Abstract book

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[1] CLINICAL P. AERUGINOSA PROPHAGES: INSIGHTS INTO THEIR ROLE VIA THEIR ACTIVITY, ABUNDANCE, PERSISTENCE

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It remains unclear how much the accessory genome contributes in the complex processes of establishment and virulence of bacterial infections. ***P. aeruginosa*** is one of the most common opportunistic human pathogens and can establish difficult-to-eradicate infections. Genome-integrated viruses, known as prophages, are frequent components of this bacterium's large accessory genome and can contribute to the virulence of ***P. aeruginosa***. However, systematic interpretations of the contributing role of prophages in the evolution and fitness of the ubiquitous ***P. aeruginosa*** in its diverse niches are still in their infancy. This study provides insights into these roles by exploring the activity, abundance and persistence of prophages belonging to ***P. aeruginosa*** from the cystic fibrosis (CF) lung. We selected a cohort of 12 CF patients with a high-resolution history of difficult-to-eradicate ***P. aeruginosa*** infections. Nanopore technology was used to sequence high-contiguity genomes of one early isolate per patient. Subsequently, we applied a strategy that combined bioinformatics, antibiotic-assisted inductions, lysate sequencing and genomics to identify intact prophages in the host genomes and assess their long-term survival in follow-up isolates. From these data, we observed that CF ***P. aeruginosa*** genomes harbour a high abundance of intact prophages which can sometimes self-induce. We identified 29 intact prophages with a wide genomic diversity and some unique prophage genomes with minimum similarity to available genomes. All induced prophages were retraced in follow-up isolates for a tested period of 4 to 9 years with minimal genomic changes. In addition to elucidating the role of prophages in ***P. aeruginosa***, we expect our findings to assist in developing novel diagnostics and phage-based therapies for ***P. aeruginosa*** infections.

[2] QUORUM SENSING MOLECULAR SIGNATURES TO DIAGNOSE P. AERUGINOSA INFECTIONS

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Purpose: *P. aeruginosa* is a concerning Gram-negative bacterium causing a range of infections, notably in surgical patients. In ICUs, it accounts for 36% of respiratory infections. ***P. aeruginosa*** is on the WHO's list of antibiotic resistant pathogens for which we most urgently need new therapies[1]. Current microbial identification techniques are slow, taking 48 to 72 hours, leading to unnecessary treatment with broad-spectrum antibiotics, exacerbating antimicrobial resistance[2].

Emerging diagnostic methods focus on bacterial molecular footprints. One such example exploits bacterial Quorum Sensing (QS), which regulates virulence factor secretion and biofilm formation using small molecules called autoinducers (AIs). AIs show promise as biomarkers for ***P. aeruginosa*** infection, offering both faster identification and prognostic information[3].
In this study, we have developed antibodies targeting four QS-related molecules in ***P. aeruginosa*** and we present their application in detecting potential biomarkers in clinical isolates and complex respiratory samples, such as sputum.

Methodology: We developed immunoreagents against AIs PQS and HHQ, along with virulence factors HQNO and pyocyanin. We used our highly sensitive ELISA assays to analyze culture media and sputum samples from patients at various disease stages.

Results: Using our ELISA assays, we successfully detected and quantified PQS, HHQ, HQNO, and pyocyanin in clinical isolates and sputum from patients with lower respiratory and urinary tract infections. These assays provided the QS molecular signature in under 2 hours, facilitating parallel processing. Diverse QS molecular signatures were observed depending on the infection stage.

Conclusions: Our immunochemical diagnostic tests for ***P. aeruginosa*** infections offer valuable tools for rapid clinical decision-making. These results suggest the potential use of QS signatures for diagnosing infections and stratifying patients by disease stage while providing additional pathological insights. We expect that further clinical validation and implementation in Point of Care devices will enhance infection management.

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[3] COLLECTIVE IMMUNITY – HOW GROUPS OF BACTERIA SENSE AND RESPOND TO DANGER

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Chemical communication shapes the outcomes of encounters between bacteria and the bacteriophage (phage) viruses that prey on them. Quorum sensing (QS), is the bacterial cell-to-cell communication process that promotes the collective undertaking of group behaviors, including anti-phage defenses. QS relies on the production, release, accumulation, and detection of autoinducer signalmolecules.

In the opportunistic pathogen ***Pseudomonas aeruginosa***, the LasI/R QS system induces the RhlI/R QS system, and these two systems control, in opposing manners, the PQS QS system that relies on the autoinducer PQS. We have previously shown that ***P. aeruginosa*** uses LasI/R and RhlI/R QS to activate the ***P. aeruginosa*** CRISPR-Cas anti-phage defense system (1). We have also uncovered a physical phage defense, in which phage-infected ***P. aeruginosa*** cells emit a warning PQS QS signal, redirecting healthy neighboring bacterial swarms away from the infected cells (2). Thus, collectives of ***P. aeruginosa*** coordinate their phage defenses via QS.

We recently discovered that phage infection of a ***lasI*** mutant, which is unable to produce PQS, restored PQS synthesis. Importantly, this caused the induction of downstream QS-target genes, including those encoding virulence factors. This demonstrates, that a ***P. aeruginosa lasI*** mutant can bypass the hierarchal QS organization in response to phage infection, thereby restoring its otherwise muted QS regulatory network (3).

Clinical isolates of ***P. aeruginosa*** frequently harbor mutations in QS genes. Thus, phage therapy against such ***P. aeruginosa*** strains may inadvertently increase bacterial virulence. This underscores the importance of uncovering phage-host interactions in the context of bacterial mutants that are relevant in clinical settings.

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[4] MECHANISM OF PHAGE SENSING AND ABORTION BY TOXIN-ANTITOXIN-CHAPERON SYSTEMS

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Mechanism of phage sensing and abortion by toxin-antitoxin-chaperon systems

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Type II toxin-antitoxins (TA) are comprised of a proteinaceous toxin that is neutralised by a proteinaceous antitoxin though formation of a tight inactive complex. Toxin-antitoxin-chaperone (TAC) additionally include a SecB-like chaperone essential for antitoxin stabilisation (1). TAC systems have been shown to mediate defence against phages (2), but the molecular mechanisms are unexplored.

We have set up a pipeline for identification and validation of TA systems though genomic neighbourhood analysis (3) and demonstrated the mechanism of phage defence by toxSAS TA discovered in the lab (4). Here we applied this toolbox to TACs. We describe ***E. coli*** HigBAC TAC system that defends against Demerecviridae and Siphoviridae. In the case of λ_{vir} the toxicity of the HigBAC is triggered though recognition of the GpV major tail protein. To trigger the toxin GpV competes with the Chaperone Addiction Domain (ChAD) of the antitoxin for binding to the chaperone. Furthermore, we describe ***E. coli*** TAC that employs an ADP-ribosyltransferase toxin to protect against Tevenvirinae. The toxin modifies mRNA to shut down cellular protein synthesis. Our results establish a framework for TAC-mediated defence.

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[5] 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. In consequence, these methods are unable to reveal the gut microbial biogeography and fine-scale spatial structure of microbial communities. Due to this lack of resolution, we are missing relevant information, which could contribute to understanding the mechanistic underpinnings of microbial interactions.

Synthesizing the information generated here will allow us to move towards spatially referenced metagenomics and metatranscriptomics to understand the 3D'omic landscape in the chicken gut further. Lastly, such a pipeline is not restricted to just studying poultry microbiota. Future aims of the project involve the expansion of 3D'omic techniques to study larger livestock such as swine.

[6] ENVIRONMENTAL PH AND COMPOUND STRUCTURE AFFECT THE POTENTIAL OF SHORT-CHAIN CARBOXYLIC ACIDS AS ANTIMICROBIAL METABOLITES

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Purpose: The history of fermentation dates back to 10,000 years ago and it remains a powerful technology for sustainable food processing. Microbial fermentation is known to naturally produce short-chain carboxylic acids (SCCA) as biopreservatives. However, the antimicrobial mechanism of SCCA has not been fully uncovered as the surrounding pH alters the acid dissociation state and the compound structure affects the hydrophobicity (log Kow), which is often ignored. This study aimed to systematically evaluate the effects of environmental pH and compound structure on the antimicrobial and antibiofilm activity of SCCA against the major food pathogen, ***Salmonella enterica***.

Methods: The minimum inhibitory concentration (MIC) of 21 structurally different SCCA was tested using a high-throughput broth dilution method at pH 4.5, 5.5 and 6.5. Optical density (OD) was determined after incubation, and the biofilm formation was analysed with crystal violet stain. After growing the ***Salmonella*** biofilm with SCCA (1st acid treatment, 1st AT), the biofilm cells were subjected to 2nd AT to determine the minimum biofilm eradication concentration (MBEC).

Results: SCCA inhibited ***S. enterica*** depending on environmental pH with 57%, 33% and 20% of the 21 SCCA reducing final cell density to 50% (MIC_{50}) at concentration < 50 mM at pH 4.5, 5.5 and 6.5, respectively. The MIC_{50} was lower for SCCA with higher pK_a (> 4.0) and hydrophobicity (log Kow > 4.0), while log Kow correlated positively with the presence of a benzene group and negatively with additional hydroxyl and carboxyl groups. Most SCCA inhibited biofilm growth at concentrations of 1.6 – 20 mM, while some SCCA increased biofilm formation up to 2.5-fold at concentrations close to MIC_{50}. The MBEC was ≤ 20 mM when the 1st AT was at least 3-4 times higher than the MIC_{50} against planktonic cells. Crotonic and caproic acids were the strongest antimicrobial and antibiofilm agents while lactic acid showed no inhibition at controlled pH.

Conclusion: This study suggests the application of rationally selected SCCA to inhibit ***Salmonella*** and can serve as a basis when designing fermentation systems to diversify the metabolite profiles in food.

[7] ENVIRONMENTAL PH REGULATES TRYPTOPHAN METABOLISM IN HUMAN GUT MICROBES

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Purpose: Microbial tryptophan (Trp) catabolism in the gut results in the generation of multiple metabolites, exerting either beneficial or harmful effects on host health. For example, gut bacteria can metabolize Trp into indole, which contributes to the progression of chronic kidney disease, or into indolelactic acid (ILA) and indolepropionic acid (IPA), associated with positive health outcomes. Human studies have indicated links between fecal pH and microbiota-derived Trp metabolites; however, the mechanistic regulation of the production is largely unknown. We aimed to explore the effect of environmental pH on bacterial Trp metabolism.

Methods: The effect of pH on bacterial Trp metabolism was assessed by culturing ***E. coli***, a primary producer of intestinal indole, and ***Clostridium sporogenes***, which produces ILA- and IPA, in chemostats at different constant pH, either in monoculture or in defined community experiments. Liquid chromatography-high resolution mass spectrometry (LC-HR-MS) was done to identify the metabolites. Additionally, fecal samples from healthy humans were collected, fecal pH were measured and Trp metabolites were quantified.

Results: Results from ***in vitro*** experiments showed that pH significantly affected Trp utilization and indole production by ***E. coli***, whereas pH did not directly influence the generation of IPA by ***C. sporogenes***. Moreover, using a continuous co-culture of both species, we revealed that low pH inhibits Trp utilization and indole production by ***E. coli***, thereby allowing more Trp available to ***C. sporogenes*** towards the production of high IPA levels. In contrast, high pH stimulated Trp utilization and indole production by ***E. coli***, thus strongly inhibiting IPA production by ***C. sporogenes***. Total bacterial cell counts showed that pH alterations influenced the relative metabolite production by regulating corresponding metabolic pathways rather than altering bacterial abundance. Corroborating our ***in vitro*** findings, we found that the levels of ILA and IPA are negatively associated with pH in human fecal samples.

Conclusions: We present mechanistic explanations for how pH in the gut regulates microbial Trp metabolic pathways. Environmental pH alterations shift the metabolic profile of Trp towards indole at high pH or towards health beneficial Trp metabolites at low pH.

[8[BACTERIA USE EXOGENOUS PEPTIDOGLYCAN AS A DANGER SIGNAL TO TRIGGER PROTECTIVE BIOFILM FORMATION

Sanika Vaidya,[1], Dibya Saha,[2], Daniel Rode,[2], Mads Frederik Hansen*,[3], Praveen Singh,[4], Eric Jelli,[5], Kazuki Nosho,[2], Hannah Jeckel,[6], Stephan Göttig,[7], Knut Drescher,[8]

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For any organism, survival is enhanced by the ability to sense and respond to a threat prior to direct exposure to the threat. Human immune cells can sense bacteria, fungi or tissue damage through many signals that range in information content from very specific to very general danger. For bacteria, danger sensing among kin cells has been previously observed, yet it is unclear whether general danger signal exist. Here we show that different bacterial species use exogeneous peptidoglycan, which is released by kin- or non-kin cells lysis in their vicinity, as a general danger signal. We find that bacteria regulate a response to even brief exposures, which results in the formation of biofilms that ultimately protect cells from a broad range of stresses, including bacteriophage predation. As peptidoglycan from Gram-negative and Gram-positive species triggered biofilm formation, we propose that this danger signal and -response is conserved among bacteria.

[9] STUDYING P. AERUGINOSA AMINOPEPTIDASE AAAA IN A DUAL SPECIES SYNTHETIC CHRONIC WOUND MODEL

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Pseudomonas aeruginosa has intrinsic multi-drug resistance and forms chronic infections that are difficult to eradicate in immunocompromised individuals, resulting in high patient mortality and a global burden of disease. ***P. aeruginosa*** is the leading cause of death following infection of the lungs of people with cystic fibrosis and is one of the main bacteria associated with chronic wound infections. This project aims to determine the role in pathogenesis of one of the virulence factors, termed AaaA, produced by ***P. aeruginosa*** that is crucial for the development of chronic infections. AaaA is an arginine specific aminopeptidase tethered to the surface of ***P. aeruginosa***, but the function it has during the establishment of the coordinated biofilm communities is currently unknown. An existing collagen based synthetic chronic wound (SCW) model was further developed to enable the study of the effect that AaaA has on ***P. aeruginosa*** when cultured with ***Staphylococcus aureus***. When comparing AaaA activity in WT ***P. aeruginosa*** to the respective Δ***aaaA*** mutant a significant difference in the amount of L-arginine cleavage was seen after 2-days incubation. To increase how realistic the infection model was, a set of ***P. aeruginosa*** clinical isolates were used which were co-isolated with ***S. aureus***. In comparison to their matched derived ***aaaA*** mutants, the difference in AaaA activity was less evident, and only appeared after 8 days. Adding these pairs to the model will enable synergy between the isolates with respect to AaaA activity to be investigated. The collagen based SCW model developed in Copenhagen was successfully studied and optimised to allow the activity of AaaA to be tracked in dual species biofilms using bacterial strains which have not previously been co-cultured together. This has allowed research to start exploring the effect AaaA plays in a dual species chronic wound.

[10] LONGITUDINAL CHARACTERIZATION OF ESCHERICHIA COLI IN THE INFANT GUT MICROBIOME

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Introduction: The infant gut microbiome undergoes dynamic changes during the first year of life, with the initial colonization by facultative anaerobes like ***Escherichia coli*** giving way to strict anaerobes. Despite being a pioneer species of the human gut, information on the establishment and maintenance of ***E. coli*** at the strain-level in the infant gut and its effect on microbial maturation and community assembly remains poorly understood. This study aims to provide a comprehensive longitudinal characterization of ***E. coli*** in the infant gut microbiome, shedding light on its diversity, prevalence, and dynamics during the first year of life.

Methods: Approximately 700 ***E. coli*** isolates were collected from 172 infants in the COPSAC2010 cohort at 1-week, 1-month, and 1-year, and whole-genome sequenced. Additionally, we combined the metagenomes from the 1-month and 1-year infant gut to analyze the phylotype and clone complexes of ***E. coli*** present in all 662 children in the cohort.

Results: We clustered the isolates into different clades based on phylogenomic and analyzed their differences in respect to the number/type of genes related to antibiotic resistance, plasmids, prophages, anti-phage defense systems, surface adhesion proteins, and secreted proteins all likely to contribute to persistence. We found that the ***E. coli*** B2 phylotype was the most prevalent and persistent in infants, and the functional profiles potentially underlying this phylotype were elucidated. We also explore the competitive dynamics between ***E. coli*** and strict anaerobes present in early life. Furthermore, we plan to explore how these dynamics were influenced by the infant's exposure history.

Conclusion: Our findings indicate that ***E. coli*** plays a crucial role in the early development of the infant gut microbiome, with the ***E. coli*** B2 phylotype being a key player. The study underscores the importance of strain-level analysis in understanding the contributions of specific microbial populations to gut development. Additionally, it highlights the complex interactions between ***E. coli*** and other bacterial species during the first.

[11] BIOFILMS AND FUNGI AS MAJOR CHALLENGES FOR THE CONSERVATION OF OUR CULTURAL AND NATURAL HERITAGE.

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Microorganisms colonize any kind of surface and environment, including all those artefacts, monuments and sites with an artistic, aesthetic, ethnological or anthropological value that constitute our cultural and natural heritage. Microorganisms are part of our heritage as much as their constituent materials – being those wood, textile, metal, stones or paintings. Upon exposure to different treatments and environments (for example, the transfer of a statue from an outdoor site to a museum indoor), the microorganisms colonizing our heritage change and evolve with dynamics that we still do not fully understand and that can largely affect the state of conservation and the future of our heritage.

Here, we present the two major challenges for the conservation of our cultural and natural heritage: biofilms and fungi. Their resistance to chemical and physical stress and treatments makes prevention the only effective approach conservators can currently use. Unfortunately, most heritage pieces are outdoor or stored in facilities with no climate control, make prevention meaningless. New approaches for biofilms and fungi removal are thus needed.

It is only with a detailed knowledge of microorganisms' interaction with our heritage material that we can propose sustainable solutions to avoid its biodegradation. As an example, we introduce a new longitudinal study to describe and monitor the evolution of the bacterial and fungal communities of six tombstones at the Assistenz Kirkegård in Copenhagen. Taking advantage of a conservation treatment that completely removed the biofilms from the six tombstones, we are collecting samples for microbiome analysis just before the treatment and for a year after. In addition, preconservation 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.

[12] SELECTION AND DOMESTICATION OF NOVEL ENVIRONMENTAL BACTERIA FOR THE VALORIZATION OF LIGNOCELLULOSIC BIOMASS

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Currently most fermentation products are produced from first-generation biomass by well characterized and genetically modifiable model strains, often referred to as cell-factories. To efficiently valorise second-generation biomass using fermentation, strains are needed that can grow on the diversity of sugars and tolerate the inhibitory compounds present in this substrate, traits that are often challenging to engineer in model organisms.

In this project we have isolated bacteria from soil samples that are tolerant to hemicellulosic biomass hydrolysate from beech wood. This has yielded several novel environmental strains that are able to grow on and tolerate this generally toxic substrate. The most interesting isolates are a novel species of ***Pseudomonas*** and a novel strain of the ***Pantoea*** genus. Both strains show relatively high tolerance to the inhibitors present and can grow on a wide range of different substrates. The genome sequences of these strains have been determined using nanopore sequencing and basic physiological features have been characterized. The ***Pseudomonas*** strain has been shown to grow in a wide pH range; from pH 3 up to a pH of 9, which could be an advantage depending on the substrate and product. Both strains showed great robustness and tolerance to high density fermentation in a bioreactor, indicating a large potential for industrial applications.

In order to use these novel strains to produce valuable compounds from lignocellulosic biomass they need to be modifiable and domesticated. To do this, a wide range of molecular tools must be identified and developed. We first established efficient transformation protocols for both strains. We have characterized a number of different constitutive and inducible promoters, a prerequisite for further development of efficient engineering tools. We are currently in the process of developing molecular tools for gene deletions and integrations to improve the industrial applicability of these novel strains.

The discovery, isolation, characterization and modification of these novel strains could help in enabling efficient production of chemicals from lignocellulosic biomass.

[13] A PILOT-SCALE MICROBIAL TECHNOLOGY TO ENHANCE PLASTIC DEGRADATION IN A RIVER

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Purpose: "Microplastics (MP) has caused worldwide concerns. Methods that can enhance the MP degradation in practical environments are highly needed and challenging. Among different MP-degrading technologies, microbial technologies are considered to be the most promising. This study aims to find better MP-degrading microbes and microbial technologies."

Methods: "We isolated bacteria from plastic wastes and ***Bacillus*** sp. Red22 is the most promising one. We also found that microbial fuel cell (MFC) is a promising technology to enhance MP-degradation. Thereafter, we integrated the ***Bacillus*** sp. Red22 and MFC into an bioaugmented-MFC and evidenced its feasibility in a practical river in Guangdong, China."

Results: "Compared to several other bacteria, ***Bacillus*** sp. Red22 showed higher efficiency in decreasing the weight and molecular weight of polystyrene microplastics (PS-MP). It can also develop multilayer-cell biofilms with high activity on the surface of PS-MP after 100 days. On the other hand, lab-scale MFC can stimulate the PS-MP degradation in both sediment and overlying water. After ecotoxicological-safety tests of the ***Bacillus*** sp. Red22, we integrated ***Bacillus*** sp. Red22, as biofilms on either cathode or anode, with MFC and applied it in a practical river for a three-month test. The results showed that either ***Bacillus*** sp. Red22 or MFC alone could simulate the PS-MP degradation in the river. In comparison, the ***Bacillus*** sp. Red22-MFC integrated equipment not only showed higher microbial extracellular electron transfer (current generation) activity in the sediment, but also stimulated the PS-MP degradation efficiency in both sediment and overlying water environments, suggesting a highly feasibility of this technology."

Conclusions: "For the first time, we combined a MP-degrading bacteria and MFC to stimulate the degradation of MP in both sediment and water environments, and we successfully demonstrated the promising feasibility of this pilot-scale biochemical technology in practical river. "

[15] PRODUCTION OF VALUE-ADDED COMPOUNDS FROM LIGNOCELLULOSIC BIOMASS

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Undoubtedly humanity faces various challenges related to the extensive use of fossil feedstocks including global warming and environmental pollution. As a step towards a more sustainable and circular economy, lignocellulosic biomass was proposed as a potential renewable feedstock for fermentations to replace fossil fuel-dependent production lines.

However, despite tremendous research efforts, the valorisation of second-generation lignocellulosic biomass by fermentation is still not industrially feasible. Different aspects make the valorisation of this biomass more difficult than once anticipated. The heterogeneity between different biomass sources, the presence of inhibitory compounds and the limited effectiveness of valorising the sugars concomitantly are challenging. Nevertheless, the anticipated benefits of valorising lignocellulosic biomass by fermentation persist.

The goal of this project is thus to precisely engineer microorganisms to be able to robustly grow and valorise different lignocellulose fractions derived during a novel fractionation process.
In the second stage, the aim is to implement the production of a value-added compound at industrially relevant titers with said microorganisms. The products will be selected to fit the profile of the used lignocellulose fraction. The cleaner cellulose fraction is aimed to be used for higher value products like amino acids while the heterogenous hemicellulose fractions will be used to produce lower-value bulk chemicals.

Finally, investigations on downstream processing steps and potential implications thereof when using lignocellulosic biomass are anticipated. Overall, we hope to contribute to the future production of sustainable chemicals from renewable recourses.

[16] COMPUTATIONAL SCREEN FOR CONSERVED RNA STRUCTURES IN CYANOBACTERIA

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Purpose: RNA structures are essential regulators in cyanobacteria, ***e.g.***, the small RNA PsrR1 regulates photosynthesis, and the glutamine synthetase riboswitch regulates expression relative to the glutamine concentration. To our knowledge, all known RNA structures in cyanobacteria were found with sequence similarity methods. Here, we conduct a phylum-wide screen for novel RNA structures with a broader search space.

Methods: We use the CMfinder tool to predict RNA structures by both sequence and structure conservation. Across 202 cyanobacterial genomes, we screen the intergenic regions adjacent to 931 ortholog genes. To estimate the false discovery rate (FDR), we repeated the screen on the shuffled intergenic search sequences. We checked for recall of known RNA structures relative to homolog hits (CMsearch) of bacterial RNA families (Rfam) and bacterial transcription terminators.

Results: Our phylum-wide screen found 825 motifs with an FDR ≤ 10%. We found overlaps between 402 motifs and known structures, thus these motifs recall several known small RNAs (3 of 14 RNA families) and cis-regulatory RNAs (7 of 16 families). We predict 423 motifs without any overlaps to known structures as novel RNA motifs, for which we derived tentative functional associations via the adjacent gene orthologs. A preliminary analysis suggests novel motifs in key pathways, such as nitrogen assimilation, photosynthesis, RNA degradation, and many more.

Conclusions: To date, our study is the most comprehensive comparative genomic screen for RNA structures in cyanobacteria. Our preliminary results provide a plethora of novel RNA structure candidates to expand the field of microbiological RNA research. Further, we implemented our workflow according to state-of-the-art computational reproducibility standards, such that the analysis can be repeated in other bacterial taxonomies.

[17] INVESTIGATION OF THE POUCH MICROBIOME AND ANTIMICROBIAL RESISTANCE IN PATIENTS SUFFERING FROM POUCHITIS

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Pouchitis is an inflammation that often develops in the ileal pouch-anal anastomosis after a restorative proctocolectomy in the surgical treatment of ulcerative colitis. Today, the first-line therapy for pouchitis is antibiotic treatment. In case of chronic inflammation, a prolonged period of antibiotic treatment is used, increasing the antimicrobial resistance (AMR) susceptibility in the patients' pouch microbiome. AMR poses as a pressing global public health concern. With the steady increase in the use of antibiotics to treat bacterial infections, there has been a simultaneous rise in the spread of AMR genes within microbial communities, rendering many drugs ineffective in combating infections. In 2019, a reported 5 million deaths worldwide were associated with AMR, and by 2050, 10 million deaths are predicted to be caused by multi-resistant bacteria.

In this project, sequencing data and metadata from a clinical study investigating the treatment of pouchitis with faecal microbiota transplantation (FMT) will be analyzed. The pouch microbiome from faecal samples of all patients was sequenced throughout the study period using both Illumina and Nanopore metagenomic DNA sequencing platforms. This allowed for a thorough analysis of the microbial composition before and after FMT treatment, enabling a comprehensive examination of the treatment effects. We aim to characterize the AMR landscape in the patients over time by employing bioinformatic methods to identify patterns and correlations in resistance gene spread and progression for each patient.

[18] CONTROL OF THE GROWTH OF ALICYCLOBACILLUS ACIDOTERRESTRIS SPORES AND BYSSOCHLAMYS ASCOSPORES USING ORGANIC ACID AND HEAT TREATMENT

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Abstract

Despite low pH, certain spore forming bacteria and moulds can grow in acid foods, posing quality and safety concerns. Our previous study found that ***A. acidoterrestris*** may cause spoilage in ambient acid dairy-fruit products. Heat resistant moulds, such as ***Byssochlamys*** species, can also spoil heat-processed acid foods. The objective of this study was to gain insights into the control of ***A. acidoterrestris*** spores and ***B. fulva*** and ***B. nivea*** ascospores under conditions relevant for ambient acid dairy products using heat and organic acids.

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of lactic acid (LA), citric acid (CA), potassium sorbate (PS), and potassium benzoate (PB) against four strains of ***A. acidoterrestris*** were determined using the microplate turbidimetric assay. LA showed a consistent 8 mg/mL MIC across all ***A. acidoterrestris*** strains; PS and PB had MICs ranging from 0.4 to 1.6 mg/mL, with CA being the least effective. The antimicrobial efficacy of these organic acids against ***B. fulva*** and ***B. nivea*** ascospores revealed very low antifungal activity for LA and CA (MIC values >100 mg/mL) and the highest activity for PS (0.8 mg/mL against both species).

The individual and combined effects of heat treatments (75 °C for 20 minutes, 85 °C for 10 minutes, and 95 °C for 30 seconds) and organic acids (0.8% LA, 0.05% or 0.08% PS) on ***A. acidoterrestris*** spore and ***Byssochlamys*** ascospore growth were investigated at 25 °C and 40 °C for 12 weeks. None of the heat treatments used alone inhibited ***A. acidoterrestris*** spore growth. However, 8 mg/mL LA combined with 85 °C for 10 min or higher showed inhibition, highlighting a synergistic effect. In contrast, the presence of only 0.5 mg/mL or 0.8 mg/mL PS inhibited ***A. acidoterrestris*** spore growth. The only heat treatment that inhibited the growth of ***B. fulva*** was 95 °C for 30 seconds. However, 85 °C for 10 min combined with 0.5 mg/mL PS synergistically inhibited ***B. nivea*** ascospores, unlike individual treatments. ***B. nivea*** ascospores showed higher susceptibility to heat and PS than ***B. fulva***. Overall, this study suggests employing combined heat and PS treatments as a viable strategy for the control of ***Alicyclobacillus*** spores and ***Byssochlamys*** ascospores in ambient dairy acid products.

[19] LACTOSE ADDITION INCREASES EXPRESSION OF FECAL MICROBIOTA BETA-GALACTOSIDASES AND THE FERMENTATIVE PRODUCTION OF BUTYRATE INDEPENDENT OF THE PRESENCE OF STARTER CULTURES

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Lactose is the major carbohydrate of bovine milk, fermented dairy, and a component of the prebiotic galacto-oligosaccharides. In the gastrointestinal tract, undigested lactose can be utilized by intestinal microbes to produce health beneficial short chain fatty acids (SCFA). Concurrent ingestion of dairy products with lactose-utilizing starter cultures could lead to the formation of the fermentation intermediate lactate, however this type of lactose-dependent cross-feeding activities of intestinal microbiota has been understudied.

The aim of this study was to investigate the impact of the addition of a lactose-utilizing starter culture on lactose fermentation by fecal microbiota. Fecal samples were collected from 10 healthy adult donors and ***in vitro*** batch fermentations were set up using the complex MacFarlane medium supplemented with 9 mM lactose. ***Streptococcus thermophilus*** and its Δ*lac*Z mutant were added to determine the contribution of starter culture on lactose utilization. Beta-galactosidase activity was determined using nitrophenyl-beta-galactosides and SDS activity staining.

In all fermentations, acetate was the major SCFA that was formed (53-65%) followed by butyrate (16-33%) or propionate (15-24%). The supplied lactose was used within 24 h of fermentation, and lactose addition consistently increased the formation of butyrate up to 1.8-fold compared to controls. Lactate accumulation was higher when lactose was present after 5 h fermentation but was completely utilized for cross-feeding by 24 h. When added, ***S. thermophilus*** or its Δ***lac***Z mutant (around 10^6 cells mL^{-1}) outnumbered the indigenous ***Streptococcaceae*** population but had no impact on lactose utilization and SCFA profiles. Lactose addition increased the activity of structurally different fecal microbiota beta-galactosidases.

Our results indicate that lactose is hydrolyzed and readily fermented by fecal microbiota for all donors, shifting fermentation profiles towards the health associated metabolite butyrate. Fecal microbiota outcompeted the added starter culture in lactose utilization which might prevent the accumulation of fermentation intermediates.

[20] INTESTINAL TRANSIT TIME AND THE GUT MICROBIOTA: CAUSALITY AND IMPLICATIONS?

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Microbial communities are highly dependent on the abiotic (environmental) factors of their surroundings, which affect both the composition and activity of the communities. However, our understanding of how specific abiotic factors of the gut affect the gut microbial community is limited. In the collaborative project named PRIMA (towards **P**ersonalized dietary **R**ecommendations based on the **I**nteraction between diet, **M**icrobiome, and **A**biotic conditions in the gut), we strive to understand how selected abiotic factors in the gut affect the microbiome and thereby host health.
A key environmental factor of the gut is intestinal transit time. This factor is subject to high inter-individual variability even in healthy subjects, and we hypothesize that it may drive differences in gut microbial composition and responses to different diets. Moreover, the extremes of intestinal transit times (i.e., constipation and diarrhea) accompany various medical conditions, including inflammatory bowel diseases, Parkinson's disease, and autism spectrum disorders. Thus, understanding the relationship between intestinal transit time and the gut microbiota may improve our understanding of microbiota/host interactions in health and disease.

We will present the concept and preliminary results from two pilot mouse studies, both focusing on the establishment of animal models for manipulated intestinal transit time. In the first pilot study, we demonstrated that suspensions of Imolope tablets (loperamide), increased intestinal transit time in conventional C56BL/6 mice in a dose-dependent manner. In the second pilot study, we transferred fecal material from two healthy human donors, one with short and one with long transit time, to germ-free Swiss Webster mice. After fecal transplantation, no difference in transit time between the two groups was observed. Thus, in our experimental setup, transit time could not be transferred through fecal transplantation, and we will continue working with the model of pharmaceutical manipulation of transit time in our future animal experiments.

[21] MICROBIAL COMMUNITY RESPONSES ACROSS THREE KINGDOMS AND DIFFERENTIAL GENE EXPRESSION OF PHOSPHORUS CYCLING GENES TO THE ADDITION OF SEWAGE SLUDGE AND SEWAGE SLUDGE BIOCHAR IN SOIL REVEALED BY TOTALRNA METATRANSCRIPTOMICS

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The rhizosphere microbiome is involved in nutrient cycling, carbon sequestration, plant health and plant growth promotion. Understanding how different factors affect the rhizosphere microbiome is crucial to predict effects from agriculture practices such as the application of sewage sludge or sludge based biochar. Sewage sludge transformation into biochar, a sterile, phosphorus-rich product containing a considerable amount of stable carbon, for use as bio-based fertilizer is a promising strategy. In this study, we aimed at unravelling the effects of the application of dewatered sewage sludge and the derived biochar on the active microbial community of the wheat rhizosphere by applying these in localized patches in rhizoboxes. We performed TotalRNA sequencing on samples taken from the patches and from the tip of the roots that passed through them. Our analyses showed changes in the microbial community of the wheat rhizosphere in all Domains from the application of the two products. The changes were more prominent in the sewage sludge treatment compared to the biochar, though localized to the patches. Additionaly, we observed differential expression of genes and pathways related to phosphorus mineralization and utilization (***i.e.,*** acid-phosphatases) and we analyzed the regulation of genes involved in carbohydrate metabolism and sulfur cycling. Our results, coupled with plant growth measurements, plant phosphorus uptake, zymography and visualization of soil pH changes using optodes will allow us to elucidate the effects of sewage sludge biochar addition to soils and to estimate its potential in real life applications.

[22] STUDYING THE PLASMIDOME OF DENMARK

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Plasmids are a key element of horizontal gene transfer, with evolutionary, industrial, and clinical relevance. In this regard, there is an interest in gaining a deeper understanding of plasmids and their dynamics. Metagenomics, the study of genetic material directly from environmental samples, serves as a powerful tool for exploring the plasmidome (the entire plasmidial content of a microbial community). In comparison to traditional methods of plasmid identification, involving culture-dependent techniques, the metagenomic approach allows us to capture the diversity and dynamics of microbial communities ***in situ***.

Using the Microflora Danica dataset, which comprises more than 10,000 metagenomic samples from various environments across Denmark, we will investigate the plasmidome of the whole country, exploring its diversity and spatial dynamics - potentially uncovering uncharacterised elements.

[23] COMPARING TWO METHODS FOR MEASURING EFFLUX IN BACTERIA

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Bacterial efflux pumps are transport proteins located in the membrane and involved in active extrusion of substrates, which can be antibiotics and biocides. Increased expression of efflux pumps associated with extrusion of several drugs are widely implicated in the development of antimicrobial resistance. Efflux is measured by different methods. In this study, an ethidium bromide (Etbr) agar assay and an active efflux (AE) assay were compared. The Etbr agar assay gives an indirect measure of efflux by estimating the intracellular Etbr concentration, whereas the AE assay gives a measure of both the accumulation and the subsequent active efflux.

The study included five clinical isolates of ***Enterococcus faecium***. For the Etbr agar assay, diluted overnight cultures were spotted on brain heart infusion agar containing 5 mg/L Etbr and following overnight incubation were imaged under UV exposure. Fluorescence intensities were calculated using Zen Blue software. In the AE assay, overnight cultures were incubated 1.5 hour with 5 mg/L Etbr, and then resuspended in buffer without Etbr, with glucose. Fluorescence and optical density were measured every 5 min for 3 hours on a BioTek plate reader.

The results showed that the AE assay provided nuances that were not evident in the Etbr agar assay. Examples: the strain that in the Etbr agar assay displayed the lowest fluorescence showed the least EtBr accumulation in the AE assay thus, the lack of EtBr accumulation was likely the main reason for the low fluorescence signal in the Etbr agar assay. Another strain displaying intermediate fluorescence in the Etbr agar assay, accumulated the highest amount of Etbr in the AE assay, but also was the most efficient at extruding the Etbr explaining the intermediate level observed in the Etbr agar assay. The strain with highest fluorescence in both assays had a high accumulation and a low active efflux. To conclude, the AE assay provides a more detailed characterization of the membrane permeability, accumulation, and active efflux, compared to the Etbr agar assay where the constant presence of Etbr fails to differentiate between accumulation and actual efflux activity.

[24] CULTIVATION AND IMAGING OF ASGARD ARCHAEA TO ILLUMINATE THE EVOLUTION OF CELLULAR COMPLEXITY

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The evolution of complex eukaryotic cells from simple prokaryotic ancestors is a remarkable milestone in the history of life, yet the starting point and initial steps in this transition remain a major open question in biology. Progress in resolving this question was achieved by the recent discovery of Asgard archaea, which represent the closest known prokaryotic relatives of eukaryotes and encode proteins that are hallmarks of eukaryotic cellular complexity. Using fluorescence microscopy of Asgard archaea in the environment, we demonstrated a spatial separation of DNA and ribosomes in their cells, implying a condensation and distinct localization of genomic material – like a nucleus. Together with the genomic data, this indicates that key building blocks of eukaryotic cellular complexity originated in a prokaryote. However, systematic exploration of the cell biology of Asgard archaea is hampered by the challenges related to their cultivation and imaging. We aim to cultivate members of the Asgard archaeal phyla, which are the closest relatives of eukaryotes using a novel approach allowing their rapid enrichment from marine sediments. We will then image their ultrastructure using innovative correlative electron and light electron microscopy techniques to reveal their cellular architecture. Discovering subcellular structures at the prokaryote-eukaryote interface will provide insights into the early stages of cellular complexity and could revolutionize our understanding of the bounds between prokaryotic and eukaryotic life forms.

[25] ON THE BIOTRANSFORMATION OF PSEUDOMONADS SECONDARY METABOLITES

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Bacterial-produced secondary metabolites (SMs) play pivotal roles in microbial interactions. While substantial progress has been made in elucidating the structures, biosynthesis, and functions of many natural products, our understanding of the fate of SMs once released into a specific niche still is limited. Questions about the transformation of SMs within microbiome communities, including the underlying mechanisms and ecological implications for both the producer and recipient microbes, are essential for expanding our comprehension of the role of SMs in nature. Therefore, I will outline our approach to developing a method for selecting minimal microbiomes with ***"biotransformation activi***ty" Furthermore, I will present novel examples of bacterial strains capable of degrading cyclic lipopeptides produced by Pseudomonads. SMs' roles in microbial ecosystems are multifaceted, and their transformation is a critical aspect of microbial interactions. Our research aims to elucidate these processes, contributing to a deeper understanding of the intricate web of interactions that govern microbial communities and their environmental impact.

This project is part of the Center for Microbial Secondary Metabolites (CeMiSt) funded by the Danish National Research Foundation (DNRF137).

[26] TWO ARE BETTER THAN ONE: INCREASED BIOFILM FORMATION AND MODULATED GENE EXPRESSION IN DUAL-TYPE COMMUNITIES OF CUTIBACTERIUM ACNES

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Cutibacterium acnes, an opportunistic pathogen in orthopedic implant-associated infections (OIAIs), comprises distinct subspecies and phylotypes (IA_1, IA_2, IB, IC, II, and III). While ***C. acnes*** can cause single and multi-typic biofilm infections, the organization of different phylotypes within a complex biofilm community remains unknown. In this study, we investigated interactions between phylotypes IB (subspecies ***acnes***) and II (subspecies ***defendans***) in co-culture experiments. Contrary to expectations co-culturing of the two strains exhibited enhanced biofilm formation on microtiter plates and titanium discs compared to mono-cultures. Fluorescence in situ hybridization revealed co-occurrence of both strains throughout the biofilm, with the IB strain being more abundant at the base of the disc. Transcriptome analysis demonstrated modulated gene expression in the dual-type biofilm, impacting metabolism, energy production, and stress responses. These findings illuminate the nature of ***C. acnes*** dual-type biofilms, suggesting synergistic relationships. The results of the study influence the understanding of OIAIs diagnosis and treatment.

[27] CHARACTERIZATION OF FIMH IN E. COLI ISOLATED FROM ULCERATIVE COLITIS PATIENTS AND HEALTHY CONTROLS

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Inflammatory Bowel Disease (IBD) represents a complex and multifactorial group of gastrointestinal disorders, with an etiology that is still not completely understood. Patients suffering of IBD often exhibit an increased prevalence of ***Escherichia coli*** (***E. coli***) within their gut microbiota compared to healthy individuals, suggesting that there is a potential link between this bacterium and the disease. This study aimed to investigate this connection by characterizing the adhesin of type 1 fimbriae (FimH) from ***E. coli*** strains isolated from fecal samples of ulcerative colitis patients and healthy controls.

Various assays were employed, including FimH typing using the MLST based CH scheme (FumC/FimH), transformation with plasmid pPKL91 harboring the fimbrial regulator FimB, assessment of adhesion to its natural receptor mannan and RNaseB, and evaluation of anti-adhesive antibodies targeting FimH. Notably, FimH from ulcerative colitis patients exhibited a higher frequency of mutations compared to those from healthy individuals. However, regardless of the FimH type, the antibodies employed in this study demonstrated an inhibitory effect on the adhesion of ***E. coli*** strains isolated from both ulcerative colitis patients and healthy controls. This observation suggests that targeting FimH with antibodies may provide a promising path for therapeutic measures for IBD, by potentially disrupting the interaction between ***E. coli*** and the gut mucosa.

[29] STAPHYLOCOCCUS EPIDERMIDIS EDNA AND POLYSACCHARIDE MATRIX PROTECTS BIOFILMS FROM PHAGOCYTOSIS BY PMNS

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Staphylococcus epidermidis is a ubiquitous skin commensal which is not usually pathogenic. Despite this, it is the leading cause of implant-associated infections. Its main virulence factor is its ability to form biofilms on these devices which are highly tolerant to antibiotics and are effective at evading the immune system. Hence, these infections can become chronic and difficult to treat. Here we investigate to what extent major ***S. epidermidis*** extracellular matrix components, extracellular DNA (eDNA) or polysaccharides, protect ***S. epidermidis*** biofilms from phagocytosis by polymorphonuclear leukocytes (PMNs). To address this, we visualise phagocytosis using time-lapse confocal laser scanning microscopy of PMNs interacting with planktonic ***S. epidermidis***, 24 h biofilms formed by ***S. epidermidis*** wildtype, and 24 h biofilms formed by mutants lacking either eDNA or polysaccharides. We also compare phagocytosis of 24 h vs. 6 h wildtype biofilms.

PMNs quickly became immobilised on 24 h wildtype biofilms and appeared unable to phagocytise the biofilm, whilst in comparison they easily phagocytised planktonic ***S. epidermidis***. Mutant biofilms (24 h) lacking either eDNA or polysaccharides were much less dense than the wildtype, and many PMN were able to phagocytise bacteria in these biofilms. We also observed multiple PMN working together to break up aggregates in the biofilm lacking eDNA. A fraction of PMN were able to phagocytise parts of young wildtype biofilms (6 h), whereas none appeared to be able to phagocytise mature wildtype biofilms (24 h). Our findings suggest that both eDNA and polysaccharides contribute to the ability of ***S. epidermidis*** biofilms to resist phagocytosis, likely due to the larger size of the biofilm and possible increased rigidity of the biofilm when both matrix components are present and in older biofilms.

[30] FROM TWO SENSORS TO A SINGLE SENSOR: BETTER UNDERSTANDING OF OXYGEN–SULFIDE INTERFACES

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In sediments and biofilms intense biogeochemical transformations may occur over sub-mm distances, leading to steep microscale chemical gradients. The introduction of microsensors has significantly advanced our present understanding of these highly stratified environments. Until now most studies have been conducted using individual sensors for the various chemical species. However, when working with multiple sensors, careful alignment of the sensor tips is essential for meaningful interpretation of the chemical gradients. For example, the determination of total dissolved sulfide (TDS) requires perfect alignment of H_2S and pH sensors for precise calculation of TDS, as the fraction of H_2S is dependent on the sample pH. Even minor misalignment of the two sensors or lateral heterogeneity of the sample can drastically change the calculated TDS values and can lead to misinterpretation of biological phenomena.

In this study, we showed how a recently developed TDS sensor and a combined H_2S/O_2 microsensor can improve the analysis of sulfidic environments, such as the oxygen-sulfide interface in photosynthetic mats. Unlike the conventional method, the TDS sensor does not rely on simultaneous pH measurement and can even measure TDS at elevated pH where little to no H_2S can be detected. The combined H_2S/O_2 sensor allows for perfect alignment of H_2S and O_2 profiles and therefore can be used to exactly pinpoint the overlapping gradients of H_2S and O_2 at exceptionally high spatial resolution. The concept and an overview of the working principle of the two sensors is shown in Figure 1.

Using these new tools, we were able to precisely measure TDS under challenging conditions such as the ones imposed by steep pH gradients in photosynthetic mats. In addition, we were able to exactly identify the oxic/sulfidic interface, eliminating measurement artifacts generated by sensor misalignment in multi-sensor setups and spatial sample heterogeneity.

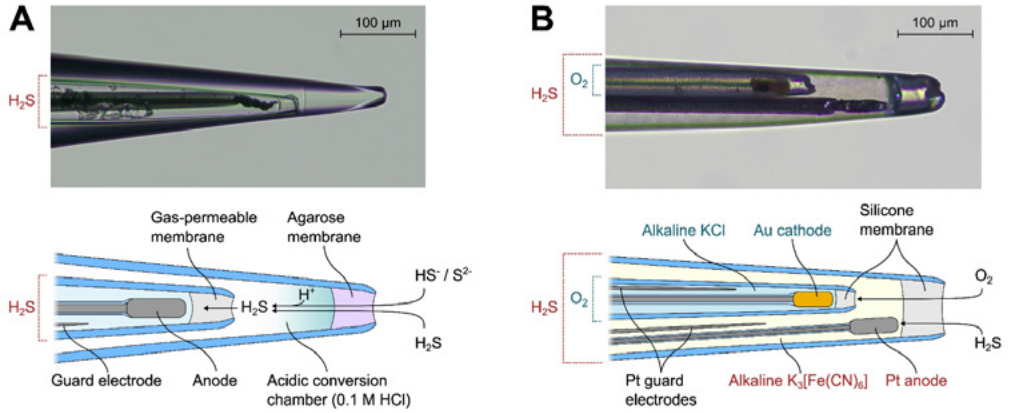

Figure 1. A: TDS microsensor, **B**: combined H_2S/O_2 microsensor.

[31] EXPLAINING THE FORCES BEHIND MICROBIAL BIOGEOGRAPHY

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Microbial life is a pervasive component of the ecosystems of planet Earth. The distribution of microbial populations at geographical scales is not uniform nor random, but shows clear signs of genetic, morphological and functional differentiation. In brief, microbes follow a biogeography and we want to understand why we observe a given microbe at a given location in time and space. 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 action is to explain and predict the microbial biogeography via the integration of the individual processes shaping the communities at scale (i.e., the environment, the ecological interactions and the migrations/stochasticity) and extending the current EU habitat classification to include microbes. To explain microbial biogeography, this action leverages the integration of multiple existing large-scale datasets covering the country of Denmark at an unparalleled resolution. The microbial communities were identified with more than 10'000+ metagenomic samples covering soils, waters and sediments from natural and man-made environments as part of the Microflora Danica (MfD) project. 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; 70+ LiDAR parameters (e.g. canopy openness, solar radiation, etc.) acquired in space and time; field-expert flora and fauna identification; macroecological habitat characterisation as well as crop and land use history.

[32] TRANSCRIPTION OF A TOXIN-ANTITOXIN LOCUS, XRE-RES, IS REGULATED BY A BALANCE BETWEEN RNA POLYMERASE AND TA COMPLEX BINDING

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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. Despite the importance of this aspect, the principles underlying transcriptional regulation and activation of TA systems remain largely unknown. We have previously determined the 2.2 Å crystal structure of the intact ***Pseudomonas putida*** Xre-RES TA complex, comprising the RES-domain NADase toxin and a Xenobiotic response element (Xre) antitoxin[1]which are important cellular regulators in prokaryotes, usually encode two proteins, a toxin that inhibits cell growth and a nontoxic and labile inhibitor (antitoxin. The structure revealed an unusual 2:4 TA stoichiometry, in which each of the toxin molecules of a central dimer interacts with a dimer of antitoxins. The Xre antitoxin belongs to the Cro-like helix-turn-helix (HTH) DNA-binding domain family, known to bind palindromic DNA sequences, suggesting that the TA complex could regulate transcription by binding of its own promoter. In support of this, transcriptional fusion studies show that the Xre-RES complex, but not the Xre antitoxin alone, represses transcription ***in vivo*** by binding to a DNA motif upstream of the ***xre*** gene. This region contains an imperfect inverted repeat overlapping with the transcriptional start site and an upstream putative σ^{70} element. Mutations in this region were found to affect transcription, suggesting that the promoter is optimized for both RNA polymerase and Xre-RES complex binding. In addition, we confirmed that the 2:4 TA complex is capable of DNA binding ***in vitro***, while the isolated Xre antitoxin is on monomeric form likely explaining its lack of DNA binding ***in vivo***. Surprisingly, the complex adapts a 2:2 TA stoichiometry at concentrations below 5nM, suggesting that transcription could be regulated by differences in antitoxin availability. Taken together, we propose a model in which transcriptional regulation of the ***xre-res*** operon is a fine-tuned balance between RNA polymerase and TA complex promoter affinity and depends on the stoichiometry of the Xre-RES complex.

1. Skjerning, R. B., Senissar, M., Winther, K. S., Gerdes, K. & Brodersen, D. E. The RES domain toxins of RES-Xre toxin-antitoxin modules induce cell stasis by degrading NAD. ***Mol. Microbiol.*** **0**, 1–16 (2018).

[33] G-QUADRUPLEXES IN THE EXTRACELLULAR MATRIX OF STAPHYLOCOCCUS EPIDERMIDIS BIOFILM

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Staphylococcus epidermidis is a commensal skin bacterium, which can be pathogenic and tolerant to antibiotics due to formation of a biofilm – multicellular community encased into extracellular polymeric matrix composed of proteins, nucleic acids, lipids and polysaccharides. Confocal microscopy revealed large amount of extracellular B-DNA and GQ-DNA quadruplexes labelled by distinct DNA-specific antibodies. The extracellular DNA (eDNA) appears to become resistant to DNase I as the biofilm matures[1]. Moreover, we detected remodeling of the eDNA in ***S. epidermidis*** biofilms treated by DNase I: while canonical B-DNA was removed, large areas of aggregated GQs were emerged.

The aim of this study was to develop a new approach to visualize GQ-DNA in ***S. epidermidis*** biofilms using DNA-binding dyes rather than immunolabelling. For the first time, we demonstrate GQ-specific Förster Resonance Energy Transfer (FRET) between DNA binding dyes SYTO™60 and TOTO™-1 in the biofilm extracellular matrix. Furthermore, our aim was to determine if GQ-DNA form a peroxidase-like DNAzyme in biofilms when binding hemin. We demonstrate and pinpoint the location of DNAzyme activity using immunolabelling and tyramide signal amplification.

SYTO™60 and TOTO™-1 are used to visualize intracellular and extracellular DNA, respectively, in a biofilm matrix[2]. In the presence of SYTO™60, excitation of TOTO™-1 resulted in FRET between the two dyes in the extracellular matrix. By using a proxy model consisting of pure B-DNA and GQ-DNA, we observed stronger FRET when both dyes bound to GQ-DNA motifs compared to B-DNA. Thus, we propose FRET between SYTO™60 and TOTO™-1 as fast method to indicate GQs in the biofilm.

GQs may be involved in the stiffening of the biofilm matrix[3], biofilm resilience to the mammalian DNase I, and binding heme. Collectively, we confirmed abundance of GQs in the extracellular matrix of ***S. epidermidis***, despite the low GC content of its genomic DNA, using immunolabelling and peroxidase activity of hemin/GQ-DNAzyme. Finally, our study demonstrates how the secondary DNA structures and the use of certain dye combinations have profound effects on the results when imaging eDNA in biofilms.

[1] Buzzo ***et al. Cell*** (2021) 184, 5740
[2] Okshevsky ***et al. J Microbiol Methods*** (2014) 105, 102
[3] Seviour ***et al. NPJ Biofilms and Microbiomes*** (2021) 7, 27

[34] BACTERIAL EFFLUX PUMPS EXCRETE SYTO™ DYES FROM BACTERIA AND LEAD TO FALSE-NEGATIVE STAINING RESULTS

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Efflux pumps (EPs) are mostly associated with transport of antibiotics and antimicrobials out of bacterial cells leading to efflux-mediated antibiotic resistance. The activity of EPs is often studied with fluorescence-based assays that detect the uptake and excretion of a fluorescent dye. Dye uptake occurs under conditions where the EP is inhibited, and the subsequent excretion is then measured after removal of the inhibitor. SYTO™ dyes are commonly used for fluorescence labelling of bacteria, and since SYTO™16 was previously used to study EP activity in mammalian cells, we hypothesized that bacterial EPs also have affinity for SYTO™16 and other SYTO™ dyes. If this is the case, it will impact the result from SYTO™ staining of live bacteria. The aim of this study was thus to determine if bacterial EPs can excrete SYTO™ dyes.

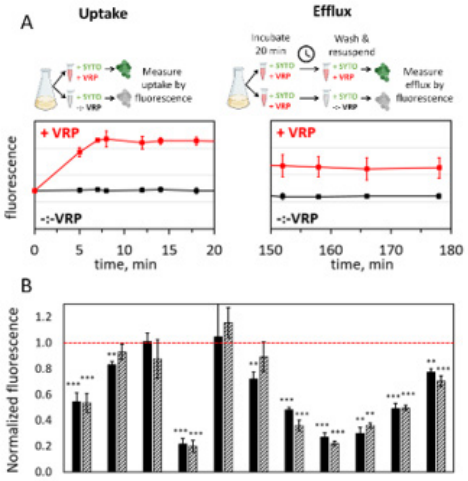

Figure 1. ***S. epidermidis*** **multidrug efflux pumps have affinity for a range of SYTO™ dyes.** A) Schematic illustration of the assay to quantify the effect of efflux pump activity on dye uptake and efflux, exemplified for SYTO™60. B) Mean fluorescence in samples with active efflux pumps relative to samples with VRP-inhibited efflux pumps (normalized pair-wise to the fluorescence in samples containing 300 mg/L VRP). Black bars reflect difference in dye uptake, and striped bars reflect difference in dye efflux. Values <1 signify that efflux pump activity impacts dye uptake (black bars) and dye efflux (striped bars) during uptake and washing, respectively. Student's t test, n=3, **=p<0.01. ***=p<0.001.

We use ***Staphylococcus epidermidis*** (***S. epidermidis***), as a model organism to address this question, because it only encodes homologues of the S. ***aureus*** EPs NorA/B/C. We used Verapamil (VRP) as the EP inhibitor and EtBr as the fluorescent dye for detection of dye uptake and subsequent excretion. After confirming EP activity with EtBr, we tested if the EP also excreted SYTO™9, 12, 13, 14, 16, 21, 24, 25, 60, and 62.

S. epidermidis cells were grown overnight in TSB with glucose, harvested and split into 4 vials per replicate. We measured dye uptake by bulk fluorescence (Figure 1A, left) in samples with/without VRP. When fluorescence only increased with VRP present, the EP had affinity for the dye. Dye efflux was quantified after 20 min (with VRP), and efflux was observed as reduced fluorescence in samples without VRP compared to those with VRP (Figure 1A, right). ***S. epidermidis*** efflux pumps had affinity for SYTO™13, 21, 24, 25, and 60. It had some affinity for SYTO™9, 16, and 62 resulting in slightly impaired dye uptake, while there was no effect on SYTO™12 and 14.

In conclusion, we show for the first time that a wide range of SYTO™ dyes are excreted from ***S. epidermidis*** cells when EPs are active. This means that such SYTO™ stains can be used to detect efflux pump activity, but importantly, it shows how EP activity affects cell staining for use in e.g. microscopy or flow cytometry. SYTO™ staining should be used with care, and we think that EP inhibitors can improve sensitivity and reproducibility of SYTO™ staining in unfixed samples.

[35] SHORT-TERM (CO-)ADAPTATION IN BIOFILMS OF LACTOCOCCUS LACTIS AND LEUCONOSTOC MESENTEROIDES IMPACTS GROWTH PARAMETERS AND INTERSPECIFIC INTERACTIONS

Heiko T. Kiesewalter*,[1], Caroline Olsen,[2], Nathalie Nina Suhr Eiris Henriksen,[3], Mads Frederik Hansen,[4], Jakob Russel,[5], Joseph Nesme,[6], Kevin R. Foster,[7], Birte Svensson,[8], Gunnar Oeregaard,[9], Jakob Herschend,[10], Mette Burmølle,[11]

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Background: In nature, bacteria coexist in polymicrobial complex communities dominated by interspecies interactions. This coexistence contributes to a constant emergence of variants, but their establishment depends on eco-evolutionary dynamics. Spatially structured and heterogenous biofilms enable niche differentiation and local interplay, supporting greater diversity. This study examines the adaptation of mono- and co-cultured ***Lactococcus lactis*** and ***Leuconostoc mesenteroides*** in a biofilm-selecting environment.

Methods: Cultures were passaged every 24 h by bead transfer for 16 days. Evolved variants and ancestors were compared in their growth metrics, competitiveness in reestablished co-cultures, and global proteomes.

Results*:* Evolution experiments displayed that, in biofilm co-cultures, ***L. mesenteroides*** predominated*,* but both species coexisted. Comparative analyses of ***L. lactis*** biofilm variants revealed an increased biofilm formation but, interestingly, increased culture yield and prolonged generation time exclusively in co-evolved variants. Furthermore, the performance of evolved ***L. lactis*** variants was evaluated when co-cultured with ***L. mesenteroides***. Biofilm assays showed a higher proportion of evolved ***L. lactis*** strains than their ancestor when co-cultivated with ancestral ***L. mesenteroides***. This effect was particularly evident in low ***L. lactis*** starting ratios. Interestingly, in co-cultures with evolved ***L. mesenteroides, L. lactis*** was strongly reduced. However, evolved ***L. lactis*** strains were more persistent than their ancestor in such conditions.
Combined, this shows that coexistence in biofilms selects for variants adapted to the biofilm life cycle and the other member's presence, reflected in their enhanced persistence and prevalence in the mixed biofilm. Preliminary proteomic analyses of mono- and co-cultures suggest that proteomes of mono- and co-evolved ***L. lactis*** variants are more distinguishable by the selected ancestor (lineage) than the cultivation method.

Conclusions: This study emphasizes spatially structured biofilms' importance in maintaining species diversity, as interspecies interactions and the biofilm setting were conditional for the emergence of novel phenotypic variants.
The Novo Nordisk Foundation funded this study, grant No. 27620.

[36] IDENTIFICATION OF BACTERIAL DEFENSE MECHANISMS AGAINST CONJUGATIVE PLASMIDS

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The PEACE (Plasmid Evolutionary Arms race) project aims to understand the microbial defense mechanisms that hinder the horizontal gene transfer (HGT) of plasmids in bacteria and shed light on how plasmids overcome these barriers.

To identify novel anti-plasmid defenses, we screened 700 clinical ***E. coli*** isolates for plasmid permissiveness using a previously established dual fluorescent reporter gene system. We measured the transfer of fluorescently tagged plasmids using a flow cytometer, allowing us to determine the ratios of plasmid donors, recipients, and transconjugants in bacterial populations. Our analysis revealed a wide range of conjugative efficiency among ***E. coli*** isolates, which we classified into two distinct groups based on the rate of plasmid transfer. All isolates underwent whole-genome sequencing and, through comparative bioinformatic analysis, we identified genomic regions encoding candidate barriers to plasmid entry. These groups exhibited significant differences in their defense system profiles and we plan to further explore the defense islands of isolates and observe variations among the groups.

Taken together, our research provides fundamental insights for biotechnology applications and addresses a critical knowledge gap in predicting the dissemination of antimicrobial resistance genes through HGT. Future work will focus on uncovering medically relevant barriers to HGT, potentially benefiting biotechnology applications that utilize or restrict plasmid transfer.

[37] AUTOINDUCER 3: A ONE-STEP CONSTRUCTION OF THE DPO RING SYSTEM AND THE FORMATION OF BOTH DPO ISOMERS.

Jacob Tofte*,[1]

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Salmonella typhimurium utilizes quorum sensing to coordinate collective behavior in a density dependent manner. In recent years autoinducer-3 has been detected in first, Vibrio Cholerae as 3,5 DPO and since E. coli as 3,6 DPO. Both isomers were proposed to play a significant role in regulation of biofilm formation and virulence which makes it an interesting target for anti-infective treatment. Since then both isomers have been detected in Salmonella typhimurium though their role in virulence regulation is yet to be revealed. Previously it was established that 3,5 DPO is the predominant isomer of the two proposed structures but to further investigate the biological effects the development of a selective synthesis of 3,5 DPO and its analogous structure was required. We will present research on a one-step construction of the DPO ring system and the formation of both DPO isomers.

[38] CLEAN WATERS AHEAD: HARNESSING SALINITY FLUCTUATIONS TO PREVENT BIOFILM FORMATION IN RO MEMBRANES

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Approximately 2.2 billion people lack access to safe drinking water, with 785 million having no basic drinking water facilities. Desalination and Reverse Osmosis (RO) purification are common in arid coastal regions with limited freshwater, but fouling and scaling limit RO efficiency, increase energy consumption and chemical use. Biofouling of RO systems takes place on a background of fluctuating salinity, yet it remains unclear how such fluctuations affect the formation and species composition of the fouling biofilms. To address this question this study examined the effect of salinity fluctuations on the activity and survival of biofilms of halotolerant and halophilic bacteria.

Two halotolerant bacterial species, ***P. fluorescens*** and ***S. adhaesiva***, were studied, and both showed a significant decrease in growth with increasing salt concentrations. However, when looking at exposure to fluctuating salt concentrations when they grow in biofilm (fluctuations between 0.5 and 10 % NaCl, with 20 minutes incubations in each concentration) no significant effect was observed. Regarding ***A. fischeri***, the most favorable conditions for its planktonic growth were ascertained at a salinity range of 2-3 % NaCl. Departing from this optimal range resulted in a significant reduction in the growth rate. When this organism underwent biofilm growth and exposed to fluctuating osmotic stress, a markedly distinct response pattern emerged. Whenever the osmotic stress included low salinity concentrations, cell survival diminished.

Fluctuating salinity can be used to suppress halophile organisms and by applying high salt concentrations the growth of halotolerant organisms can be limited. The halotolerant bacterium, ***P. fluorescens***, demonstrated the ability to survive exposure to salinities outside the permissive range for growth. Consequently, for this organism, repeated exposure is unlikely to result in the biofilm's demise. However, it may hinder the formation of biofilm. Understanding such variations in response patterns is crucial for elucidating the ecological strategies and survival mechanisms of microorganisms in saline environments.

[39] GENOMIC MOBILISATION BY RRNA OPERON RECOMBINATION – ANOTHER ROUTE OF PHAGE TRANSDUCTION?

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Multiple rRNA operons in bacteria can facilitate homologous recombination of both horizontally acquired and genomic DNA (Lan & Reeves, 1998). Indeed, homologous recombination between rRNA operons can cause large genomic rearrangements in Salmonella (Liu & Sanderson, 1998) and E. coli (Hill & Harnish, 1981). Here we show that Staphylococcus aureus rRNA operons also facilitate homologous recombination and that this can promote a novel route of phage mediated transduction, here called recombined transduction. Transduction is the process whereby bacterial DNA is packaged and transferred between cells by phages. Here we show that large DNA circles formed by homologous recombination of two neighbouring rRNA operons flanking a prophage can be packaged by the phage when induced, leading to recombined transduction at a higher rate than the basal level of generalised transduction. Furthermore, this phenomenon seems to occur more widely, with similarly structured sequences identified in Salmonella. Our results further blur the lines between the core genome and mobile genetic elements and establishes another form of phage mediated transduction, recombined transduction.

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[40] BIOLOGICAL NITRIFICATION INHIBITION – INTEGRATING WHEAT GENETICS, MICROBIAL ECOLOGY, AND NATURAL PRODUCT CHEMISTRY TO IMPROVE CROP PRODUCTION.

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Climate change is a key challenge facing mankind. Anthropogenic activities contribute to nitrous oxide (N_2O) emissions, a long-lived and highly potent greenhouse gas accumulating in the atmosphere at an increasing rate. Agricultural fertilised soils are the primary source of N_2O emissions, where about 50% of fertiliser input in farmland is lost due to the microbial process of nitrification performed by nitrifiers that utilise fertiliser N for their growth. Reducing nitrification in agricultural soils efficiently reduces greenhouse gas emissions, improves crop yield by increasing fertiliser N use efficiency and reduces eutrophication from excessive N input. The synthetic nitrification inhibitors currently in use are inefficient and very costly. The release of nitrification inhibitors from plants, through a process called biological nitrification inhibition (BNI), represents an attractive and sustainable approach to reducing nitrification and increasing the farmer economy.

This project aims to further BNI knowledge in wheat agriculture by integrating wheat genetics with plant metabolite natural chemistry and its impact on nitrifier microbial diversity and activity in soils. We will establish a high-throughput platform for identifying BNI capacity in old landraces and currently used wheat cultivars with the aim of breeding wheat lines with an improved BNI efficiency. Here we will present our preliminary data from old Iranian wheat landraces demonstrating that different wheat varieties have variable BNI efficiency based on nitrifier response and its links to the root exudate chemistry.

[42] AN APPROACH TO UNCOVER DNA METHYLTRANSFERASES IN METAGENOME-ASSEMBLED GENOMES

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DNA methylation is a universal epigenetic regulation mechanism across all domains of life. In bacteria, it modulates a range of biological processes, including host defense mechanisms, cell cycle regulation, gene expression, and virulence. This modification is facilitated by DNA Methyltransferases, which transfer methyl groups from S-adenosyl-1-methionine to DNA. These enzymes dictate the methylation patterns of bacterial genomes in a motif-specific manner, which often differ among species and strains. Recent advancements in sequencing technologies have made it possible to detect methylation signals, thereby enabling the prediction of genome-associated methylation motifs and methylation patterns.

A key step towards understanding the bacterial epigenome and the mechanisms behind genomic methylation patterns lies in identifying the DNA methyltransferases dictating these patterns. Here, we explore an approach to 1) identify putative DNA methyltransferase genes in metagenome-assembled genomes 2) classify these putative methyltransferases by their respective restriction-modification system Type and predict features of their target methylation motif.

[43] PHAGE-ENCODED XENOGENEIC INTERFERENCE MODULATES QUORUM SENSING AND VIRULENCE IN PSEUDOMONAS AERUGINOSA

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Bacteriophages (phages) are viruses that infect and kill bacteria. To protect against phage infection, bacteria have evolved phage defense systems such as phage receptor modifications, nucleases, and xenogeneic silencers. The ***Escherichia coli*** encoded H-NS is the most well studied xenogeneic silencer. It acts as a global transcriptional repressor in addition to repressing phages and other horizontally acquired elements such as phage defense systems and virulence islands. The human pathogen ***Pseudomonas aeruginosa*** encodes two H-NS homologs called MvaT and MvaU that likewise serve as global regulators controlling quorum sensing (QS), virulence as well as repressing phages. As phages infecting ***E. coli*** encode proteins that antagonize H-NS as a mechanism to allow phage propagation, we asked if ***Pseudomonas*** phages employ a similar strategy by expressing ***mvaT*/*U***-like genes to inhibit MvaT/U function and alleviate phage repression.

To examine this, we identified ***mvaT/U***-like genes carried on genomes of uncultured phages and expressed them ectopically in ***P. aeruginosa***. Culturing the cells, we observed that expression of some of the phage-encoded ***mvaT/U***-like genes led to increased production of the virulence factor pyocyanin, which is one of the key phenotypes of Δ***mvaT*** or Δ***mvaU*** mutants. This suggests that the phage-encoded ***mvaT/U***-like gene products inhibit MvaT and/or MvaU. As pyocyanin is regulated by QS, we are currently quantifying the effect of the phage-encoded MvaT/U homologs on the level of the QS autoinducers 3-oxo-C12-HSL and PQS in ***P. aeruginosa***. Additionally, we will determine the effect of the phage-encoded ***mvaT/U***-like genes on phage proliferation, by quantifying phage burst sizes during infections of cells expressing the phage-encoded MvaT/U homologs.

Potentially, phages may employ xenogeneic interference as a stealth mechanism to avoid phage repression by xenogeneic silencers of the host. While xenogeneic interference could be utilized in a phage therapy context to circumvent MvaT/U-mediated phage repression, it presents a double-edged sword as it could lead to increased virulence of ***P. aeruginosa.***

[44] PERMISSIVENESS TOWARDS RESISTANCE PLASMIDS AND PLASMID FITNESS EFFECT VARY ACROSS AEROMONAS FROM RESIDUAL WATERS

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Aeromonas is prevalent in wastewater and some are opportunistic pathogens of humans and animals. Plasmids are also frequently detected in ***Aeromonas***, making them good candidate indicators of the dissemination potential of antimicrobial resistance between humans and the environment. However, there is a knowledge gap on whether the ability to acquire and maintain conjugal plasmids is homogenously distributed within this genus. Therefore, the aim of this study was to investigate a set of diverse ***Aeromonas*** strains isolated from residual water systems in Denmark for their ability for uptake of resistance plasmids and for plasmid maintenance, evaluated as the cumulated effect of plasmid segregational loss and plasmid-induced fitness cost/benefit on their growth. Based on this information, we aimed to assess the contribution of wastewater treatment plant (WWTP) discharges to the spread of antibiotic resistance in the environment.

Aeromonas spp. were isolated from both raw sewage and effluents of several WWTPs in Denmark, and from upstream and downstream locations of the receiving water bodies. The taxonomic diversity of the retrieved ***Aeromonas*** covered 13 species with ***A. media*** predominant in all samples. Solid matings were performed between diverse ***Aeromonas*** strains and model donors carrying the broad host range plasmid. ***A. media, A. veronii***, ***A. salmonicida***, ***A. caviae*** and ***A. allosaccharophila*** can obtain plasmids from several donors with relatively high transfer rates. Environmental ***Aeromonas*** were more permissive towards pKJK5 than pTR4, but with obvious inter-strain variability. Through a 24-hour competition experiment without selection, we observed that pKJK5 tended to be lost from most of the 106 permissive isolate cultures. However, 9 isolates demonstrated net fitness benefits from plasmid carriage, which implies high likelihood of long term plasmid persistence.

Overall, we conclude that the ability of ***Aeromonas*** to take up and carry plasmids makes them potentially important vectors for resistance dissemination, with some isolates playing a disproportionally large role as reservoir of conjugal plasmids. WWTP discharges may enhance the dissemination of antibiotic-resistant Aeromonads into surface water, thereby increasing the risk of spreading antibiotic resistance in the environment.

[45] THE IMPACT OF ENVIRONMENTAL FACTORS ON ECOLOGICAL PATTERNS OF MICROBIAL SUCCESSION WITHIN THE FIRST SIX YEARS OF LIFE

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The healthy embryo is considered essentially sterile and the first and very important colonization is established by the infant's early contact with the environment. The bacterial composition of the gut is very dynamic during the first year of life. Initially the composition is dominated by early colonizers, while these are later outcompeted by bacterial taxa characterizing a more mature composition. Despite the role of colonization patterns in structuring the human gut microbiota being increasingly appreciated, we know little about the history dependency of initial community assembly and its implications for the maturation trajectory of the infant gut microbiota.

$COPSAC_{2010}$ is an ongoing Danish cohort study of 738 unselected pregnant women and their 700 children followed from pregnancy week 24 with deep clinical phenotyping and microbiome characterization. Here we used this longitudinal cohort of 700 Danish children to study the gut microbiota dynamics during their first 6 years of life. Using a time-course gene set analysis, we analyzed a complete series of samples by 16S rRNA gene sequencing (n = 2699) from 1 week to 6 years and identified four main trajectory clusters for individual genera in the developing gut microbiota and the formation of distinct community states. Using Dirichlet multinomial mixtures modeling 7 clusters and three phases can be identified, an initial colonization, transition, and stabilization.

Utilizing the developmental trajectories, we derived deviation and fluctuation scores for each child. Subsequently, we correlate these scores with various environmental factors, which include rural vs. urban living, duration of breastfeeding, antibiotic intake, and exposure to siblings or pets. These environmental factors can substantially increase in deviation and fluctuation. The initial colonization patterns of microorganisms in the gut can be influenced by environmental exposures, leading to alterations in community types and assembly over time. Thereby, these initial changes can manifest a lasting impact on the compositions in the infant gut. Our results highlight the distinct ecological patterns observed within our cohort and how the environmental factors influence the developmental trajectories and microbial dynamics in early life.

[46] THE IMPORTANCE OF SPATIAL ORGANIZATION AND MATRIX PRODUCTION IN MULTISPECIES BIOFILMS DURING PHAGE PREDATION

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Bacteriophages (phages) are viruses that specifically infect and kill their host bacteria, resulting in a constant threat to the survival of bacterial populations. Most of our knowledge on bacteria-phage interactions is rooted either in single-species experiments conducted in planktonic cultures or in culture-free sequencing-based approaches. In terms of community context, these two approaches represent the extremes of controlled and complex systems, respectively. That leaves a gap as we do not understand how the dynamics of phages and hosts shift in a multispecies context of medium complexity, where a moderate number of species are present.

Here, we challenged a three-species community consisting of ***Vibrio anguillarum, Kluyvera cryocresens*** and ***Escherichia coli*** with the lytic ***E. coli***-specific coliphage T7. By administrating the bacteria in a sequential manner, we were able to grow a segregated biofilm resulting in a spatial organization of the two other species on top of an ***E. coli*** biofilm. By utilizing confocal laser scanning microscopy, we found significantly increased survival of ***E. coli*** after exposure to the T7 phage when grown in our multispecies biofilm compared to a ***E. coli*** monospecies biofilm. Moreover, by growing dualspecies biofilm we observed that ***V. anguillarum*** would confer a high level of ***E. coli*** survival, while ***E. coli*** together with ***K. cryocresens*** would be eradicated by the T7 phage.

Currently, we are constructing different mutants of ***V. anguillarum***, each lacking a matrix-associated gene or gene cluster, in order to identify matrix components involved in the observed protection of ***E. coli***. Although, we have not yet pinpointed a specific matrix component being essential for the protection, our results indicate that the protection is reliant on the biofilm-associated polysaccharides produced by ***V. anguillarum*** shielding ***E. coli*** from the T7 coliphage.

[47] MYXOBACTERIA AS OFF-FLAVOUR PRODUCERS IN RECIRCULATING AQUACULTURE SYSTEMS: ISOLATION AND INFLUENCE OF NUTRIENTS ON OFF-FLAVOUR GENERATION

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Purpose:Earthy off-flavors in aquaculture products reduce consumer preferences for farmed fish and cause economic losses for farmers of Recirculating Aquaculture Systems (RAS). These microbiologically produced compounds have mainly been attributed to Cyanobacteria and ***Streptomyces***, but recent molecular studies indicate that the more obscure myxobacteria may be the leading bacterial group responsible for earthy off-flavors in aquaculture. For the first time we succeeded in isolating these bacteria from RAS, enabling their production of geosmin (**1**) and 2-MIB (**2**) to be studied in detail by Gas Chromatography-Mass Spectrometry (GC-MS).

Methods: Samples from a Danish RAS were used for the isolation. Isolated strains were identified through 16S rRNA gene amplicon sequencing and subsequently whole genome sequenced. The isolates were cultivated in different nutritional media from which **1** and **2** were extracted and analyzed through GC-MS. Production of **1** and **2** in the different media were normalized to cellular production by enumeration using qPCR targeting the 16S rRNA gene.

Results: For the first time, isolation of myxobacteria from RAS was successful. The isolates produced insignificant levels of **2**. The concentration of **1** was striking for both isolates in rich media (>1500 ng/L). When normalizing the **1** produced to the cell count of the sample, it was evident that **1** is not produced constitutively, but depend on nutritional factors. There was a significantly higher cell-specific production in the minimal medium for both isolates. Comparing to a study from Klausen et al. (2005), who isolated different ***Streptomyces*** from Danish fish ponds, and found that the **1** production of those isolates ranged from 0.13-35 ag **1** $cell^{-1}$ h^{-1}, the production here ranges between 2-99 ag **1** $cell^{-1}$ h^{-1}.We can conclude that the **1** production of these myxobacteria is of the same magnitude as ***Streptomyces***, and likely has a significant contribution to off-flavors in RAS-reared products.

Conclusion: Myxobacteria isolated from RAS are prolific producers of the off-flavor compound **1**. Their production is of the same magnitude as of ***Streptomyces***. The production is not constitutive but depends on nutritional composition. This study provides further knowledge of how the rearing water composition will influence the earthy off-flavor production of these bacteria.

[48] EFFECTS OF AGRICULTURAL PRACTICES ON SOIL PROTIST COMMUNITIES

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Protists are an important component in soil food webs providing services such as nutrient cycling, soil fertility maintenance and population control through predation. Nevertheless, the main drivers of the protist community structure remain poorly understood. Hence, we conducted a large-scale field experiment examining the diversity of soil protists to gain further insight into the impact of factors such as season (spring vs. fall), soil type/location (Foulum vs. Høje Taastrup) and a wide range of agricultural practices including the choice of crop (spring barley and winter wheat), the use of different types of fertilizer (pig slurry vs. conventional fertilizer), three different nitrification inhibitors and tillage regime (conventional tillage vs. no-tillage) on protist community composition. The experiments were carried out at two field locations (Foulum vs. Høje Taastrup) with different soil types during 2020 – 2022 with sampling in spring and fall. The genetic diversity of the protist community in 270 environmental soil samples was assessed by metabarcoding of DNA using protist specific primers targeting the V9 region of the 18S rRNA gene. We found diverse protist communities within the phyla of ***Cercozoa***, ***Amoebazoa*** and ***Cillophora*** with a dominance of Cercozoa. Soil samples subjected to organic fertilizer exhibited a distinct microbial signature and using the PERMANOVA test we found that location, fertilizer and tillage regime, in that order, were vital factors in shaping the protist community structure. This knowledge will help unravel the factors that shape the protist community structure and will lead to a better understanding of soil food web.

[49] NOVEL GENUS OF BACTERIOPHAGES TARGETS DANISH SOFT ROT ISOLATES AND REPRESENT PROMISING BIOCONTROL AGENTS

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The bacterial diseases black leg and soft rot in potatoes causes heavy losses of potatoes worldwide. Bacteria within the genus ***Pectobacteriacea*** are the causative agents of black leg and soft rot in Potatoes. The use of antibiotics in agriculture is heavily regulated and no other effective treatment currently exists, but bacteriophages (phages) have shown promise as potential biocontrol agents. In this study we aimed to isolate phages targeting Danish soft rot bacteria. Danish soft rot bacteria were isolated from tubers and plants symptomatic with soft rot or black leg disease. Using organic waste, we isolated 19 phages targeting different species within Pectobacterium. Here we focus on seven of these phages representing a new genus primarily targeting ***P. brasiliense***; phage Ymer, phage Amona, phage Sabo, phage Abuela, phage Koroua, phage Taid and phage Poppous. TEM image of phage Ymer showed siphovirus morphology, and phages belong to the class ***Caudoviricetes,*** with double-stranded DNA genomes varying from 40kb to 42kb. A hostrange experiment with 59 bacterial isolates from Danish tubers and plants symptomatic with soft rot or black leg disease, showed phages to primarily target ***P. brasiliense***. Interestingly the seven phages displayed difference in hostrange even within species level, with two of the phages being able to infect two or more species of ***Pectobacterium***. All phages were able infect 8, or more, out of 17 isolates of ***P. brasiliense. In silico*** analysis of the ***P. brasiliense*** genomes showed difference in genes encoding antiphage systems as well as in genes involved in the outer membrane, even in closely related isolates, which correlated with host range results. None of the phages encodes any integrase or other genes typically associated with lysogeny. Based on the genome analysis together with the host range results these phages could have potential as biocontrol agents against soft rot and black leg in potatoes and should be tested further.

[50] MOUND COMPARTMENTS AND SOIL NUTRIENTS, BUT NOT SYMBIOTIC PODAXIS FUNGI, DRIVE MICROBIAL LANDSCAPES IN TRINERVITERMES TERMITE COLONIES

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Termites are important ecosystem engineers across the globe and play key roles by modulating microbial communities within and outside their mounds. Microbial diversities within termite mounds are generally lower than surrounding soils, likely due to termite-associated antimicrobial compounds and active sanitary behaviors. Microbial symbionts of termites can also influence the microbial landscape within termite mounds, where symbionts can inhibit or out-compete other microbes. Certain clades of the arid habitat specialist fungal genus ***Podaxis*** (Agaricomycetes; Agaricaceae) are symbiotic with grass-cutting ***Trinervitermes*** termites and may affect mound-associated microbiomes. To test this, we characterized fungal (ITS) and bacterial (16S) communities within and outside 39 ***Trinervitermes*** mounds with and without ***Podaxis*** fruiting bodies across a 1000km transect in South Africa. We predicted that ***Podaxis*** would be a dominant member of the fungal communities in mounds and that its presence would reduce microbial diversity and tested if soil elemental composition affected ***Podaxis*** presence. As expected, we observed less diverse fungal communities, but not bacterial communities, within than outside mounds, and differed by sampling regions and mound compartments. ***Podaxis*** sequences were found in 46 of 49 mounds, but in relatively low abundance, and neither the presence of fruiting bodies or ITS sequences were associated with microbial community diversity or composition. There was, however, an overall association between ***Podaxis*** fruiting body presence and elemental composition; albeit, with different elements playing a role in different geographic regions. Finally, we observed multiple significant associations between elemental amounts and fungal and bacterial taxa, indicating that soil element composition is a key driver of community compositions. Taken together, our findings suggest that ***Podaxis*** is not a major driver of microbial community composition in ***Trinervitermes*** mounds, which in contrast is most strongly affected by termite microbial filtering and regional elemental compositions.

[51] MICROBIOTA'S IMPACT ON INFERTILITY

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Infertility, affecting 10% of the global population, is defined as a year of unprotected sexual intercourse without conception. The intricate factors contributing to infertility include age, lifestyle, nutrition, and environment. Overlooked in infertility diagnostics is the role of microbiota, particularly in the female reproductive tract. This study investigates the impact of zoonotic infections on the vaginal microbiota composition which has yet to be fully explored. In this study, vaginal samples are collected from individuals with confirmed infertility and healthy controls in collaboration with fertility clinicians. With the utilization of High-throughput Nanopore sequencing of 16S rRNA genes, a characterization of the vaginal microbiota profiles is achieved. Furthermore, metagenomic analysis is performed to elucidate potential functional changes in the vaginal microbiota associated with zoonotic-infections, which could include alterations in microbial community structure, virulence factor profiles, diversity in taxonomic composition, and relative abundances of specific taxa. This would be the first step to incorporate microbiota analysis into clinical infertility diagnostics unveiling dysbiosis-related infertility causes affecting conception and reproductive technologies outcomes.

[52] THE GLOBAL REPRESSORS MVAT AND MVAU REGULATE CRISPR-CAS ACTIVITY IN PSEUDOMONAS AERUGINOSA BY CONTROLLING GROWTH RATE

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CRISPR-Cas is an adaptive immune system of bacteria and archaea that protects against foreign genetic material, including plasmids and bacteriophages. CRISPR arrays store the memory of previous infections as short nucleotide sequences called spacers. These are transcribed and processed into mature CRISPR-RNAs that guide the Cas-mediated cleavage of intruding foreign genetic elements. Yet, despite intensive research on CRISPR-Cas function, our knowledge of the physiological factors controlling CRISPR-Cas activity is still limited. The human pathogen ***Pseudomonas aeruginosa*** employs a cell-cell signaling system, called quorum sensing, to activate the expression of CRISPR-Cas at high cell density. Additionally, lowering growth rate increases CRISPR-Cas activity in ***P. aeruginosa***. In ***Escherichia coli***, the conserved global regulator H-NS is known to repress CRISPR-***cas***. While ***P. aeruginosa*** harbors two H-NS homologs, MvaT and MvaU, their role in CRISPR-***cas*** regulation has not been explored. In this study, we investigate whether MvaT and MvaU regulate CRISPR-***cas*** in ***P. aeruginosa*** PA14. We show that in the absence of both H-NS homologs, CRISPR-Cas activity is increased by an order of magnitude, as measured by the loss of a CRISPR-targeted plasmid normalized to a control plasmid. Even though this suggests that MvaT and MvaU inhibit CRISPR-Cas activity, our preliminary data shows no significant difference in ***cas3*** mRNA or Csy4 Cas protein abundance between a Δ***mvaT*** Δ***mvaU*** mutant and parental strain as determined by RT-qPCR and Western blotting, respectively. Importantly, the Δ***mvaT*** Δ***mvaU*** mutant had a significantly lower growth rate as compared to the parental strain. Thus, while our data shows increased CRISPR-Cas activity in the absence of both MvaT and MvaU; this could be a direct consequence of the decreased growth rate in the Δ***mvaT*** Δ***mvaU*** mutant, as a slower growth rate has previously been demonstrated to enhance CRISPR-Cas activity in ***P. aeruginosa***.

[53] VISCOSIN FROM P. FLUORESCENS SBW25 IS REGULATED BY THE RECOGNITION OF INTERSPECIES AND INTERKINGDOM MOLECULES THROUGH LUXR RECEPTORS

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Viscosin is a cyclic lipopeptide (CLP) produced by ***Pseudomonas fluorescens*** SBW25 that facilitates bacterial plant-root colonization due to its biosurfactant properties. The biosynthesis of viscosin is regulated by two LuxR-type regulators, ViscAR and ViscBCR, but the exact regulatory mechanisms remain unclear. The production of CLPs in several Pseudomonads is regulated through quorum-sensing, where a LuxR transcriptional regulator binds to an acyl homoserine lactone (AHL) signaling molecule, forming a complex that induces the transcription of the target genes. Although ViscAR and ViscBCR belong to the LuxR family, they lack the conserved amino acids typically required for binding to AHLs. However, recent research suggests that in addition to binding to their cognate AHLs, several LuxR receptors can also interact with other compounds, including plant phenolics like arbutin, salicin, phenyl as well as flavonoids.

We hypothesize that plant phenolics and/or AHLs in complex with ViscAR/BCR regulators, or other uncovered orphan LuxR regulators in the SBW25 genome may trigger up-regulation of viscosin biosynthetic genes. To investigate this, we tested a range of plant phenolics and quorum-sensing molecules as potential triggers for viscosin biosynthesis using a bioreporter assays. We determined that ViscAR up-regulates the expression of ***viscA*** in the presence of salicin, ***viscB*** in the presence of C6-HSL, and self-regulates its expression in the presence of 3OC6-HSL. Additionally, ViscBCR regulates ***viscA*** and ***viscAR*** expression via an unknown mechanism. Moreover, we discovered an orphan LasR-homolog that directly regulates ***viscA*** in the presence of 2-benzoxazolinone (BOA). An ***in silico*** structural analysis of ViscAR showed a non-archetypical ligand-binding domain potentially able to bind to AHLs and plant phenolics. The observed induction translated into significantly increased swarming motility of SBW25 in the presence of salicin, C6-HSL and 3OC6-HSL.

This study shows that SBW25 can sense its environment and respond to both interspecies and interkingdom signals of importance for its dispersal.

[54] WHOLE-CELL BIOSENSORS FOR DETECTION OF BACTERIAL AND PLANT SIGNALS PRESENT IN THE SOIL MICROBIOME

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The soil microbiome inhabiting the plant rhizosphere and the interactions between microbes and plant roots play essential roles in plant health and resilience. Most inter- and cross-species interactions between microbes and plants can are mediated by key secondary metabolites (SecMet). However, it remains challenging to capture these chemical interaction events ***in situ***. Therefore, our understanding of how plant-beneficial rhizobiomes are formed and how they function is limited. The aim of the project is to design and implement bacterial whole-cell biosensors to not only detect various SecMets, but also record the detection event in genetic memory devices such that we retain the spatial and temporal dynamics of the SecMet production. As opposed to typical sense-and-report biosensors utilizing e.g., fluorescent reporters, we store the detected signal as irreversibly "flipped" DNA sequences on the sensor plasmid for subsequent analysis. Our development and finetuning of biosensors for detection of the ***Streptomyces coelicolor*** produced antimicrobial compound actinorhodin and the plant flavonoid naringenin will be presented. Actinorhodin production is induced by various stresses (predatory, iron depletion etc.) and is thought to play a role in biocontrol. Plant cells can excrete naringenin to remove pathogenic bacteria and fungi and limit oxidative stresses, but also to recruit symbiotic rhizobial microbes. Both sensors are designed based on the regulatory mechanisms natively involved in either the production or degradation of the target SecMets. Sensor-containing microbes included in the plant rhizosphere together with native soil microbes will enable recording the presence of these important SecMets during different environmental conditions.

[55] LINKING BIOGENIC HIGH-TEMPERATURE ICE NUCLEATING PARTICLES IN ARCTIC SOIL AND STREAMS TO THEIR MICROBIAL PRODUCERS

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Clouds impact global climate by regulating precipitation and radiative balance. Aerosols, including bioaerosols, influence clouds and precipitation. Despite their prevalence, biological aerosols, and especially their sources are underexplored, affecting Earth's energy budget uncertainties through aerosol-induced cloud changes. The Arctic is highly vulnerable to climate change due to Arctic amplification and features persistent mixed-phase clouds that impact the energy balance. Ice crystals modify the content of supercooled liquid water, with ice nucleation being a crucial process driven by ice nucleating particles. This study focuses on biogenic INPs. Research gaps remain regarding their presence in soil and potential transport into freshwater systems. Arctic soil is housing diverse microbial communities, and may hold a significant amount of INPs, but specific INP-producing taxa are unexplored. This research aims to fill these gaps by analyzing soil taxonomic composition and quantifying INP concentrations and size distributions in soil and adjacent freshwater samples from Northeast Greenland streams. It seeks to reveal the connection between soil and freshwater systems in INP dynamics, impacting cloud formation, precipitation patterns, and Arctic climate dynamics. Findings show that soil samples had high freezing temperatures generally above -8°C. The composition of INPs varied widely across locations, including bacterial and fungal sources, challenging conventional expectations. In streams, INP concentrations were abundant, suggesting potential significance in atmospheric processes. Multiple sources, including terrestrial runoff, glacial outwash, and autochthonous production, could contribute to INPs in the streams. The findings underscore the complexity of Arctic INP dynamics and their potential impact on cloud formation and climate. This study advances Arctic INP understanding and their role in regional climate processes. Given rapid Arctic transformations due to climate change, grasping INP dynamics and impacts is vital for precise climate modeling and predictions. Further investigations into INP sources, transport, and climate consequences in shaping the Arctic's climate are crucial.

[56] EMPOWERING ANTIBIOTICS IN THE AMR LANDSCAPE: INSIGHTS FROM DENDRIMER CONJUGATION IN ALI SYSTEMS

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In the shadow of the AMR crisis, reigniting the antibiotic pipeline has emerged as a pivotal strategy. Despite the urgent need, there has been a decrease in finding new types of natural or synthetic antibiotics over the past forty years. Of the 65 antibiotics introduced in the last two decades, only four were genuinely new pharmacological classes, whilst the remainder were merely derivatives or modifications of pre-existing compounds. This challenge arises from the need to bypass bacterial resistance, which, paradoxically, is also the target.

Inspired by a 2006 study that successfully integrated propranolol with a known drug delivery polymer, DAB-PAMAM dendrimer, for enhanced cellular uptake, we advanced this approach by conjugating the antibiotic ciprofloxacin with PAMAM dendrimers. This strategy aims to enhance the antibiotic's effectiveness by preventing its ejection from cells.

The screening results were promising as ***Escherichia coli*** showed MIC values of 1.25 µg/µl and ***Pseudomonas aeruginosa*** 1-2 µg/µl. ***Staphylococcus aureus*** exhibited values, oscillating between 2-4 µg/µl. MIC values, which lowered by a factor of 2 when tested in media other than MHB. The compounds did not result in cytotoxicity nor hemolysis.

An important outcome of these experiments was the insight that tackling AMR requires not only identification of suitable candidates, but also require that standardized screening methodologies are in congruence with clinical needs.

Therefore, we employed an air-liquid interface (ALI) culture set-up to mimic respiratory epithelial cells' natural environment. By introducing ***P. aeruginosa,*** a severe cause of respiratory infections, we sought to compare its behavior with standard lab screenings, aiming to bridge the gap between conventional lab methods and clinical needs inside the antimicrobial development pipeline.

[57] PROBING THE DARK MATTER OF BACTERIAL GENOMES: THE COMPLETE SULFIDE OXIDATION PATHWAY IN CABLE BACTERIA HIDDEN AMONG HYPOTHETICAL PROTEINS

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In aqueous surface-sediments around the world, centimeter-long filamentous bacteria called cable bacteria have been found. These bacterial filaments are highly effective sulfide-oxidizers as they spatially separate the two half reactions of sulfide oxidation and oxygen reduction by transporting electrons through conductive fibers in the filaments. The electric potential gradient, changes in pH, and the separation of substrates create a unique ecosystem in which cable bacteria thrive.

It is known that cable bacteria utilize the reverse dissimilatory sulfite reductase (Dsr) pathway to oxidize sulfide. They contain and express ***dsrABC***, ***aprAB***, and ***sat***, but other key genes of the pathway have not been identified in cable bacteria genomes yet, including the marker genes ***dsrEFH***. This study set out to identify unknown components to the central metabolism of all cable bacteria. To achieve this, high-quality genomes of cable bacteria were obtained by Oxford Nanopore sequencing and from the NCBI database. Comparative genomic analysis was then performed to identify the genes shared among all cable bacteria, and these were compared to expression data from single cable bacteria species. The resulting list of highly expressed and highly conserved genes contained 42 proteins annotated as “hypothetical proteins”, and the structures of these proteins were predicted with AlphaFold2. Three of these protein structures had high structural homology to known DsrEFH proteins and were further analyzed to identify conserved catalytical residues and possible quaternary folds. Subsequently, the genes were inserted into a plasmid and transformed into ***E. coli*** for expression and analysis with persulfurization assays. The results showed that the three genes are expressed together and can receive sulfur from TusA and transfer it to DsrC. These results strongly indicate that this newly identified version of DsrEFH is functionally equivalent to known DsrEFH complexes. In addition, these genes are found in many sulfate reducers and sulfur disproportionaters, which might indicate a possibility for these organisms to function as sulfide oxidizers.

[58] DOMESTICATION OF PANTOEA SP. THROUGH GENOME-SCALE METABOLIC MODELLING AND A GENETIC TOOLBOX

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The genus ***Pantoea*** comprises a versatile group of Gram-negative bacteria belonging to the family of ***Erwiniaceae***. The species in this genus have been isolated from several environments, such as water, soil, plants and animals. The ubiquity and versatility of ***Pantoea*** isolates make it an ideal genus for exploring niche-specific adaptation and opportunism, as well as for developing commercially relevant therapeutic, agricultural and environmental products.

Our fundamental understanding of ***Pantoea***, however, remains limited due to the absence of a standardized genetic toolbox for this genus. Additionally, only approximately 30 genome-sequenced strains are available to date and no genome-scale metabolic model is available.
In this study, a ***Pantoea*** strain was isolated from soil samples and characterized as a newly discovered strain; ***Pantoea*** sp.. To further develop ***Pantoea*** strains into fully optimized microbial chassis, an in-depth, systems-level understanding of metabolism is required, for which genome-scale metabolic models are essential. We have developed a draft genome-scale metabolic model using CarveMe (Machado et al., 2018). The model is curated using additional data derived from wet-lab experiments.

Additionally, Synthetic biology and metabolic engineering require the ability to alter the genome of the strain in question. Limited research has been conducted in strain engineering tools specific for ***Pantoea*** strains. Preliminary experimental results are promising for the application of Lambda-Red recombineering, which is an easy and efficient method for generating genetic modifications.
In conclusion, this study aims to provide a base-level understanding of ***Pantoea*** as a genus and pave the way for its application in various fields.

[59] THE DYSBIOSIS OF THE ACNE SKIN MICROBIOME AND ITS DECLINE AFTER ISOTRETINOIN TREATMENT

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Acne vulgaris is a complex disease that can cause skin inflammation. The onset of the disease involves various factors, including the skin microbiome. However, knowledge about the skin microbiome in acne remains scarce. In addition, the impact of isotretinoin (ISO), the most efficient treatment of severe acne, on the skin microbiome is poorly understood. This study aimed to investigate differences between acne and healthy skin microbiomes along with the effects of ISO treatment. We collected skin swabs from 36 healthy individuals, 32 pre-ISO acne patients and 14 post-ISO patients. Each sample was analysed using target-specific amplicon-based sequencing to assess the general bacterial, staphylococcal and ***C. acnes*** composition. Furthermore, droplet digital PCR was used for absolute quantification of staphylococcal and ***C. acnes*** populations. Pre-ISO, acne microbiomes displayed reduced diversity within the ***C. acnes*** population, characterised by increased relative abundance of IA_1 strains and decrease of type II strains. Within the staphylococcal population an increased diversity was detected, notably an increased relative abundance of ***Staphylococcus aureus***, a potential harmful pathogen. Both staphylococcal (1.4-fold) and ***C. acnes*** (5.7-fold) populations were reduced in quantities. Post-ISO treatment, staphylococcal and ***C. acnes*** populations were significantly reduced (8.6-fold and 28.2-fold, respectively). In summary, the acne microbiome dysbiosis was characterized by divergent changes in ***C. acnes*** (diversity decrease) and staphylococcal populations (diversity increase). Furthermore, ISO treatment mainly affected ***C. acnes*** but also reduced staphylococci. Other organisms could -to some extent- take over the space; ***Streptococcus, Corynebacterium*** and ***Micrococcus*** had increased relative abundances, in a patient-specific manner. Results may suggest a potential benefit from skin probiotics for timely restoration of the healthy skin microbiome in acne patients treated with ISO.

[60] HOT SPOTS IN ARCTIC SOILS: ARE ANCIENT ARCTIC SETTLEMENTS POSSIBLE RESERVOIRS FOR PATHOGENIC AGENTS?

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The history of Greenland is marked by repeated waves of Paleo-Inuit immigration from North America. From the 10th to 15th century, Norse settlers immigrated from Europe and flourished in southwest Greenland with the introduction of domestic livestock. The different Inuit and Norse cultures created middens by dumping and accumulating domestic waste. Today, animal bones, excrements, mollusc shells and other artefacts associated with past human occupation are a valuable resource for archaeologists to study these past societies' diets, habits, life and death. However, these archaeological features might also represent unique microbial reservoirs of organisms from mixed origins within an organic rich matrix. A latent source of microbes, that is currently exposed to climate change. Drastic increase in temperature observed in Greenland leads midden material to be carried away by enlarged melting water from snowdrifts. Rising sea levels and loss of sea ice also accelerates the erosion of middens that are most often situated along the coast and might be washed out into the sea. This raises the question whether these sites represent a disease emergence threat. Especially that the increase in plant production in West Greenland is opening for the development of sheep farming in the Nuuk area, exactly where sheep farms were situated during the Norse era and abandoned for the past 500 years. In the VEO project, we aim to evaluate if ancient Arctic settlements are possible hot-spots for pathogenic agents, and if potential pathogens may spread to the surrounding environment. Using metagenomics, we compared the microbial communities of middens from different age and location in West and South Greenland (Paleo-Inuit, Norse and Modern Inuit middens) to pristine surrounding soils. We found that even after hundreds of years, the middens harbor a distinctive microbial signature enriched in host-associated and/or pathogenic bacteria belonging to ***Clostridium*** and other firmicutes; commensal bacteria from human faecal microbiome ***C. massilliamazoniense***, ***Ramboutsia hominins*** and ***Eubacterium tenue***, food poisoning agent ***C. perfringens***, opportunistic pathogens ***Paeniclostridium sordellii*** causing toxic shock in humans, or agents associated to rare infant or adult botulism such as ***C. baratii*** and ***C. thermobutyricum***.

[61] CHARACTERISATION OF EFFLUX PUMP REGULATION AND ACTIVITY IN PSEUDOMONAS AERUGINOSA

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Purpose:
The emergence of antimicrobial resistant pathogens, harboring the tools to cause treatment failure towards conventional antibiotics, is an ever-growing problem globally. Bacteria have evolved a plethora of different mechanisms to attain resistance towards clinically available antibiotics. One such mechanism is the increase in gene expression of efflux pumps, when the bacteria is exposed to antibiotics or other effector molecules.

In this study we focus on the clinically relevant bacteria ***Pseudomonas aeruginosa***. ***Pseudomonas aeruginosa*** possesses multiple RND-type efflux pumps in which four have been shown to result in significant clinical phenotypes, MexAB, MexCD, MexEF and MexXY. Using deletion mutants for these efflux pumps, we systematically determined the substrate specificity of each efflux pump to a varied selection of clinically useful antibiotics. Furthermore, analysis of a large scale RNA-seq database for our clinical isolates showed that high efflux pump expression, confers an increased MIC to the corresponding drugs, but not in all cases. Finally, we characterised the ability of antibiotics to induce efflux pump expression, and we show that in some cases this induction can cause cross-tolerance towards other unrelated antibiotics.

[62] INA GENE EXPRESSION IN PSEUDOMONAS SYRINGAE R1079 IS AFFECTED BY AEROSOLIZATION

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Airborne ice nucleation active (INA) microorganisms have great impact on atmospheric processes, being involved in cloud formation and consequently affecting cloud properties and the climate. Moreover, atmospheric dispersion of microorganisms plays an important role in the microbial biogeography. Ice nucleating proteins (INpros) promote ice formation by providing a structure where water molecules arrange in an ice-like structure. Therefore, allowing phase transition through heterogenous freezing at temperatures as high as -2ºC. Although the importance of INpros for climate and environment is clear, the external factors and molecular mechanisms controlling their synthesis are not completely understood.

The present study investigates the effect of aerosolization on ***Ina*** gene expression. A series of experiments were conducted using a model INA bacterium ***Pseudomonas syringae*** R10.79. Cells in the stationary growth phase were aerosolized from an 0.1 wt% NaCl solution using a Sparging Liquid Aerosol Generator. The aerosolized cells were passed through a stainless-steel flow tube, where they were mixed with a controlled relative humidity (RH) airflow using an Aerosol Conditioning System. The aerosolized particles were then recollected either in 0.1wt% NaCl solution with a BioSampler impinger or on polycarbonate filters. The obtained samples were analysed for viability using flow cytometry and ice nucleation activity by droplet freezing assay.

The study shows that aerosolization of cells triggers the synthesis of new INpros (10 out of 13 times) once the cells are reintroduced in a liquid environment, which happens in a time frame of 50 min. This caused an increase from one to three orders of magnitude in the numbers of cells carrying INpros. Supporting this observation, INpro synthesis in cells collected on the filter did not increase. In addition, new INpro-synthesis was independent of the RH cells were exposed to while airborne. Therefore, our findings suggest that ***Ina*** gene expression is induced as a stress response during aerosolization and drying. Further studies of this process can help predict how INpros would impact future weather, particularly relevant in the current context of climate change.

[63] HORIZONTAL GENE TRANSFER IN PLANT-BASED FOOD

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Bacteria can acquire exogenous plasmids via horizontal gene transfer, enhancing the dissemination of antibiotic resistance genes. Considering the emerging antibiotic resistance crisis, it is important to investigate the ability of different microbial communities to receive and transfer relevant plasmids, in new areas such as microbial communities found in raw vegetables and plant-based food. Plasmid transfer to microbial communities on food is relevant in the context of biosafety in food processing environments, as interspecies interactions could potentially serve as vectors that spread detrimental genetically-based traits like resistance genes. In this study we investigated the transfer of the IncP1 plasmid pB10 from ***Pseudomonas putida*** KT2440 and ***Escherichia coli*** Nissle 1917 to native microbial communities present in raw, unprocessed vegetables: spinach, potatoes and carrots. The presence of transconjugants was confirmed using a dual fluorescence gene reporter system. Donors emitted red fluorescence (mCherry) while repressing the green fluorescence (GFP) originating from the plasmid, whereas transconjugants emitted green fluorescence only. Preliminary results have shown the presence of transconjugants in all three vegetable microbial communities with different frequencies. The next step is to sort and identify the transconjugants on species level to unravel which food derived microbial community members can take up plasmids and potentially be involved in their further spread. This knowledge is relevant with regards to the choice of specific bacterial strains to use as biological control agents, to ensure a higher level of biosafety and prevent, to the extent of it being possible, frequent plasmid transfer the food products we consume.

[64] OPTIMISATION OF PARAGEOBACILLUS THERMOGLUCOSIDASIUS FOR CLIMATE-POSITIVE ACETONE PRODUCTION

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The world is currently facing a climate crisis facilitated by greenhouse gas emissions. The chemical industry is a major contributor to this issue, as conventional chemical productions are energy-intensive and mostly rely on petrochemical-based feedstocks. Microbial production provides a promising sustainable alternative, as it requires less energy and can utilise renewable feedstocks. However, the most prominent issue with microbial approaches lies with the economic viability of biochemicals. In the case of bulk chemicals, which have lower profit margins, most large-scale bio-based productions struggle to compete with the higher yields and lower production costs of their fossil fuel-based counterparts. If sustainable bio-based productions are to make an impact on the global market, it is vital to improve their feasibility and competitiveness in large-scale industrial settings.

Thermophilic fermentation can provide advantages to the development of the biochemical market, as high-temperature productions offer several benefits. This includes significantly reduced process costs, lower contamination risks, and easier extraction of volatile compounds. Amongst thermophilic species, ***Parageobacillus thermoglucosidasius*** is a promising candidate for bulk-chemical production. Here, we present the development of a strain of ***P. thermoglucosidasius*** that is optimised for the conversion of acetic acid into acetone. This is accomplished through metabolic engineering, omics-based analysis, and the application of adaptive evolution-based strategies. The aim is to optimise growth, acetate tolerance, and acetone production in ***P. thermoglucosidasius*** to facilitate the development of large-scale sustainable acetone production, and to empower the development of other chemical production strategies that intend to take advantage of the thermophilic aspects of this species.

[65] LONG-TERM WARMING-INDUCED TROPHIC DOWNGRADING IN THE SOIL MICROBIAL FOOD WEB

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Presentation will be based on the results recently published in the article with same title Dahl et al. 2023[1] as well as new (unpublished) results from a larger follow-up study covering a year's cycle of seasonal changes.

Purpose: To understand how the soil microbial food web players respond to soil warming. From the premise, that microbially driven processes are responsible for most of soil organic carbon (SOC) mineralization, and that warmer conditions – in cold and temperate regions – promote microbial activity, which subsequently leads to the prediction that global warming will accelerate SOC decomposition and enhance carbon release to the atmosphere in such regions.

Methods: To address this, we applied double-RNA metatranscriptomics to obtain comprehensive profiles of the soil (micro-)biota, by the analysis of the small subunit ribosomal RNA (SSU rRNA) and viral RNA. We studied a grassland ecosystem in Iceland where geothermal activity has consistently warmed the soil (+6 degrees C above ambient) for more than 50 years, thus representing the world's largest scale (temporal and spatial) natural soil warming experiments.

Results: Our method allowed us to obtain broad community profiles of the soil microbial food web, including bacteria, archaea, fungi, 'protists', Metazoa and viruses. When compared to ambient soil temperature conditions, we found pronounced differences in taxa abundances within and between trophic modules of the food web under warmed conditions. Specifically, we observed a 'trophic downgrading' at elevated temperature, with soil fauna decreasing in abundance, while predatory bacteria and viruses became relatively more abundant. We propose that the drivers for this shift are previously observed decreases in quantity and quality of microbial biomass and soil organic carbon, and the increase in soil bulk density (decrease in soil porosity) at elevated temperature.
Conclusions: We conclude that a trophic downgrading may have important implications for soil carbon sequestration and nutrient dynamics in a warming world.

1 **Dahl** *et al*. 2023. Long-term warming-induced trophic downgrading in the soil microbial food web. *Soil Biology and Biochemistry*. Doi: 10.1016/j.soilbio.2023.109044.

[66] COMPARATIVE ANALYSIS OF BIOFILM MATRIX PROTEOMES OF XANTHOMONAS RETROFLEXUS WILD TYPE AND ΔFAP MUTANT

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Biofilms are bacterial communities consisting of bacteria enclosed in a self-produced matrix. This extracellular polymeric substance (EPS) matrix is a major contributor to biofilm-associated emergent properties, which cause the biofilm lifestyle to differ greatly from the planktonic lifestyle. The EPS composition varies between biofilms composed of different bacteria, but the matrix most commonly consists of exopolysaccharides, nucleic acids, proteins, and lipids. Functional amyloid fibers belong to a specific group of proteins frequently found in biofilm matrices. They can have diverse structural and functional roles and have been associated with various properties, most notably increased biofilm formation, rigidity, and hydrophobicity. Fap amyloids were first identified in ***Pseudomonas fluorescens*** UK4 and have been reported to be involved in aggregation and increased biofilm formation. A fap operon was identified in an environmental ***Xanthomonas retroflexus*** strain frequently used for experiments to assess community dynamics in multispecies biofilm settings. This project aims to compare the matrix proteomes of a wild type and Δ***fap*** mutant grown under flow conditions. We propose using a commercially available flow cell (Convertible Flow Cell® CF-CAS0003, IBI Scientific, USA) for biofilm cultivation under flow conditions using peristaltic pumps. LC-MS/MS-based shotgun proteomics will be used for comparing the matrix proteomes.

The study's main objective is to compare the proteomes and assess whether differences in proteome composition can be observed beyond the expected absence of amyloid fibers in the Δ***fap*** mutant. Additionally, we believe this work will serve as a framework for matrix proteomics using mainly commercially available means.

[67] DEOXYHEXOSES AS OVERLOOKED FERMENTATION SUBSTRATES FOR FOOD MICROBES

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Fucose and rhamnose are naturally occurring 6-deoxyhexoses and are released by enzymatic and chemical hydrolysis from fucosylated oligosaccharides or dietary fibers in the gut (e.g., pectin). Only a few intestinal microbes can utilize deoxyhexoses to support intestinal colonization and microbial growth. The key fermentation metabolite produced from deoxyhexoses is propionate. Propionate is an important short-chain fatty acid (SCFA) in the intestine and is actively used as an antimicrobial in food preservation due to its broad inhibitory effect against food pathogens. Inspired by fermentation processes in the intestine, it is the aim of this study to develop consortia of food-grade microbes that produce propionate from fucose and/or rhamnose.

From deoxyhexose metabolism, a few species of ***Bifidobacteriaceae*** (e.g., ***Bifidobacterium infantis***) and ***Lactobacillaceae***, e.g., ***L. rhamnosus*** produce 1,2-propanediol (1,2-PD), an intermediate of deoxyhexose-based cross-feeding, which can further be metabolized to propanol, propanal, and propionate strains.

In a screening, we identified strains of ***B. infantis***, and ***L. rhamnosus*** that were able to produce 1,2-PD from fucose and rhamnose while strains of ***L. reuteri*** and ***L. coryniformis*** used 1,2-PD to produce propionate. Next, consortia of ***Lactobacillaceae*** and ***Bifidobacterium*** strains were established and were grown in bioreactors at pH 6.5 and anaerobic conditions. When cultivated together ***L. rhamnosus*** and ***L. reuteri*** produced propionate, while only 1,2-PD accumulation was observed for co-cultures of ***B. infantis*** and ***L. reuteri*** or ***L. coryniformis***.

Together, this study indicates the potential of propionate production by ***Lactobacillaceae*** in the presence of fucose or rhamnose. Our results expand the profile of fermentation metabolites produced by ***Lactobacillaceae*** two-strain consortia, which might find application in biopreservation.

[68] MICROBIAL DEGRADATION OF DIFFERENT CARBON COMPOUNDS AND THEIR IMPACT ON THE MICROBIAL CRYOCONITE COMMUNITY ON THE GREENLAND ICE SHEET

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Cryoconite holes are water-filled depressions that form on the surface of glaciers or ice sheets due to the presence of inorganic and organic debris that absorbs solar radiation and leads to melting of the surrounding ice. They are considered the most active microbial habitat on snow-free, melting ice and harbor a diverse microbial community that is adapted to the harsh environmental conditions including cold, intense solar radiation and nutrient limitation.

Cryoconite material was incubated with four different ^{14}C labeled compounds (^{14}C phenanthrene; ^{14}C dodecane; ^{14}C glucose and ^{14}C cellulose) in two concentrations (1 and 100 ppm) at -1°C with an 18/6-hour light/dark cycle. In the low concentration, glucose was fast mineralized by the cryoconite microbiome and the accumulated $^{14}CO_2$ produced a first-order shaped mineralization curve, while an S-shaped mineralization curve was observed for the higher concentration. The mineralization of cellulose was lower, but the best fit of the mineralization data was still found with a first-order model. Dodecane was mineralized following an S-shaped curve for both concentrations, however, in comparison to the high concentration, the curve for the low concentration showed a faster and steeper increase. The last compound, phenanthrene (a three ring polyaromatic hydrocarbon), showed a much lower mineralization, which did not exceed 2% of added ^{14}C being mineralized for both added concentrations. Culturable cryoconite microorganisms were shown to increase on R2A and 1/10 TSA after incubation with all four carbon sources, most predominantly with addition of 100 ppm glucose, followed by 100 ppm cellulose and phenanthrene. Interestingly, incubation of the cryoconite material with the different carbon compounds also showed an effect on the abundance of slowly growing colonies. Finally, DNA and RNA were co-extracted from samples taken at several time points during the incubation and analyzed for changes in the microbial community.

[69] RECORDING MICROBIAL SIGNALS IN SOIL: DEVELOPING GENETIC MEMORY DEVICES FOR DETECTION OF SPECIALIZED METABOLITES IN MICROBIOMES

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Microbes reside in complex microbiomes, where secreted specialized metabolites play a major role in interspecies communication. However, investigating the role of specialized metabolites in microbial signaling is currently limited to methods that are disruptive and affected by detection limits. The aim of our research is to engineer soil-dwelling microbes capable of recording the presence of bioavailable specialized metabolites ***in situ*** in soil and rhizosphere microbiomes. This approach provides an ultrasensitive, non-disruptive alternative to existing methods, which additionally allow for both spatial and temporal investigations of signals ***in situ***. The sensors function by inferring an irreversible genetic switch in the presence of the metabolite in question, which is catalyzed by a tightly controlled integrase.

We have developed a handful of different sensors responding to pico-nanomolar concentrations of ecologically relevant metabolites. Each sensor has been tuned to avoid leaky expression in the absence of inducing metabolite in ***E. coli***. Sensor sensitivity and responsiveness were analyzed with flow cytometry to generate response curves. Additionally, we have transferred our sensors to the soil- and rhizosphere compatible bacterium ***P. putida*** KT2440 and determined their effectiveness with flow cytometry. Finally, we have changed the output to genetic memory and verified the efficiency and orthogonality of the sensors with qPCR.

Whole-cell biosensors hold an immense potential to detect and record signals in natural environments. They are highly tunable genetic devices that serve as a promising alternative method for ***in situ*** investigations of natural products in complex environments such as soil- and plant microbiomes.

[70] ISOLATION AND CHARACTERIZATION OF ROBUST THERAPEUTIC BACTERIOPHAGES TARGETING VANCOMYCIN RESISTANT ENTEROCOCCI

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Enterococci are some of the most common healthcare associated infections with ***Enterococcus faecium*** accounting for a third of all nosocomial invasive infections in Europe in 2020. Furthermore, the prevalence of vancomycin resistant Enterococci (VRE) infections is increasing in both Denmark and Europe. Enterococci are a part of the ESKAPE pathogens, a group of bacteria determined by the WHO as having the most urgent need for development of new antimicrobials. This is due to enterococci inherent resistance towards multiple antibiotics alongside their great capacity for acquiring new resistance genes. It is therefore crucial to identify potential alternative treatments. For this project Statens Serum Institut has identified and mapped the resistance profiles of the 20 most prevalent vancomycin resistant ***E. faecium*** and vancomycin resistant ***E. faecalis*** from nosocomial infections in Denmark. These VRE strains are used for isolating new phages, which will be characterized and evaluated for their phage therapy potential. At present, five phages have been isolated from wastewater from the Biofos Wastewater treatment plant in Avedøre, Denmark with more underway. Isolated phages have been sequenced and will be investigated for possible novel modifications using Oxford Nanopore Technology. Their therapeutic capabilities will be investigated through host range experiments, determination of burst size and lysogeny, efficacy on biofilms, and synergy/antagonism with antibiotics as well as other phages. Suitable phages from the project are aimed to be made available for therapeutic use through implementation in international bacteriophage collections and hopefully result in new treatments against the most prevalent VRE in Denmark.

[71] EXTRACELLULAR G-QUADRUPLEX/HEMIN COMPLEXES IN STAPHYLOCOCCUS EPIDERMIDIS BIOFILMS ENHANCE PEROXIDASE ACTIVITY

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Abstract

Bacterial metabolic processes have evolved biocatalysts such as superoxide dismutase, catalase, and peroxidases which function under oxidative stress, conferring survival advantages especially in bacterial biofilms. Peroxidases catalyse the reduction of hydrogen peroxide to water, and they can function as natural defense mechanisms against endogenous and exogenous peroxide stress in low oxygen tensions characteristic of biofilms. Recent findings have shown that bacterial biofilms have an abundance of non-canonical nucleic acid structures such as G-quadruplexes, however their physiological roles and survival advantage conferred on the bacteria remain unclear. Abiotic experiments utilizing G-quadruplexes and redox active hemin have exhibited peroxidase properties, basically as DNAzymes. We have developed a novel model for demonstrating the role of G-quadruplex/hemin complexes as peroxidase inducers in bacteria. In ***Staphylococcus epidermidis***, higher tolerance to hemin (5 – 200 µM) was observed in cells treated with a multimeric G-quadruplex (5 µM) and G-quadruplex/hemin complexes conferred an inductive peroxidase activity (> 4 fold) in comparison with untreated bacteria. Furthermore, biofilm experiments demonstrated similar enhanced peroxidase activity with ***S. epidermidis*** cells grown with G-quadruplex/hemin. These properties show that G-quadruplex/hemin complex can assist bacteria in peroxide detoxification, and can be vital in balancing physiological processes with respect to regulation of oxidative stress and the control of activities of reactive oxygen species.

Key words: Iron-porphyrin complexes, reactive oxygen species, extracellular DNA,

[72] UNLOCKING MICROBIAL DARK MATTER: A METAGENOME ENGINEERING APPROACH

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Microbiologists have only obtained <1% of ***Bacteria*** (and ***Archaea***) species in pure cultures while the remaining species have aptly been coined as the 'Microbial Dark Matter'(MDM). Evidently, accessing MDM culturability will pave the way for new avenues in microbial ecology and biotechnology, and we believe one reason to this unculturability is harmful generation of reactive oxygen species (ROS) at laboratory conditions. Many environmental communities experience low fluxes of electron donors, thus generating low amounts of ROS. By contrast, laboratory conditions present a high flux of electron donors, leading to high generation of ROS, while simultaneously isolating members from one another. We hypothesize that some members of environmental communities lack some (any or all) ROS defenses. Thus, any attempt to culture said members fails at standard conditions as ROS accumulates and hampers growth. The aim of the Remicult project is to develop a biological, gene delivery system for metagenome engineering of environmental communities. We intend to remediate culturability of members that lack ROS defenses. This delivery system employs vector constructs that harbor genes for ROS-scavenging (H_2O_2) and a fluorescent marker for cell sorting. We have a now constructed broad-host vectors harboring an operon insert, consisting of a fluorescent marker gene fused with an ***E. coli kat***G gene. Furthermore, we have established an ***E. coli*** model for H_2O_2 MIC in ***ΔkatG/ΔkatEG*** (catalase) mutants and observed recovered growth along fluorescent signal in complementation experiments of mutants harboring the vectors. The Remicult delivery system has now been applied to soil bacteria to evaluate any remediating effect of culturability of the proposed (unculturable) species. Here, preliminary growth results show that sorted soil transconjugants can withstand the same H_2O_2 concentrations as used in the model, whereas soil isolates harboring control vector are highly challenged. Thus, generated H_2O_2 may play important role for culturability of wild type bacteria under laboratory conditions. Here, ongoing growth experiments with soil isolates harboring either vector, inoculated in standard media (without H_2O_2 addition) will reveal the true potential of this metagenome engineering concept.

[73] EFFECT OF STRAW BIOCHAR ON EXTRACELLULAR ENZYME ACTIVITY IN SANDY SOILS DURING BARLEY GROWTH

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Purpose: The application of biochar on coarse sandy soils to improve crop yields, water holding capacity and microbial community dynamics, is a subject of active exploration. Soil extracellular activities are relevant for the decomposition of organic matter by microbial communities. Testing those enzyme activities will provide a better understanding of the response of the soil microbial communities to biochar amendment. The aim of this research was to assess the effects of the application of biochar on the extracellular enzymatic activity of coarse sandy soils at different depths in a mesocosm column experiment.

Methods: Spring barley was sown in 30 cm diameter PVC cylinders filled with two types of coarse sandy soils collected from two different Danish localities. In 145 cm PVC cylinders, the upper 25 cm contained soil rich in organic matter while the next 50 cm contained coarse sandy soil amended with different concentrations of straw biochar (0%, 1%, 2%, and 4% wt biochar) produced at 600 °C. The lower most part (70 cm) contained unamended sandy soil. The experiment was set up in four replicants and was carried out in April 2022. Soils samples were collected at the end of the experiment in August 2023, and subjected to the 4-methylumbelliferone (MUF) microplate assays to analyze the activity of seven extracellular hydrolases related to carbohydrate degradation.

Results: We showed that the activity of all the enzymes analyzed was significantly higher in the organic rich surface soil compared with the lower depths of the soil column. The biochar increased the α-D-glucosidase activity, suggesting the potential presence of degradable organic matter remains from the straw biochar feedstock. In contrast, the biochar amendment had a negative impact on the phosphatase activity, indicative of phosphorous deficiency in soils without biochar.

Conclusion: These distinct variations in glucosidase and phosphatase activity underscore biochar's capacity to enhance nutrient availability, benefiting plant growth and modifying soil microbial communities.

[74] CONVERSION OF METHANE TO ORGANIC ACIDS BY GAMMAPROTEOBACTERIAL METHANOTROPHS OF LAKE AND POND ECOSYSTEMS

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Aerobic gammaproteobacterial methanotrophic bacteria (gMOB) play a crucial role in regulating methane emission at the oxic-anoxic interfaces of freshwater ecosystems, including lakes, ponds, and wetlands. Under O_2-limiting conditions, gMOB might shift from aerobic metabolism to fermentation, resulting in secretion of extracellular organic acids, which could then serve as growth substrates for non-methanotrophic microbes.

To find out whether gMOB of lakes convert methane to organic acids, we isolated strains representing three relevant gMOB genera from boreal lake water columns, i.e., strains ***Methylobacter*** sp. S3L5C, ***Methylomonas paludis*** S2AM, and ***Methylovulum psychrotolerans*** S1L, and subjected them to laboratory experiments and genomic analyses (1,2). The isolates could convert methane into organic acids, including acetate, formate, and malate, while additionally, S1L and S2AM produced succinate, S2AM produced lactate, and S3L5C produced propionate (1,2). Moreover, genes linked to organic acid production were found in the genomes of the isolates as well as in the metagenome-assembled genomes representing ***Methylobacter*** spp., ***Methylomonas*** spp., and ***Methylovulum*** spp. from lake and pond ecosystems (1,2).

In conclusion, our findings show that the conversion of methane to organic acids is a common trait among gMOB in lakes and ponds, emphasizing their significant role in channeling methane-carbon into the microbial food chains of these freshwater ecosystems (2).

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[75] MICROBIAL INSIGHTS INTO PEATLAND CARBON CYCLING: UNVEILING METHANE PRODUCERS AND CONSUMERS FOR SUSTAINABLE ECOSYSTEM MANAGEMENT

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Peatlands house unique microbial communities that play an important role in carbon cycling due to the presence of the methane producing archaea (methanogens), and methane oxidizing bacteria (methanotrophs). Although just over 170,000 ha of original peatland areas are cultivated in Denmark corresponding to 7 percent of total agricultural area, they are responsible for more than half of the total CO_2 emission regarding soil cultivation. While CO_2 release will be mitigated by rewetting, the anaerobic conditions of the raised water table, and factors such as age of degradation or accumulation of nutrients, may create conditions where methanogens thrive. In this case, the production of methane may outweigh the capture of CO_2. To avoid this biogeochemical compromise, we need to understand the carbon cycling microorganisms found in peatlands as well as their abundances and habitat preferences. This study aims to investigate the diversity of carbon cycling bacteria and archaea in degraded and intact peatlands.

The Microflora Danica project has collected 10,000 samples across Denmark that will be queried to identify characteristic of carbon cycling bacteria and archaea found in different peatlands. We have profiled the samples using SingleM, which uses single-copy marker genes for taxonomic classification and calculation of bacterial and archaeal relative abundances. Currently, we have identified a variety of methanogen and methanotroph species in our 527 wetland samples and observe distinct differences depending on the wetland classification. Additionally, soil cores from Store Vildmose including depth profiles will be analyzed using metagenomics. Metagenome-assembled genomes recovered from the Microflora Danica genome database and soil cores will be explored to determine metabolic differences between key methanogen and methanotroph species relevant to their habitat types.

[76] COMPLEXITY ENHANCES EVOLUTIONARY PRESSURE IN MULTISPECIES BIOFILMS

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Multispecies biofilms are predicted to be evolutionary hotspots, making them a unique source of information about the impact of diversity on co-evolution and adaptation. Therefore, we set up a biofilm evolution experiment focusing on two competing strains – ***Pseudomonas defluvii*** and ***Pseudomonas brenneri***, isolated from wastewater facilities – while increasing complexity by adding up to three additional wastewater strains. The experiment lasted for 18 days, during which different combinations (single, dual and multispecies) were analyzed with various techniques: CFUs, sequencing, phenotypic screening, and microscopy. ***P. defluvii*** abundance was low when co-cultured with ***P. brenneri***, and further reduced in multispecies combinations. However, phenotypic variation frequency of ***P. defluvii*** was highest in mono-cultures, and it dropped with increased diversity. Although genes involved in biofilm metabolism and chemotaxis were the most frequently targeted in ***P. defluvii***, mutations were different in variants isolated from mono-, dual- and multispecies biofilms. These results suggest that the complexity in the biofilm community impacts diversification over extended periods of time (18 days). Moreover, this is strain-dependent, as ***P. brenneri*** displayed constant phenotypic variation and mutations throughout the combinations. Based on their phenotype, several evolved ***P. defluvii*** variants were selected, and these showed increased performance when grown with ***P. brenneri***. This demonstrated the impact of diversity on evolution in complex biofilms by proving that interspecific genotypic variation found in biofilm formation and interspecies interactions compensates for intraspecific changes over extended periods of time. Moreover, co-evolution patterns are still hard to predict, as shown by the different behaviors in ***P. defluvii*** and ***P. brenneri***, highlighting the relevance of this type of research.

[77] UNRAVELING THE ROLE OF MINERALS IN ANTIBIOTIC RESISTANCE GENE PROPAGATION: IMPLICATIONS FOR ENVIRONMENTAL HEALTH

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During bacterial life journey as single cells, they may encounter environmental stresses that results in some physiological changes in the individual cells towards forming multicellular communities, forming spores, sharing resistant genes, etc. Soil includes a range of different surfaces compositions and characteristic which can attract different bacteria for induction of bacterial communities, the so-called biofilms. Biofilms protect cells from stress and provide a niche for horizontal gene transfer (HGT) to share antibiotic resistant genes (ARG). We here aimed to address the effect of different soil minerals on bacterial life journey and investigated (1) Bacterial cell survival and membrane integrity of two soil bacteria (2) The ability of bacteria to take up circular DNA pre-adsorbed to mineral surfaces through HGT and (3) Evaluation of biofilm development on minerals using metabolic activity assay, Scanning electron microscopy (SEM) and Optical Photothermal Infrared (O-PTIR) Spectroscopy.

We find that mineral surface structure, surface charges, and wettability play a major role in attraction or repulsion of bacterial cells and that both bacteria got injured when encountered to highly positively charged nano minerals.
Our results confirmed that bacteria through HGT can incorporate DNA adsorbed to the mineral surfaces and that the rate of DNA transformation was related to mineral type. Hydrophilic negatively charged and hydrophobic positively charged minerals resulted in the highest number of transformants, demonstrating that the transformation is independent on mineral charge and mineral wettability.

SEM images showed that clusters were formed on positively charged surfaces while scattered adherences were observable for negatively charged surfaces. O-PTIR was done to investigate whether the substrate influenced matrix composition and showed that the intensity of the matrix production of biofilms on positively charged surfaces was higher than the rest of the minerals.
This research illuminates the multifaceted interactions between minerals, bacteria, and ARG, offering insights into the mechanisms underlying ARG propagation. This enhanced understanding may pave the strategies to control gene spread, contributing to the management of antibiotic resistance in natural environments.

[78] INTESTINAL FAECALIBACTERIUM PRAUSNITZII ABUNDANCE CORRELATES WITH THE EFFECT OF HIGH-DOSE THIAMINE ON CHRONIC FATIGUE IN PATIENTS WITH IBD IN REMISSION

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Abstract:
Gut microbiome analysis in previous studies has shown that IBD patients with fatigue symptoms were characterized by a significant reduction in butyrate-producing bacteria in the colon and a less diverse microbiome. Although some gut bacteria can produce thiamine in the gut environment, many butyrate-producing bacteria, including ***Faecalibacterium prausnitzii***, appear to depend on dietary thiamine or cross-feeding from other bacterial species in the colon. In some situations, a lack of thiamine in the colon may thus reduce the number of butyrate-producing bacteria, and thereby butyrate levels in the colon. Recently, a randomized, double-blinded, placebo-controlled crossover trial revealed that high-dose oral thiamine significantly reduced chronic fatigue in IBD patients in remission. Here, we analyzed the microbiota and short-chain fatty acids concentrations in fecal samples from that study to test the possibility that the beneficial effect of thiamine on chronic fatigue was explained by induced changes in the microbiome, including increased levels of butyrate-producing bacteria and butyrate. However, we found no differences in butyrate nor butyrate-producing bacteria between controls and chronic fatigue patients or during the treatment by comparing placebo vs. treated patients. Interestingly, a negative correlation between ***F. prausnitzii*** relative abundance in fecal samples and IBD fatigue score change was seen in thiamine-treated patients with chronic fatigue.

[80] INVESTIGATING THE ICE NUCLEATION ACTIVE MICROORGANISMS IN ARCTIC SEA ICE

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Over the past 40 years, the Arctic has warmed three times faster than the rest of the globe notably because of drastic decline in the sea ice extent. By linking the ocean to the atmosphere and controlling energy transfer between them, Arctic sea ice is a hot spot for climate change. Cloud radiative properties and lifetime are additional key factors controlling Arctic climate. Recently, cloud formation has been linked to the presence of biological Ice Nucleating Particles (bioINPs). As Arctic seawaters are known to be among the primary sources for atmospheric bioINPs associated with high activity, and given the accelerating rate of sea ice melt, there is a need to understand sea ice as a potential contributor to the atmospheric INP pool in the Arctic.

In March 2023, 4 first-year sea ice cores were sampled in Malene Bight (Greenland). After being completely thawed, the cores were sequentially filtered. These were resuspended in sterile sea water. All suspensions as well as the total sea ice samples contained INPs active >-15°C. The larger fractions (>70 and >20 µm) displayed activity at highest temperatures suggesting INPs were mainly produced by large eukaryotes. Indeed, a large part of sea ice biomass is composed of filamentous microalgae and pennate diatoms colonising the brine channels. Consequently, we set out to isolate and culture these microorganisms by washing the biomass off the filters into F/2 medium. So far, none of the enrichments showed activity. Two main candidates producing bioINPs were observed in the samples, one was identified as the centric diatom ***Melosira arctica***. This species forms long strands and is usually found attached to the bottom of the ice hanging in the water column making these microalgae vessels in the complex interplay between sea ice and water. Experiments are currently underway to induce ice-nucleation activity in the enrichments and isolates and identify which of these eukaryotes is responsible for bioINP observed in the sea ice samples. Axenic algal cultures will be prepared to exclude the possibility of bacterial activity. Overall, this study suggests that eukaryotic microorganisms are responsible for high concentration of bioINP in sea ice and aims to identify those producing potent bioINP in the Arctic sea ice in order to decipher the impact of sea ice microbiota on cloud formation and climate.

[82] 1000+ NEW COMPLETE GENOMES AID DISCOVERY OF NATURAL PRODUCTS

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The lack of financial incentive from the pharmaceutical industry has led to a stagnation in the discovery of new antimicrobial compounds, creating a severe threat to human health from multi-resistant bacterial pathogens. Advances in cost-efficient sequencing equipment and bioinformatics tools enable the identification of biosynthetic gene clusters with desirable properties, leading to the discovery of new secondary metabolite compounds and producer strains from ***Actinomyces*** bacteria.

In order to investigate the biosynthetic potential in ***Actinomycetes***, we have developed a comprehensive and efficient pipeline for generating a dataset of more than 1000 Actinomycete genomes sequenced using Nanopore and Illumina sequencing platforms. The pipeline spans the entire process from sampling to benchmarking of the assembled genome.

By predicting the domains required for synthesizing natural products using antiSMASH (antibiotics & Secondary Metabolite Analysis Shell) and comparing them to known biosynthetic pathways we will be able to expand our knowledge of the properties, underlying mechanisms and in turn discover currently unknown features of sustainable natural products.

[83] TWO PLASMIDS, ONE PHAGE: UNDERSTANDING THE ENTRY OF THE PLASMID-DEPENDENT PRD1-LIKE BACTERIOPHAGE

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Abstract

PRD1-like bacteriophages are membrane-containing icosahedral viruses that infect bacterial cells containing IncP, IncN, or IncW conjugative plasmids. Given the broad host range nature of the PRD1-like bacteriophages, this phage family provides a promising tool to fight conjugation-mediated antimicrobial resistance spread and bacterial infections. Previous studies reported that the mating pair formation (MPF) complex encoded by the conjugative plasmid is the entry point of the PRD1-like phage. Nonetheless, the molecular mechanism remains unclear, including which proteins among the 10 or more components constituting the MPF complex enable the phage entry. To better understand the first step of infection, we first isolated and sequenced the phage resistant mutants of two parental strains carrying two different IncP conjugative plasmids, ***Salmonella enterica*** Typhimurium MHM112/pKJK5::***gfp*** and ***Pseudomonas aeruginosa*** PAO1/RP1. Among the four resistant mutants of ***S.*** Typhimurium MHM112/pKJK5::***gfp***, three carried the same mutation, Leu136Pro in TrbG, and remained conjugation-proficient as a proxy of the MPF complex assembly. For ***P. aeruginosa*** PAO1/RP1 counterparts, one out of the four isolated mutants retained the ability to conjugate, had a 5-residue insertion to the C-terminus of TrbJ, and conferred cross-protection against Pf3, another plasmid-dependent filamentous bacteriophage. Overall, these findings suggest that the TrbG and TrbJ proteins encoded by the plasmids are the interacting points between the PRD1-like phage and the bacterial host. In addition, while most phage-resistant mutations led to a loss of conjugation ability, there exist mutations to escape the PRD1-like phage predation without sacrificing the plasmid-transfer conjugation.

[84] INTERROGATING MICROBIAL DYNAMICS: COMPREHENSIVE ASSESSMENTS USING THE GALLERIA MELLONELLA IN VIVO MODEL

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Purpose: The escalating significance of virulence and antibiotic resistance in clinical isolates underscores the imperative for a high-throughput, in vivo screening methodologies. The Galleria mellonella larvae animal model (GM) emerges as a promising tool for conducting extensive screenings to assess the virulence of bacterial isolates within a clinical contexts.

Methods: Our methodology was meticulously refined to mitigate external variables and maintain consistent standards in both larval and bacterial handling. The optimization process encompassed parameters such as larvae weight, quality, and the precise quantity administered during the injection. Through these measures, we established a robust injection protocol and devised a systematic approach for subsequent pathogenicity assessment, done by the survival count of the inoculated larvae.
Additionally, our methodology extended to the tracking of bacterial growth in vivo as well as treating, allowing for correlation analyses with in vitro data and virulence outcomes.

Results: Using a standardized high-throughput Galleria mellonella (GM) model, we assessed numerous bacterial strains and clinical isolates, revealing significant variations in virulence and growth. The GM larvae proved effective for treatment evaluation with injected clinical isolates. Discrepancies in growth and clearance rates between in vivo and in vitro conditions underlines the importance of employing an animal model for comprehensive assessments.

Conclusions: The Galleria mellonella model emerges as a potent in vivo tool for extensive screening encompassing growth, virulence, and treatment evaluations. Its favorable cost-effectiveness and adherence to ethical principles render the GM model exceptionally well-suited for large-scale screenings of clinical isolates. The GM model's scalability, combined with expedited results, enhances its utility, providing a standardized and ethically streamlined way for comprehensive testing.

[85] A BROAD-HOST-RANGE EXPRESSION PLATFORM TO FACILITATE CHASSIS SCREENING

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Microbes are extremely valuable for the bioproduction of proteins and compounds, with applications in the medical, environmental, and industrial fields. Host selection in biotechnological research is usually limited to a few model organisms, which are well-characterized and have an extensive molecular toolbox. However, model organisms can have endogenous physiology that is unfitting for the desired application, for example, notwithstanding the harsh conditions of industrial processing methods. Fortunately, nature has provided a vast landscape of organisms through evolution, each with unique phenotypic traits adapted to varied environments. The biotechnological field could greatly benefit from finding simple metabolic engineering approaches suitable for non-model organisms and from exploring their potentially advantageous traits, such as high temperature or low pH resistance. In this study, we provide a modular, single vector-based expression platform, centered around the well-known promoter system tetR-pTet, inducible by anhydrotetracycline. This system has been presented in several studies with different modifications to improve its functionality in specific organisms. However, here, we show that a single version can be compatible with a wide range of eubacteria. In all the studied microbes, the promoter system was shown to be tight and titratable. It enables easy screening of recombinant proteins and pathways in both mesophilic and thermophilic Gram-negative and Gram-positive hosts. Overall, this platform enables simple screening of heterologous expression and production in a broad variety of hosts, supporting the exploration of previously unconsidered hosts.

[86] IMPACT OF PLASMID INCOMPATIBILITY GROUPS ON CONJUGATION DYNAMICS UNDER BIOCIDE EXPOSURE

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Purpose: Evidence suggests that biocides at sub-inhibitory concentrations in aquatic environments may stimulate the dissemination of antimicrobial resistance via conjugation. Currently, we lack an understanding of the types of chemicals that exert that effect, the influence of the incompatibility group of plasmids, and donor/recipient combinations that display it.

Methods: To achieve this goal, we conducted conjugation assays using conjugative plasmids, RP4, R27, and R1, belonging to IncP-1, IncHI1, and IncFII, respectively. The plasmids were harbored by ***E. coli*** MG1655/***P. putida*** KT2440 (only RP4) while plasmid-free ***E. coli*** MG1655 Nal^R Rif^R/***P. putida*** KT2442 Rif^R was used as the recipient strain. Cell densities at approximately 10^8 CFU/ml of the donors and recipients were used in a 1:1 ratio and mating was carried out in PBS to avoid growth bias. Two different sub-inhibitory concentrations of biocides were used to expose the cells during mating. Non-exposure controls (at the beginning of the experiment t_0 and end-of-experiment time-point t_8) were used as the baseline for the detection of conjugal stimulation. The transconjugants, recipients, and donors were enumerated using a Most-Probable-Number method in a 96-well microplate format.

Results: For the three plasmids harbored in ***E. coli*** and for RP4 in ***P. putida***, we did a complete assessment for six biocides at two different exposure concentrations below the minimal inhibitory concentration. In addition, ***E. coli*** harboring RP4 was further screened against six other biocides. Exposure to these biocides resulted in statistically significant stimulation of horizontal gene transmission when exposed to copper sulphate and silver nitrate. By increasing the number of replicates, we also found that both triclosan and chlorhexidine were capable of stimulating conjugation. Furthermore, we observed inhibition of conjugation for the R27 and R1 suggesting plasmid-dependent interactions with the biocides.

Conclusions: The findings give valuable insight into the risk of AMR plasmid dissemination associated with exposure to environmental concentrations of biocides and lay the foundations for doing an environmental risk assessment.

[87] QUORUM SENSING INHIBITORS BIND TO VIBRIO VULNIFICUS SmcR AND PROMOTE ITS DEGRADATION

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Quorum sensing (QS) is widely studied in pathogenic marine Vibrio species because this cell-cell signaling system regulates virulence genes, including biofilm formation and production and secretion of cytotoxins and proteases. In Vibrio species, QS autoinducer signaling culminates in production of a central TetR-type master transcription factor present in each species and collectively called the LuxR/HapR family, which have crucial roles in regulating genes required for colonization and infection of host organisms. Thus, LuxR/HapR and its homologues present key targets for designing therapeutics to block QS in Vibrio species. We previously showed that the small molecule PTSP (3- phenyl-1-(thiophen-2-ylsulfonyl)-1H-pyrazole) specifically targets Vibrio vulnificus SmcR but fails to inhibit Vibrio cholerae HapR, with moderate inhibition of other LuxR/HapR homologs from various Vibrio species. We hypothesized that variations in conservation of the ligand binding pocket of the LuxR/HapR family determine the efficacy of PTSP inhibition. Here, we used structure-function analyses to identify PTSP-interacting residues in the ligand binding pocket of SmcR that are required for PTSP inhibition of SmcR activity in vivo. Genetic screens identified substitutions at eight residues that were sufficient to reduce or eliminate PTSP-mediated SmcR inhibition, three of which are divergent in HapR. X-ray crystallography analysis of the SmcR-PTSP structure confirmed these residues as important for PTSP binding in the pocket. We show that SmcR is degraded rapidly by proteases in vivo in the presence of PTSP, and substitutions of these key PTSP-interacting residues in the binding pocket stabilized or increased protein levels in the cell. This mechanism of inhibition is observed of all thiophenesulfonamide compounds tested against V. vulnificus. From this study, we conclude that thiophenesulfonamides specifically bind to the binding pocket of LuxR/HapR proteins, leading to protein degradation and eliminating downstream gene regulation.

[88] DUPLEX ddPCR FOR NASOPHARYNGEAL PNEUMOCOCCAL DETECTION

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Isolating and culturing Streptococcus pneumoniae from clinical specimens is a complex procedure requiring precision and expertise. With the advent of molecular diagnostics, notably qPCR, there has been a marked improvement in detection capabilities compared to traditional culturing. This pilot study aimed to evaluate the efficacy of duplex ddPCR in detecting pneumococcal-specific genes, LytA and PiaB, in nasopharyngeal swabs from asymptomatic children. A total of 160 samples, comprising 80 culture-positive and 80 culture negative specimens, were analyzed. Our findings indicated a 36% increase in pneumococcal detection using ddPCR compared to the conventional culturing method. Notably, samples positive for both LytA and PiaB genes via ddPCR were decisively identified as pneumococcal-positive, reinforcing the established prominence of these genes. Furthermore, ddPCR demonstrated superior sensitivity, especially in samples with reduced total DNA concentrators, potentially due to its ability to distinguish amidst extraneous bacterial DNA. In conclusion, duplex ddPCR emerges as a promising diagnostic tool for pneumococcal gene detection, showcasing its advantages over traditional culturing techniques in specific contexts. Future endeavors will focus on refining the assay and expanding its application to sterile sites, paving the way for broader clinical integration.

[89] DEVELOPMENT OF RAPID TYOING METHOD FOR VANCOMYCIN-ENTEROCOCCUS FAECIUM

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Purpose: The aim of this research task is to develop and test a rapid typing method suitable for immediate typing of most vancomycin-resistant Enterococcus faecium (VREfm). DNA sequence targets and primer sequences for PCR tests that specifically identify the most prevalent types of VREfm have been identified. It is desired to establish these sequences for real-time typing of VREfm in diagnostic samples, as well as screening samples. Furthermore type-specific primers for MLST sequence type 117 are being developed. The identification of targets and primer/probe sequences will be conducted with the RUCS tool developed by the Center for Genomic Epidemiology.

Methods: Previously published PCR assays for vanA, vanB and an outbreak cluster of ST80 was introduced in the laboratory. A ST117-specific PCR was designed using RUCS (Rapid Identification of PCR Primers for Unique Core Sequences). The method utilizes whole genome sequence (WGS) data for identification of specific primer and probes for PCR. vanA and vanB genes were identified from WGS data using KmerFinder.

Results: The vanA and vanB testing agrees with the expected data, despite laboratory challenges. 36 VREfm isolates are tested, of which 10 samples differ due to technical problems. The remaining 26 samples agree with the expected outcome, which is satisfactory. Furthermore, 31 VREfm isolate underwent evaluation, comprising 15 categorized as ST117 and 16 as ST80. Except one isolate there was complete accordance between the two methods for typing. One isolate typed as ST80 by WGS was positive in both the ST117 and the ST80 PCR assay. Repeating both PCR and WGS showed the same results.

Student: Cloudia
Supervisor: Michael Kemp, Karen Angeliki Krogfelt

[90] REVOLUTIONIZING SEPSIS DIAGNOSIS AND ANTIBIOTICRESISTANCE DETERMINATION: NANOPORE SEQUENCING FOR RAPID HIGH-THOUGPUT PATHOGEN IDENTIFICATION AND ANTIBIOTIC RESISTANCE PROFILING IN BLOODSTREAM INFECTIONS

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Sepsis is an immune response disorder caused by an infection that has often spread to the bloodstream. To survive, it is crucial to initiate antibiotic treatment immediately. Rapid diagnostics are essential to provide effective antibiotics. However, current methods rely on culturing the pathogen(s), which has a long turnaround time. Until a pathogen identification is available, broad-spectrum antibiotics are administered, which promotes antibiotic resistance towards last-resort antibiotics. According to WHO estimates, up to 10 million deaths annually will be due to treatment failure of resistant pathogens by 2050. We have demonstrated that Nanopore sequencing is a fast and reliable method to investigate the pathogens involved in bloodstream infections. It could change the way sepsis is diagnosed and treated. In collaboration with the Department of Clinical Microbiology at Aalborg University Hospital, this project aims to investigate whether Nanopore sequencing can predict the antibiotic resistance profile of pathogens in a comprehensive database approach. The project will build a database containing genomes of the pathogens detected in positive cultures from patients at Aalborg University Hospital in a high-throughput Whole Genome Sequencing (WGS) approach. This will be done for pathogens of selected species isolated in the past two years (2021-2022). The project will also involve the development of a protocol for high-throughput DNA extraction and Nanopore sequencing. Additionally, it will analyze the genetic mechanisms of the phenotypic resistances.

Keywords: Nanopore HT Sequencing, Bloodstream infections, Sepsis diagnostics, Antibiotic resistanc

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