One of the SOB are cable bacteria, multicellular filaments that spatially separate sulfide and oxygen by long distance electron transfer. When these bacteria grow in the rhizosphere of aquatic plants, they protect them by keeping toxic sulfide away from the roots. There are many different species of cable bacteria, but their ecophysiological adaptation and environmental distribution is unknown.

We hypothesized that sulfide and oxygen concentrations were major regulating factors for cable bacteria distribution, and therefore expected to find different species with distinct adaptations, i.e. niche differentiation, in the contrasting rhizospheres of TT and ST Spartina alterniflora. Initial results showed that cable bacteria were more abundant in the ST rhizosphere, while the TT rhizosphere seemed to select for species with a terminal oxidase complex (COX). This suggests that sulfide concentration rules the abundance of cable bacteria, but oxygen availability might select for cable bacteria with the COX system. Further studies of oxygen and sulfide concentrations along Spartina transects while monitoring gene expression and species abundance will hopefully improve understanding species differentiation of cable bacteria, so that these may someday be used to promote the growth of aquatic plants for e.g. salt marsh restoration and coastal protection.

[P86] Advanced antibiotic screening with the predictive power of genome mining and phenotypic data.

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The Tiny Earth framework is addressing the antimicrobial resistance crisis through a crowdsourcing-initiative, isolating and screening of bacteria from soil for the production of antibiotics. Globally this initiative has led to 26,380 bioactive isolates, but so far only 297 complete genome sequences and 28 known bioactive compounds identified. The recent developments in DNA sequencing technology enables easier generation of complete bacterial genomes at scale and bioinformatics tools can be used for screening the genomes for the gene clusters involved in the synthesis of antibiotics. The Tiny Earth isolate collection thus represents a lot of untapped potential. Tiny Earth Denmark is a branch of this crowdsourcing-initiative, which works with high-school students around Denmark to find new bioactive isolates. We extracted DNA from bioactive isolates and sequenced the DNA using nanopore sequencing, assembled genomes and analysed their genomes for biosynthetic gene clusters using antiSMASH. We linked this with phenotypic screening data to form a unique dataset for exploring the predictive power of bioinformatics and link it with real lab data. Thereby, determining filtering criteria's there prioritize microbial isolates with the most promising traits. We generated 276 genomes and showed that the isolated bacteria's produce a large variety of broad- and narrow-spectrum antibiotics against the tested WHO-identified priority pathogenetic bacteria's. And integrating genomic potential with phenotypic screening data can serve as a filtering criteria for selecting best bioactive isolate candidates.

[P87] Interspecific interactions alter functionality and promote the key-stone species in a synthetic four-species community

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Biofilms are highly diverse ecosystems that harbor multiple interacting species. These interspecies interactions lead to emergent properties influencing the community structure and functionality. Here, we aimed to investigate how interspecific interactions shape the community dynamics and functionality of a synthetic four-species biofilm community.

We focused on four soil isolates—Stenotrophomonas rhizophila, Xanthomonas retroflexus, Microbacterium oxydans, and Paenibacillus amylolyticus—previously shown synergistic interations in biofilm formation. We used Arabidopsis thaliana as host plants to evaluate bacterial impacts, combining molecular analyses (quantitative PCR, amplicon sequencing) with fluorescence in situ hybridization (FISH) for analysis of spatial organization.

Initially, the four species were introduced either individually or in combination to Arabidopsis plants grown in non-sterile conditions. The four bacterial species when combined protected the seedlings from prolonged drought, whereas individual strains did not. Moreover, the four-species inoculation altered the diversity of the indigenous rhizosphere microbiome. We next introduced the four species to sterile Arabidopsis roots. We observed enhanced root colonization and plant growth promotion by the four species compared to individual inoculations. P. amylolyticus, a weak root colonizer on its own, was significantly more abundant in the community. Co-localization analysis identified P. amylolyticus as a focal species, and pull-out experiments—where P. amylolyticus was removed—identified it as a functional keystone species. These results reveal that interspecific interactions were essential for the keystone species to establish and promote plant growth.

In conclusion, interspecies bacterial interactions drive community function and should be considered in the design of synthetic communities for biotechnological applications.

[P88] The effect of probiotic cell-free supernatant and live biofilm on in vitro biofilm growth of pathogens isolated from urinary catheters

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Catheter-associated urinary tract infections (CAUTI) that occur in long-term catheterized patients may lead to severe, persistent infections or bacteremia. They are difficult to eradicate and control, as they are caused by multispecies biofilms that may have higher resistance to antibiotics. Recently, there has been a growing interest in using probiotics, like probiotic lactic acid bacteria, to battle against pathogenic biofilms. Here, a four-species in vitro biofilm model on catheters was developed, involving clinical strains of Escherichia coli, Pseudomonas aeruginosa, Klebsiella oxytoca, and Proteus mirabilis isolated from urinary catheters. And two probiotics, Lactobacillus plantarum, and Lactobacillus rhamnosus were used to obtain cell-free supernatant (CFS) and probiotic biofilms. We investigated the individual interactions between two probiotics and pathogen biofilms. Both L. plantarum and L. rhamnosus cultures grew well and lowered pH in 50%MRS for 48 hours. The pH of 48-h L. plantarum, and 48-h L. rhamnosus cultures decreased from 6.5 to approximately 4. Mono-species biofilm formation of all pathogens was inhibited in 25%, 50%, and 75% CFS, while only 10% CFS can lead to pathogen biofilm formation. That may be pH-dependent. Furthermore, the absolute biomass of the four-species pathogen biofilm was higher than that of all mono-species pathogen biofilms through crystal violet quantification. Moreover, E. coli, P. aeruginosa, and K. oxytoca were either inhibited or killed in mono-species pathogen biofilms when 48-h L. rhamnosus biofilm existed. While E. coli and K. oxytoca were observed in the four-species pathogen biofilm. Thus, our study shows inhibitions of probiotic cell-free supernatant and live biofilm on pathogen biofilm growth and certain protection within four-species pathogen biofilm.

[P89] Gene expression in biofilms with different metabolic activities unveiled by RNA sequencing

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Background: The heterogeneity of metabolic activity in biofilms is related to applying different types of antibiotics for anti-biofilm therapy. And metabolically inactive, slow-growing, or non-growing bacterial cells in biofilms have been regarded as the most tolerant part of eradicating biofilms. Therefore, visualizing bacterial metabolic activity in biofilms and comparing gene expression in biofilms with different metabolic activities will contribute to understanding biofilm properties and developing anti-biofilm strategies. This study aims to investigate the gene expression heterogeneity of biofilms with different metabolic activities.

Method: Adenosine triphosphate (ATP) is highly relevant to bacterial metabolic activity. Here, an ATP reporter has been inserted into the chromosomes of two good biofilm makers, Escherichia coli AR3110 and Kluyvera cryocrescens. The expression of fluorescence was first measured in planktonic bacterial cells. Then biofilms were grown in microfluidic systems and the ATP heterogeneity of biofilms was imaged by using a Confocal Laser Scanning Microscope (CLSM). Next, the active bacterial cells and inactive cells will be sorted into two subpopulations based on fluorescence intensity, and then mini-RNA sequencing will be performed. Based on sequencing results, we will be interested in finding out the significant differential expressional genes between active cells and inactive cells in biofilm.

Results: This ATP reporter works well in E. coli with good fluorescence under the microplate reader, CLSM, and Flow cytometer. Subpopulations with different fluorescence intensities were found in planktonic bacteria after growing in M9 for 48 h. The next steps are to sort out the subpopulations in biofilms based on fluorescence and then perform mini-RNA sequencing, which are ongoing experiments.

[P90] INNOLYSINS: Novel antibacterials against Salmonella

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Salmonella is among the leading causes of foodborne bacterial disease worldwide. Severe complications are associated with high mortality rates, particularly in developing countries. Increasing antibiotic resistance and the ability of Salmonella to hide inside macrophages as persisters call for novel antibacterial solutions targeting Salmonella infections. Endolysins, phage-encoded enzymes degrading the peptidoglycan, are showing promise as effective antibacterials. Nevertheless, the outer membrane of gram-negative bacteria, such as Salmonella, limits the effectiveness of endolysins, as their access to the peptidoglycan is restricted. Innolysins, endolysins fused to phage receptor-binding proteins, have shown capable of overcoming this problem, allowing them to reach the peptidoglycan and exert antibacterial activity against both Escherichia and Campylobacter. Here, we engineered different Innolysins libraries with over 500 theoretical variants for screening their antibacterial activity towards S. Typhimurium. We identified a top candidate, Innolysin InF9, leading to a 6-log reduction in the bacterial cell count when used at a concentration of 2.5 uM. The antimicrobial spectrum of this Innolysin and its ability to disrupt biofilm is currently being investigated. To further customize Innolysins, we will fuse InF9 to cell-penetrating peptides to target Salmonella persisters and determine the antibacterial activity in vitro and in vivo. Overall, we are expanding the use of Innolysins as novel antibacterials against Salmonella and have identified a potent Innolysin, InF9, which is a promising novel antibacterial in humans.

[P91] Myxobacteria in Recirculating Aquaculture Systems: Pungent Predators?

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Tainting of fish flesh due to the off-flavor compound geosmin is a long-known problem in land-based fish farming in Recirculating Aquaculture Systems (RAS). These microbiologically produced compounds have historically been attributed to Cyanobacteria and Actinobacteria, but recent molecular studies indicate that the more obscure myxobacteria may be the leading bacterial group responsible for earthy off-flavors in RAS (Lukassen et al., 2021). These bacteria are ubiquitous on the planet (accounting for 2% of the total OTUs in Earth Microbiome Project) (Wang et al., 2021) and have a very peculiar lifestyle - they feed cooperatively through predation on other bacteria. For the first time, we succeeded in isolating these bacteria from RAS, enabling their production of geosmin to be studied in detail by GC-MS. But what is the use of this secondary metabolite from the bacteria's point of view? Apart from geosmin-producing myxobacteria, we have also isolated and characterized a new genus that does not have the geosmin synthase gene, geoA. This further mystifies the role of this compound. Does it provide some fitness to the bacteria having the gene? Transcriptomic studies of myxobacteria have shown that geoA is upregulated during predation of other bacteria. To further investigate a potential relationship between geosmin production and predatory activity, we performed a predation assay with myxobacteria isolated from RAS, using general bacteria isolated from RAS as prey. The volatiles produced during predation was measured in real time using Proton Transfer Reaction-Mass Spectrometry (PTR-MS). Myxobacteria isolated from RAS predated readily on most, but not all, selected RAS bacteria. This trait likely gives them an important role in the shaping of the RAS microbiome. Uncovering the relationship between predation and geosmin production can provide useful insights, helping to obtain a better RAS management through reducing off-flavor problems directly at the bacterial source.

[P92] Methicillin-resistant Staphylococcus pseudintermedius – multiresistance and clonal diversification

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Staphylococcus pseudintermedius is the most important staphylococcus in dogs, causing a range of infections, most often skin and soft tissue infections. In addition, it has a certain zoonotic potential. In recent years, several methicillin-resistant S. pseudintermedius (MRSP) have emerged, which in addition to β-lactam resistance have acquired resistance to many other antibiotic classes, making infections with these strains very difficult to treat with antibiotics. Some MRSP strains are resistant to all antibiotics registered for use in dogs.

The first case of MRSP infection in dogs in Sweden was reported in 2006 and since then new MRSP have been reported yearly. In this investigation, we subjected 356 MRSP from dogs (345) and cats (11) from 2012-2021 to whole genome sequencing to look for genomic relatedness and detect antibiotic resistance and virulence genes.

MRSP sequence type (ST) 71 was the first clone to emerge and spread throughout Sweden, but subsequently, we observed other clones becoming dominant, ST258, ST45, 265, and ST551, while simultaneously, a genomic diversification took place with emergence of several new STs, which appeared to have evolved independently. All isolates carried several antibiotic resistance genes. Most isolates were resistant to macrolides, lincosamides, aminoglycosides, tetracyclines, and sulphonamides. In addition, several clones had developed mutations in the grlA and gyrA genes, conferring resistance to fluoroquinolones. The most recent dominant clone, ST551 was the most resistant clone, resistant to all antibiotics registered for veterinary use, except for fucidic acid.

A variety of virulence factors associated with attachment, toxins, immune evasion, and quorum sensing was found in all isolates.

The emergence of these virulent, multidrug resistant MRSP clones is a serious health hazard for dogs, leaving few or no options for antibiotic treatment. This calls for preventive measures and the use of treatment not using antibiotics.

[P93] The environmental plasmidome of Denmark

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Plasmids are ancillary DNA molecules that play an important role in shaping microbial communities by extending the traits of their hosts. However, the collection of known plasmids is largely populated by sequences recovered from culturable microbes, leaving a large gap of knowledge especially regarding the environmental plasmids. In the past decade advancements in metagenomics allowed the in situ recovery of microbial communities and their plasmidomes. Increasing the knowledge base of plasmids will allow us to peer into their overall diversity and specific contributions to the microbial communities beyond model organisms and systems.

Using the Microflora Danica data collection, spanning 10,000+ complex metagenomes sampled across Denmark, we recover 46,186 putatively complete plasmids and 699,973 plasmidial contigs. We clustered the plasmidome of Denmark with plasmids from public databases into Plasmid Taxonomic Units (PTUs) and doubled the known plasmidial diversity. We showed that 99% of the Danish plasmids do not cluster with previously known plasmids (70% Average Nucleotide Identity and 50% Alignment Fraction of shortest plasmidial sequence in a pair) and 59.3% of the Danish plasmidial genes coded for hypothetical proteins; more than double of those from databases. We focused on plasmids harboring full rRNA operons and reported 613 cases (115 and 498 from the Danish plasmidome and databases, respectively); more than 30 times the cases previously reported in literature. Lastly, we corroborated literature culture-based findings in an environmental setting by highlighting two examples: i) a plasmid used as an extension of the rRNA gene repertoire in the genus Bacillus and ii) a plasmid as the potential sole source of rRNA in the family Acetobacteraceae. Our study vastly increases the set of plasmids recovered from environmental metagenomes, bridging the significant knowledge gap regarding plasmid diversity in natural environments.

[P94] Administration of dietary lactate shows changes in short-chain fatty acids profiles of pre-diabetic subjects

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Fermented dairy products that are rich in dietary lactose and lactate, are widely consumed. Intestinal bacterial fermentation of lactose can lead to the formation of lactate, which may successively be transformed into short-chain fatty acids (SCFA), which are associated with health benefits. Lactate has been associated with regulation of energy and fatty acid metabolism and is used by gut microbes for cross-feeding. Recent research suggests that lactate consumption may influence appetite regulation, but the impact of oral lactate on the gut microbiome remains poorly understood.

We analyzed data obtained from twelve pre diabetic volunteers enrolled into an intervention study that consumed either a lactate solution or a placebo. Fecal samples were collected at two time points post-intervention to assess changes in fecal SCFA profiles and microbiota composition were determined.

Our results show that dietary lactate can cause a short-term shift in SCFA profiles. While changes of the proportions of butyrate and acetate were subject-dependent, the proportion of propionate increased consistently in most subjects after the intervention. These changes may reflect changes in lactate-dependent propionate and butyrate pathways, like the acrylate pathway.

This study suggests that oral lactate administration temporarily affects gut microbiota and SCFA production, with potential implications for the development of microbiome-targeted therapies.

[P95] Auxin alone is not responsible for wheat root hair elongation in soil by Pseudomonas brassicacearum S3C01

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Root hairs (RHs) are an important instrument for plant growth, as they improve uptake of nutrients and water from the soil. However, evidence shows that domestication and modern crop breeding have likely shortened the length of RHs, which may cause limitations in crop adaptations to more sustainable low-input agriculture. The plant root microbiota contains many important functions for crop health, including the ability to modulate root growth in vitro, but the ability of root-associated bacteria to elongate root hairs in the soil environment is unknown.

Here, we use a novel bread wheat (Triticum aestivum) mutant that is stunted in RH elongation to explore the diversity and mechanisms of RH elongation in soil by root-associated. We hypothesized that microbial auxin production would restore RH elongation in the mutant line.

From a strain collection of 48 Pseudomonas isolates, we identified five strains producing auxin in vitro. Inoculation of the auxin-producing isolate P. brassicacearum S3C01 onto the mutant grown in soil increased RH length up to 69%, measured by stereo- and scanning electron microscopy. We used a 12-strain wheat synthetic microbial community to increase complexity of the system and test the ability of P. brassicacearum S3C01 to modulate RH elongation in the presence of natural microbial interactions, and no difference was observed. Ultimately, the addition of pure auxin failed to rescue the mutant to the same degree as P. brassicacearum S3C01. Our results indicate that microbial mechanisms beyond auxin signaling are capable of rescuing stunted RH growth.

Further work with our experimental system will test the influence of P. brassicacearum S3C01 on processes associated with RH elongation, such as the concentration of reactive oxygen species and calcium dynamics. Using the wheat RH mutant line, we aim to uncover novel microbial mechanisms capable of elongating RHs even in crops genetically predisposed to short RHs.

[P96] Inhibition of Pseudomonas aeruginosa quorum sensing by chemical induction of the MexEF-oprN efflux pump

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The cell-to-cell communication system quorum sensing (QS), used by various pathogenic bacteria to synchronize gene expression and increase host invasion potentials, is studied as a potential target for persistent infection control. To search for novel molecules targeting the QS system in the Gram-negative opportunistic pathogen Pseudomonas aeruginosa, a chemical library consisting of 3,280 small compounds from LifeArc was screened. A series of 10 conjugated phenones that have not previously been reported to target bacteria were identified as inhibitors of QS in P. aeruginosa. Two lead compounds (ethylthio enynone and propylthio enynone) were re-synthesized for verification of activity and further elucidation of the mode of action. The isomeric pure Z-ethylthio enynone was used for RNA sequencing, revealing a strong inhibitor of QS-regulated genes, and the QS-regulated virulence factors rhamnolipid and pyocyanin were significantly decreased by treatment with the compounds. A transposon mutagenesis screen performed in a newly constructed lasB-gfp monitor strain identified the target of Z-ethylthio enynone in P. aeruginosa to be the MexEF-OprN efflux pump, which was further established using defined mex knockout mutants. Our data indicate that the QS inhibitory capabilities of Z-ethylthio enynone were caused by the drainage of intracellular signal molecules as a response to chemical-induced stimulation of the MexEF-oprN efflux pump, thereby inhibiting the autogenerated positive feedback and its enhanced signal-molecule synthesis.

[P97] Quantifying the impact of microbes associated with fungus-farming termites on plant biomass decomposition in savanna ecosystems.

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Biological processes associated with nutrient accumulation and cycling are foundational for ecosystem stability and biodiversity build-up. Decomposition is strongly affected by temperature and water availability, leading to accumulation of plant biomass in drier ecosystems such as savannas. Fungus-farming termites cultivate Termitomyces fungi that, along with gut bacterial microbiomes, degrade plant polysaccharides to completion. These termites collect ground water to maintain high humidity within fungus gardens, allowing year-round decomposition in dry savanna. The roles of the termites and Termitomyces in decomposition are well-established, but it is not known if microbes recruited from the surrounding soil to fungal gardens also contribute. To help close this gap, we conducted a standardized teabag index experiment to estimate decomposition parameters in fungus gardens, in termite mound soil, and in soil away from mounds for 10 Macrotermes bellicosus colonies collected from woody savanna in the Ivory Coast. This revealed that fungus garden decomposition rates were slightly higher than in mound soil but lower than outside soil, which is likely because it excludes the main plant-biomass degrader Termitomyces. Nutrient analyses showed significantly higher carbon and nitrogen content in fungus gardens than soils, and isotope analyses indicated differences in their plant origins. Ongoing analyses using droplet digital PCR and amplicon sequencing of bacterial and archaeal (16S rRNA) and fungal (ITS) genes will establish microbial density, community composition, and community richness. This will help test if communities within fungus gardens are most likely recruited from the surroundings or coopted termite gut microbes. Collectively, our work will improve our understanding of the role of termite-associated microbes beyond Termitomyces in decomposition and nutrient cycling, and potentially reveal new microbial contributions to the farming termite symbiosis.

[P98] Identification of early expressed Phapecoctavirus AV126 proteins that inhibit the growth of Extended Spectrum Beta-Lactamase E. coli

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During phage infection, interactions between early expressed phage proteins and host components enable efficient reprogramming of the host metabolism towards phage progeny production. However, the identities of phage proteins, their bacterial targets involved in this reprogramming, and the underlying molecular mechanisms are poorly understood. We aim to elucidate the host reprogramming of ESBL032, an extended-spectrum beta-lactamase (ESBL) producing E. coli strain, by Phapecoctavirus AV126. 153 early expressed phage genes were identified by RNA-seq and bioinformatical analyses, and genes with known functions were discarded. Thus, 95 genes were cloned, and by inducing expression from a low-copy number vector, we identified 8 genes inhibiting the growth of ESBL032 on a plate spot assay. A liquid growth assay confirmed that expression of some proteins prevents growth while others inhibit growth. Bioinformatic analyses revealed that these proteins are small (48 to 252 aa) with no or limited structural similarity to known proteins. However, Gp4 contains a PhoH domain suggesting ATPase activity, which may indicate a function as an RNA-helicase in a PhoH2 complex. In addition, phase contrast microscopy revealed that Gp1 and Gp5 promote filamentous cell morphology, suggesting inhibition of cell division, although their bacterial targets are yet to be identified. Furthermore, protein purification in combination with structure prediction using AlphaFold3 suggested that Gp2 forms stable homodimers and homotrimers. We are currently verifying these exciting new phage proteins and functions to uncover their interactions with host targets and elucidate new mechanisms of host reprogramming.

[P99] Free-living nitrogen fixing bacteria colonize wheat mucilage and contributes to nitrogen uptake in wheat

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Plants host a myriad of microorganisms colonizing different niches above and below ground. Some of those colonizing the roots (rhizosphere) protect the plants against abiotic and biotic stressors and aid in nutrient uptake. However, the rhizosphere consists of both lateral roots and energy-rich root tips, making it a complex environment. At the root tips, plants excrete mucilage, a substance rich in polysaccharides, to facilitate penetration through soil. In aerial roots in ancient maize, this mucilage layer hosts free-living nitrogen fixers (FLNF). However, it is unknown whether wheat mucilage is colonized by FLNF and whether they contribute to nitrogen (N) uptake in wheat. Here, wheat was grown in aeroponics to isolate FLNF from the mucilage and determine the bacterial community composition using 16S rRNA amplicon sequencing. Plants were exposed to 15N in a closed environment, and 15N content of plant shoots and roots were measured to determine the contribution of microbial nitrogen fixation. In addition, plants were grown in soil under high and low N fertilizer levels to determine the importance of FLNF colonizing mucilage in N uptake for wheat. We show that different FLNF colonize wheat mucilage and contribute to N uptake in wheat. Taken together, FLNF might hold an underexplored potential for a sustainable N supply in future agriculture.

[P100] Key bacteria and environmental factors associated with the restoration of cesarean section perturbed gut microbiome

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Background & Aim: Cesarean section (CS) changes the initial seeding and early development of the infant gut microbiome. Long-term gut differences associated with the CS delivery have been associated with a higher risk of childhood asthma. It is hypothesized that with restoration of a CS-like gut microbiome, children born by CS could have a reduced risk of asthma comparable to those born by vaginal delviery. However, whether CS-associated microbiome changes can be reversed by environmental exposures or ecological interactions, and thereby ameliorate disease risk, is unclear. In this study, we describe early-life environmental and ecological predictors of the restoration in the 1-year gut microbiome with the aim to enhance our understanding of the natural microbiome restoration process in the context of CS-associated risk of asthma.

Methods: Gut microbiota from stool samples collected from 700 children in the Copenhagen Prospective Studies on Asthma in Childhood2010 (COPSAC2010) cohort at age 1 week, 1 month, and 1 year were analyzed by 16S rRNA gene sequencing. A CS score was predefine in our prior study by constructing a cross-validated sparse partial least squares (sPLS) model on gut microbial composition at 1 year of age predicting delivery mode (vaginal/CS). The model identified the gut microbial composition at 1 year of age most associated with CS delivery. By reversing the CS microbial score, we define this restoration score as our outcome measure to characterize the restoration of 1-year gut microbiome. Thus, a higher restoration score means a child's microbiome resembles being born vaginally, while a low restoration score means that a child retains a CS-like gut microbiota composition. This score was used as either a continuous score or dichotomized (above and below median value).

Results: At age 1 week, higher diversity was observed for higher 1-year restoration score (Linear regression, Chao1 index, P=0.086; Shannon diversity, P=0.018; PD index, P=0.021). At 1 month of age, the same directionality was observed, but it was not significant (Chao1 index, P=0.178; Shannon diversity, P=0.390; PD, P=0.120). The gut microbiota composition differed between children with high vs low 1-year restoration scores (above vs below median values). Children with low 1-year restoration scores appeared to have less mature compositions compared to those with high 1-year restoration scores. At 1 week of age, the compositional difference was most obvious (F=7.8, R2=1.5%, P=0.001). At 1 month of age, there was still a difference between children having high and low restoration scores, but F value and R2 decreased compared to those at 1 week (F=3.3, R2=0.6%, P=0.025).

We next investigated individual taxa at 1 week and 1 month to determine which members of the microbiota that were driving the observed compositional differences. This differential abundance analysis, performed at species level, showed associations of several early gut microbiome taxa with the restoration score. At 1 week of age, children with higher restoration scores at 1 year had higher relative abundances of Sutterella wadsworthensis and Neglecta timonensis (Fig.2A, FDR adjusted P<0.05). Additionally, high restoration score was also found associated

with high relative abundance of Bifidobacterium longum, Bacteroides fragilis, Bacteroides vulgatus, Bacteroides dorei, Bifidobacterium pseudocatenulatum, Parabacteriodes distasonis, Streptococcus gallolyticus, Phassolarctobacterium succinatutens and Prevotella sp. (P<0.05). On the other hand, we found that Clostridium perfringens at 1 week was negatively associated with the restoration score at 1 year and was the most differentially abundant species found. Compared to the gut microbiome at 1 week of age, the 1-month gut microbiome showed less significant differences according to the 1-year restoration score. Bifidobacterium pseudocatenulatum, Bifidobacterium bifidum, Bifidobacterium adolescentis, Collinsella aerofaciens, Streptococcus parasanguinis, Odoribacter splanchnicus, and Streptococcus equinus were associated with higher restoration score (P<0.05).

We next examined the relationship between early life environmental factors and the 1-year restoration score. We found that especially having older siblings was associated with a higher restoration score in both the full cohort and in the CS stratum (Full cohort, 0.381 [0.227;0.536], P<0.001; CS stratum, 0.366 [0.028;0.705], P=0.034). Interestingly, the age of the youngest older sibling was negatively associated with the restoration score; i.e. the younger the older child was the higher the restoration score was at 1 year (Full cohort, -0.135 [-0.224;-0.046], P=0.003; CS stratum, -0.194 [-0.397;0.009], P=0.061). Having cats and being born in a rural area were positively associated with the restoration score in the CS stratum (cat in the home, 0.44 [0.050:0.83], P=0.0274; rural area, 0.371 [0.023; 0.718], P=0.036) while being breastfed for more than 6 months was negatively associated with the restoration score (any breastmilk for 6 months, -0.405 [-0.760;-0.049], P=0.026).

Multivariate analyses were used to identify species and environmental factors jointly associated with the 1-year restoration score in the full cohort (Fig.4) and in the CS stratum (Fig.S2). We employed three sPLS models in both strata, which were modeled on only gut microbiome, only environmental factors, and combined gut microbiome and environmental factors, respectively. These models had fair performance (model on only gut microbiome, Spearman rho 0.21, P<0.001; model on only environmental factors, 0.16, P<0.001; model on combined gut microbiome and environmental facors, 0.24, P<0.001). In the full cohort, the gut microbiome was enriched for Bifidobacterium longum, Parabacteroides distasonis, Sutterella wadsworthensis, and Neglecta timonensis in children with higher restoration scores at 1 year of age, while Clostridium perfringens was depleted at 1 week of age. Of these, Bifidobacterium longum was the most abundant and prevalent (see Fig 2), while Parabacteroides distasonis and Sutterella wadsworthensis were less so. Neglecta timonensis was only present in few samples (prevalence 12.42%), but had a strong association with the 1-year restoration score. Clostridium perfrigens contributed the most to the model with a strong negative loading towards the restoration score. In the model with environmental factors, only maternal antibiotics at birth and having older siblings were selected by the model to have negative and positive loadings with the 1-year restoration score, respectively. The model on combined gut microbiome and environmental factors showed a better performance than each individual model, predicting 1-year restoration score with 7 variables selected, including the same five species selected by the model on only the gut microbiome, but also Vellonella parvula and having older siblings.

At 1 month of age, models had moderate performance on predicting restoration scores (model on gut microbiome, 0.1, P=0.023; model on environmental factors, 0.15, P<0.001; model on combined gut microbiome and environmental factors, 0.16, P<0.001). The combined model had a slightly better performance than each individual model, but the inclusion of the gut microbiome didn't improve the model much compared to the model on only environmental factors. In this combined model, 3 environmental factors and 14 species were selected. Antibiotics to mother and CS were negatively associated with the restoration scores, while having older siblings was positively associated with the restoration score and had the strongest positive loading. Clostridium perfringens, Neisseria meningitidis, Helicobacter pylori, Acinetobacter baumannii, Actinomyces sp., and

Klebsiella quasipneumoniae were negatively associated with restoration scores, while Lactobacillus paracasei, Odoribacter splanchnicus, Bifidobacterium adolescentis, Escherichia Shigella sp., Streptococcus parasanguinis, Streptococcus equinus, Bifidobacterium pseudocatenulatum, and Collinsella aerofaciens were positively associated with restoration scores. Of these, having older siblings and Clostridium perfringens were selected in 1-week combined model in the same directionality.

In the CS stratum, the models had lower performance compared to models in the full cohort probably due to the reduced sample size (Fig.S2). The comparatively best model was on the combined 1 week gut microbiome and environmental factors, the spearman rho between the predictions and the restorations scores was 0.19, P=0.064. However, the models on the 1 month gut microbiome and environmental factors were not able to predict the restoration scores with the cross-validated correlation between the predictions and the restorations scores around 0.00.

We further performed a differential abundance analysis to investigate differences in species-level taxon abundances between kids with older siblings vs those without and compared these with the previously presented taxa associated with the 1-year restoration score, followed by a comparative analysis of logFC between having older siblings and the association with restoration score(Fig.5, Table S12-17).

At 1 week, out of 140 species, 85 showed consistent associations with having older siblings and restoration scores, either positively associated with both factors or negatively associated with both, indicating a similar directional trend. 22 bacteria were significantly associated with having siblings at 1 week of age, of which 5 were associated with restoration score at 1 year as well. All these were directionally correlated between siblings and restoration score including jointly higher abundances of Bifidobacterium longum and Bifidobacterium pseudocatenulatum; and lower abundances of Actinomyces bowdenii, Clostridium tarantellae, and Clostridium perfringens. At 1 month of age, 94 out of 146 species showed consistent associations. 16 species were significantly associated with having older siblings and 4 of them were also associated with 1-year restoration score. After comparing the logFC of these 4 bacteria, we found the same directionality of association between siblings/restoration and individual taxa; having older siblings enriched those positively associated with restoration score and depleted those negatively associated with restoration score.

In the CS stratum, 87 out of 137 species, and 73 out of 136 species showed consistent associations with having older siblings and restoration scores, at 1 week and 1 month time point, respectively, but less significant compared to the associations in the full cohort. Nevertheless, Bifidobacterium longum was still found in higher abundance in both analyses. We can also see the same directionality at 1 year of age both in the full cohort and in the CS stratum, which further suggests a positive role of having older siblings on the restoration of the gut microbiome by such very early influences, which could be due to close contact with the sibling in the first few weeks of life.

Having found that older siblings is a potential protective factor that may facilitate the restoration of a perturbed gut microbiome, we next investigated how having older siblings impacts a CS-perturbed gut microbiome. To accomplish this, we associated alpha diversity and the 1 week microbial-only score described above with having older siblings.

We performed a mediation analysis to investigate the possible mediating role of the 1-week gut microbiome between having older siblings and the restoration of the gut microbiome by 1 year. Here, we used the cross-validated predictions from the model of the 1-week gut microbiome vs the 1 year restoration score and expressed it as a 1-week microbial score representing the degree to which a child's 1-week microbiome looked like it would be restored by 1 year of age.

After adjusting for delivery, the results showed a significant mediation effect of older siblings on the 1-year restoration score through the 1-week microbial score (ACME=0.002373, P=0.004) but also a strong direct effect of older siblings on the 1-year restoration score (ADE=0.01782, P<0.001). The indirect pathway accounted for a portion of 11.2% of the total effect. This suggests that older siblings may contribute to the restoration partially through influencing the very early gut microbiome.

Conclusion: Higher microbial diversity at 1 week as well as higher abundances of several bacteria including Bifidobacterium longum, Bacteroides fragilis, and Bacteroides vulgatus were associated with a higher restoration score at 1 year, suggesting a role promoting restoration of a CS perturbed gut microbiome. Similarly, having older siblings was also associated with the restoration score via higher abundance of restoration-associated bacteria like Bifidobacterium longum and Bifidobacterium pseudocatenulatum, and lower abundance of bacteria associated with prolonged perturbation including Actinomyces bowdenii and Clostridium perfringens. Our findings suggest protective effects from harboring specific beneficial bacteria early and an impact of older siblings on restoring a healthy gut microbiome development in CS-born infants. These could represent new targets for early interventions to restore a CS-perturbed gut microbiome. Further research is needed to validate these findings and explore the therapeutic potential of microbiome restoration."

[P101] Diversity and Culturability of Microbial Communities in Cryoconite from the Western Margin of the Greenland Ice Sheet

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On glacier surfaces, cryoconite holes serve as stable microcosms that shelter life from harsh environmental extremes, supporting high microbial diversity. Despite their ecological significance, these and other microbial habitats on glaciers and ice sheets remain understudied. To better understand the biodiversity and culturability of microorganisms in cryoconite, we investigated the culturable fraction of two visibly distinct types of cryoconite from the Greenland ice sheet: submerged cryoconite (hereafter cryoconite) from water-filled holes and dispersed cryoconite directly exposed to air.

Through 16S rRNA gene amplicon sequencing (V3-V4), microbial diversity was assessed for both raw sample material and enriched cultures obtained under various culture conditions (aerobic vs. anaerobic, 5 °C vs. 15 °C, and regular vs. diluted R2A or TSA media).

We found that Actinobacteria were more abundant in cryoconite (38.8%) than in dispersed cryoconite (18.8%), while Cyanobacteria were more prevalent in dispersed cryoconite (38.8%) compared to cryoconite (10.8%). However, plate culturing indicated that the number of cells may be an order of magnitude higher in cryoconite (>105 CFU/g dry weight cryoconite) compared to dispersed cryoconite (104-105 CFU/ g dry weight). The two types of cryoconite shared approximately 68.9% of the amplicon sequence variants (ASVs) between each other.

Only about 9% of the above-described ASVs were found in the culturable fraction of the bacterial microbiome. Notably, a higher number of unique bacterial taxa were cultured under lower nutrient conditions. However, all cultures were primarily dominated by bacteria belonging to the Microbacteriaceae family (58.5%-100%), most of which were related to the genus Cryobacterium (40.1%-100%). In submerged and dispersed cryoconite, approximately 28% and 10% of the bacterial community was represented by Microbacteriaceae, primarily consisting of two unique ASVs found exclusively in low nutrient conditions.

[P012] Actinomycetes Automation: a literate programming approach for medium-throughput robotic conjugation of Streptomyces spp.

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Streptomyces play a crucial role in agriculture and medicine, but progress is hampered by the difficulty modifying these strains. Traditional cloning methods, while efficient, generate vast bioinformatic data that necessitates new synthetic biology platforms for exploration. However, cloning methodologies have not kept pace with advances in DNA sequencing, leading to labor-intensive screening and prolonged hands-on time. Our team at DTU Biosustain has developed the CRISPR-BEST system for Streptomyces spp., achieving higher efficiencies and parallelization but still faces challenges due to labor-intensive bottlenecks.

Large biofoundries, which combine synthetic biology with automation, offer potential solutions with their high-throughput capabilities. However, they face significant barriers, especially in academia and start-ups, due to high costs, the need for specialized skills, and lack of standardized protocols. We present a cost-effective, adaptable workflow using the Opentrons 2 platform for automated interspecies conjugations of Streptomyces spp. and heat-shock transformation of E. coli. ET12567/pUZ8002. This workflow is validated with S. coelicolor M1152, S. albidoflavus J1074 and S. venezuelae DSM 40230, paving the way for more efficient engineering of Streptomyces spp.

[P103] The influence of tropodithietic acid (TDA) production on the DNA packaging in gene transfer agents (GTAs) in marine bacteria

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Horizontal gene transfer (HGT) is one of the main driving forces of prokaryote evolution, yet some non-canonical mechanisms remain not fully characterized. One of these mechanisms is the release of gene transfer agents (GTAs), which transfer DNA in a phage-like protein capsid. However, unlike transducing phages which package their own genomes, GTAs package short, host-genomic DNA fragments. GTA production is also tightly regulated within the bacterial population as the producing cells are lysed upon GTA release. This curious phenomenon demands an answer to the ecological purpose of the GTAs and whether GTAs have a packaging bias towards genomic regions to fulfil this purpose.

Our perspective on this question starts with a connection between tropodithietic acid (TDA), a bacterial secondary metabolite with a plethora of effects, and GTA production. TDA has antibacterial properties, may function as a quorum sensing signal and in some species, its production is increased in bacteria forming a biofilm. Our group examined this metabolite in Phaeobacter piscinae S26 and found that the production and release of GTAs increased when TDA production was abolished. Furthermore, the removal of TDA production also affected the cell morphology and motility of the bacterium.

We were the first to apply long-read Nanopore sequencing to the DNA packaged in GTAs of P. piscinae (PpGTAs). Interestingly, we found that the presence or absence of TDA production influences the DNA packaging bias of PpGTAs. To expand our analysis, we currently work on isolating GTAs from other TDA-producing marine bacteria and their TDA-deficient mutants to examine if the influence of TDA on GTA release is consistent across species. Furthermore, we will sequence the GTA-packaged DNA to see if TDA influences packaging bias with similar trends as in P. piscinae S26. Clearly, TDA is not only an antibacterial molecule but also plays an important role in the physiology and evolution of the producing bacterium.

[P104] CO2MOS: CO2 Metabolism Optimisation Strategy to prevent CO2 loss during methanol assimilation

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Methanol is a promising alternative to conventional carbon sources used in bio-production processes, given its status as a multi-million-ton bulk chemical as well as the extensive research dedicated to the large-scale production of green methanol from renewable resources. Bacteria that can use methanol as feedstock are called methylotrophs. Among the methylotrophs, Bacillus methanolicus is particularly interesting due to its potential to become an industrial workhorse. Currently one major limitation for bio-production using B. methanolicus as a host is that approx. 50% of all carbon is lost as CO2 during growth on methanol.

To this end, flux balance analysis (FBA) was performed using the genome-scale metabolic model (GEM) reconstruction of B. methanolicus with the tetrahydrofolate (THF) and bacillithiol (BSH) dissimilatory pathways. The analysis revealed CO2 production from the three formaldehyde dissimilatory pathways, which might be considered as potential targets for strain engineering. These are methylenetetrahydrofolate dehydrogenase/cyclohydrolase gene in the THF dissimilatory pathway, bshA involved in BSH synthesis for the BSH dissimilatory pathway and glucose-6-phosphate isomerase gene in the dissimilatory ribulose monophosphate cycle. Based on these findings, experiments were conducted to determine the effect of formaldehyde supplementation on the growth rate of B. methanolicus. A logistic decay correlation between formaldehyde concentration and growth rate was observed, with IC50 estimated at 1.57 mM. Using this information, the regulation of formaldehyde detoxification in B. methanolicus will be elucidated through comparative transcriptomic analysis of strains cultivated under normal conditions and formaldehyde stress. The end goal is to knock out the three deletion targets and analyze each for phenotype and their amino acid synthesis.

[P105] ECO-COATING: Harnessing Marine Bacteria for Biofilm Engineering

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Microbial colonization of submerged surfaces in marine environments is vital for both ecological processes and industrial applications. While it contributes to nutrient cycling and habitat formation, it also poses challenges such as biofouling and corrosion. Investigating bacterial biofilm formation on marine materials can provide insights into microbial interactions with synthetic coatings designed to withstand environmental stressors.

This study focuses on biofilm engineering by sourcing microbial "building blocks" from the marine environment and repurposing them into stable, beneficial biofilms. We employed marine bacteria from parts of unmanned underwater vehicles (UUVs) coated with PPG PSX® 700, a durable siloxane-based coating commonly used in marine and industrial settings. A total of 160 UUV isolates were screened for adhesion and growth on untreated polystyrene (uPS) and treated polystyrene (tPS) under various media formulations.

Notably, uPS induced higher adhesion rates, outperforming tPS. The top 30 adhering isolates were further validated on PSX700, where uPS consistently predicted adhesion on PSX700. Nine UUV isolates were selected based on their adhesion profiles and taxonomy, leading to the assembly of seven distinct multispecies communities. Two communities, COM1 and COM4, demonstrated stability after five days of incubation on both PSX700 and uPS, maintaining all constituent members at the end of incubation. Notably, both communities included isolate ABV4_151 (Alteromonas sp.), which exhibited high biofilm biomass, placing it among the top three isolates in our screening. This suggests that ABV4_151 is a promising partner in developing effective biofilm communities.

This study demonstrates the potential of marine-derived bacteria in engineering stable and beneficial biofilms. These findings provide valuable insights for industries seeking to mitigate biofouling while leveraging microbial processes for enhanced performance in marine environments.

[P106] Establishing a Genome-scale Metabolic model of Pseudomonas hemicellulosum LS4

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Pseudomonas hemicellulosum LS4 is a novel species in the Pseudomonas genus that was originally isolated for growth on hemicellulosic beech wood hydrolysate, a promising feedstock for fermentation, as it is derived from waste streams. The use of this feedstock is limited due to its complexity and toxic compounds. However, little is known about this species and therefore there is need for a comprehensive study of its genome, growth conditions, molecular tools and metabolic pathways.

The scope of this research, is to construct a genome scale metabolic model (GEM) for P. hemicellulosum. GEMs computationally describe the metabolic reactions occurring in an organism, based on the genome as well as experimental data. The GEM will be used to facilitate future metabolic engineering efforts to e.g. maximize product yields. More specifically, we will be able to simulate different conditions and various knock-outs or upregulated genes or pathways that enhance valorization of the lignocellulosic feedstock.
For the construction of the GEM, the automated tool CarveMe will be used, which utilizes a top-down approach to eliminate reactions from a universal model, based on genetic evidence through a process called carving. The model will be reconstructed through the Flux Balance Analysis (FBA), where the organism's growth serves as an objective function, in an assumed steady state. CarveMe's integrated gapfilling tool will be used to supplement missing reactions, which is particularly helpful for organisms, where limited physiological data is available.

The model will be technically validated using MEMOTE. Furthermore, experimental validation will be performed, using growth curves in biolog plates, and the model will be curated based on the experimental results. This study aims to contribute to further understanding P. hemicellulosum and establish tools that will enhance its use in industrial applications.

[P107] Effects of biochar on microbial communities in coarse sandy subsoils

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Finely ground biochar has been shown to enhance the water retention of coarse sandy subsoils and mitigate drought-related yield reductions, while its impact on indigenous microbial communities remains unclear. The aim of this study was to assess the effect of biochar amendment on microbial communities from coarse sandy subsoils from two distinct Danish sites. The subsoils were amended with different biochar treatments (1-4% wt) and incubated in a mesocosm column experiment with spring barley growing in the topsoil. We sampled subsoil at 50 cm depth four times during 14 months of incubation. The activity of three extracellular enzymes related to organic matter decomposition and the temporal dynamics of prokaryote and fungal diversity using 16S rRNA and ITS2 gene sequencing, respectively, were analyzed. Our findings revealed that the biochar amendment impacted the extracellular enzyme activity of both subsoils, which exhibited distinct enzyme activity profiles. After 14 months, the biochar decreased the phosphomonoesterase activity, indicating bioavailable phosphate in soils. Both subsoils harbored different microbial communities, in which biochar increased the prokaryotic diversity while the fungal diversity hardly changed. The highest amount of biochar amendment (4% wt) caused the strongest effect in the microbial community profiles. We found a significant rise in the relative abundance of the bacterial genus Iamia and an unknown archaeon genus member of Marine Group II. These insights offer novel perspectives on the responses of the subsoil microbial communities to biochar amendment that will be useful to address side effects of biochar addition to soil, develop more sustainable agricultural practices, and prepare for future climate changes with extended periods of draught.

[P108] Genetic Engineering of Novel Environmental Bacteria for the Valorization of Lignocellulosic Biomass

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Almost all fermentation products produced today are derived from first-generation biomass using cell factories, derived from well-characterized and genetically engineered model strains. To increase the scale and sustainability of the manufacturing of these fermentation products, it is necessary to start valorizing second-generation biomass. For this to be feasible, strains that can metabolise diverse carbon sources and tolerate the toxic compounds in hemicellulosic hydrolysates are needed.

We are working towards domesticating one such novel strain; the newly isolated Pseudomonas hemicellulosum LS4. P. hemicellulosum LS4 was originally isolated for growth on hemicellulosic hydrolysate derived from beech wood. The strain exhibits a relatively high tolerance to these substrates and can grow on a wide range of different carbon sources. Several engineering tools have already been established, such as efficient transformation protocols and compatibility with a range of plasmids and different promoters. However, given the novel nature of the strain, further engineering tools need to be established in order to investigate the strains properties and engineering it to become an efficient cell factory.
In this study, we have successfully established a protocol for Tn7 transposon-mediated integration, providing an efficient method for specific and stable integration into the genome. We have further established an efficient (100%) counter-selection protocol based on SacB counter-selection, which allows for removing e.g. helper plasmids prior to further engineering efforts.

We are currently exploring other engineering techniques including both lambda-Red recombineering and potentially various CRISPR techniques.

The project will contribute to the domestication of P. hemicellulosum LS4 through the development and establishment of various genetic engineering tools, which is needed in order to engineer P. hemicellulosum LS4 into an efficient cell-factory for the valorization of hemicellulosic hydrolysates.

[P109] Synthetic bacterial community mimics the assembly patterns of natural communities on wheat roots grown in soil

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Root-associated bacteria provide important nutrients and can alleviate biotic and abiotic stresses in the plant. Originating from the soil and the seed, they form a structured bacterial community, and the intimate host-microbe interactions have led to the view of the plant and its microbiota as a metaorganism. However, it is still not clear how the bacterial community assemble on the roots, due the high complexity in the soil. Here, we designed a synthetic community (SynCom) comprising 13 genera isolated from wheat roots, to study the community assembly on roots. We inoculated the SynCom into a gamma-irradiated soil with a low bacterial abundance and diversity, prior to wheat seedling transplantation, and tracked the community development on the roots over a 4-week period using 16S rRNA amplicon sequencing. This was compared to seedlings inoculated with a richer and more diverse natural soil community, as well as to plants grown in the field. Despite the low diversity of the SynCom, we did not find any changes in root and shoot length inoculated with the SynCom, as compared to inoculation with the natural community. The temporal dynamics of most SynCom members mimicked those of corresponding genera in the natural soil community, and the community found on field-grown plants. We demonstrate that a reductionist approach can be used to study bacterial community assembly on plant roots in soil and provide a conceptual framework for future studies which can shed light on factors determining assembly processes on the root and interactions among root-associated bacteria.

[P110] Establishment of Microbial Cell Factories Tolerant to Hemicellulosic Hydrolysate

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Lignocellulosic biomass is considered one of the most abundant sources of biomass found on the planet and is full of fermentable sugars that can be valorized in fermentation processes. However, a pretreatment process of the lignocellulosic biomass is needed to release these fermentable sugars and during this process, inhibitory compounds such as furan aldehydes, weak acids and aromatic compounds are also released. To remove the inhibitory compounds several detoxification methods can be used, but most of them are either too expensive or considered as not being environmentally friendly. However, a strategy involving microbial degradation of the inhibitory compounds could ameliorate some of these caveats.

In this study, we aim to establish bacterial cell factories capable of degrading toxic compounds pre-sent in hemicellulosic hydrolysates through heterologous expression of degradation pathways. Sev-eral plasmids, encoding genes for the degradation pathways of furfural and aromatic compounds such as vanillin will initially be generated through USER cloning. The generated plasmids will subse-quently be tested in our pre-engineered Escherichia coli cell factories through growth coupling and/or HPLC. Non-functional pathways will be further investigated using a dual-reporter biosensor system, after which we will attempt to resolve potential issues with bottleneck enzymes.

When the pathways have been shown to function in the heterologous host, they will be integrated into the genome of the pre-engineered host organism, after which a fermentation will be performed using hemicellulosic hydrolysate derived from wheat straw.

[P111] Development of genetic tools functional across bacterial species

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Microbes have diverse metabolic capabilities and physiological traits that can be leveraged to valorize waste, minimize environmental impacts, and create high-value products. For example, some organisms naturally tolerate high temperatures or low pH, while others support valorizing sustainable feedstocks, like CO2 and nonfood biomass. Microbes can be applied in numerous fields, such as in the sustainable production of bulk chemicals, in the food industry, and for bioremediation. Unfortunately, unconventional microbes with properties of interest are understudied and often have very few compatible genetic tools, required for strain study and engineering. Besides, the existing tools for commonly used organisms often only work for closely related species.

In this research, we focus on developing tools functional across different bacterial species, both gram-positives and negatives and mesophiles and thermophiles, through two different approaches. One approach focuses on developing broad-host range transformation vectors capable of evading the natural immune system of bacteria by removing the targets of the restriction-modification system. The second approach is comparing gene expression regulation across species by creating a promoter library with more than 65.000 variants to be analyzed in different organisms. The aims are to create plasmids that are easily transferable for a wide range of bacteria to ease the establishment of initial DNA transformation protocols and to increase the availability of broad-host range promoters. Together, these efforts will enable the implementation of more advanced molecular tools and high-throughput screening approaches across several bacterial species. The ultimate goal is accelerating the domestication of unconventional microbes for various sustainable microbial applications.

[P112] Clonal colonization and dynamics of Escherichia coli in the developing infant gut

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The infant gut microbiome undergoes rapid changes in the first year, transitioning from facultative to strict anaerobes, with Escherichia coli as a key pioneer species. This study explores the clonal prevalence, dynamics, and evolutionary patterns of E. coli in the infant gut and its role in microbial development, a topic still not fully understood. We analyzed 690 E. coli isolates from 172 infants in the COPSAC2010 cohort, sampled at 1 week, 1 month, and 1 year, clustering them into 98 distinct clone complexes. Using metagenomics sequencing data from approximately 500 and 660 children from the cohort at 1 month and 1 year, respectively, we identified dominant E. coli phylotypes and clone complexes. Key gene modules, including those involved in siderophore production and sialic acid degradation, were linked to the prevalence of the top 10 clone complexes. Additionally, we tracked within-child evolution, uncovering adaptive shifts associated with lacY, relevant to lactose utilization and anaerobic respiration. Our analysis reveals a correlation between E. coli colonization and dynamics from 1 month to 1 year and gut maturation, as well as disease outcomes such as diarrhea and asthma, alongside environmental influences. These findings provide new insights into strain-level dynamics and evolution of E. coli in the developing infant gut, with implications for gut health and disease.

[P113] Gp38 adhesins of Straboviridae phages target specific extracellular loops of the OmpA receptor

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While bacteriophages present a promising alternative to antibiotics in treating antibiotic-resistant infections, their high specificity poses a challenge for phage therapy. It is therefore essential to analyze the first step in phage infection: phage adsorption. The receptor binding protein (RBP) Gp38 of Straboviridae phages is of particular interest due to its modular structure consisting of conserved glycine-rich motives (GRMs) interrupted by hypervariable segments (HVS). These HVS enable the phage to bind to outer membrane protein receptors by interacting with their extracellular loops. This distinct interaction, combined with the high variability of the HVS, provides an opportunity to investigate the molecular determinants of adhesin-receptor interaction. Performing a targeted isolation approach, we established a collection of Straboviridae phages belonging to three different genera, Tequatrovirus, Mosigvirus, and Krischvirus, all expressing a Gp38 adhesin protein. We identified the outer membrane proteins OmpA, Tsx, OmpF, and TolC as receptors for our phages and observed that Gp38 similarity correlated with receptor recognition. Focusing on OmpA, a holistic analysis combining in silico adhesin-receptor binding predictions, phage plaque assays on E. coli expressing OmpA variants with diverse outer loop sequences, and a host range analysis across the diverse E. coli reference collection (ECOR) revealed the influence of the Gp38 variants on phage infectivity. We concluded that the Gp38 variants of phages FL08 and AV119 bind to several diverse OmpA outer loop variants, whereas the Gp38 of phages FL12, FL18, and FL20 bind only to a limited number of outer loop variants, resulting in a narrower host range of these phages. In summary, we demonstrated that the binding of Gp38 to specific receptor outer loops influences the phage's host range and overall identified specific molecular interactions leading to host binding of phages in our Straboviridae phage collection.

[P114] The Complicated Relationship between Phage Resistance and Antibiotic Susceptibility in S. aureus

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Combination or sequential treatment using lytic bacteriophages and antibiotics is a promising strategy against antibiotic resistant bacteria. The use of phages can instigate an evolutionary trade off, in which bacterial antibiotic susceptibility can be increased. In Staphylococcus aureus the wall teichoic acid of the cell wall functions as the main phage receptor, and wall-related mutations have been identified in phage-resistant strains. Thus, phage-resistance might come at the cost of changed susceptibility to wall-targeting antibiotics. To investigate, we evolved the S. aureus USA300 strain JE2 against different lytic staphylococcal phages and assessed the impact of phage-resistance on susceptibility to wall-targeting antibiotics. We found that while the majority of phage-resistant strains were more susceptible to a range of wall-targeting antibiotics, a small subset showed increased resistance to these antibiotics. In some cases, these divergent changes in susceptibility correlated with mutation genes relating to cell wall synthesis, while in other cases mutations were solely linked to cytoplasmic processes. Additionally, most phage-resistant clones exhibited increased delta-hemolysis, although there was no common mutation to explain this. Our results indicate varied paths to phage-resistance and divergent effects of phage resistance on antibiotic susceptibility and other phenotypes. The intricacy of phenotypic changes without direct genetic explanation led us to investigate intrinsic pathways related to persistence and epigenetic alterations through transcriptomic analysis. Results confirm a complexity of phage resistance that can evolve through either genomic or non-genomic mechanisms, a tendency that will be explored further in future work.

[P115] Staphylococcus aureus adaptation to vancomycin drives changes in bacteriophage susceptibility

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The increase in infections caused by antibiotic resistant bacteria is alarming and alternatives to conventional antimicrobials are needed. Bacteriophages are increasingly being considered as an option, because of their specificity towards bacterial species as well as efficiency in eradicating bacteria. However, little is known about interactions between antibiotics and phages, and if collateral resistance emerges during antibiotic treatment. To this end, we studied how cell-wall targeting antibiotic exposure might affect phage susceptibility through alterations of cell wall structures used as phage receptors. We evolved 10 lineages of vancomycin-intermediate Staphylococcus aureus (VISA) from the methicillin resistant Staphylococcus aureus (MRSA) USA300 lineage. These isolates were genetically, transcriptionally, and phenotypically characterized. From our analysis, we found that epistasis, i.e. interplay between mutations and the order of mutations occurring, affected collateral resistance towards antibiotics other than vancomycin and towards bacteriophages. We further analyzed decoration of wall teichoic acid, the main staphylococcal phage receptor, with N-acetylclucosamine, and found a correlation with phage resistance, providing a link between antibiotic resistance and phage susceptibility, potentially important for future phage therapy. Furthermore, we characterized whether phage resistance development was apparent only in vitro. Therefore, we turned to an in vivo Galleria mellonella infection model. Indeed, larvae infected with the MRSA USA300 wild type could be rescued by phage, while phage therapy had no effect on the survival of larvae infected with phage-resistant VISA strains. This has implications for bacteriophage and phage-antibiotic combination therapies in the future, since vancomycin is a last-resort antibiotic used for treating MRSA infections.

[P116] Impact of the c-di-GMP metabolic network on lipid A modulation in Acinetobacter baumannii

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Acinetobacter baumannii endure various environmental stressors, including antibiotic exposure and desiccation. Cationic antimicrobial peptides (CAMPs) have emerged as promising therapeutic agents due to their ability to target the conserved lipid A component in the outer membrane of Gram-negative bacteria, leading to cell lysis. However, several Gram-negative pathogens, including A. baumannii, bolster their outer membrane by modifying lipid A with additional acyl chains that enhance protection against CAMP-induced lysis. In A. baumannii, lipid A is essential for providing resistance to polymyxin-based CAMPs and promoting survival in desiccation environments. Cyclic di-GMP (c-di-GMP), a bacterial second messenger, is known to regulate numerous processes such as biofilm formation, motility, and virulence. Our study shows the potential connection between the c-di-GMP signaling network and lipid A in A. baumannii. To this end, eleven A. baumannii mutant strains were created, each with a single deletion of a c-di-GMP metabolism gene. Proteomics analyses were conducted to compare each mutant strain with the wild-type A. baumannii, in both planktonic and biofilm modes, with a focus on differentially expressed proteins. LpxL, a key enzyme involved in lipid A acylation, was identified as the most downregulated protein in one mutant strain with a deletion in a gene encoding diguanylate cyclase activity, showing a log2FC of -4.39 (P-value = 0.00005) in planktonic mode and -4.4 (P-value = 0.000157) in biofilm mode. This strain, along with others, was further characterized for antibiotic susceptibility against colistin, meropenem, and tobramycin as well as its tolerance to desiccation. It exhibited the lowest susceptibility to colistin, meropenem, and desiccation, confirming the synthesis of defected lipid A. These results suggest a link between the c-di-GMP metabolic network and lipid A modification, enabling A. baumannii to adapt to stressful conditions.

[P117] LuxR regulators modulate viscosin production and swarming motility in Pseudomonas fluorescens SBW25 in response to plant metabolites

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Cyclic lipopeptides (CLPs) such as viscosin produced by Pseudomonas fluorescens SBW25, play key roles in biofilm dynamics, swarming motility and plant root colonization. However, the regulatory mechanisms controlling viscosin biosynthesis in response to environmental signals, in particular plant metabolites, are not fully understood. In this study, we explored the role of LuxR-family regulators, specifically three novel LuxR solos homologous to QscR, PsoR, and LasR, along with the local regulators ViscAR and ViscBCR in controlling viscosin-associated phenotypes in P. fluorescens SBW25. The LuxR solos were found to function independently of quorum sensing and through in silico protein modeling and docking analyses, predicted to interact with plant phenolic glycosides, namely salicin, helicin and esculin. Experimental assays showed that these plant metabolites enhance expression of the viscosin biosynthesis genes viscA and viscB, mediated through interactions with the local LuxR-type regulators ViscAR and ViscBCR. Furthermore, these interactions led to increased viscosin production and swarming motility by SBW25. This work highlights the importance of LuxR family regulators in facilitating interkingdom communication and adaption of Pseudomonas to plant environments. Understanding the regulatory complexity controlling secondary metabolite production will provide new insights into their ecological functions and roles in plant-microbe interactions.

[P118] A Thiophenesulfonamide Binds Vibrio vulnificus SmcR and Promotes its Degradation

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In Vibrio species, quorum sensing signaling culminates in the production of the master transcription factor SmcR that regulates genes required for host infection. Previously, we identified a small molecule thiophenesulfonamide inhibitor called PTSP that targets the SmcR family of proteins and blocks activity in vivo. Here, we use structure-function analyses to identify eight PTSP-interacting residues in the ligand binding pocket that are required for PTSP inhibition of Vibrio vulnificus SmcR. We find that binding of PTSP to SmcR drives allosteric unfolding of the N-terminal DNA-binding domain and, in this state, SmcR is degraded by the ClpAP protease in V. vulnificus and other Vibrio species. We also discover that SmcR degradation controls the timing of the phenotypic switch between high and low cell density. These studies implicate ligand binding as a mediator of SmcR protein stability and function, which dictates the timing of quorum sensing gene expression in Vibrio pathogens.

[P119] Compensating for rare codons in bacterial recombinant protein production: a versatile tool for tRNA expression

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Microbial protein synthesis represents a fundamental asset for the protein production industry. While microorganisms have been optimized throughout the years to perform at their best and produce the highest yield possible, limitations still exist.

One of the greatest challenges that remains to be solved is codon bias, i.e., the host's preference for a subset of synonymous codons during translation. As the heterologous proteins produced are in most cases not native to the production strain used, their sequences can contain rare codons, leading to inefficient or impaired protein synthesis.

To address the problem of codon bias in Escherichia coli we have developed pCODE, a standardized, easily applicable tool to endow the cell with more copies of the six rarest tRNAs, compensating for their scarcity. The efficiency of the tool was tested by building an extensive set of reporter proteins, represented by GFP variants containing rare codons in the gene sequences. Effects of position, type, number, and distribution of the rare codons was assessed. After expression with the pCODE plasmid, cells show enhanced protein expression ability.

To broaden the usefulness of the pCODE plasmid, we assembled a toolkit that includes two different origins of replication and two antibiotic marker variants. Moreover, we developed a version of the plasmid where expression of the supplementary tRNAs can be conveniently activated at need, in order to optimize cellular resources according to the production stage.

The pCODE plasmid represents a valuable tool for heterologous protein production improvement both for research and industrial application.

[P120] Rapid genetic response to stress: IS elements, prophages, RNAs, and REPIN

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Microbial genomes are constantly reshaped by mobile genetic elements (MGEs), resulting in genetic configurations that can confer new phenotypic traits such as antibiotic resistance and novel metabolic capabilities. Traditional genomic sequencing offers a static snapshot of a genome, lakking insights into the dynamic processes of genomic evolution and MGE activity. We employed single-strain mobilome sequencing on Escherichia coli K-12 substrain MG1655 under various stress conditions, including UV light, SDS detergent, nalidixic acid, tetracycline, cetrimide, and copper. This approach allowed us to quantify the activity of various genetic elements, such as extrachromosomal circular DNA (eccDNA) from insertion sequences (IS), RNA genes, the UV-inducible e14 prophage, and repetitive extragenic palindromic sequences (REP). Among the stressors tested, copper and SDS were the most significant inducers of eccDNA formation from IS elements. Additionally, we observed increased levels of hypothetical RNA/DNA heteroduplexes involving ribosomal and transfer RNAs, as well as Rhs-nuclease proteins, particularly under copper and SDS stress. This method provides valuable insights into the genetic responses to environmental stress and the implications for genome plasticity. The mobilization of IS elements in response to copper and other stressors also sheds light on the co-selection of heavy metals with antibiotic resistance genes and MGEs.

[P121] Renewed insight into Ackermannviridae phage biology and applications

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The Ackermannviridae phage family has garnered increasing attention due to their ability to infect a broad range of pathogens, including those affecting humans, animals, and plants within the Enterobacteriaceae family. Central to their host infectivity, these phages express a branched complex of multiple Tail Spike Proteins (TSPs), each targeting diverse polysaccharide receptors. Through computational analysis, we identified a conserved tsp gene cluster and characterized TSP diversity in 99 Ackermannviridae phages. Notably, receptor-binding domains strongly correlate with phage genera and can be exchanged between TSPs, and even show similarities with distantly related lytic and lysogenic phages. We further experimentally identified specific receptors for the TSPs of agtrevirus AV101 and kuttervirus S117. To manipulate the host range, we engineered the TSP complex of S117, replacing each of its four TSPs. To further expand host recognition, a fifth TSP was inserted into the genome to interact with the original TSP complex. Interestingly, while the acquisition of this additional TSP did not expand the host range, it generated novel TSP variants through genetic alterations in the tsp gene cluster, modifying host recognition. Our studies also revealed that S117, like other Ackermannviridae phages, can transduce genomic and plasmid DNA, highlighting potential safety concerns for therapeutic use. Overall, this investigation into the TSP diversity and their complex structure offers valuable insights into the evolutionary biology of Ackermannviridae phages and their potential applications.

[P122] Early expressed proteins of mosigvirus AV110 interact with diverse cellular targets of ESBL E. coli

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During phage infection, the interplay between early expressed phage proteins and host components allows phages to take over the metabolism of their bacterial host. However, our understanding of these interactions remains limited. Here, we aim to elucidate this interplay between Mosigvirus AV110 and its host ESBL098, an Escherichia coli strain producing extended-spectrum beta-lactamase (ESBL). To identify early expressed proteins, we compared predicted genes of phage AV110 to the previously identified early genes of phage T4 as the two phages are related (61% nucleotide identity). Among 90 early proteins of T4, 63 proteins of AV110 could be proposed as early expressed. We further reduced this number by discarding genes with known functions. Thus, 30 genes were cloned, and by inducing expression from a low-copy number vector, we identified five genes inhibiting the growth of ESBL098 on plates. A growth curve confirmed that expression of gene1, gene2, and gene3 prevents growth, whereas expression of gene4 and gene5 reduces the growth rate. Bioinformatics analyses revealed that these proteins are small (59 to 326 aa). Predicting protein structures suggests that gene1 is part of a protein complex. Gene2 may function as a dCTPase/dUTPase involved in nucleotide metabolism and DNA modification. Gene3 and gene4 contain DNA binding domains and show structural similarity to transcriptional regulators. In addition, gene3 also has an ATPase domain. Lastly, gene5 is structurally similar to a phage-encoded quorum-sensing anti-activator. We are currently verifying these exciting new phage functions to reveal their interacting host targets and elucidate new mechanisms of host takeover.

[P123] Establishment of microbial cell factories tolerant to lignocellulosic hydrolysate

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The common practice for producing fermentation-based products is currently to use 1st-generation biomass, i.e. sugars from edible food sources. However, when using these sugars to produce bulk chemical replacements, long distance transportation fuels or bioethanol, the cost of feedstocks can be affected, resulting in price fluctuations that can impact food markets. Furthermore, to keep up with demand for these high sugar content crops, such as sugarcane, deforestation of threatened and fragile ecosystems has increased, along with other environmental issues stemming from sugarcane farming. It is therefore important to reduce the dependency on 1st-generation biomass, and look for more sustainable substrates, such as lignocellulosic biomass.

Lignocellulosic biomass is a waste product of many industries, and is currently primarily being burned or used as feedstock for livestock. However, if we can engineer a bacterial strain to efficiently valorize lignocellulosic biomass, we can minimize the use of 1st-generation biomass, and work our way towards a more sustainable future.

Prior to this project engineered strains of Escherichia coli was subjected to adaptive laboratory evolution (ALE), in order to improve its tolerance to hemicellulosic hydrolysate.

In this project, the fitness of individual ALE strains will be analyzed, after which their capability of still being able to produce a product of interest will be verified. The most promising strains will be re-sequenced in order to identify mutations of interest. Potential causal mutations, most likely to influence fitness during growth on hemicellulosic hydrolysate will be reintroduced into the starting strains through reverse engineering.

The overall objective is to contribute to the development of an efficient E. coli based cell-factory tolerant to hemicellulosic hydrolysates, thereby facilitating a more sustainable production of bulk chemical replacements, reducing environmental impact and improving resource utilization.

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