[PA39] TRANSCRIPTOMIC FINGERPRINT OF BACTERIAL INFECTION IN LOWER EXTREMITY ULCERS

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Lower extremity ulcers, such diabetic foot and leg ulcers, represent both humanistic and economic burdens to society. These ulcers commonly develop infections, which can lead to chronic, recurring wounds and increased risks for amputation. This study examined RNA-sequencing data from clinically infected lower extremity ulcers (n = 44) from two sources to compare whether clinical parameters, such as infection severity score or ulcer duration, reflect the physiological environment of the wound. We demonstrated that clinical infection severity score or ulcer duration did not explain the variability among the ulcers and identify high transcriptomic variability among samples with the same clinical classification. Samples with high proportions of bacterial RNA, however, exhibited a consistent shift in gene expression, towards increased immune response and inflammation. In terms of predicting wound physiology, the proportion of bacterial reads was a better predictor of wound physiology than clinical observations. We applied k-means clustering to the data and identified two clusters, one of which contained all of the samples with high amounts of bacterial RNA. We the utilized a support vector classifier to develop a fingerprint of 20 genes, including immune-associated genes such as *CXCL8*, *GADD45B*, and *HILPDA*, which accurately identified samples with signs of infection via cross-validation. This suggests that a transcriptomic fingerprint may be applicable not only as a tool for increasing host-bacterial interactions in these ulcers, but also as a potential classification method for ulcer infection state.

[PA40] MECHANISM BEHIND ANTIBIOTIC ENHANCED PLASMID TRANSFER AND IDENTIFICATION OF COMPOUNDS WHICH INHIBIT THIS MECHANISM.

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Conjugation is considered a major contributor to the diversity of bacterial genomes and the emergence of new antimicrobial-resistant pathogens. This process is highly efficient and depends on the presence of conjugative plasmids. These plasmids provide the necessary genes for the DNA transmission including the transfer (*tra*) genes involved in the type IV secretion system. In addition, previous work has shown that transfer frequency of a cefotaxime (CTX) resistance plasmid was enhanced significantly when the donor was pre-grown in CTX. However, it is currently still unknown how CTX induces the conjugative transfer of resistance plasmids. In this study, we aim at identifying the genes and regulatory pathways involved in the conjugative spread of resistance plasmids in *E. coli* subjected to CTX. In addition, we intend to identify compounds which prevent plasmid conjugation. To test this hypothesis, we will predict a regulatory pathway for CTX exposure using two approaches, expression assays for individual genes previously identified as part of the pathway and protein-protein expression assay to reveal the interaction between the identified proteins. This will provide an overview of the genes and proteins involved in CTX induced conjugation. Additionally, individual deletions of identified genes will be performed to verify their involvement in conjugation and *in vivo* confirmation will be performed using *C. elegans*. Finally, a compound library will be screened to identify compounds that prevent antibiotic induced conjugation; and mode of action of identified compound will be elucidated. Identifying the regulatory pathway involved in conjugation will identify targets, and help, on the long term, to create new antimicrobial agents.

[PA41] HUMAN MILK OLIGOSACCHARIDES INDUCE COMPOSITIONAL CHANGES IN THE GUT MICROBIOTA OF CONVENTIONAL MICE AND SPECIFICALLY INDUCE AN ACUTE YET REVERSIBLE INCREASE IN THE ABUNDANCE OF THE GENUS PHOCAEICOLA.

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Species within the Bacteroidales order are generally dominant members of the established human microbiota. During weaning of the child, most individuals experience a shift from a bifidobacteria-dominated microbiota to a microbiota dominated by Bacteroidales and Clostridiales. Members of the Bacteroidales order can utilize Human milk oligosaccharides (HMOs) in *in vitro* studies. To investigate whether specific HMO's affect the abundance of naturally occurring Bacteroidales in a complex mammalian gut environment, we conducted a study in conventional mice (n=40) administered three different HMO's, namely 6'sialyllactose (6'SL), 3-fucosyllactose (3FL) and Lacto-N-Tetraose (LNT), in their drinking water (5 %) for 8 days. Enumeration of Bacteroidales in fecal samples by culturing showed that mice receiving any of the three HMO's had increased absolute abundance of this bacterial order from two days onwards compared to controls. The increase in Bacteroidales was reverted within one day of removing the 3FL from the drinking water. Analysis of 16s rRNA gene sequences of fecal samples revealed a significant change in microbial composition from experimental day 0 to day 8 for mice administered 3FL and 6'SL, which for the 6'SL group further resulted in a decreased Shannon index. The observed changes for mice administered 3FL were driven by an increase in abundance of the genus *Phocaeicola* (formerly *Bacteroides* genus). Short-chain fatty acid analysis revealed a decrease in fecal acetate and butyrate levels in mice administered 3FL and 6'SL which correlated with the seen decrease in Shannon index. This study points towards an interesting dynamic of HMO-driven selection in the gut microbiota affecting structure and function of the microbiota.

[PA42] MOBILIZATION OF ANTIBIOTIC RESISTANCE GENES DIFFER BY RESISTANCE MECHANISM

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Screening for antibiotic resistance genes (ARGs) in, especially environmental, samples with (meta)genomic sequencing is associated with false-positive predictions of phenotypic resistance. This stems from the fact that most ARGs require being overexpressed before conferring resistance, which is often caused by decontextualisation of said ARGs by mobile genetic elements (MGEs). Overexpression of ARGs can be caused by strong promoters often present in IS elements and integrons and by being placed on multicopy plasmids. Here, we screen all complete bacterial RefSeq genomes for ARGs. The genetic contexts of detected ARGs are investigated for IS elements, integrons, plasmids, and phylogenetic dispersion. The ARG-MOB scale is proposed which indicates how mobilized detected ARGs are in bacterial genomes. Antibiotic efflux genes are rarely mobilized and it is concluded that these are often housekeeping genes that are not decontextualized to confer resistance through overexpression. Even 80% of β-lactamases have never, or very rarely, been mobilized in the 15,790 studied genomes. In this study, ARGs in all complete bacterial genomes are classified by their association with MGEs, using the proposed ARG-MOB scale. These results have consequences for the design and interpretation of studies screening for resistance determinants, as mobilized ARGs pose a more concrete risk to human health. An interactive table of all results is provided for future studies targeting highly mobilized ARGs.

[PA43] ADAPTIVE LABORATORY EVOLUTION AND INDEPENDENT COMPONENT ANALYSIS DISENTANGLE COMPLEX VANCOMYCIN ADAPTATION TRAJECTORIES

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Infections with methicillin-resistant *Staphylococcus aureus* MRSA are commonly treated with the last-resort antibiotic vancomycin, however strains with intermediate resistance to vancomycin termed VISA are spreading globally and are associated with treatment failure.

VISA strains are difficult to detect in the clinic due to their low-level resistance, and common VISA genetic markers are not available. Indeed, vancomycin adaptation in MRSA is highly diverse, resulting from the stepwise accumulation of mutations in a large number of genes. Many of these genes belong to the same regulatory pathways or encode similar functions. This led us to question whether individual factors in VISA could be grouped under common evolutionary pathways, which would facilitate the study and consequently the detection and treatment of infections with VISA.

We characterized the phenotypic, mutational, and transcriptional landscape of 10 lineages of MRSA strain JE2 evolved in parallel to vancomycin. Despite a common ancestor and fixed evolution settings, vancomycin adaptation across lineages was diverse. However, using independent component analysis of transcriptional profiles, we retrieved two divergent pathways of adaptation to vancomycin central to the activity of the VraR regulon, and associated with either high or low oxacillin susceptibility.

Our results narrow the large adaptive diversity in VISA to two distinct pathways, which can help guide future research efforts into the detection of VISA and alternative therapies to VISA infections. Furthermore, we describe numerous methods for studying the evolution of complex resistance mechanisms.

[PA44] UNIQUE MICROBIOTA IN ATOPIC DERMATITIS SKIN

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The skin barrier dysfunction in atopic dermatitis (AD) is associated with microbial imbalance, which is affected by the immunological status of the host and the local microenvironment. Here we characterize the microbial community composition and distribution in AD and healthy skin to pinpoint the important host-microbial interdependence.

Skin biopsies and tape strips from the hand back, elbow pit and chin of 37 AD patients and corresponding controls were analyzed by DNA metabarcoding, confocal microscopy and cultivation.

The skin microbiota was distributed as scattered single bacterial cells and aggregates. In the stratum corneum, bacteria was attached to anucleated corneocytes, while hair follicles harbored vast bacterial aggregates. Cultivation revealed specific species interchanges within the genera *Staphylococcus, Corynebacterium, Bacillus* and *Rothia,* and an increased bacterial load in the superficial AD skin. *Staphylococcus aureus* was significantly correlated to AD epidermis, and was less prominent in dermal microbiota. Epidermal microbiota was highly variable, while dermal microbiota composition was more conserved. Consequently, the dermal microbiota was more similar between habitats, but remained different between AD and healthy skin.

In summary, we found a common homologous dermal microbiome also within AD skin. Nevertheless, as dermal microbiome is more likely shaped by internal host factors, minor compositional changes may therefore be immensely informative for individualised treatment. In the superficial skin, specific species interchanges could be seen as potential biomarkers. Although, *S. aureus* was strongly correlated to epidermal AD skin, other species may show significance in the pathogenesis.

[PA45] A SYSTEMATIC SCREENING OF A LARGE COLLECTION OF CLINICAL PSEUDOMONAS AERUGINOSA ISOLATES REVEALS STRAIN- AND ANTIBIOTIC-SPECIFIC BIOFILM TOLERANCE PATTERNS

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Pseudomonas aeruginosa is one of the most important pathogens in CF patients, causing lifelong chronic lung infections that are a major determinant of morbidity and mortality. Chronic bacterial infections are associated with biofilm formation, a sessile lifestyle in which bacteria are embedded in an extracellular matrix and thus protected from adverse stresses. Antimicrobial therapy most often fails despite the absence of genotypic resistance, and it is generally accepted that biofilm-grown bacteria are 10- to 1,000-fold more resistant than planktonic cells, which are classically used for standard antimicrobial susceptibility testing. However, we are only at the beginning to understand the reasons for biofilm recalcitrance, and systematic approaches to describe biofilm tolerance are lacking. In this study, we investigated a large and highly diverse collection of >250 clinical *P. aeruginosa* isolates for their biofilm resistance profiles towards the antibiotics ciprofloxacin, tobramycin, and colistin. We discovered characteristic patterns of drug-specific killing activity and identified groups of strains exhibiting high, as well as strains exhibiting low tolerance when grown under biofilm conditions. Our results indicate cross-tolerance of individual strains to ciprofloxacin and tobramycin killing. Furthermore, our results suggest that there is a biofilm-specific adoption of a metabolic state that drives a general tolerance, which seems to be variantly expressed among clinical isolates. Although much more remains be learned about the molecular mechanisms underlying biofilm tolerance, our data provide valuable insights for the development of novel treatment strategies to combat chronic biofilm infections.

[PA46] ISOLATION OF NOVEL EPISOMES INVOLVED IN ANTIGENIC VARIATION IN MYCOPLASMA GENITALIUM

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Mycoplasma genitalium is a neglected yet prevalent sexually transmitted pathogen, capable of causing acute and chronic infections in humans, which can ultimately lead to infertility. While complete clearance by the immune system is possible, the bacterium can persist by generating virtually infinite protein variants of the immunodominant cytadhesins MgpB and MgpC. This process occurs by recombination of regions found within the *mgpB/mgpC* genes with 9 homologous DNA repeats scattered around the genome, named MgPa repeats. We hypothesize that *M. genitalium* can generate circular intermediates or episomes and recombine them in multiple MgPa repeats before re-inserting in the original position, thus increasing antigenic variation potential. To investigate the existence of these episomes, we inserted a selection marker and an *Escherichia coli* replication origin downstream of the *mgpC* gene. Extrachromosomal DNA was extracted and transformed into *E. coli* for propagation and characterization. In total, 50 different episomes were isolated and confirmed via restriction analysis. Eight of them were further characterized by Sanger sequencing to determine the origin of these molecules and trace recombination events. Our results indicate that episomal DNA is originated by recombination of multiple repeats within the *mgpB* and *mgpC* genes and that acquire numerous genetic variants by DNA shuffling, suggesting that *M. genitalium* can use extrachromosomal DNA to generate antigenic variants.

[PA47] THE IMPORTANCE OF TAXONOMIC CLASSIFICATION SOFTWARE AND MACHINE LEARNING ALGORITHMS FOR THE PREDICTION OF COLORECTAL CANCER

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Colorectal cancer (CRC) is the development of cancer in the rectum or colon and represents a rising global burden ranking third in terms of incidence and second in terms of mortality. It is estimated the global burden of CRC will increase by 60% to 2.9 million new cases and 1.5 million new deaths by 2040. Due to the burden of CRC several countries, including Denmark, have implemented a national screening program for early detection of CRC. The method, iFOBT, implemented in Denmark measures hemoglobin in stool, and in case of a positive test, the patient is invited to a colonoscopy for final diagnosis. However, the iFOBT has a high false-positive rate (FPR) of 45%, which resulted in 9,800 unnecessary colonoscopies in 2019, amounting to approximately 43 million DKK and 7,350 hours.

In recent years, the combination of machine learning algorithms (MLA) and shotgun metagenomics have established strong associations between the gut microbiota and cancer status in patients, representing a potential new tool for CRC screening. In this thesis the impact of three taxonomic classification software (MetaPhlAn3, Kraken2, Kaiju) and four MLA's (Neural Net, XGBoost, Random Forest, LASSO) on CRC prediction were tested. Kraken2 resulted in the best prediction of CRC, a significantly better prediction than Kaiju. Furthermore, XGboost and Random Forest performed on average better than other MLA's. Also, CRC prediction could be achieved with as few as 100,000 reads, when using Kraken2.

[PA48] FROM DAYS TO HOURS: DEVELOPMENT AND IMPLEMENTATION OF METAGENOMIC DNA-SEQUENCING IN CLINICAL MICROBIOLOGY DIAGNOSTICS

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Traditionally, diagnosis of bacterial or fungal infections rely on culture-based methods. This usually takes 1-3 days and has poor sensitivity, especially if antimicrobial therapy has been initiated before the clinical samples were obtained. Due to the long turnaround time, critically ill patients are administered empiric broad-spectrum antibiotics prior to pathogen identification, and internationally, it is estimated that ~20% of patients receive ineffective treatment that may cause excess mortality. Ideally, a real-time diagnostic of the causative pathogen is preferable. This will also give the opportunity for the use of targeted antimicrobial therapy administered early in the clinical setting. Finally, this will also minimize the use of broad-spectrum antibiotics.

To overcome the diagnostic delay in current methods, we are exploring the use of DNA sequencing on the MinION platform for pathogen identification in critically ill hospitalised patients with septicaemia. This has the potential to revolutionise the routine clinical microbiology diagnostics by lowering turnaround times from multiple days to <6 hours. We will utilise methods developed for archaeogenetics (the study of ancient DNA) that target ssDNA in the library preparation to enrich microbial DNA with the aim of obtaining higher sensitivity at a reduced cost. Furthermore, we will use methods from machine learning to separate DNA profiles of diseased from that of healthy individuals to increase analysis specificity.

[PA49] FOUR-SPECIES CLINICAL UROPATHOGEN BIOFILM MODEL TO STUDY CATHETER ASSOCIATED URINARY TRACT INFECTION

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Catheter associated urinary tract infections (CAUTI) are a common clinical concern as they can lead to severe, persistent infections or bacteremia. CAUTI are difficult to eradicate, as most CAUTI cases are caused by multi-species biofilms that are tolerant to various antibiotics. New strategies to tackle CAUTI have been proposed, including antibiotic combination treatments, surface modification and probiotic usage. However, those strategies were mainly assessed on mono- or dual-species biofilm that could hardly represent the clinical CAUTI cases where 2-4 or even more species are normally involved.

We have developed a four-species *in vitro* biofilm model on catheter material involving clinical strains of *Escherichia, Pseudomonas, Klebsiella* and *Proteus* from indwelled catheters. Inter-species cooperation, competition as well as their response to antibiotics and probiotics were quantitatively assessed by CFU enumeration and qPCR. The four species were chromosomally tagged with genes expressing different fluorophores, enabling visualization of the spatial organization and attachment to the catheter material at individual species level, using confocal microscopy and image analysis. Both synergetic and antagonistic interactions were found among members in our model biofilm and those interactions affected the strains' fate upon exposure to antibiotics and probiotics as mono- or multi-species biofilms.

Our study emphasizes the species complexity of the model biofilm and highlights the necessity of evaluating treatment and control regimes in a multispecies setting. Results of antibiotic and probiotic responses from our multi-species biofilm are instructive to clinical usage of antibiotics and probiotics.

[PA50] EXPLORING THE PHENOTYPIC AND GENOTYPIC DIVERSITY OF PSEUDOMONAS AERUGINOSA, CLINICAL ISOLATES

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Pseudomonas aeruginosa is an opportunistic human pathogen. Responsible for infecting immunocompromised patients, often seen in individuals with cystic fibrosis, urinary infection, or patients with chronic wounds. The large genome size and the genetic complexity of *P. aeruginosa* contribute to the pathogenicity and their ability to adapt and thrive under different environmental conditions. Unfortunately, treating *P. aeruginosa* infection has been extremely difficult due to a high intrinsic and acquired antibiotic resistance. The clinical isolates of *P. aeruginosa* used in this study were donated by two patients with chronic venous leg ulcers. The patients were admitted to Copenhagen Wound Healing Centre, Bispebjerg Hospital.
In this study, 16 clinical isolates of *P. aeruginosa* were analyzed together with PAO1 to determine the phenotypic and genotypic diversity among the isolates and explore whether the isolates originated from the same *P. aeruginosa* strain exclude the possibility of cross-infection had occurred at the hospital. In the phenotypic analysis, the colony morphology and the growth rate were determined, together with antimicrobial susceptibility testing using tobramycin, ciprofloxacin, meropenem, and polymyxin B. Furthermore, whole genomic sequencing was performed to investigate the genotypic diversity. Hence, multi-locus sequence typing and phylogenetic analysis were conducted.
The phenotypic and genomic analysis revealed one clonal lineage per patient, and the phylogenetic analysis excluded the possibility that cross-infection had occurred between the two patients. The result indicates one clonal lineage per patient, and the phylogenetic analysis excludes the possibility of cross-infection.

[PA51] BIOFILM FORMATION IN THE UDDERS OF DAIRY COWS WITH CHRONIC MASTITIS

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Background

Bovine mastitis is one of the most paramount diseases in the dairy industry and has adverse effects on the economy, the use of antibiotics and animal welfare.

Some cases of mastitis cannot be eradicated using traditional antibiotic treatments and some cows suffer from recurrent or chronic infections. Biofilm formation in the udders can be a possible explanation of why these infections reoccur and are difficult to treat.

In this PhD study, several research questions will be investigated to uncover the role of biofilm in mastitis and the microbial composition in the udders.

Materials and methods

Tissue samples will be collected from udders from dairy cows with chronic mastitis and healthy dairy cows. The bacterial composition in udders from healthy dairy cows and dairy cows with chronic mastitis will be characterized by cultivation, MALDI-TOF and 16S PCR. The distribution and location of bacteria and potential biofilm will be elucidated through PNA FISH and confocal laser scanning microscopy.

Results

The results from these different investigations will provide a deeper understanding of the pathogens responsible for chronic mastitis infections and the potential role of biofilm in chronic mastitis in dairy cows.

Conclusion

This PhD study aims to contribute to the limited research of biofilm in bovine mastitis and to acquire a greater understanding of bacteria's role in chronic mastitis. This could lead to new and optimized treatments, decreased use of antibiotics and improved animal welfare in the dairy industry.

[PA52] THE EARLY LIFE SKIN MICROBIOTA AND ATOPIC DERMATITIS - A BIRTH COHORT STUDY

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In skin, the most common inflammatory immune-mediated disease is atopic dermatitis (AD), with at least 230 million suffering from this disease worldwide. AD has the highest prevalence in the first years of life, with more than 20% being diagnosed with AD in some countries and can be distressing for both the child and their families. However, no cure exists and current treatment is only improving symptoms.
We hypothesize that risk of disease in children is heavily influenced by early life bacterial colonization and immune training. The aim of this study will be to elucidate if the early life skin microbiota associate with risk of later atopic dermatitis and disease severity.
To accomplish this we will use data from two deeply phenotyped longitudinal birth cohorts (COPSAC2010, and EAT). A total of 850 infants in these independent studies underwent longitudinal sampling of the skin microbiota before and after the onset of disease, alongside detailed reporting of environmental exposures, and clinical phenotyping.
The skin swap samples have been analyzed with 16s rDNA gene amplicon sequencing of the V3V4 region. The long-term perspective of this project is a new therapeutic approach for AD, intervening before disease initiation. Data analysis is ongoing and preliminary data will be available at the time of the DMS congress.

[PA53] A RAPID, COST EFFICIENT AND SIMPLE METHOD TO IDENTIFY SARS-COV-2 VARIANTS OF CONCERN BY SANGER SEQUENCING PART OF THE SPIKE PROTEIN GENE

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Around New Years, 2020, it became excruciatingly clear that monitoring variants of the SARS-CoV-2 virus was important to evaluate ongoing COVID19 pandemic. Several strategies exist for monitoring these variants, including whole genome sequencing and RT-qPCR. Here, we suggest using a technology as old as time in molecular biology: Sanger sequencing. While inferior to other technologies at first glance, a number of advantages exist for Sanger sequencing in monitoring variants of SARS-CoV-2. Namely, the lack of multiplexing of samples, the low and linear cost of operation, and the availability of and familiarity with the technology in developing and developed countries alike. Our method is efficient when used at an existing RT-qPCR based COVID19 testing facility, where it requires no new equipment, no extra cold chain, minimal training of lab personel and no bioinformatics support. We present a complete protocol for sample preparation and automated analysis of the resulting sequencing data, with protocols and software made freely available, without uploading sensitive data. Relying on a single well-placed primer set from the ARTIC protocol for WGS, this method is able to differentiate all known Variants of Concern, and identify key mutations on the SARS-CoV-2 spike gene that confer increased infectioness or immune system evasion. Since the implementation of this protocol in January 2021, this method has been used for routine screening of positive samples in the DTU COVID facility and hospitals in Denmark, as well as in a number of other countries.

[PA54] OVERCOMING CHALLENGES IN ANTIMICROBIAL RESISTANCE PREDICTION FROM GENOMIC DATA

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Antimicrobial resistance (AMR) is an emerging global threat to human health, projected to cause more than 10 million annual deaths by 2050[1]. To improve patient outcomes and manage antibiotic resources, rapid and accurate diagnostics are needed.

To this end, machine learning (ML) methods for the prediction of AMR from genomic data are increasingly being developed. However, best practices need to be followed to ensure model accuracy and generalizability when applying ML techniques to genomic data. The high dimensionality of genome representations commonly used for ML carries the risk of overfitting, training data is limited and sampling of microbial isolates from the underlying population may be biased[2].

Using the AMR reference database ARESdb[3] – a unique resource of over 84,000 bacterial isolates with genotypic and phenotypic information sampled broadly in time and space – we investigate the generalization properties of published techniques to predict AMR. We observed significant improvements in robustness and accuracy of AMR prediction from NGS data[4] when using tailored sampling and ML model ensembling methods.

In summary, we demonstrate that accurate AMR prediction from genomic data in clinical settings can be achieved through algorithmic improvements, exhaustive method validation and sustained sampling efforts.

References

1. https://amr-review.org/
2. https://doi.org/10.1128/JCM.01405-18
3. https://doi.org/10.1016/j.gpb.2018.11.002
4. https://doi.org/10.3389/fcimb.2021.610348

[PA55] LIPOTEICHOIC ACID AND MEMBRANE INTEGRITY IS ESSENTIAL FOR STAPHYLOCOCCUS AUREUS TOLERANCE TO D-SERINE

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D-serine is the most prevalent D-amino acid in human blood serum and urine. Being a non-uropathogenic bacterium, *Staphylococcus aureus* is sensitive to D-serine, and combinations of D-serine and β-lactam antimicrobials show promising synergistic effects against *S. aureus*. However, the mechanism behind the bacteriostatic effect of D-serine against *S. aureus*, and which factors that influence D-serine tolerance, has not been enlightened. In a screen for D-serine sensitivity, we found members of the lipoteichoic acid pathway, YpfP and LtaA, and novel β-lactam auxiliary factors AuxA and AuxB as essential during D-serine stress. Inactivation of each individual gene reduced D-serine tolerance by 8-32 fold but could be countered by the addition of D-alanine. Transmission electron microscopy of D-serine treated cells revealed that gene-inactivated mutants have trouble completing their septal cell wall and split into separate daughter cells after ended cell division. Suppressor mutations against D-serine in *aux*-inactivated mutants arose primarily in genes encoding members of the type II fatty acid biosynthesis pathway. This could work to counteract a destabilized membrane as *aux* mutants carry subpopulations with membranes permeable by propidium iodide with no difference in viability to wild type cells. Additionally, we show that AuxA and AuxB interact with one another and with members of lipoteichoic acid, peptidoglycan synthesis and cell division pathways. Our findings imply a direct link between staphylococcal membrane integrity and cell division events and suggests the integrity of the cytoplasmic membrane and its incorporated lipoteichoic acids as key for *S. aureus* tolerance to D-serine.

[PA56] POTENTIAL FOR ZOONOTIC SPREAD OF MULTI-RESISTANT CLOSTRIDIOIDES DIFFICILE

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Background

Clostridioides difficile is a toxin-forming bacillus causing severe enteric infection. PCR ribotype 078 (RT078) and its sequence type 11 (ST11), is associated with a rising number of infections in young and healthy patients. Farm animals have been identified as RT078 reservoirs. Intensive antibiotics use in farms leads to high resistance and a selective pressure for spreading to humans.

Aim

This project aims to investigate the zoonotic potential of *C. difficile* and its role in horizontal transfer of antimicrobial resistance genes.

Methods

In total 330 fecal samples were collected during 2020 from nine Danish pig farms. Isolates were whole genome sequenced (Illumina), to determine multilocus sequence type (MLST), toxins and resistance genes. core genome MLST (cgMLST) was used for comparison with 600 human clinical isolates from same period.

Results

C. difficile was isolated in 12 samples (~4%). All isolates were toxigenic (*tcdA+, tcdB*+), four also binary toxin (*cdtA/B*) positive. Five sequence types (ST) were found (numbers in brackets): ST11(4), ST6(3), ST7(1), ST13(1), ST36(1), ST49(1), all also common in humans. cgMLST distance between pig and the closest human isolate varied from two to 49, the four ST11 being closest to the humans, i.e. two or three alleles different. Nine isolates contained at least one resistance gene and in total nine different genes were observed. Most common were genes towards vancomycin and tetracycline, which were also found in humans.

Conclusions

ST11 was the most common overlapping type in porcine and humans in Denmark and three isolates were within possible cgMLST transmission range. Resistance genes indicate that *C. difficile* plays a role in zoonotic resistance gene reservoir and exchange.

[PA57] QUANTIFYING ENTEROTOXIGENIC E. COLI INFECTING PHAGE FROM FAECES

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Background

Post-weaning diarrhoea (PWD) is a serious disease in piglets commonly associated with enterotoxigenic *E. coli* (ETEC) as the pathogen, causing significant financial loss to farmers worldwide. Traditional prevention methods involving vaccines, the prophylactic use of antibiotics or zinc oxide as feed additive have been inefficient or problematic because of the spread of antibiotic resistance and environmental pollution. AVANT (Alternatives to Veterinary ANTimicrobials) is a collaboration project between five academies and eight companies funded by the European Union, which aims to develop economically sustainable solutions for PWD.

Our work

In AVANT we support the transition from the laboratory to *in vivo* studies of a phage product, based on previously isolated lytic phages specifically targeting our collection of European ETEC strains. Here we develop a quantitative PCR (qPCR) based method to monitor the phage product in pre-clinical experiments. Traditionally double-layer agar plates are used to determine the plaque forming unit (PFU), that is the concentration of infective phage particles in a given sample. Unfortunately, this technique can be time consuming, have low throughput and sensitivity. Our rapid qPCR protocol, instead, can precisely quantify the phages from the administered product in the piglets' faeces, and distinguish them from native phages already inhabiting the piglet gut. Additionally, using a correction coefficient generated with spiked faecal samples, we can convert the number of phage particles estimated by qPCR into viable PFU counts. These results will consolidate the use of phages against ETEC, thus reducing the spread of antibiotic resistance in line with WHO's One Health program.

[PA58] ANTIVIRULENCE TROJAN HORSE (CRISPR-CAS IN PHAGE) APPLICATION AGAINST PSEUDOMONAS AERUGINOSA INFECTIONS

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Medical science is pitted against evolutionary forces acting upon pathogenic bacteria. With the increasing prevalence of antibiotic resistance, an alternative strategy is to exploit our understanding of population dynamics of social traits in pathogens. *Pseudomonas aeruginosa*, a major cause of community- and hospital-acquired infections, cooperates and maximises its fitness during infection by communicating through quorum sensing (QS) signals to synchronously control the expression of virulence factors such as elastase, siderophores and phenazines, which aid in disruption of eukaryotic cell junctions, iron acquisition and immune evasion.

Here we developed a series of CRISPR-Cas13-based antivirulent systems, capable of sensing such virulence activators and eliminating cells expressing the target genes necessary for elastase (*lasB)*, pyoverdine (*pvdD*) and pyocyanin production (*phzM* & *phzS*). By employing this approach, we ensure a strong bactericidal effect upon recognition of the target genes regardless of their location, making sure that the expression profile does not become hypervirulent during treatment.

As a Trojan horse-like strategy, we generated a phage-based delivery system which packs the programmed CRISPR-Cas13 constructs into phage capsids to be delivered into pathogenic populations. The development and proof of principle of this technology offers an unorthodox alternative to both antibiotics and phage therapy, enabling strain-specific elimination and dampened virulence, while minimising the potential collateral damage to host cells.

[PA59] TINY EARTH DENMARK: STUDENT-SOURCING ANTIBIOTIC DISCOVERY

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The search for new antibiotics is urgent - as already today - ten thousand people die every year due to infections caused by resistant bacteria in Europe alone. This is predicted to rise to 10 million worldwide by 2050. The lack of economic gain has caused many stakeholders to refrain from antibiotic research. Tiny Earth is a global student-sourcing initiative that aims to create awareness of antimicrobial resistance and find new antibiotics in soil samples collected all over the world.
We have adapted this concept with the main goal of engaging high school students in the search for new antibiotics, creating awareness of antimicrobial resistance, demystifying STEM research, and ultimately hoping to inspire the students to pursue an education in science.
All the metadata associated with the isolates are entered into the expanding Tiny Earth Database, providing scientists worldwide with an extensive dataset for future investigations.
The isolates themselves are funneled into our research lab for further investigations.

[PA60] THE GUT MICROBIOME AND GROWTH IN EARLY LIFE

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Today, nearly every fifth child or adolescent in Denmark is dealing with overweight or obesity and being obese in childhood increases the risk of being obese as an adult, which is further associated with the increased risk of several health threatening conditions. However, there is a lack of evidence on interventions proven effective in preventing children with a healthy normal weight from developing obesity. Previous studies have shown that obese adults have a different gut microbiota compared to lean adults. The gut microbiota is known to be very susceptible in the first years of life from the beginning of neonatal colonization, whereas later in life it stabilizes and demonstrates great plasticity as many factors may induce smaller fluctuations without further complications to health status. This has led to the hypothesis that the critical time window for optimal modulation of the microbiota is limited to the early years when the composition is still being established. The aim of the study is to unravel patterns in the early life gut microbiota associating with weight development and give rise to a better understanding and potential of new strategies against childhood obesity, with the gut microbiota as key modulating factor. To test this hypothesis, data on 700 children's growth from birth to 10 years of age from the COPSAC2010 birth cohort will be used. This includes longitudinal measures of body mass index combined with assessments of body composition from dual-energy X-ray absorptiometry scans, which will be analyzed together with 16S rRNA gene sequenced fecal samples collected at five different timepoints. Data analysis is currently ongoing but preliminary results will be ready by the time the congress is taking place.

[PA61] THE ROLE OF EARLY LIFE GUT VIROME IN HEALTH AND DISEASE

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In recent years, we have started to understand the important role that the human microbiome represents in the first year of life for determining susceptibility to the development of asthma, allergies and other chronic diseases. However, most microbiome research has focused on the bacterial communities inhabiting the human body, while the role of the inhabiting viruses in the gut and their association with health and disease remains uncertain. The human gut contains at least as many viruses as bacteria, and while only a fraction of these infect human cells, most, so called bacteriophages (or phages), infect bacteria. By infecting specific populations of bacteria, phages can alter microbiota structure, and contribute to maintaining intestinal homeostasis or dysbiosis. The scope of this study is to assess the role and contribution of phages to the gut microbial ecosystem and its influence in the early immune training of the child to disentangle the associations with later development of asthma and allergy. To do so, we analysed fecal viromes (all DNA viruses found in the samples) of 647 infants at 1 year of age, all deeply phenotyped from birth in the COPSAC2010 cohort. In a long-term perspective, this will allow us to improve our understanding of virome-disease associations, the relationship with their bacterial counterpart, and how the virome can be modulated in a therapeutic window for early intervention. Data analysis is ongoing and preliminary data will be available at the time of the DMS congress.

[PA62] PHAGE INFECTION RESTORES PQS SIGNALING OF A PSEUDOMONAS AERUGINOSA LASI QUORUM-SENSING MUTANT

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Chemical communication between bacteria and between bacteria and the bacteriophage (phage) viruses that prey on them can shape the outcomes of phage-host encounters. Quorum sensing (QS), the bacterial cell-to-cell communication process that promotes collective undertaking of group behaviors including anti-phage defenses, enhances bacterial survival in the face of phage attack. QS relies on the production, release, accumulation, and detection of signal molecules called autoinducers. 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 called PQS. A *P. aeruginosa* Δ*lasI* mutant is impaired in PQS synthesis, leading to accumulation of the precursor molecule HHQ. We show that, in response to phage infection, the *P. aeruginosa* Δ*lasI* mutant restores *pqsH* expression, enabling conversion of HHQ into PQS. Moreover, downstream QS-target genes including those encoding virulence factors are induced. Additionally, phage-infected *P. aeruginosa* Δ*lasI* cells transiently exhibit superior growth compared to uninfected cells. Clinical isolates of *P. aeruginosa* frequently harbor mutations in particular QS genes. Thus, phage infection of such *P. aeruginosa* strains may increase bacterial virulence, underscoring the importance of characterizing phage-host interactions in the context of bacterial mutants that are relevant in clinical settings.

[PA63] INTESTINAL MICROBIOTA IN PATIENTS WITH ULCERATIVE COLITIS AND HEALTHY CONTROLS FROM GHANA AND DENMARK

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Introduction

Ulcerative colitis (UC) is a relapsing non-transmural inflammatory disease that is restricted to the colon and is characterized by flare-ups of bloody diarrhea. In this study we aim to investigate intestinal bacterial diversity in healthy controls and patients with UC with and without active disease, from Ghana and Denmark.

Method

This study included 18 healthy controls, 9 UC patients with active and 9 with inactive disease from Ghana. Additionally, 19 healthy controls, 8 UC patients with active and 8 UC patients with inactive disease from Denmark were included in the study. Microbiota diversity analysis relied on sequencing of ribosomal small subunit genes targeting prokaryotes and eukaryotes.

Results

When analysing the taxonomy-results for prokaryotes and eukaryotes, cluster and principal component analysis shows Danish healthy controls clustered together, but separate from healthy controls from Ghana, which also clustered together.
When analyzing prokaryotes taxonomy-results for Shannon diversity index (SDI), there are significant differences between Danish healthy controls and patients (mean 2-2.7) in comparison to the corresponding groups from Ghana (mean 1.5-1.8), p= 0.0056. The SDI of the eukaryotes ranges between 0-3.1 in the Ghana group, while in the corresponding Danish group it ranges between 2.4-3.2, the difference is non-significant (p=0.138).

Conclusion

Overall, healthy controls and patients with UC from Denmark have increased intestinal prokaryotic and eukaryotic diversity in comparison to corresponding groups from Ghana. Increased variance of the SDI in the Ghana group explains increased differences between individuals in their intestinal microbiota in comparison to the Danish group.

[PA64] TARGETED SCREENING OF PROBIOTICS WITH ANTI-VIRULENCE ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS IN ATOPIC DERMATITIS

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Atopic dermatitis (AD) is a common inflammatory skin disease that affects many children and adults worldwide, and most patients are colonized by the bacterial pathogen, *Staphylococcus aureus*. *S. aureus* contributes to AD severity through the expression of genes encoding virulence factors and exoproteins, which is controlled by the *agr* quorum-sensing system. Other staphylococci produce auto-inducing peptides (AIPs) able to inactivate the system, indicating that *agr* is an inter-species communication system. We hypothesize that probiotic bacteria are able to reduce *S. aureus* toxin production through repression of the staphylococcal quorum-sensing system and that some of the reported benefits of probiotics in AD may be associated with such an activity. We screened a probiotic bacteria collection against *S. aureus* and identified strains with antibacterial and anti-virulence activity, i.e. quorum sensing inhibiting activity. Probiotics are promising candidates that can be used in anti-virulence therapies against *S. aureus* infections and potentially be applied in the topical treatment of AD.

[PA20] MICROFLORA DANICA: GENETIC ANALYSIS OF NITROUS OXIDE RELATED GENES IN DANISH SOIL SAMPLES

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Nitrous oxide (N_2O) is a very potent greenhouse gas. Its greenhouse effect is about 300 times greater than that of CO_2 and is a significant contributor to the decomposition of the ozone layer. N_2O is produced through several pathways and e.g., by ammonia oxidizers in the process of reducing NO_3^- to N_2. A relationship between the abundance of genes coding for N_2O-producing enzymes and N_2O released has already been established. This allows us to study the soil samples collected and sequenced in the Microflora Danica project, to investigate the effects different conditions can have on N_2O production and reduction. In this project we are exploring the abundance of N_2O related genes with the type of habitat (nature, urban and agriculture), the season, soil pH and more. The result from these investigations could give us greater insight into N_2O production under different conditions and guidelines on what to adjust to reduce further greenhouse gas emission.

[PA21] CABLE BACTERIA WITH ELECTRONIC CONNECTION TO OXYGEN ARE SWARMED BY OTHER BACTERIA

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Cable bacteria are long, filamentous bacteria which can transfer electrons over centimetre distances. In freshwater sediment enrichments we observed diverse motile bacteria swarming around segments of cable bacteria in the anoxic zone. The swarming was transient, occurring only when the cable bacterium extended all the way to oxygen, millimetres away. Cell tracking of the swarming bacteria showed that they spend most of their time within 50 µm. Metagenome sequencing of glass chambers selecting for motile bacteria show that chemoorganotrophic and lithotrophic bacteria are differentially more abundant when cable bacteria are present, relative to the cable free controls. Swarming cells increase their swimming speed near the cable bacterium, and the detection frequency by fluorescence in situ hybridization is increased, indicating high ribosome content. This indicates that the interaction with the cable bacteria is metabolically positive to the swarming cells. The swarming ceases immediately then the filaments is cut with a dissection laser microscope. Preliminary Raman microscopy of swarming cells suggests the redox state of their cytochrome c is more oxidized near the cable bacteria. These results strongly suggest some type of electron exchange whereby the swarming bacteria take advantage of long distance electron transfer by cable bacteria.

[PA22] MICROBIAL SINGLE-CELL RNA SEQUENCING TO DIG INTO PLASMID MANAGERIAL CAPABILITIES OF BACTERIAL POPULATIONS

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In isogenic population of bacterial cells that are exposed to identical conditions, gene expression can be highly heterogenous. Such heterogeneity can be a means to optimizing fitness e.g. as bet-hedging strategies or division of labor. Plasmids can have a huge impact on the fitness of bacteria and plasmid maintenance is a dynamic process with continuous plasmid loss and acquisition. They affect the transcriptional programming of the cell and sometimes, engage in cross-talk with the rest of the host's genome, hereby reducing their fitness cost. To assess the heterogeneity in the bacterial response to plasmid loss and plasmid invasion, we are using a single-cell transcriptomics approach. Single-cell transcriptomics was first developed for eukaryote cells but were not easily adapted to bacteria due to their extremely low mRNA abundance, lack of mRNA polyadenylation and thick cell walls. The recently developed microSPLiT (microbial split-pool ligation transcriptomics) approach allows the characterization of thousands of bacterial cell's transcriptomes through combinatorial barcoding system. Our preliminary data, using this technique demonstrates that transcriptionally distinct subpopulations of Pseudomonas putida arise when carrying the highly conjugative plasmid pKJK5, with a differential transcription of certain genes, including the chaperone ClpB, activating the plasmid replication.

[PA23] DO NITRIFICATION INHIBITORS AFFECT NON-TARGET SOIL MICROORGANISMS?

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Nitrification inhibitors (NIs) are suggested as one of the remedies to reduce agricultural GHG emissions due to their potential to reduce nitrate leaching, improve nitrogen use efficiency of fertilizers and reduce N_2O emissions. Prior to widespread use of NIs in Danish agriculture, there is a need for determining non-target effects of NIs on soil organisms. Therefore, we investigated commonly used NIs (DMPP, N-Lock and Piadin) in combination with three fertilizers (pig slurry, nitrogen sulphur chemical fertilizer and urea-ammonium nitrate) for their effects on soil fungal and bacterial communities. The effects were tested in two locations with different soil types – sandy clay (Højbakkegaard, KU) and clayey sand (Foulumgaard, AU). Sampling took place in May 2020 four weeks after application of NIs and again in September 2020 after harvest of spring barley. Soil microbial activity was measured by MicroResp, while fungal and bacterial communities were studied by amplicon sequencing of ITS and 16S rRNA regions, respectively.

Preliminary results from the 2020 growing season showed differences in the soil microorganism populations between the two agricultural sites and different fertilizers by all techniques. MicroResp and DNA analysis found no effects of NIs on microbial activity and bacterial diversity, however, several fungal genera were differentially abundant.

In the growing season of 2021, the studies are continued in fields with winter wheat under conventional tillage and no tillage management.

[PA24] FUNGAL-ASSOCIATED MOLECULES ACTIVATE THE NUNF GENE REQUIRED FOR NUNAMYCIN AND NUNAPEPTIN PRODUCTION IN PSEUDOMONAS FLUORESCENS IN5

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The Greenlandic strain *Pseudomonas fluorescens* In5 synthesizes the antifungal cyclic lipopeptides, nunamycin, a nine amino acid peptide that belongs to the syringomycin class, and nunapeptin, which is a 22 amino acid peptide belonging to the tolaasin group. Genes encoding nunamycin and nunapeptin are located on a large genomic island with a genetic organization similar to that of islands encoding similar cyclic lipopeptides *e.g.* syringomycin and syringopeptin. Regulation of syringomycin and syringopeptin has been shown to be dependent on the two component global regulatory system GacS/GacA and specific transcription factors SalA, SyrF, and SyrG. Furthermore, plant signal molecules have been shown to activate synthesis of syringomycin. We have previously shown that a specific transcription factor, NunF, positively regulates the synthesis of nunamycin and nunapeptin in *P. fluorescens* In5 and that the *nunF* gene may be upregulated by fungal associated molecules. Here, we use promotor fusions to show that the specific activator NunF is dependent on the global regulator GacA and in contrast to GacA is regulated by fungal associated molecules and low temperatures, and contrariwise synthesis of GacA but not NunF is stimulated by plant signal molecules. This led us to hypothesize that *P. fluorescens* In5 is a fungal associated rhizobacterium with transcription factor encoding genes that respond to the presence of fungi and oomycetes, and we present a model for how synthesis of nunamycin and nunapeptin is regulated by fungal or oomycete associated molecules.

[PA25] EARTHWORMS INCREASE EXTRACELLULAR ENZYME ACTIVITIES AND SHAPE BACTERIAL COMMUNITIES IN SOIL

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Earthworms are important for functioning of soil ecosystems, and their presence can be used as an indicator of soil health. The feeding and digging activities are known to stimulate soil microbial activity. The aim of our study was to reveal how the presence of earthworms modify the genetic diversity of soil microorganisms. We investigated this by comparing soil without earthworms, soil with geophage *Aporrectodea* spp., feeding on soil, and soil with detritivore *Lumbricus* spp., feeding on detritus. Experimental pots with 500 g of sandy loam soil were sampled after 0, 14 and 28 days. At each sampling time the microbial activity, bacterial abundance and community composition were determined by extracellular enzyme activity assay on seven carbon substrates, quantitative PCR, and metabarcoding of 16S rRNA genes. No significant difference between the earthworms was found. In contrast, presence of earthworms significantly increased the activities of α-glucosidase ($p=0.003$), endo-β-glucanase ($p=0.028$), chitinase ($p=0.035$) and β-xylosidase ($p=0.052$), although only for a limited time. We also found that earthworms decreased alpha diversity ($p=0.03$). Beta diversity showed that addition of earthworms was a significant factor ($p=0.003$) in shaping bacterial communities. Furthermore, earthworms enriched the soils with several bacterial taxa, mainly belonging to *Bacteroidia* and *Gammaproteobacteria* classes, suggesting that the presence of earthworms favors these specific bacteria.

[PA26] UPREGULATION OF PURINE METABOLISM GENES HAS RECIPROCAL EFFECTS ON P. AERUGINOSA FITNESS, VIRULENCE AND ANTIBIOTIC RESISTANCE.

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Pseudomonas aeruginosa is the major pathogen in cystic fibrosis lung. In the current research, we studied the adaptation of *P. aeruginosa* to exponential growth in nutrient rich LB medium and the trade-offs that accompanied the adaptation. Mismatch repair-deficient *P. aeruginosa* cultures were propagated for more than 800 generations and the evolved cultures showed a significant increase in fitness. Genome and transcriptome sequencing of starting and evolved cultures demonstrated that approximately 10% of the evolved clones had a non-synonymous mutation in the gene encoding allantoinase. We showed that this mutation resulted in the accumulation of intracellular allantoin, which led to the upregulation of genes involved in the uptake and metabolism of purines and to increased respiration. Since it was previously described that faster growing bacteria show lower resistance to antibiotics and that higher respiration leads to lower tolerance to antibiotics, we studied the resistance and tolerance of *P. aeruginosa* allantoinase mutant to tobramycin. Killing curves showed that *P. aeruginosa* allantoinase mutant had lower tobramycin resistance but unchanged tolerance. Interestingly, the genes upregulated in *P. aeruginosa* allantoinase mutant were also upregulated under biofilm conditions in highly virulent *P. aeruginosa* clinical isolates. Using mouse acute peritonitis infection model, we revealed that the upregulation of purine metabolism genes in *P. aeruginosa* allantoinase mutant leads to 10-fold increase of bacteria load in blood of the infected mice. Thus, we showed that the upregulation of genes involved in purine uptake and metabolism increases *P. aeruginosa* fitness and virulence and decreases the resistance to antibiotics.

[PA27] CRISPR-CAS SYSTEMS ARE WIDESPREAD ACCESSORY ELEMENTS ACROSS PLASMIDS

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Many prokaryotes encode CRISPR-Cas systems as immune protection against mobile genetic elements (MGEs) yet, intriguingly, a number of MGEs have also recruited CRISPR-Cas components. With a few exceptions, MGE-encoded CRISPR-Cas loci are largely uncharted and a comprehensive analysis of their distribution, prevalence, diversity, and biological function is lacking. Here, we systematically investigated CRISPR-Cas loci across the largest curated collection of wildtype bacterial and archaeal plasmids. We found that CRISPR-Cas systems are widely but heterogeneously distributed across plasmids and, in comparison to host chromosomes, their mean prevalence per Mbp is significantly higher and their distribution is markedly distinct. Furthermore, plasmid-spacer contents exhibit a strong targeting bias towards other plasmids, while the corresponding host chromosome arrays are enriched with virus-targeting spacers. This trend dominates across the diversity of CRISPR-Cas subtypes and host taxa, highlighting the genetic independence of plasmids and suggesting a primary recruitment of CRISPR-Cas for mediating plasmid-plasmid conflicts. Altogether, our results show that CRISPR-Cas loci are frequent accessory components of many plasmids, an overlooked phenomenon that possibly facilitates their dissemination across microbiomes.

[PA28] INVESTIGATING THE ORIGIN OF A PREVIOUSLY UNDESCRIBED DNA-MODIFICATION IN MYOVIRIDAE PHAGES.

Sif Christine Lykke Hougaard[1], Nikoline Olsen[1], Lars H Hansen[1]

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It is known that phages hold the greatest diversity of DNA modifications observed in nature compared to any other organism. Phage DNA modifications include but are not limited to; a variety of methylations, more complex modifications like $preQ_0$ deazaguanine, and sugar-derived modifications like glycosylations and arabinosylations in T-even phages. These modifications regularly act as a counter-resistance towards sequence specific anti-viral defense systems such as the R-M and CRISPR-Cas.

Escherichia phage ukendt is a newly isolated *Myoviridae*-phage which, through nanopore sequencing, was shown to have four modification motifs, one of them (GTAC) cannot be explained by any known methylases in the host or phage genome. A restriction assay using the enzyme rsaI, (GT/AC) which can cut methylated-cytosine and-adenosine residues, could not digest phage ukendt DNA indicating that the phage either possess another type of methylation or a more complex modification. Furthermore, preliminary results indicate a broad resistance towards diverse anti-phage defense systems.

This project aims to investigate the nature of the GTAC modification in phage ukendt and its biological implications by targeted genome editing and classical microbiological assays.

[PA29] WHY DO THEY PLAY TOGETHER? DISCOVERING POSITIVELY INTERACTING PAIRS OF BACILLUS SUBTILIS AND PSEUDOMONAS SOIL ISOLATES.

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In nature, bacterial biofilms are almost exclusively polymicrobial, comprising entire consortia of different species all interacting in complex networks for the benefit of the entire biofilm. In practice, we use this knowledge in our preparations of probiotic supplements, starter cultures, and plant biofertilizers. However, when *Bacillus subtilis* is isolated from the roots of plants it does not readily recolonize the rhizosphere under controlled conditions, a phenotype accompanied by little biofilm formation. Therefore, we have developed a microscopy-based high-content screening method to evaluate the air-liquid biofilm formation of *B. subtilis* when cocultured with soil isolates and have screened a library of 720 *Pseudomonas* soil isolates in two different types of rich medium to identify candidates that enhance *B. subtilis* biofilm. Using an in-house bioimaging pipeline, we determined which *Pseudomonas* isolates engaged in positive interactions with *B. subtilis*. To understand the molecular mechanisms behind the positive interactions, we are currently examining if the candidate biofilm facilitating soil isolates can complement biofilm-deficient mutants of *B. subtilis* and are evaluating the transcriptional landscapes within coculture biofilms. Upregulated genes in either species will be later subjected to knock-out mutations to determine their relevance in a coculture biofilm setting.

Our study will potentially reveal generalities in cross-species interactions and may aid the development of microbial consortia for plant biocontrol.

[PA30] SYNTHETIC COMMUNITY INVASION DEPENDS ON SECONDARY METABOLITE PRODUCTION IN BACILLUS SUBTILIS

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Isolates of the *B. subtilis* species complex produce a plethora of biologically active molecules. Among those, lipopeptides (LPs) have been extensively studied under *in vitro* conditions, revealing inhibition of various other microbes, impact on motility and colonization, signaling and cellular differentiation. Despite the vast knowledge about *B. subtilis* secondary metabolism, there is still a gap in understanding the role of those compounds on the ecology of the producers and the resident communities *in situ*. To address these questions, *B. subtilis* wild type (WT) and mutant derivatives impaired in LPs production were introduced into a synthetic bacterial community using a soil-mimicking matrix. Population dynamics and community metabolomes were assessed over two weeks. Interestingly, neither the WT nor the mutants had major impact on the synthetic community assembly. However, assessment of *B. subtilis* growth dynamic revealed that the *sfp* (lacking all LPs) and *srfAC* mutants declined drastically compared with the WT strain. Interestingly, inoculation of *B. subtilis* strains capable of surfactin production as well as purified surfactin alters the chemo diversity of the community. Synthetic bacterial community gene expression upon *B. subtilis* (WT or *sfp*) invasion was assessed using RNAseq to gain insight into the role of LPs as gene modulators at the community level. Overall, our results highlight that LPs, and more specifically surfactin, determine the invasion success of *B. subtilis* in a simplified bacterial community, suggesting a broad spectrum of action for this natural product.

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

[PA31] INVESTIGATING THE PIPERACILLIN/TAZOBACTAM (TZP) RESISTANCE MECHANISMS IN 28 CLINICAL UROPATHOGENIC ESCHERICHIA COLI RESISTANT TO TZP BUT SUSCEPTIBLE TO CEPHALOSPORINS.

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Background
bla$_{TEM-1}$ encodes a β-lactamase that cleaves β-lactam antibiotics rendering it useless. Penicillin/β-lactamase inhibitor (P/BLI), such as piperacillin/tazobactam (TZP), normally clears an infection caused by uropathogenic *Escherichia coli* (UPEC) since tazobactam can inhibit the TEM-1 β-lactamase. However, hyperproduction of TEM-1 β-lactamase can cause resistance to TZP since the inhibitor tazobactam cannot inhibit all the produced β-lactamase. This leads to excess β-lactamase free to inactivate piperacillin. The aim of this study was to investigate the prevalence of TEM-1 β-lactamase hyperproduction in clinical UPEC isolates and to uncover the TZP resistance mechanism of each isolate.

Methods
28 isolates resistant to TZP but susceptible to cephalosporins, were subjected to whole genome sequencing and coverage of β-lactamase genes was estimated relative to coverage of the seven *E. coli* MLST genes. Minimal inhibitory concentration of TZP was determined by broth dilution method.

Results
Sequence analysis identified 15 out of 28 isolates having TEM-1 hyperproduction as the cause of TZP resistance. Eight out of these 15 isolates had promoter variations leading to stronger *bla*$_{TEM-1}$ promoters. The seven other isolates had a *bla*$_{TEM-1}$ copy number >12. The remaining isolates carried: *bla*$_{OXA}$ gene (six isolates), inhibitor resistant β-lactamases (two isolates), hyperproduction of *bla*$_{SHV}$ (two isolates), and a mutated *bla*$_{CTX-M-27}$ (one isolate).

Conclusions
This highly adaptive resistance mechanism, hyperproduction of TEM-1 β-lactamase, can confer resistant to TZP. Therefore, it is important to monitor the prevalence and this mechanism in order to retain the successful use of TZP to treat severe UPEC infections.

[PA32] ELICITATION OF BACILLUS SUBTILIS SECONDARY METABOLITES THROUGH BIOTIC AND ABIOTIC FACTORS.

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The soil bacterium *Bacillus subtilis* produces a variety of secondary metabolites (SMs), which play a key role in its interactions with the surrounding environment. Among these SMs are the two antimicrobial lipopeptides surfactin and plipastatin that are important for *B. subtilis* to colonize and protect plants. Here, we aimed to identify how the production of surfactin by *B. subtilis* is influenced by biotic and abiotic factors. Using biosynthetic gene cluster (BGC) promoter coupled reporter strains, we followed gene expression in a plate reader assay and developed a screen to reveal how biotic and abiotic factors influence surfactin- and plipastatin related BGCs. Our screen determined that the addition of sucrose, a component of the root exudate, to LB medium significantly induced the expression of surfactin BGC, while supplementation of xylan decreased the expression of BGC for surfactin. Furthermore, biotic factors also influenced production of surfactin in *B. subtilis*, both expression of surfactin BGC as well as the level of surfactin was found to be increased upon growing in the supernatant of certain soil isolates. Next, our experiments are focused on the interaction betweein *B. subtilis* and the wheat microbiome, and the influence of wheat exudates on the production of antimicrobial lipopeptides that are required for plant protection by *Bacilli*.

This project is part of INTERACT within the Collaborative Crop Resiliency Program (NNF19SA0059360) funded by the Novo Nordisk Foundation.

[PA33] POTENTIAL GENES INVOLVED IN BACTERIAL PERSISTENCE

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Bacterial persistence is a sub-population phenomenon, where only a fraction of a population exhibit high tolerance against different stress factors. This sub-population of persister cells are genetically identical to the rest of the population, hence, their survival is not caused by any resistance genes or other kind of mutations, but rather by an activation of a preexisting genetic repertoire. Although several genes have been linked to persistence, the first step in the persister switch remains elusive. The aim of this project is to identify genes that are involved in the persister phenotype, as these might represent potential targets for new antimicrobials. By making a transposon-mutant library, followed by screening for cells exhibiting a high persister level, eight transposon-mutants were found to have a significant higher persister fraction compared to the wild type. In total seven genes were identified to be affected in the transposon-mutants isolates, and surprisingly a variation in the relA gene were identified in seven out of the eight isolates. The relA gene codes for the bifunctional RelA protein that regulates the cellular level of the global transcription-regulator (p)ppGpp. A SNP was found to impact the hydrolysis domain of the RelA protein, having an impact of the hydrolytic binding pocket of (p)ppGpp, which might diminish the hydrolysis ability of the enzyme. This might lead to an elevated level of (p)ppGpp in the cells, that might be the first signal to initiate the persister switch. Hence, the control of cellular (p)ppGpp production might be a potential target for developing new antimicrobials.

[PA34] HYDROGEN CYANIDE PRODUCTION BY PSEUDOMONAS FLUORESCENS IN5: A DEFENSE MECHANISM OR A LATE-NIGHT SNACK?

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Hydrogen cyanide (HCN) is a toxic molecule produced by many pseudomonad species. Due to its broad-spectrum antimicrobial activity, HCN has been associated with the suppression of several plant pathogens and is therefore considered as a key biocontrol trait. To date, the Greenlandic bacterium *Pseudomonas fluorescens* In5 has been mostly studied for its potential as a biocontrol agent owing to its antifungal properties. Although *P. fluorescens* In5 is able to produce HCN, its antifungal activity has been attributed to the production of cyclic lipopeptides rather than HCN. In addition, HCN biosynthesis in *P. fluorescens* In5 is highly depended on nutrient availability, such as nitrogen (N) and iron (Fe), and not the presence of fungi or fungal-associated compounds. Moreover, several studies have shown that numerous *Pseudomonas* species are able to degrade and use cyanide as nitrogen source. Based on these findings, we hypothesized that the HCN produced by *P. fluorescens* In5 does not act as a defense compound but rather as a storage of nutrients, a “late-night snack” available to the bacterium when nutrient levels are low. To test this, *P. fluorescens* In5 knockout mutants of key genes involved in HCN synthesis and metabolism will be constructed. The mutants will then be tested for HCN production in rich and minimal media, and their ability to degrade and utilize cyanide (free form or Fe-complexes) as the only nitrogen source will be examined. Finally, the *P. fluorescens* In5 wild type and mutants will be co-cultured with other rhizosphere bacteria in soil microcosms to verify that only strains capable of producing and assimilating cyanide complexes are able to grow and survive under low N and Fe conditions.

[PA35] COOPERATIVE ANTIBIOTIC RESISTANCE FACILITATES HORIZONTAL GENE TRANSFER

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The rise of β-lactam resistance among pathogenic bacteria, due to horizontal transfer of plasmid encoded β-lactamases, is a current global health crisis. Markedly, β-lactam hydrolyzation by β-lactamases, not only protects the producing cells but also sensitive neighboring cells cooperatively. How such cooperative traits effect plasmid transmission and maintenance is currently poorly understood. Here we experimentally show that β-lactamase expression and extracellular activity was higher when encoded on plasmids compared with chromosomes, resulting in elevated rescue of sensitive non-producers. This facilitated efficient plasmid transfer to the rescued non-producers and expanded the potential plasmid recipient pool and the probability of plasmid transfer to new genotypes. Social conversion of non-producers by conjugation was efficient yet not absolute. Our results suggest that cooperative antibiotic resistance especially promotes the fitness of replicons that transfer horizontally such as conjugative plasmids.

[PA36] COMPARATIVE GENOMICS REVEALS PROPHYLACTIC AND CATABOLIC CAPABILITIES OF ACTINOBACTERIA WITHIN THE FUNGUS-FARMING TERMITE SYMBIOSIS

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Actinobacteria, one of the largest bacterial phyla, are ubiquitous in many of Earth's ecosystems and often act as defensive symbionts with animal hosts. Members of the phylum have repeatedly been isolated from basidiomycete-cultivating fungus-farming termites that maintain a monoculture fungus crop on macerated dead plant substrate. The proclivity for antimicrobial and enzyme production of *Actinobacteria* make them likely contributors to plant decomposition and defence in the symbiosis. To test this, we analysed the prophylactic (biosynthetic gene cluster [BGC]) and metabolic (carbohydrate-active enzyme [CAZy]) potential in 16 termite-associated *Actinobacteria* and compared these to soil-dwelling close relatives. Using antiSMASH, we identified 435 BGCs, of which 329 (65 unique) were similar to known compound gene clusters, while 106 were putatively novel, suggesting ample prospects for novel compound discovery. BGCs were identified among all major compound categories, including 26 encoding the production of known antimicrobial compounds, which ranged in activity and modes of action that might suggest broad defensive potential. PPR analysis revealed 823 (43 unique) CAZymes coding for enzymes that target key plant and fungal cell wall components (predominantly chitin, cellulose, and hemicellulose), confirming a substantial degradative potential of these bacteria. Comparison of termite-associated and soil-dwelling bacteria indicated no significant difference in either BGC or CAZy potential, suggesting that the farming termite hosts may have co-opted these soil-dwelling bacteria due to their metabolic potential but that they have not been subject to genome change associated with symbiosis.

[PA37] INVESTIGATION OF A 3'UTR REGULATORY ELEMENT FROM THE MRNA ENCODING THE MAJOR PNEUMOCOCCAL VIRULENCE FACTOR PSPA

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Streptococcus pneumoniae is an opportunistic pathogen that frequently resides in the nasopharynx of healthy individuals asymptomatically, but under certain circumstances becomes infectious and causes pneumonia and severe invasive pneumococcal diseases (IPD), such as meningitis and bacteremia. Pneumococcus has several factors that are critical for its virulence, including the polysaccharide capsule, and the Pneumococcal surface protein A (PspA). Several studies have also evidenced small non-coding RNAs (sRNAs) as critical regulatory elements of bacterial pathogenesis. Many putative sRNAs have been identified in pneumococcus, however, knowledge of their biological function and their role in pathogenesis is very limited. The aim of this project was to uncover sRNA-mediated control of virulence gene expression in pneumococcus.
In this project we have investigated the regulatory RNA F5, which was first identified in an RNA-sequencing study by Mann et al., and shown to be implicated in virulence due to its importance for bloodstream survival. We have uncovered that F5 is a 3'UTR-derived regulatory RNA transcribed with the gene encoding the major virulence factor PspA, and that it negatively regulates the expression of the protein chaperone ClpL, which is also known to modulate virulence. Furthermore, we have uncovered that F5 is processed from the *pspA-F5* transcript during heat stress, but that it is more efficient at regulating ClpL when part of the full transcript. Further understanding the molecular mechanisms behind this *pspA*-3'UTR-derived regulatory RNA, and other pneumococcal regulatory RNAs, is very important to fully understand pneumococcal pathogenesis, and is therefore very valuable to future drug development.

[PA38] QUORUM SENSING AUTOINDUCER-3 IN SALMONELLA TYPHIMURIUM : FROM ITS BIOSYNTHESIS TO ITS IMPACT ON CELL PHYSIOLOGY

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Quorum sensing (QS) is a communication system between bacterial cells using small, secreted molecules (auto-inducers, AIs) to coordinate population wide-behaviors in a cell-density dependent manner. In *Escherichia coli* and *Vibrio cholerae*, AI-3 has been reported to play roles in virulence and biofilm formation, and recently, the structure of AI-3 was proposed to be either 3,5- or 3,6-dimethylpyrazin-2-ol (DPO) (Figure 1). However, its role in the major human pathogen *Salmonella* Typhimurium (STM) remain largely unknown. In this study, we first developed a method for measuring the presence of DPO in STM supernatant by UHPLC-MS/MS and determined that both L-threonine and L-threonine-3-dehydrogenase enzyme (Tdh) were essential for DPO production in STM. This STM production of active DPO was verified using a model containing the known receptor –VqmA- and gene activated –*vqmR*- of DPO in *V. cholera*. With that knowledge, we showed STM biofilm production was repressed in the presence of DPO (at 100 µM), whereas virulence behavior in a mice infection model was promoted by biosynthesis of this proposed AI-3. Through future experiments, including an silico study and screening experiments (transcriptomic, isothermal titration calorimetry), we hope to reveal the potential receptor for DPO in STM as well as pathways impacted by this newly described AI-3.

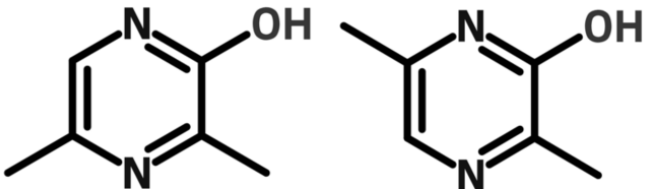

Figure 1 : DPO molecule. On the left, 3,5-dimethylpirazin-2-ol and on the right, 3,6-dimethylpyrazin-2-ol

Keywords : *Salmonella* Typhimurium, Quorum sensing, autoinducer, biofilm, virulence

[PB01] DISCOVERY OF MULTIPLE ANTI-CRISPRS HIGHLIGHTS ANTI-DEFENSE GENE CLUSTERING IN MOBILE GENETIC ELEMENTS

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Many prokaryotes employ CRISPR–Cas systems to combat invading mobile genetic elements (MGEs). In response, some MGEs have developed strategies to bypass immunity, including anti-CRISPR (Acr) proteins; yet the diversity, distribution and spectrum of activity of this immune evasion strategy remain largely unknown. Here, we report the discovery of new Acrs by assaying candidate genes adjacent to a conserved Acr-associated (Aca) gene, *aca5*, against a panel of six type I CRISPR-Cas systems. We uncover 11 type I–F and/or I–E *acr* genes encoded on chromosomal and extrachromosomal MGEs within *Enterobacteriaceae* and *Pseudomonas*, and an additional Aca (*aca9*). The *acr* genes not only associate with other *acr* genes, but also with genes encoding inhibitors of distinct bacterial defense systems. Thus, our findings highlight the potential exploitation of *acr* loci neighborhoods for the identification of previously undescribed anti-defense systems.

[PB02] HOW CIPROFLOXACIN INDUCE CONJUGATION IN ESCHERICHIA COLI

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Previous studies have shown that sub-inhibitory concentrations of ciprofloxacin (CIP) increase plasmid conjugation frequency. While the mechanisms of plasmid transfer via type 4 secretion systems (T4SS) are well understood, it is unknown which regulatory networks that leads to antibiotic CIP induced increased conjugation. In this study, we used a transposon library to identify genes involved in this response. In addition, different mutants in SOS response were constructed to investigate if the SOS response was involved.

Keywords: Antibiotic induced conjugation, Ciprofloxacin, SOS response, $bla_{ctx\text{-}M\text{-}1}$, resistance plasmid, *E.coli*

[PB03] PHAGE-INDUCIBLE CHROMOSOMAL ISLANDS PROMOTE GENETIC VARIABILITY BY BLOCKING PHAGE REPRODUCTION

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Phage-inducible chromosomal islands (PICIs) are a widespread family of highly mobile genetic elements that disseminate virulence and toxin genes among bacterial populations. Since their life cycle involves induction by helper phages, they are important players in phage evolution and ecology. Here we report an unexpected role for PICIs in bacterial virulence and antimicrobial resistance by promoting the phage- mediated transduction of chromosomal or plasmid DNA. PICIs can interfere with the lifecycle of their helper phages at different stages resulting frequently in reduced phage production after infection of a PICI-containing strain. Since phage defense systems have been recently shown to be beneficial for the acquisition of exogenous DNA via horizontal gene transfer, we hypothesized that PICIs could provide a similar benefit to their hosts and tested the impact of PICIs in recipient strains on host cell viability, phage propagation and transfer of genetic material. Presence of PICIs generates favorable conditions for population diversification and the inheritance of genetic material being transferred, such as antibiotic resistance and virulence genes. Our results show that by blocking phage reproduction, PICIs protect the bacterial population from phage attack, increasing the survival of the general bacterial population as well as the transductant cells. Moreover, our results also demonstrate that PICIs reduce the frequency of lysogeny lysogenization by temperate phage infection, creating a more genetically diverse bacterial population with increased bet-hedging opportunities to adapt to new niches. In summary, our results identify a new role for the PICIs and highlight them as important drivers of bacterial evolution.

[PB04] DETERMINANTS OF PHAGE HOST RANGE IN PORCINE ENTEROTOXIGENIC ESCHERICHIA COLI.

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Enterotoxigenic *Escherichia coli* (ETEC) are one the main cause of porcine post-weaning diarrhoea. They are characterized by fimbriae (F4, F18, F5, F6, and F41) and enterotoxins (ST1, ST2, and LT), mainly carried by plasmids. Phages have been proposed as a strategy to control ETEC, but efficient and long-lasting solutions require a thorough understanding of phages ecology and bacterial diversity. Surprisingly, only few phages targeting ETEC have been reported and their host range determinants (HRD) is still unclear.
Here, we set a collection of 84 ETEC strains, recently retrieved from European pigs with post-weaning diarrhoea, to study HRD of newly isolated phages from wastewater, pig manure, gut and fecal samples. Next-generation sequencing was used to sequence the phage-host pairs. Fimbriae F4 (46/84 strains) and F18 (34/84 strains), and two or more enterotoxins were identified in the majority of the ETEC strains, with the ST2, LT combination being the most frequent (28/84 strains). By intraspecies comparative genomes analysis, we found that the most common virotype (F4, ST2, LT) was associated with the O149 serotype. Genome-wide association studies of the newly isolated phages ET_P21B (infecting 31/84 strains), ET_P22 (infecting 16/84 strains) and ET_P102 (infecting 12/84 strains) and of T-odd coliphages helped identifying the HRD. For example, phage ET_P21B infected 31/84 ETEC strains by recognizing the O-antigen of O149 serotype, with few exceptions that might be linked to internal resistance mechanisms. Despite the importance of receptor recognition, it is only with a functional enrichment analysis that we can now propose candidate genes for HRD. This collection will help researchers studying phage fitness and ETEC diversity.

[PB05] AMINOGLYCOSIDE RESISTANCE IN E. COLI WITH ALTERED MEMBRANE LIPID COMPOSITION

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Aminoglycosides are a potent broad-spectrum activity class of antibiotics that act primarily by disrupting bacterial protein synthesis through binding to the ribosome leading to protein inhibition or release of aberrant proteins. In order to reach the target, the drugs must reach the cytoplasm. In gram-negative bacteria, the cell envelope consists of an outer and inner membrane, separated by the periplasmic space, forming a formidable permeability barrier for both hydrophilic and hydrophobic compounds. Membrane lipids form the bulk of the phospholipid bilayer that serves as a matrix and support for a vast array of proteins. The ability to alter membrane lipid composition of the cellular envelope is crucial for bacterial survival and adaptation in response to environmental stress. Aminoglycoside entry in gram-negative bacteria occurs directly across the membrane and is comprised of 3 distinct stages. In order to further our understanding of how aminoglycosides cross the bacterial membrane and the importance of the membrane phospholipid composition, the sensitivity of *E. coli* lipid mutants to the aminoglycoside gentamicin is being investigated. These mutants have an altered membrane lipid composition caused by mutations in key lipid metabolism enzymes. Gentamicin resistant strains of the lipid mutants were generated in an adaptive laboratory experiment (ALE), in which bacteria are grown in an antibiotic gradient for 20 days. Whole genome sequencing is being performed on the gentamicin resistant lipid mutants in order to determine what genes are involved in the resistance mechanisms that occurred and gain insight into how bacteria with an atypical membrane adapt to aminoglycoside challenge.

[PB06] DISTRIBUTION AND ABUNDANCE OF PLASMID-SPECIFIC BACTERIOPHAGES IN WASTEWATER SYSTEMS

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Plasmid-specific phages are viruses that specifically infect bacteria carrying conjugal plasmids, using the conjugal pilus as receptor. As pili differ across plasmid classes, the infection range of plasmid-specific phages has specificity. The source, fate, and abundance of these phages in the environment are largely uncharacterized and there is a lack of experimental platforms to study them.

We aimed to (i) provide an experimental platform to quantify the abundance of plasmid-specific phages for several conjugal plasmid classes and (ii) describe the distribution of such phages in wastewater systems and (iii) identify what controls their distribution.

We introduced four model plasmids, belonging to different plasmid incompatibility groups (IncP1, IncN, IncH, IncF), into an avirulent *Salmonella enterica*, which displays a low background abundance of somatic phages in wastewater. These strains were used in double layer agar assays with water samples from contrasting sources.

Depending on the location and plasmid class, phage abundance ranged from below detection limit to about $5x10^3$ pfu/mL. Hospital wastewater contained significantly more IncP1-, but less IncF-, specific phages than domestic wastewaters, potentially reflecting differences in plasmid loads. The comparison between influent and effluent of wastewater treatment plants revealed a reduction in phage density by 1.5 log, but no significant removal from primary clarification.

These data help understand phage-plasmid interactions, which is relevant as plasmids can accelerate the dissemination of antimicrobial resistance (AMR), and may suggest novel ways to control AMR.

[PB07] EXPRESSION OF THE CYCLIC LIPOPEPTIDE VISCOSIN IN PSEUDOMONAS FLUORESCENS SBW25 IS MODULATED BY MICROBIAL INTERACTIONS

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Numerous secondary metabolites are reported to play a key role in microbial interactions. Viscosin is a secondary metabolite produced by the soil bacterium *Pseudomonas fluorescens* SBW25 with biosurfactant and antimicrobial properties. However, the role of viscosin in mediating microbial interactions and how microbial interactions impact viscosin production is unknown. In this study, we present the first report on bacteria that induce changes in viscosin production and growth of SBW25 by a high-throughput microtiter assay based on a dual-fluorescence bioreporter system to continuously monitor *viscA* gene expression using mCherry and cell density levels using GFP in *P. fluorescens* SBW25. Microbial co-cultures of *P. fluorescens* SBW25 with 88 rhizobacteria isolated from wheat were established. It was observed, that the strains more phylogenetically related to SBW25 reduce both growth and *viscA* expression. In order to determine whether the expression of *viscA* could be further enhanced by bacterial excreted signaling molecules, supernatants were collected from nine phylogenetically diverse strains. Assays demonstrated that *Ensifer adhaerens* A8 improves both the relative expression of *viscA* and the growth of SBW25. However, the highest *viscA* relative expression level was obtained during an interaction between *E. adhaerens* A8 and SBW25. Furthermore, we show that regulation of viscosin does not appear to have a positive feedback loop on the relative expression of *viscA* when added to *E. adhaerens* A8 pure culture. Based on these results, we conclude that *viscA* expression is induced by unknown molecules produced by SBW25 that stimulate the production of *viscA* enhancing molecules in *E. adhaerens* A8.

[PB08] VIABILITY STUDY OF ICE NUCLEATING ACTIVE BACTERIA IN FREEZING CLOUD DROPLETS

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Bacteria such as *Pseudomonas syringae* belong to the most active bacterial ice-nucleating particles. Several studies report a high ice nucleation efficiency of these bacteria with freezing temperatures as high as -2°C. Although the freezing temperatures are well studied, little is known about the viability of *P. syringae* cells after freezing under atmospheric conditions.

In this study, we investigate the survival of the ice nucleating active bacteria *P. syringae* in freezing droplets. To simulated atmospheric conditions, bacteria-containing water droplets are levitated in an electrodynamic balance and cooled down to temperatures of -25°C. After freezing, the droplets are extracted from the electrodynamic balance and the bacterial viability is determined by colony counting. To understand the effect of the ice nucleating proteins on the viability, bacteria with a high number and a low number of proteins are studied separately.

We find that the survival rate of ice nucleating active bacteria depends on the growth rate of ice in supercooled water droplets. Bacteria with a higher number of INA proteins trigger the freezing at higher temperatures, which leads to a slower growth rate of the ice crystals. This results in a higher survival chance of the bacteria, while freezing at low temperatures reduces the survival probability dramatically.

In further studies, we determine the atmospheric conditions where cells express and synthesize INA proteins while airborne by linking the gene of a green fluorescent protein (GFP) to the INA gene promoter prior to the aerosolization.

[PB09] SUBTYPES OF TAIL SPIKE PROTEINS PREDICTS THE HOST RANGE OF ACKERMANNVIRIDAE PHAGES

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The *Ackermannviridae* family comprises phages encoding up to four tail spike proteins (TSPs) that each recognizes a specific receptor of their bacterial hosts. Here, we performed a comprehensive in-silico analysis to determine the diversity of the TSPs in 99 *Ackermannviridae* phages. Based on sequence similarity, we assigned all TSPs as TSP1, TSP2, TSP3, or TSP4 and then further classified into subtypes. We observed that each TSP subtype were specifically associated with the genera (*Kuttervirus*, *Agtrevirus*, *Limestonevirus,* and *Taipeivirus*) of the *Ackermannviridae* family. Further analysis showed that the N-terminal XD1 and XD2 domains in TSP2 and TSP4 were preserved in all TSP2 and TSP4 subtypes. In contrast, the C-terminal receptor binding modules were only conserved within TSP subtypes, except for some *Kuttervirus* TSP1s and TSP3s that were similar to specific TSP4s. A conserved sequence motif present in TSP1, TSP3, and TSP4 of *Kuttervirus* phages may allow for recombination between the receptor binding modules. By experimentally determine the host recognition of three of the four TSPs expressed by kuttervirus S117, we could show that TSP1 and TSP2 bind to O:21 and O:157 O-antigens, respectively, whereas TSP3 found in 51 other *Kuttervirus* phages was shown to bind O:4 and O:9 O-antigens. Finally, we used previous data to predict the receptor for TSPs of the same subtypes, thus allowing us to predict the host range of numerous uncharacterized *Ackermannviridae* phages. Overall, our study demonstrates that comprehensive *in silico* and host range analysis of TSPs can predict host recognition of *Ackermannviridae* phages.

[PB10] SENSOR-BASED MONITORING OF MYCOTHIOL REDOX POTENTIAL AND DNA DAMAGE RESPONSE IN CORYNEBACTERIUM GLUTAMICUM UPON EXPOSURE TO OXIDATIVE STRESS

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Excessive amounts of reactive oxygen species (ROS) can cause irreversible damages to essential cellular components such as DNA. Genetically encoded biosensors targeting oxidative stress and DNA stress have emerged to a powerful analytical tool to assess physiological states in a non-invasive manner. However, to the best of our knowledge, a combinatorial biosensor set-up targeting both types of stress in one cell has not been realized so far.
In this study, we aimed to combine the redox biosensor protein Mrx1-roGFP2 with a transcriptional biosensor for DNA damage based on the P_{recA} promoter fused to a reporter gene (*e2_crimson*) in *Corynebacterium glutamicum*. This combination was selected based on *in vitro* experiments, which revealed that E2_Crimson is stable in an oxidized environment and its presence does not disturb the functionality of the Mrx1-roGFP2 sensor protein. Based on this, the redox biosensor strains *C. glutamicum* WT_Mrx1-roGFP2 and the mycothiol-deficient mutant strain *C. glutamicum* Δ*mshC*_Mrx1-roGFP2 were equipped with the DNA stress reporter plasmid pJC1_P_{recA}- *e2_crimson*. Exposure of the double-sensor equipped *C. glutamicum* WT strain to hypochlorite resulted in an oxidative shift of the MSH redox potential, accompanied by an induction of the DNA stress response. In absence of mycothiol, the major non-enzymatic antioxidant utilized by *C. glutamicum* to counteract ROS formation, the induction of the DNA stress response was more pronounced. Besides the importance of the antioxidant mycothiol for the protection of DNA, our results reveal the compatibility of Mrx1-roGFP2 with E2_Crimson as second a fluorescent reporter in *C. glutamicum* for different combinatorial biosensor set-ups in the future.

[PB11] PHAGE BINDING AND INFECTION OF E.COLI

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Bacteriophages used for therapy are often isolated directly from the environment and selected based on their ability to kill the bacterial strain causing the infection. Yet, a more efficient approach may be to genetically modify phages to specifically target the pathogen. But to customize phages, knowledge of phage binding specificity and infection efficiency as well as the molecular process of phage binding is essential. Therefore, a phage collection isolated from the environment using the *E. coli* reference strain collection (ECOR) as isolation host, was characterized. Host ranges were determined on the ECOR collection and found to be diverse. In accordance, diverse receptors were identified using a receptor mutant collection, containing knockout mutations in common phage receptor genes. For future work, we will have special emphasis on the *Tevenvirinae* phages, a group of *E. coli* phages, containing the receptor binding protein gp38 on the tip of their long tail fibers. Gp38, has a modular structure containing conserved glycin rich motives and hypervariable loops, which can be mutated easily and are binding to bacterial receptor outer loops. An analysis of gp38 in our phage collection will be performed and phage binding efficiencies will be quantified using GFP fused to gp38. Further, bacterial factors leading to resistance and sensitivity to phages will be investigated by comparative genomics of the ECOR collection.

[PB12] SCREENING FOR HIGHLY TRANSDUCED GENES IN STAPHYLOCOCCUS AUREUS REVEALS BOTH LATERAL AND SPECIALIZED TRANSDUCTION

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Bacteriophage-mediated transduction of bacterial DNA is a major route of horizontal gene transfer in the human pathogen, *Staphylococcus aureus*. Transduction involves packaging of bacterial DNA by viruses and enables transmission of virulence and resistance genes between cells. To learn more about transduction in *S. aureus*, we searched a transposon mutant library for genes and mutations that enhanced transfer mediated by the temperate phage, ϕ11. Using a novel screening strategy, we performed multiple rounds of transduction of transposon mutant pools selecting for an antibiotic resistance marker within the transposon element. When determining the locations of transferred mutations, we found that, within each pool of 96 mutants the screen had selected for just 1 or 2 transposon mutant(s). Subsequent analysis showed that the position of the transposon, rather than inactivation of bacterial genes, was responsible for the phenotype. Interestingly, from multiple rounds we identified a pattern of transduction that encompassed mobile genetic elements, as well as chromosomal regions both upstream and downstream of the phage integration site. The latter was confirmed by DNA sequencing of purified phage lysates. Importantly, transduction frequencies were lower for phage lysates obtained by phage infection rather than induction. Our results confirm previous reports of lateral transduction of bacterial DNA downstream of the integrated phage, but also indicate a novel form of specialized transduction of DNA upstream of the phage. These findings illustrate the complexity of transduction processes and increase our understanding of the mechanisms by which phages transfer bacterial DNA.

[PB13] EXPANDING SYNTHETIC BIOLOGY TOOLBOX FOR CORYNEBACTERIUM GLUTAMICUM

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Synthetic biology is an emerging research field that aims to design and build new functional biological parts and tools which significantly contribute to the advance of cell factory engineering. Several synthetic logic gates and toggle switches have been developed for model organisms such as *Escherichia coli*. However, for *Corynebacterium glutamicum,* a industrially important workhorse, which is also a promising platform strain for biomanufacturing, such tools are scarely reported. To expand the use of genetic switches in *C. glutamicum*, we firstly characterized five TetR family repressors (BetI, HlyIIR, PhlF, SrpR, TetR) and their corresponding synthetic promoters, and then investigated the respond function between the promoters and repressors. Afterwards, we designed and built an inverted-control of gene expression system. As a proof of concept, we showed that the reporter GFP was suppressed with the addition of the inducer IPTG, on the contrary, GFP was expressed when the inducer was absent. This inverted switch represents a new manner to regulate gene expression in *C. glutamicum*.

[PB14] COMMON, UNIQUE AND NOVEL BIOSYNTHETIC GENE CLUSTERS IN THE STAPHYLOCOCCUS GENUS

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Introduction

Members of the *Staphylococcus* genus are common colonizers of the human microbiota. Some staphylococcal species are almost ubiquitously found throughout the body while others have adapted to a specific niche. Staphylococci and other bacteria produce a wealth of secondary metabolites that are used for both communication and competition. Bacteriocins are peptide-based molecules with antimicrobial activity that are employed by bacteria to compete for nutrients and survival. The genes that code for bacteriocins and other secondary metabolites are located on operons in the chromosome or on plasmids and are referred to as biosynthetic gene cluster (BGCs).

Methods

Analysis of the entire reservoir of BGCs encoded by the staphylococcal pangenome facilitate a better understanding of staphylococcal interactions and the biosynthetic potential. Comparison of the staphylococcal BGC reservoir with previously characterized antimicrobial compounds produced by staphylococci can facilitate discovery of novel antimicrobials and assist in dereplication.

Results

We have conducted a pangenome analysis of all available staphylococcal genomes using antiSMASH and analysed the residing BGCs.
By screening a selection of commensal bacteria for antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA), we have found bacterial isolates with prospective and uncharacterized BGCs coding for putative bacteriocins that are currently under investigation for their antimicrobial potential as therapeutics.

[PB15] ANTIBIOTIC RESISTANCE AND INSERTION SEQUENCES IN ENVIRONMENTAL KLEBSIELLA PNEUMONIAE KP3B

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Here, we report a new strain of *Klebsiella pneumonia* KP3b collected from plastic litter in Zanzibar, Tanzania. We have whole genome sequenced the isolate using Nanopore long-reads technology in combination with Illumina short-read sequencing. Assembly resulted in two circular contigs: one chromosome (5,331,576 bp) and one plasmid (230,544 bp). Both were annotated with PGAP. By Resfinder analysis, it was found that the isolate displays 17 resistance genes towards fosfomycin, β-lactams, aminoglycosides, fluoroquinolones, trimethoprim, tetracyclin and including 2 copies of the ESBL gene $bla_{\text{CTX-M-15}}$ in association with the antibiotic resistance-associated insertion sequence (IS) IS*Ecp1*. Surprisingly, this module $bla_{\text{CTX-M-15}}$-IS*Ecp1* was found both on the chromosome and on the plasmid. This is, to our knowledge, the first report of an MDR isolate with two copies of a CTX-M-gene located on different genomic molecules. Multiple IS (n=18) from different families were found and many are known to be strongly linked to antibiotic resistance (IS*26*, IS*5075*, IS*Ecp1*). Two potential new IS candidates have been found to and will be uploaded to IS database, ISFinder.

Keywords : antibiotic resistance, insertion sequence, *Klebsiella pneumoniae*

[PA01] CRISP – PHAGE BIOCONTROL IN PLANTA OF PATHOGENIC PECTOBACTERIACEA

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Pathogenic bacteria from the family *Pectobacteriaceae* can cause disease in several plants that are important for global agriculture. In potato plants, they cause blackleg and soft rot symptoms that leads to a great annual loss in the potato harvest. This is not only an economic loss, that can be devastating especially in third world countries, but also a waste of resources. An effective treatment against soft rot *Pectobacteriaceae* would decrease food waste and unnecessary CO_2 emission.
Many treatment mechanisms have been employed to control soft rot *Pectobacteriaceae*, however current control methods carry a negative environmental impact, and worse still are largely ineffective
In this project, we aim to develop phages as a green tech alternative to current control methods and elaborate the use of phages as biocontrol *in planta.* We will use Danish isolates of soft rot-causing bacteria and phages isolated from relevant environmental samples to investigate implementation of phage-control *in planta.* Futhermore, we will test the efficiency of the phages as biological control agents in plants, soil and under storage and shelf conditions. We aim to describe a method that will apply phage-biocontrol to efficiently decrease disease symptoms, and thereby, loss of potatoes.
Promising results with bacteriophages as control agents has already been shown but we hope to elaborate the method and achieve even greater results.

[PA02] CRISP – CIRCUMVENTING RECURRENT INCIDENCES OF SOFT-ROT PECTOBACTERIACEAE

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Soft rot *Pectobacteriaceae* is a family of rod shaped gram-negative bacteria belonging to the order *Enterobacterales* that are able to infect a wide range of crops worldwide. The most damaging species within *Pectobacteriaceae* are members of the genera *Dickeya* and *Pectobacterium*. In potatoes soft rot *Pectobacteriaceae* are the causative agent of soft rot in tubers and black leg in plants. Treatment mechanisms are limited and primarily based on good agricultural practice. Bacteriophages (phages) have shown to be effective as biological controls agents against soft rot in potatoes, in previously studies. To elaborate the method of application together with improving the success rate, more research is needed. This project aim to find and isolate bacteriophages against soft rot *Pectobacteriaceae* for disease management in potatoes. By isolating bacterial pathogens of soft rot *Pectobacteriaceae* using diseased potatoes from all over Denmark, we will get an estimation of the prevalent soft rot *Pectobacteriaceae*, both within tubers and fields. By using these isolates together with classical phage isolation methods, selective phage isolation methods, stability assays and genomic interpretation, we aim to create a cocktail of effective phages relevant against soft rot *Pectobacteriaceae* in Danish agriculture of potatoes. To investigate the success of application we will carry out several *in planta* experiments using potato plants and tubers in field and storage conditions.

[PA03] ADAPTATION OF BACILLUS THURINGIENSIS TO PLANT COLONIZATION AFFECTS DIFFERENTIATION AND TOXICITY

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Although certain isolates from the *Bacillus cereus* group (*Bacillus cereus sensu lato*) are used as plant biologicals, safety concerns remain due to pathogenic traits. For example, toxin production might shift as an adaptive survival strategy in natural niches (the soil and plant rhizosphere). Therefore, it is crucial to explore bacterial evolutionary adaptation to the environment. Herein, we investigated *Bacillus thuringiensis* (Cry-) adaptation to the colonisation of *Arabidopsis thaliana* roots, and monitored changes in cellular differentiation in experimentally evolved isolates. Isolates from two populations displayed improved iterative ecesis on roots, reduced biofilm formation on abiotic surfaces, diminished swimming, but increased swarming, in addition to enhanced haemolysis and toxicity against insect larvae. Molecular dissection and recreation of a causative mutation revealed the importance of a non-sense mutation in the *rho* transcription terminator gene. Finally, transcriptome analysis revealed how Rho impacts various *B. thuringiensis* genes involved in carbohydrate metabolism and virulence. Our work suggests that evolved multicellular aggregates have a fitness advantage over single cells when colonising plants, creating a trade-off between swimming and multicellularity in evolved lineages, in addition to unrelated alterations in pathogenicity.

[PA04] EFFECT OF SPATIAL DISTRIBUTION ON KIN DISCRIMINATION IN BACILLUS SUBTILIS

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Bacillus subtilis strains employ a combinatorial kin discrimination system that allows them to co-exist with closely related strains while inhibiting more distant relatives. This is advantageous for competing in overlapping ecological niches as well as in avoidance of cheating, as close relatives are more likely to be cooperators. A wide variety of kin-discriminating factors (including secondary metabolites (SM)) have been identified by use of swarming assays where strains will either merge or create a boundary depending on their compatibility. However, the mechanism of kin discrimination may in fact be more complicated than first anticipated and divergent kin discrimination patterns might exists depending on the culturing conditions.

We hypothesize that different types of growth/biofilm forms (e.g. floating pellicle, agar grown colony, or submerged biofilms) display distinct patterns of kin discrimination likely due to mechanistic interactions as well as regulation of expression, and that this might allow further fine-tuning of kin discrimination to the given environment where strictness of kin discrimination is more or less advantageous.

In this study, we investigate and characterize the kin discrimination patterns in different biofilm forms of 13 *B. subtilis* isolates from allo- and sympatric niches. We aim to identify the role of SM as a part of the combinatorial kin discrimination system with focus on the effects of spatial distribution and the heterogeneity of biofilms.

[PA05] NUTRIENT-LIMITED SUBARCTIC CAVES HARBOUR DIVERSE AND COMPLEX SOIL MICROBIOMES

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Subarctic regions are particularly vulnerable to climate change, yet little is known about nutrient availability and biodiversity of cave ecosystems in these regions. To improve our understanding of life in these habitats we characterized environmental variables, and bacterial and invertebrate communities of six subarctic caves in Northern Norway. Our results show a minuscule diversity of trogloxenes (accidentally occurring) invertebrates in these caves. However, bacterial communities were diverse and compositionally different with more complex associations within these nutrient poor caves compared to the nutrient rich surface soil. Cave soil microbiomes were less variable between caves than between surface communities, suggesting that caves represent a controlled environment that collectively host uniform microbial communities. The more diverse microbial communities within caves might be the result of high niche specialization and high levels of interdependencies for nutrient cycling among bacterial taxa. The strong environmental filtering of complex cave microbiomes and their stable nature suggest that environmental changes, e.g., faster melting of snow as a result of global warming can have a detrimental impact on these communities. The comparative exploration of cave and surface microbiomes is a first step to understand the long-term environmental variables that shape the biodiversity of these vulnerable ecosystems.

[PA06] IMPACT OF ROOT DIAMETER ON RECRUITMENT OF PSEUDOMONAS TO THE WHEAT RHIZOSPHERE

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Rhizosphere engineering via plant breeding is a promising avenue to utilize endogenous plant-beneficial pseudomonads to stabilize the food supply. By manipulating the plant factors that drive rhizosphere development, we can selectively promote the growth of beneficial root-associated pseudomonads to improve crop productivity. Root diameter is a plant breeding target that may potentially promote a more diverse rhizosphere, but its impact on rhizosphere functioning is unknown. Here we show that thinner root diameter of winter wheat positively impacts *Pseudomonas* taxonomic diversity as well as their antifungal activity and cyclic lipopeptide (CLP) production. Through 16S rRNA gene sequencing, we found that a thin-rooted wheat cultivar recruited significantly more unique pseudomonads and showed increased diversity and evenness compared to a thick-rooted cultivar. High throughput *in vitro* antifungal and biosurfactant assays demonstrated an increased capability of pseudomonads isolated from thin roots to produce antifungal compounds and CLPs. Despite this, genome mining for secondary metabolite gene clusters showed no difference in the number of putative antifungal metabolites and CLPs between the two cultivars. Indeed, a group of *Pseudomonas* exclusive to the thin-rooted cultivar had significantly fewer antifungal and CLP gene clusters than groups found on the thick-rooted cultivar. Our results support the *lean rhizosphere model* where simplified genomes are favored in order to promote survival of plant-beneficial bacteria and the expression of their traits. The knowledge gained from these results will guide future plant breeding strategies that stimulate the growth of plant-beneficial pseudomonads in the rhizosphere.

[PA07] ANTIMICROBIAL SUSCEPTIBILITY PATTERNS DIFFER BETWEEN HOMOFERMENTATIVE LACTOBACILLI, LIGILACTOBACILLI AND LACTILACTOBACILLI SPECIES

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Microbiological cut-offs provided by the European Food Safety Authority is a pragmatic tool to distinguish antimicrobial resistant from susceptible industrial relevant strains. The current cut-offs are provided based on fermentation pattern for several lactobacillus species.

We aimed to set tentative epidemiological cut-offs (ECOFFs) for the interpretation of resistance in industrial relevant obligate homofermentative species. 51 strains were tested including two species belonging to newly defined genera (*Ligilactobacillus salivarius*, *Lactilactobacillus sakei*). The strains are from different sources, origins and time periods. Type strains were included. Minimal inhibitory concentrations (MIC) were determined for nine antimicrobial agents using the ISO standard method.

The MIC distributions differed between species for seven of the nine antimicrobial agents.

ResFinder was used to search for known antibiotic resistance genes. Correlation between phenotypic and genotypic resistance were observed for one *L. sakei* strain that exhibit a higher tetracycline MIC (> 64 mg/ml) than the rest of the population (2-4 mg/ml) and encoded both a *tet*(L) and *tet*(M) gene.

For *L. salivarius* and *L. sakei*, most of the strains exhibited kanamycin, streptomycin and vancomycin MICs above the current cut-offs. No known antibiotic resistance genes were found. The two species have recently been assigned new genera and the MIC distributions support this.

The data support setting ECOFFs at species level, to better distinguish between resistant and susceptible strains within species, especially, for the newly defined genera *L. salivarius* and *L. sakei.* More data on antimicrobial susceptibility in industrial relevant non-pathogens are needed.

[PA08] SHORT-TERM CO-EVOLUTION OF LACTOCOCCUS LACTIS AND LEUCONOSTOC MESENTEROIDES IN BIOFILM ACCELERATES VARIANT EMERGENCE AND COEXISTENCE

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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 and evolution of mono- and co-cultured *Lactococcus lactis* and *Leuconostoc mesenteroides*. Evolution experiments displayed that, in planktonic co-cultures, *L. mesenteroides* was predominated and tended to outcompete *L. lactis,* but in biofilms, both species coexisted. Interestingly, comparative analyses of *L. lactis* biofilm variants revealed an increased culture yield, biofilm formation, and prolonged generation time exclusively in co-evolved variants.
Finally, the performance of evolved *L. lactis* variants when co-cultured with *L. mesenteroides* was evaluated. Biofilm assays showed a higher proportion of evolved *L. lactis* strains than their ancestor when co-cultivated with ancestral *L. mesenteroides*. Interestingly, in co-cultures with evolved *L. mesenteroides, L. lactis* was strongly reduced. However, in these conditions, evolved *L. lactis* strains could persist better than their ancestor.
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.

This study emphasises spatially structured biofilms' importance in maintaining species diversity, as interspecies interactions and the biofilm setting were conditional for the emergence of novel phenotypic variants.

[PA09] WHY ISOLATING LACTIC ACID BACTERIA FROM POTATOES?

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Potato (*Solanum tuberosum*) is both an important food crop and a crucial starch source. The side streams of the potato starch industry accumulate potato protein in significant amounts. The accumulated proteins are of high quality with potentials for diverse feed and food applications. Emulsifying and antioxidant peptides have recently been identified in the main potato protein, patatin. However, these peptides need liberation to be functional which requires precise proteolytic cleavage.

Lactic acid bacteria (LABs) have adapted to diverse nutrient rich environment including diverse plant niches where some LABs depend on peptide availability and uptake. Interestingly, LABs may generate sufficient peptide supply by hydrolyzing surrounding proteins, but often display high substrate selectivity. Therefore, LABs isolated from potato may have the ability to hydrolyzing potato protein including patatin.

Here, we isolate microbes with focus on LABs from potato samples in different processing steps. Whole genome sequence data indicates novel extracellular proteases of LABs isolated from potato. Further analysis will show if proteolytic cleavage will target potato proteins and liberate functional peptides from e.g. patatin.

[PA10] BACILLUS VELEZENSIS STIMULATES RESIDENT RHIZOSPHERE PSEUDOMONAS STUTZERI FOR PLANT HEALTH THROUGH METABOLIC INTERACTIONS

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Trophic interactions play a central role in driving microbial community assembly and function. In gut or soil ecosystems, successful inoculants are always facilitated by efficient colonization, however, the metabolite exchanges between inoculants and resident bacteria are rarely studied, particularly in the rhizosphere. Here, we used bioinformatic, genetic, transcriptomic, and metabonomic analyses to uncover syntrophic cooperation between inoculant (*Bacillus velezensis* SQR9) and plant-beneficial indigenous *Pseudomonas stutzeri* in the cucumber rhizosphere. We found that the synergistic interaction of these two species is highly environmental dependent, the emergence of syntrophic cooperation was only evident in a static nutrient-rich niche, such as pellicle biofilm in addition to the rhizosphere. Our results identified branched-chain amino acids (BCAAs) biosynthesis pathways are involved in syntrophic cooperation. Genome-scale metabolic modeling and metabolic profiling also demonstrated metabolic facilitation among the bacterial strains. In addition, biofilm matrix components from *Bacillus* were essential for the interaction. Importantly, the two-species consortium promoted plant growth and helped plants alleviate salt stress. In summary, we propose a mechanism in which synergic interactions between a biocontrol bacterium and a partner species promote plant health.

[PA11] MICROBIAL PREDATOR-PREY INTERACTIONS AFFECTED BY WHEAT RHIZOSPHERE MICROBIOME EXUDATES

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The world food supply is at risk due to climate change and the increasing human population. There is therefore an increasing need for improving the yield of existing agriculture land and crops.

Microorganisms, both bacteria and fungi, in the soil matrix influence the performance and fitness of plants both positively and negatively. Root exudates are exceeded into the rhizosphere and leads to an increase in bacterial and fungal biomass. Predatory protist in the rhizosphere feed on the microorganisms and contribute to degradation of root exudates and microbial biomass. This recycling of nutrients, provided by the predatory protist, can lead to improved growth of the plant and thereby an increase in crop yield. The specific predator-prey mechanisms in the rhizosphere are insufficiently understood, yet it is important to understand these mechanisms and effects of predator-prey interactions in soil and rhizosphere in order to be able to manipulate them to increase crop yield.

The focus of my project is to unravel the role of protists in the soil microbial loop and how protists can improve plant growth. Specifically, the objectives are to unravel the mechanisms of the interactions between protists and their prey: bacteria and fungi. This is initially being approached by unravelling how wheat genotype affects protist community, and how the protist community affects the different wheat genotypes.

[PA12] ROLE OF VISCOSIN IN PLANT ROOT COLONIZATION AND MODULATION OF THE PLANT MICROBIOME UNDER REAL SOIL CONDITIONS

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The importance of microbial functions for plant health and performance is unquestioned. Beneficial rhizosphere bacteria such as *Pseudomonas* sp. promote plant growth and provide protection against pathogens. In the rhizosphere, plant-associated pseudomonads are producers of a large number of bioactive compounds, including cyclic lipopeptides (CLPs) which are particularly well-known for their antimicrobial activities. However, the role of CLP in the interaction between beneficial bacteria and plants remains underexplored especially in natural systems. In this study, the model strain *Pseudomonas fluorescens* SBW25 producing the CLP viscosin was used to unravel the impact of viscosin on bacterial colonization potential and microbiome assembly at the plant root. Four varieties of winter wheat were inoculated with either the SBW25 wild-type strain or a *viscA* mutant deficient in viscosin production. The ability of both strains to colonize the roots of each variety was quantified using qPCR analysis and plant parameters including plant biomass and height were assessed. Our results indicate that viscosin has different effects on root colonization of different genotypes of wheat. In order to study the impact of viscosin on microbial assembly, rhizobiome 16S rRNA gene amplicon sequencing was performed. This study will provide new insights into the natural importance of viscosin and specifically the role of viscosin for colonization of plant roots and in shaping the microbial communities associated to different wheat varieties.

[PA13] IMPACT OF PSEUDOMONAS FLUORESCENS SBW25 VISCOSIN PRODUCTION ON SYNTHETIC COMMUNITY ASSEMBLY IN VITRO AND IN PLANTA

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Pseudomonads have been shown to produce a group of biosurfactant compounds known as cyclic lipopeptides (CLPs). The widely studied CLP, viscosin, is shown to have several functions e.g. antimicrobial properties and involvement in biofilm formation. However, the importance of viscosin production on community assembly in different environments is poorly understood. We hypothesize that the effect of viscosin production on community assembly is environment-dependent, with no effect on pelagic community succession, where no biofilms are formed, and with a significant effect on community assembly on plant roots, due to increased root colonization competence by biofilm formation.

Here, we investigated the effects of viscosin on community assembly by applying a viscosin-producing model strain, *Pseudomonas fluorescens* SBW25, and a non-viscosin producing mutant (ΔviscA) to a six-strain synthetic community, created from strains isolated from the wheat rhizoplane. This synthetic community, containing wildtype or mutant, was applied to two gnotobiotic systems: liquid M9 medium and wheat plants (seed coating) grown in sterile sand. We tracked the development in community composition over time by 16S rRNA gene amplicon-sequencing. For the *in planta* assay, we determined the effects of viscosin production on plant root and shoot growth.

If microbial engineering is to succeed as a sustainable alternative to the use of agro-chemicals, we must understand the microbial ecology and community assembly under the highly variable biotic and abiotic conditions profound in natural soil systems. Our results increase the understanding of viscosin impact on community assembly and expands the knowledge of viscosin's role in microbial ecology.

[PA14] EXTRACELLULAR VESICLE FORMATION IN LACTOCOCCUS LACTIS IS STIMULATED BY PROPHAGE-ENCODED HOLIN-LYSIN SYSTEM

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Gram-positive bacterial extracellular membrane vesicles (EVs) have been drawing attention in recent years. However, mechanistic insights are still lacking on how EVs are released through the cell walls in Gram-positive bacteria. In this study, we characterized underlying mechanisms of EV production and provide evidence for a role of prophage activation in EV release using the Gram-positive *Lactococcus lactis* as a model.

By applying standard EV isolation procedures, we observed the presence of EVs in the culture supernatant of a lysogenic *L. lactis* strain FM-YL11, for which the prophage-inducing condition led to an over 10-fold increase in EV production in comparison to the non-inducing condition. In contrast, the prophage-encoded holin-lysin knock-out mutant YL11ΔHLH and the prophage-cured mutant FM-YL12, produced constantly low levels of EVs. Under the prophage-inducing condition, FM-YL11 did not show massive cell lysis. Defective phage particles were found to be released in and associated with holin-lysin induced EVs from FM-YL11, as demonstrated by transmission electron microscopic images, flow cytometry and proteomics analysis.

Findings from this study provide additional insights into the EV production mechanism involving prophage-encoded holin-lysin system. The knowledge on bacterial EV production can be applied to all Gram-positive bacteria and other lactic acid bacteria with important roles in fermentations and probiotic formulations, to enable desired release and delivery of cellular components with nutritional values or probiotic effects.

[PA15] ISOLATION AND CHARACTERIZATION OF BACTERIOPHAGES TARGETING BACTERIA FROM THE WHEAT FLAG LEAF

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With emerging climate changes and a growing population, the world's food production must increase significantly in the coming years. Meeting these challenges will require a comprehensive understanding of crop biology, but also their microbiome. All plants are host to an enormous number of diverse microorganisms, capable of both promoting growth and killing their hosts. However, our knowledge of how to control this microbiome is limited. Here, we report the isolation and characterization of bacteriophages with the potential to manipulate the wheat microbiome towards beneficial community compositions.

In this study, we initially sequenced and characterized six *Erwinia* spp and four *Pseudomonas* spp isolated from the flag leaf of wheat. Using these as hosts, we isolated and sequenced 19 phages from municipal wastewater and organic waste. We characterized their host range against a larger library of *Erwinia* and *Pseudomonas* strains isolated from wheat and assessed their potential for *in vivo* microbiome manipulation.

[PA16] WHEAT RHIZOSPHERE INTERACTOME: CARBON-13 LABELING OF METAPROTEOME AND META-METABOLOME

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Crop health and yield are linked to microbial activity in the soil, but the influence of the microbial community and its effects on plant growth are still poorly understood. We investigated the direct interaction between wheat *(Tricitum aestivus)* and the rhizosphere microbiome.
Wheat plants were grown in controlled environments with $^{13}CO_2$ enriched atmospheres. ^{13}C incorporated into plant constituents was in time expelled by the wheat roots as ^{13}C-labelled root exudates. These were then metabolized and incorporated by rhizosphere microbes, labelling molecules along the pathway from plant tissue to secondary soil metabolites. Rhizosphere samples were flash frozen, lyophilized, and split into batches for nucleic acid, peptide and metabolite extraction.
Subsequently, DNA and RNA were co-extracted, and metagenomes were sequenced on an Oxford Nanopore MinIon. Peptides and metabolites were extracted and following captured using HPLC-MS Orbitrap systems. Labelled peptides and metabolites will be identified and mapped to the metagenome.
Combining the metagenome, proteome and metabolome will thus provide an accurate overview of the wheat-microbe-soil interactome, through the tracking of labelled compounds from root exudates to microbial proteins and biotransformation products. The goal is identification of proteins and metabolites containing the label, and by extension, any loci and genetic pathways associated with these. This will provide a novel and robust overview of key microbial species related to root exudate biotransformation in the wheat rhizosphere. This knowledge will allow future strategic manipulation of plant-associated microbial communities to support high plant productivity.

[PA17] INVESTIGATING THE FERMENTATION CHARACTERISTICS OF THE YEAST WICKERHAMOMYCES ANOMALUS

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In the pursuit of novel flavour and aroma it is an increasing trend to look at alternative yeasts for fermentation of known products such as beer, cider and wine. With this in mind, a culture collection has been established by taking samples from Danish plant materials. One of the isolates has been identified as *Wickerhamomyces anomalus*, which is known to be present in some wines and to contribute with flavor and aroma characteristics. The objective of the current work has been to characterize the fermentation properties of *W.anomalus* and to assess its suitability for fermented beverage production.

To characterise the yeast, different parameters have been investigated and compared to *Saccharomyces cerevisiae*, as the latter is the most used yeast in food and beverage fermentations. We found its optimum growth conditions to be at around 35 degrees and with pH close to 6. Under aerobic conditions growth rates were up to 0.42$\boldsymbol{h^{-1}}$both in laboratory medium (YPD) and in beer wort. In comparison. We found that the strain was able to consume glucose, maltose and fructose sequentially, which suggests that glucose repression occurs. Furthermore, in addition to production of several different organic acids, low concentrations of ethanol (7 g/L) were produced even under aerobic conditions, suggesting that the strain is crab-tree positive.

Current results suggest that *W. anomalus* is a viable candidate for fermentation of beverages. Results indicate a similar growth rate as that of Saccharomyces cerevisiae and it is able to produce different types of organic acids dependent on the environmental stimulus, however it has a lower ethanol production, which is beneficial for low alcoholic drinks and could have potential in cider, beer and wine production.

[PA18] A BIOINFORMATICS PIPELINE FOR ROUTINE SPOILAGE DETECTION IN DAIRY PRODUCTION FROM METAGENOMIC OXFORD NANOPORE READS.

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Food and feed safety is a main concern for food authorities, centers of disease control, departments of agriculture and public health laboratories, specifically the surveillance of and acting on epidemiological outbreaks of food-borne pathogens such as Salmonella, Listeria, Vibrio, E. coli, Shigella, Campylobacter and Cronobacter reported by hospitals and doctors. Additionally, food spoilage is a major concern for large food producing companies as this can have a direct impact on revenue margins, the environment, food going to waste and last but not least brand reputation. Historically, the identification of food pathogens and spoilers involved culturing bacteria from suspected sources using specialized bacterial growth media to isolate the causal agent, followed by strain typing, a workflow that can take several days or weeks for some slow growing agents, if it is at all possible. At this stage, the damage has been done, and the impacts are wide. For this reason, there is a strong industry trend towards NGS-based approaches to sample characterization, namely whole-genome sequencing of isolates and taxonomic profiling of bacterial communities. Food quality laboratories are now routinely equipped with desktop sequencing machines from Illumina or IonTorrent and portable devices from Oxford Nanopore to provide the sequences. Here we demonstrate how a bioinformatic analysis of metagenomic sequencing reads from an Oxford Nanopore sequencer can be performed using the CLC Genomics Workbench premium, a workflow that has been developed in a collaboration between QIAGEN and Arla Foods for the detection of spoilers and pathogens in dairy production.

[PA19] TANNINS TO CONTROL CLOSTRIDIUM PERFRINGENS IN VITRO

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As the use of antibiotics is being restricted in animal husbandry, certain bacteria that used to be well controlled seem to thrive and create problems. Economically important diseases like gas gangrene and necrotic enteritis, where C. perfringens may play an important role, and which may cause sudden death in pigs are in many case not diagnosed in advance of the fatal course of the disease. In herds with this type of problems, a feed component that inhibits C. perfringens should therefore be used in a constant supply to all pigs at all times. Plant extracts with high content of tannins are well known modifiers of the microbiota and experience from many farms has shown that tannins may protect farm animals against pathogenic Clostridia as well as protect protein from microbial degradation. While hydrolysable and condensed tannins are defined by their common ability to precipitate protein, high antioxidant capacity and astringent ability, they differ in many chemical and biological characteristics.

The aim of this study was to evaluate the Non-Inhibitory and Minimum Inhibitory Concentrations (NIC and MIC) of hydrolysable and condensed tannin extracts against 9 strains of Clostridium perfringens. We applied standard photometric procedures for evaluation of MIC of extract from Sweet Chestnut (Castanea sativa, Mill.) (CE), Mimosa (Acacia sp.) (ME) and Quebracho (Schinopsis balansae Engl.) (QE). The extracts were tested in a dilution series of 8 concentrations from 10 to 0.08 mg/ml (10,000 – 80 ppm). The NIC and MIC values are calculated via fitted Gompertz curves.

Of the 9 types tested it was possible to obtain full dataset of 6. The preliminary results show that extract from Sweet Chestnut consistently were more efficient in its inhibition of C. perfringens than the 2 other extracts. The average MIC of Sweet Chestnut, Mimosa and Quebracho extracts were 290 ppm (90-760 ppm), 1,140 ppm (400-1,620 ppm) and 2,300 ppm (650-6,310 ppm) respectively. The Castanea extract therefore on average needed 4 to 8 times lower dosage compared to Mimosa and Quebracho extracts.

Selected plant metabolites may have a new and more deliberate role to play in the future of antibiotic restricted production of pigs.

[PB58] EXPERIMENTAL EVOLUTION OF BACILLUS SUBTILIS ON ARABIDOPSIS THALIANA ROOTS REVEALS FAST ADAPTATION AND IMPROVED ROOT COLONIZATION IN THE PRESENCE OF SOIL MICROBES

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The soil-dwelling bacterium *Bacillus subtilis* is known to promote plant growth and protect plants against disease. However, despite representing an important ecological niche of *B. subtilis*, knowledge about the evolutionary adaptation of this bacterium to the plant root environment is limited. In this study, we employed experimental evolution to study adaptation of *B. subtilis* to *Arabidopsis thaliana* roots. We found that *B. subtilis* rapidly adapted to the plant roots, as evidenced by improved root colonizers already after 12 successive transfers. Further phenotypic characterization of evolved isolates from transfer 30 revealed that increased root colonization was associated with robust biofilm formation in response to the plant polysaccharide xylan. Additionally, several evolved isolates from independent populations were impaired in motility, a redundant trait in the selective environment. We further found that two selected evolved isolates outcompeted the ancestor during competition on the root but suffered a fitness disadvantage in a non-selective environment, demonstrating an evolutionary cost of adaptation to the plant root. Finally, increased root colonization by an evolved isolate was also demonstrated in the presence of resident soil microbes. Our findings provide novel insights into the adaptation of *B. subtilis* to an ecologically relevant environment and reveal evolutionary consequences that are fundamental to consider when evolving strains for biocontrol purposes.

[PB59] CANDIDATUS ACCUMULIBACTER: A REFINED PHYLOGENY REVEALS THE EXISTENCE OF NOVEL SPECIES, THEIR POTENTIAL FUNCTION, AND THEIR GLOBAL DISTRIBUTION.

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Abstract: *Candidatus* Accumulibacter is the first microorganism identified as polyphosphate-accumulating organism (PAO), essential for phosphorus (P) removal from wastewater. However, the current taxonomic of this lineage is flawed, with the majority of the publicly available genomes or 16S rRNA gene sequences classified as "Candidatus Accumulibacter phosphatis", despite notable phylogenetic divergence. The use of the *ppk1* marker gene for fine-scale differentiation into "clades" has further increased the confusion in taxonomic assignment. Here, we provide a reassessment of the phylogeny of *Ca*. Accumulibacter, using a comparison between genome-, *ppk1*, and 16S rRNA gene-based phylogenies from a comprehensive dataset. With this approach, several novel species were identified and some were targeted by new species-specific FISH probes. The MiDAS4 global survey, containing samples from 667 wastewater treatment plants (WWTPs) located in 33 different countries, was used to investigate their geographical distribution and factors that may influence their abundance. *Ca*. Accumulibacter had a higher relative abundance in WWTPs with P removal and was globally distributed, indicating the process design as a major driver for their presence. Metabolic annotation and FISH-Raman microspectroscopy confirmed the potential for the PAO metabolism in all *Ca*. Accumulibacter species, with detection *in situ* of the typical PAO storage polymers. The annotation revealed fine-scale differences in the nitrate/nitrate reduction pathways, giving some insights into niche differentiation, and explaining to some extent how they could contribute to overall nutrient removal.

[PB60] DIVERSITY AND PHYSIOLOGY OF THE UNCULTURED AND ABUNDANT GENERA IN SAPROSPIRACEAE FAMILY ACROSS GLOBAL WASTEWATER TREATMENT SYSTEMS

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The *Saprospiraceae* family is a very diverse family within the phylum Bacteroidetes, commonly present in high abundance in wastewater treatment plants (WWTPs) worldwide, but little is known about their function and importance. The genera described are characterized by rod-shaped or filamentous morphology and an aerobic heterotrophic metabolism with their potential involvement in nutrient removal in WWTPs. Here we used MiDAS4 global survey, including samples from 480 WWTPs located in 30 countries, to analyze the abundance and global distribution of members of the *Saprospiraceae* family. In addition, we retrieved 32 high-quality metagenome assembled genomes (MAGs) from Danish WWTPs for metabolic reconstruction, we designed novel fluorescence *in situ* hybridization (FISH) probes for visualization, and in combination with Raman microspectroscopy we detected and confirmed important physiological features. FISH revealed rod-shaped morphologies for all analyzed genera, present mostly inside the flocs. The genomic potential revealed diverse metabolism for several undescribed genera including genus OLB8, indicating possible degradation of polysaccharides, amino acids and other carbon substrate, and partial denitrification. FISH-Raman showed presence of glycogen and PHA, also confirmed by the metabolic potential of MAGs, while none of the genera contained polyphosphate. These results provide the first overview of some of the most abundant *Saprospiraceae* genera present in WWTPs across the world, revealing their potential involvement in degradation of complex macromolecules, such as polysaccharides and proteins, and in nutrient removal.

[PB61] MAPPING THE ECOPHYSIOLOGY OF SEVERAL PUTATIVE POLYPHOSPHATE-ACCUMULATING ORGANISMS

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Phosphate (P) is a vital resource, but it is limited in its natural occurrence so recycling will become important to support the increasing demand. By using the Enhanced Biological Phosphorus Removal (EBPR) process in wastewater treatment plants (WWTPs), recycling of P from the wastewater is one way to combat this challenge. The EBPR process is controlled by polyphosphate-accumulating organisms (PAOs), such as the *Candidatus* Accumulibacter, *Tetrasphaera,* and *Dechloromonas* genera, which all store P intracellularly, despite of diverse metabolisms, depending on alternating anaerobic and aerobic conditions.
New mass balance studies of biomass from full-scale plants with EBPR indicate that several unknown PAO exists, which may be important for the removal of P.
The aim was to investigate the ecophysiology of *Rhodoferax*, *Tessaracoccus,* and *Sulfuritalea*, to determine whether they are PAOs or not. We used 16S rRNA gene sequencing to find the abundance, metagenomics to retrieve high-quality metagenome assembled genomes (MAGs) for annotation of metabolic pathways, and quantitative FISH-Raman analysis of polyphosphate (polyP) and other intracellular storage polymers to verify hypotheses.
Rhodoferax and *Sulfuritalea* were observed in both Danish and global plants with up to 5.8% and 2.3% of total biomass, respectively. *Tessaracoccus* was found to contribute up to 2.6% in Danish plants. FISH-Raman analysis of *Tessaracoccus* and *Rhodoferax* showed signs of polyP. The chance of these two being PAOs was further supported by the retrieved MAGs, which showed the present of *Pit*, *PstSCAB*, and *PhoU*.
By elucidating the ecophysiology of the remaining PAOs that contribute to phosphorus removal, we can enable surveillance and better control of the plants.

[PB62] TIME SERIES ANALYSES OF ANAEROBIC DIGESTERS AT WWTPS REVEAL HIGH STABILITY AND FACTORS AFFECTING COMMUNITY COMPOSITION.

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Anaerobic digestion (AD) at wastewater treatment plants (WWTPs) is carried out by groups of microorganisms, active in complex metabolic networks. Recent studies of community structure have shown that there are two large fractions, growing(G) and non-growing (NG), the latter being up >40% of bacteria, all immigrating from influent and surplus activated sludge. Therefore, it is important to focus on G fraction to better understand ecology and ecophysiology of process-critical species.
The aim was to describe community composition and stability in mesophilic and thermophilic AD, and to investigate fate of specific G species during a change from meso- to thermophilic conditions, using MiDAS database.
AD samples were obtained every 2 weeks at Danish WWTPs from 2015-19. Bacteria and archaea were identified by 16S rRNA gene amplicon sequencing. MiDAS 3 taxonomy was used with species-level resolution.
Microbial communities at species-level showed clear differences between AD reactor types. NG taxa, such as *Microthrix* and *Tetrasphaera*, were only present because they were continuously added by food streams. Between G species, almost only uncharacterized genera and species were among most abundant in both mesophilic and thermophilic ADs. For all reactors, a high level of community stability was observed, with only minor changes over the 5 years period. However, this was not the case with NG fraction, which showed large fluctuations. A detailed study of an AD at Aalborg East WWTP, which was perturbated from mesophilic to thermophilic conditions, showed clear changes in community. This concludes importance of focus on G bacteria fraction when investigating ADs, and importance of temperature changes on top abundant bacteria.

[PB63] BACTERIOPHAGE-ENCODED 7-DEAZAGUANINE DNA MODIFICATIONS AS ANTIVIRAL DEFENCE MECHANISM AND TARGET.

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Bacteriophages have evolved numerous strategies to circumvent the antiviral defences of their bacterial hosts. The study of these systems and escape mechanisms have historically yielded many crucial and valuable biotechnological tools such as restriction enzymes and CRISPR-Cas systems. Representative *E. coli* phage CAjan uses a recently described 7-deazaguanine DNA modification system, wherein certain canonical guanosine bases are replaced with dpreQ_0, to protect from host restriction systems[1].

Recently, several novel and diverse putative antiviral systems have been predicted computationally followed by biological verification[2]. Here, we present a screening of interactions between a selection of novel antiviral systems against deazaguanine phages, which utilize two different 7-deazaguanine modifications - dpreQ_0 and dG+. Our data suggest that 7-deazaguanine modifications can provide antiviral protection against several of them. This hints at novel and undiscovered interactions between phage defence mechanisms and the bacterial immune response. Understanding these features is of interest to biotechnological application of phage derived systems, phage therapy and phage biocontrol.

1. Hutinet G, Kot W, Cui L, et al. 7-Deazaguanine modifications protect phage DNA from host restriction systems. *Nat Commun 2019 101*. 2019;10(1):1-12. doi:10.1038/s41467-019-13384-y

2. Gao L, Altae-Tran H, Böhning F, et al. Diverse Enzymatic Activities Mediate Antiviral Immunity in Prokaryotes. *Science*. 2020;369(6507):1077. doi:10.1126/SCIENCE.ABA0372

[PB64] BIOMINING SYNERGY: CAN INDIGENOUS BACTERIA WORK TOGETHER TO DECALCIFY MAGNESITE ORES?

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Magnesium is one of the most used metals with growing interest to process low-grade Mg ores and Mg-containing by-products. However, they contain a high level of impurities, mostly silicon, Fe and CaCO3, that limits metal recovery. By producing exopolysaccharides that chelate these impurities and organic acids dissolving CaCO3, bacteria are innovative biotechnological tools for the pre-treatment of these raw materials for improved Mg recovery. In this study, we aim to characterize potential synergetic interactions of low-grade magnesite ores indigenous bacteria, using both metagenomic approach and consortia isolation. We obtained 63 metagenomic-assembled genomes with the ability to produce a wide range of organic acids based on metabolic predictions. We also isolated microbial consortia positive for CaCO3 dissolution, which members were closely related to *Pseudomonas brenneri* and *Microbacterium sp.* Further co-culture experiments will allow us to quantify their synergetic effects, biofilm and organic acids production for Mg ores impurities removal.

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[PB16] ASSESSING ILLUMINA, NANOPORE AND PACBIO SEQUENCING PLATFORMS AT RECOVERING HIGH-QUALITY GENOMES FROM COMPLEX MICROBIAL COMMUNITIES

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Cheap short-read DNA sequencing has led to a massive increase of genome reference databases, although many genome drafts from environmental systems are highly fragmented due to strain heterogeneity, micro-diversity, and repeats that cannot be resolved with short reads. This poses an issue, as the absence of genes in incomplete and fragmented genomes fails to provide important genomic information about microbial species. Here, we show that combining short reads (Illumina) with Nanopore R9 sequencing of anaerobic digester sludge enables to acquire vastly greater numbers of more complete, high quality metagenome assembled genomes, compared to a short-read-only approach (86 vs 8 high quality genome drafts). We found PacBio circular consensus sequencing to also be an effective platform for genome-centric metagenomics (74 high quality drafts), albeit at higher costs per genome draft, compared to the hybrid Illumina-Nanopore approach (59.7 vs 23.4 $ per high quality draft).

[PB17] BIOFILM SUCCESSION AND MICROBIAL SECONDARY METABOLITE DYNAMICS IN A NATURAL MARINE COMMUNITY

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Microbial secondary metabolites may be strong biotic drivers of microbial community assembly, yet their role in natural biofilm succession remain unknown. Biotic interactions across taxa can result in the inhibition or sustenance of specific organisms. Both synergistic microbial interactions and mutualistic interactions involving eukaryotic organisms and bacterial symbionts may in part be mediated by microbial secondary metabolites. An example of such mutualistic cross-domain interaction involves species of the proficient biofilm-forming genus *Phaeobacter*, which are capable of producing the secondary metabolite tropodithietic acid (TDA). *Phaeobacter* are often associated with microalgae and the marine filter-feeding bryozoan, *Electra pilosa*. To uncover the potential involvement of microbial secondary metabolites, including TDA, in the assembly of marine biofilm communities, we incubated large-surface area plastic elements in a Danish fjord with high biofouling capacity and monitored the biofilm succession over a five-month period. Plastic elements were colonized by TDA-producing *Phaeobacter* spp. and by *E. pilosa* from day 30. The dynamics of potential microbial secondary metabolite production and the taxonomic diversity of the biofilm community will be assessed by targeted amplicon sequencing of biosynthetic gene clusters (BGCs) encoding non-ribosomal peptide synthetases (NRPSs) and the 16S and 18S rRNA genes, respectively. Presence of TDA production will be evaluated by transcriptomics and mass spectrometry imaging. This study will bridge the knowledge gap that exist between biofilm assembly and the potential natural role of secondary metabolites during succession of natural marine biofilms.

[PB18] THE POTENTIAL FOR POLYKETIDE BIOSYNTHESIS IN NATURAL MICROBIOMES SCALES WITH BACTERIAL TAXONOMIC RICHNESS AND DIVERSITY

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Antibiotics are one of the most significant discoveries in modern medicine and they are an integral part of our daily life. Yet, we are running headfirst into a global antibiotic resistance crisis, as estimates suggest that by 2050 more people will succumb to infections by antibiotic resistant microorganisms than to cancer. Most of the antibiotics currently in use are natural products derived from microorganisms, e.g. bioactive polyketides (PK) and non-ribosomal peptides (NRP). However, the distribution and diversity of such compounds in natural microbiomes remains obscure. The aim of this study was to identify the most promising natural microbiomes in terms of biosynthesis potential, and to uncover which environmental and community variables drive this potential. We performed targeted sequencing of conserved ketosynthase (KS) domains of PK synthases and adenylation (AD) domains NRP synthetases on DNA extracted from soil, marine sediments, and estuarine surface waters. The potential to produce different NRPs was highest in marine sediments (OBU_{AD} richness: 656±58), and the KS biosynthesis potential was highest in soil (OBU_{KS} richness: 388±67). Moreover, KS richness was correlated with the taxonomic (16S rRNA gene) diversity and richness across all microbiomes (R=0.66, p=9.3e-11). Using linear regression models, we were able to predict the KS richness based on the environment type and the bacterial richness and diversity with good accuracy (RMSE = 55 OBU_{KS}). Hence, future efforts in the discovery of novel bioactive polyketides should focus on microbiomes exhibiting a high degree of taxonomic richness and diversity. Ultimately, this will allow us to predict the most promising microbiomes to mine for novel anti-infective agents.

[PB19] AMPLICON BASED DIRECT-GENEFISH FOR SELECTIVE EXTRACTION OF BIOSYNTHETICALLY GIFTED ENVIRONMENTAL MICROORGANISMS.

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Nature represents a rich reservoir of microorganisms with useful properties that humankind can exploit. Metagenomic and amplicon sequencing studies have demonstrated that various microbiomes hold an enormous genetic potential to biosynthesize bioactive natural products such as antibiotics. However, due to cultivation recalcitrance, it is difficult to transform this genetic potential into an array of isolated bioactive molecules. This work aims to access the biosynthesis potential of the unculturable majority through the development of a method that selectively extracts community members with a high biosynthetic gene content prior to downstream clone library preparation and screening. The method is based on amplification of conserved ketosynthase (KS) and adenylation (AD) domains using degenerate primers and subsequent labelling of amplicon probes for direct-gene fluorescence *in situ* hybridization (FISH). We used two biosynthetically gifted bacterial model strains: the Gram-positive soil dwelling *Streptomyces coelicolor*, and the Gram-negative marine bacterium *Pseudoalteromonas rubra*. Sequencing of AD and KS amplicons from the two model strains revealed that only ≈10% of the KS and AD domains present in the genomes were amplified. Nonetheless, after optimization of the in solution direct-geneFISH procedure, both *P. rubra* and *S. coelicolor* were selectively labelled with the amplicon FISH probes. Coupled to fluorescence assisted cell sorting (FACS), this new method will allow for selective extraction of community members with a high potential for natural product biosynthesis.

[PB20] BACTERIAL BIOFILMS AS A SUSTAINABLE AND STABLE FOULING CONTROL TOOL FOR SUBMERGED SURFACES

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Biofouling poses a challenge for human health and efficiency of technical and operational processes. In marine vessels, biofouling shortens the lifetime of immersed surfaces and entails large associated costs, i.e. increased fuel consumption or re-coating of ship hulls. Such challenge inspired us to assemble bacterial films, natural or engineered, with future prospects in the protection of underwater surfaces from micro- and macrofouling.
Our focus was to assemble bacterial biofilm communities, stable and adaptable to environmental conditions. We screened a collection of 46 isolates, sampled either from two marine vessel hulls (Helsingør, Denmark) or from algae in a rocky intertidal (Sydney, Australia). Moreover, *R. denitrificans* (RD) and *D.shibae* (DS) were also included for adhesion screening since they are genetically tractable, harmless and early colonizers with antifouling properties. The screening comprised four levels of complexity: mono-, dual-, triple- and multispecies combinations.
We found 7 four-species communities with highly robust and reproducible adhesion, all containing either RD or DS, facilitating the insertion of functional modules if engineering of the marine isolates failed. Biofilm productivity was generally greater at 24°C, although longer incubation increased yield at lower temperatures. The Helsingør communities proved greater resilience to changing temperatures, producing 3-fold more biofilm at 4°C than 10 or 24°C. Additionally, some communities reduced barnacle settlement by 50 % on PVC surfaces.
We envision these communities as a promising protective film with unique self-assembling and self-repairing features and a potential alternative to current synthetic coatings.

[PB21] CHEMOTAXIS MAY ASSIST MARINE HETEROTROPHIC BACTERIAL DIAZOTROPHS FIND MICROZONES SUITABLE FOR N2 FIXATION IN THE PELAGIC OCEAN

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Heterotrophic bacterial diazotrophs (HBDs) are ubiquitous in the pelagic ocean, where they have been predicted to carry out the anaerobic process of nitrogen fixation within low-oxygen microenvironments associated with marine pelagic particles. However, the mechanisms enabling particle colonization by HBDs are unknown. We hypothesized that HBDs use chemotaxis to locate and colonize suitable microenvironments, and show that a cultivated marine HBD is chemotactic towards amino acids and phytoplankton-derived dissolved organic matter (DOM). Using an *in situ* chemotaxis assay, we also discovered that diverse HBDs at a coastal site are motile and chemotactic towards DOM from various phytoplankton taxa and, indeed, that diazotrophs constituted a larger part of the motile fraction of bacterioplankton compared to the bulk bacterioplankton community. Finally, three of four HBD isolates and 16 of 17 HBD metagenome assembled genomes, recovered from major ocean basins and locations along the Australian coast, each encoded >85% of proteins affiliated with the bacterial chemotaxis pathway. These results document the widespread capacity for chemotaxis in diverse and globally relevant marine HBDs, and show that chemotaxis is a prevalent trait among marine HBDs. We suggest that HBDs could use chemotaxis to seek out and colonize particles that can harbor low-oxygen micro-environments suitable for nitrogen fixation. Chemotaxis in HBDs could therefore affect marine nitrogen and carbon biogeochemistry by facilitating nitrogen fixation within otherwise oxic waters, while also altering particle degradation and the efficiency of the biological pump.

[PB22] TROPODITHIETIC ACID, A SECONDARY METABOLITE WITH ANTIBIOTIC ACTIVITY, DRASTICALLY CHANGES THE PHYSIOLOGY OF THE PRODUCING ORGANISM

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Ever since the discovery of penicillin, humanity has exploited microbial secondary metabolites (MSMs), particularly those with antibiotic or other drug properties. Originally their ecological role was presumed to be mainly as “weapons of mass destruction”, designed to kill off competing ecological neighbours. However, we now know that MSMs work in a variety of other ways, e.g. as nutrient scavengers or as signaling molecules. Tropodithietic acid (TDA) is one such MSM produced by members of the globally occurring marine *Roseobacter* group. Aside from being a potent antibiotic, TDA is also proposed to be a signaling molecule which alters global gene expression. However, the role and importance of TDA for the producing bacteria is still not fully understood. In an attempt to unravel this, we constructed a scarless in-frame deletion mutant deficient in TDA biosynthesis. Compared to wild type, the mutant showed increased motility, temporal changes in biofilm formation, and altered cell morphology. To underpin the underlying mechanisms, we conducted a proteomics comparison of wild type and mutant. Here, we found several changes in proteins involved in flagellar assembly and chemotaxis, cell wall/membrane/envelope biogenesis, and energy conversion among others. In conclusion, TDA production significantly influences the physiology of the producing host. We speculate that these physiological changes may be important during colonization of ecological niches.

[PB23] MICROBIOMES ASSOCIATED WITH THREE DIFFERENT MICROALGAE

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Microalgae are becoming increasingly important as sustainable sources of fish feed, pigments, antioxidants, and biofuels among other things. During the last few decades, it has become evident that microalgae harbor a microbiome in their vicinity, the so-called "phycosphere". Similar to the gut microbiome of humans or the rhizosphere microbiome of plant roots, the microalgal microbiome can affect algal growth and health in positive and negative ways, and it appears that the algal microbiome is vital to algal growth and health since axenic (sterile) algae grow poorly compared to non-axenic algae. While a few interactions between members of the microbiome and their algal host have been elucidated, it is still largely unknown how microalgae and their microbiomes interact, if microbiomes are host-specific, and which species comprise the microbiome.

In this study, the composition of the microbiomes of three microalgae, *Isochrysis galbana, Tetraselmis suecica,* and *Thalassiosira weissflogii* was assessed by amplicon sequencing of the 16S rRNA gene. Both the fraction of the microbiome attached to the microalgal host (captured on 5 µm filters), and the free living fraction (captured on 0.2 µm filters) was assessed, and differences and similarities between fractions as well as algal host evaluated. Additionally, four different DNA extraction methods were applied for evaluation of any effects on microbiome composition, DNA yield, and DNA quality from the extraction method.

[PB24] ASGARD ARCHAEA, THE ANCESTORS OF THE EUKARYOTIC CELL?

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According to the endosymbiont theory, eukaryotic cells evolved more than 2 billion years ago from a merger of a bacterial and an archaeal host cell. In 2015, the closest living relatives of these archaeal ancestors of all Eukaryotes, the Asgard archaea, were identified through environmental genomics analyses. The analyses of their reconstructed genomes highlighted the presence of genes encoding homologs of eukaryotic signature proteins involved in membrane trafficking, vesicle formation and cytoskeleton.
So far, only one Asgard archaea representative has been cultivated, after a 15 years long procedure, thus stressing the need for cultivation-independent procedures for studying Asgard archaea cellular structure.
We aim to detect and enrich Asgard archaea cells with cultivation-independent techniques, applying coupled FISH and FACS technologies, to enrich specific Asgard archaea lineages and to investigate their morphology and cellular ultrastructure, clarifying their structural similarities with eukaryotic cells.
We designed CARD-FISH probes specific for Lokiarchaeota and Heimdallarchaeota phyla, and used these to successfully visualize Asgard archaea cells in Aarhus bay sediments. Their observation through super-resolution microscopy revealed a clear separation between DNA-signal and ribosome-FISH signal, suggesting a peculiar location of DNA in Asgard archaea, that might be due to intracellular compartmentalization or membrane invagination.
Now, our focus is on the optimization of the procedure for cell extraction from sediments, and of the MiL-FISH protocol, to obtain stained Asgard archaea cells, cleaned from sediments residues, and thus suitable to be separated from other cells and enriched through FACS.

[PB25] BIOTURBATION IS A KEY DRIVER OF MICROBIAL COMMUNITY ASSEMBLY IN MARINE SEDIMENTS

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The marine subsurface biosphere is the habitat for more than half of the oceanic prokaryotes. In this system, microorganisms inhabiting the surface seabed are buried into the subsurface sediment due to the continuous deposition of material from the water column. Despite sheltering a plethora of bacteria and archaea, the evolution of this deep biosphere is largely unknown. Recent publications have shown that the community in such sediments is made up of rare members of surface communities that persist and become predominant with increasing depth. It is hypothesized that the assembly of the subsurface community takes place at the lower boundary of the bioturbation zone, which marks the transition between the mixed surface sediment rich in substrates and the more substrate-depleted subsurface sediment.

In this study, we investigate the changes in microbial community composition and genome evolution across the bioturbated zone of a marine sediment by coupling high-resolution 16S rRNA amplicon sequencing with metagenome-assembled-genome (MAG) analysis. Sediment cores were sampled at marine station M1 and M5 in Aarhus Bay and subsampled with centimeter resolution for bulk RNA and DNA extraction. A total of 617 MAGs were binned and classified by taxonomy and genomes of candidate persister clades identified. The total microbial community showed a distinct change in composition at the lower part of the bioturbation zone suggesting that the main site for the assembly of subsurface communities is this transition between the energy-rich bioturbated zone and the underlying energy-poor non-bioturbated sediment. The genes evolving under positive selection indicating adaption to the energy-poor subsurface sediment remains to be determined.

[PB26] THE ENIGMA OF NITROGEN FIXATION IN OXYGEN MINIMUM ZONES- A BROAD DIVERSITY BUT LOW ACTIVITY IN THE NORTHERN BENGUELA UPWELLING SYSTEM

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Nitrogen (N) is an essential nutrient and is limiting for primary production in large parts of the ocean. Diazotrophs can circumvent this shortage due to their ability to fix dinitrogen gas (N_2). Over recent years, a large diversity of non-cyanobacterial diazotrophs have been discovered, many of them from oxygen minimum zone (OMZ) waters. While diazotrophs are usually present in OMZs, they only seem to fix N_2 at minimal rates, which may be a result of the unusual community of N_2 fixers typically found in most OMZs. This study investigates the Northern Benguela upwelling region OMZ waters community composition of diazotrophs based on extensive sequencing and isotope speciation. Consistent with a previous study, we detected a slight N deficit in the OMZ, but isotope data did not indicate active or past N_2 fixation. The diazotroph community was overall dominated by non-cyanobacterial microbes, mostly related to gamma-proteobacteria. Uniquely, however, we found a strikingly high diversity of Cluster III diazotrophs within OMZ waters, not observed in other OMZ. Moreover, in contrast to other OMZ, we found present of *Trichodesmium* and UCYN-A in the surface water connected to the OMZ waters. The detected diversity of Cluster III diazotrophs and the presence of cyanobacterial diazotrophs, potentially active, indicate that we are far from understanding the role of diazotrophs in OMZ waters. Understanding the difference between this OMZ and other ones will, however, be relevant for understanding the N budget in OMZ waters and the response of N_2 fixation to future climate change.

[PB27] FABRICATION OF MICROSTRUCTURED SURFACE TOPOLOGIES FOR THE PROMOTION OF MARINE BACTERIA BIOFILM

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Marine bacteria of the *Roseobacter* group have the beneficial effect to compete and inhibit the growth of other antagonistic bacteria when they form biofilms. Thus, their use in large-scale aquaculture units can decrease the need for antibiotics. In this study, we hypothesize that micro-patterned surfaces may promote the biofilm formation of those marine bacteria, due to the increased contact area between the cells and the surface material. Our aim is to fabricate biofilm optimal micro-patterned surfaces, which can promote the growth and biofilm formation of *Roseobacter* group and investigate relevant length scales for surface morphology and chemistry.

In a preliminary study, silicon surfaces, comprising arrays of pillars and pits with different diameters and depths, were produced by UV lithography and deep reactive ion etching on single-side polished silicon wafers. The resulting microscale topologies were characterized using optical profilometry and scanning electron microscopy. Screening of the bacterial biofilm on the patterned surfaces was performed using green fluorescent staining and confocal laser scanning microscopy. Different series of experiments were conducted by changing several parameters (growth time, rpm, growth media). Preliminary results indicate that there is a correlation between the surface morphology, and the spatial organization of the bacterial biofilm.

Our results indicate that further investigation leading to optimization of surface topology and surface chemistry will allow us to microfabricate polymer material surfaces where biofilm colonization is enhanced. Such surfaces will enable the introduction of beneficial bacteria in a variety of industrial processes including aquaculture.

[PB28] SPATIAL DISTRIBUTION, DIVERSITY, AND ACTIVITY OF MICROBIAL PHOTOTROPHS IN THE BALTIC SEA

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Microbial plankton is essential for ocean biogeochemistry. As part of the microbial community, both oxygenic phototrophs (OP) and anoxygenic phototrophs (AP) are widely distributed in the ocean and may play a significant role in carbon fixation and oxygen production. However, comparative studies of microbial OP and AP have received very little attention, despite their different roles might be important in various marine environments, especially in oxygen minimum zones (OMZ). We explored the spatial distribution of the microbial community in the Baltic Sea, including an OMZ region, with a particular focus on the distribution and activity of OP and AP. We found that specific bacterial groups dominated surface and intermediate depths, the OMZ, and deep waters, respectively. Salinity, temperature, oxygen, and depth were significant factors explaining the microbial community composition and distribution. A high diversity of OP and AP was observed, including OP-Chlorophyta, Diatoms, Cyanobacteria and Cryptomonads, and AP-Proteobacteria and Chloroflexi. OP was more abundant, as well as photosynthetically active at most stations compared to AP. OP preferred to live, and showed more photosynthetic activity, in higher salinity, warmer, oxic and upper waters, while AP did not show so many preferences, however, AP were more photosynthetically active in relatively colder and deeper waters. The Baltic Sea is exposed to multiple climate change related stressors, such as warming, decreasing salinity, and deoxygenation. This study contributes to understanding and interpretation of how microbial community, especially phototrophic groups, might shift in their distribution and activity in a changing ocean like the Baltic Sea.

[PB29] NITROUS OXIDE CYCLING IN THE EQUATORIAL ATLANTIC

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Nitrous oxide (N_2O) is a greenhouse gas in our atmosphere and contributes to the depletion of ozone within the stratosphere. The ocean plays a role as a net source which is why it is crucial to examine marine N_2O cycling to better assess the global atmospheric budget. Especially regions identified as hotspots for marine N_2O emissions such as the tropics due to occurring upwelling events. This work is the first to present an entire transect across the equatorial Atlantic to investigate the N_2O cycling during the mature phase of the equatorial upwelling. The computed investigations reveal a high degree of N_2O variability along the transect. Surface saturations range between 74.8% - 163.5% with an increasing eastward trend. The subsurface shows enhanced N_2O concentrations with an eastward gradient and a distinct maximum of 23.3 nmol L^{-1} at 400 m depth which are associated with South Atlantic Central Waters. However, due to a clear stratification and thus a restricted exchange with overlying waters, concentrations accumulated at depth and could not reach the surface. A positive correlation of AOU with N_2O identifies microbial nitrification as the predominant process for N_2O formation. Hence, the observed variability of the N_2O distribution can be explained by a combination of biological and hydrographic conditions. The presented results are in agreement with previously reported data for the tropical Atlantic measured during the off-season for upwelling events. Yet, sea-to-air flux densities are much smaller compared to the upwelling event due to a pronounced exchange with the subsurface where N_2O accumulates. Thus, this work further quantifies the seasonal and spatial variability of N_2O in the equatorial Atlantic.

[PB30] INFLUENCE OF MICROBIAL SECONDARY METABOLITES ON THE INTERACTION BETWEEN TWO MARINE BACTERIA, PSEUDOALTEROMONAS PISCICIDA AND PHAEOBACTER SP.

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Microbial secondary metabolites play a significant role in shaping microbial communities, and confers important physiological and ecological functions for the producing organism. The most renown secondary metabolites are famous for their antimicrobial activities and a common assumption is that they are produced to gain a competitive advantage over other species. Two co-existing bacteria that both produce potent secondary metabolites with antibiotic effects are bacteria from the *Phaeobacter* and *Pseudoalteromonas* genera. Several *Phaeobacter* species can produce the antibiotic tropolone tropodithietic acid (TDA) which is antagonistic against many different bacteria. Despite this, *Phaeobacter* and *Pseudoalteromonas* species can be isolated from the same environmental niche. Furthermore, preliminary experiments have indicated that the species *Pseudoalteromonas piscicida* is not sensitive to TDA and that the species *Phaeobacter* sp. is not completely inhibited by *P. piscicida*.

The purpose of this project is to determine how the two antibiotics producing species can co-exist and potentially interact trough secondary metabolite production. This is done by unraveling biosynthetic gene clusters and potential secondary metabolites that both *Pseudoalteromonas piscicida* and *Phaeobacter* sp. can produce using long-read genome sequencing technology and bioinformatics. Furthermore, we will establish an experimental setup for studying the co-cultivation of the two microorganisms and finally, we will examine secondary metabolites being produced in both mono- and mixed cultures on solid media using mass spectrometry imaging.

[PB31] AMMONIA-OXIDIZING ARCHAEA METABOLISM AT NANOMOLAR OXYGEN CONCENTRATIONS.

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Ammonia-oxidizing archaea (AOA) play a major role in the first step of nitrification, when they utilize oxygen to oxidize ammonia to nitrite. AOA inhabit a wide variety of ecosystems, including environments where oxygen is limited, for instance oxygen minimum zones in the ocean or marine hypoxic sediments, despite the fact they need oxygen to survive. It is still unknown why AOA can survive and even reach high abundances in the prokaryotic community in such environments if they need oxygen for their main metabolism.

The mechanisms behind their survival in oxygen-limited ecosystems are still unknown. To explore the ecophysiology of AOA in oxygen limitation, incubation experiments with different AOA strains were performed at nanomolar oxygen concentrations. Oxygen was measured continuously with (nanomolar range) oxygen optodes. Simultaneously, nitric oxide (NO) was also measured with microsensors. Oxygen and NO production during oxygen limitation was detected. Incubations with ^{15}N labelled isotopes were performed, and molecular nitrogen (N_2) and nitrous oxide (N_2O) coupled to oxygen production was detected through Isotope Ratio Mass Spectrometry, suggesting thy have a role in the mechanism of AOA to cope with oxygen limitation.

These novel insights are important to understand how some ammonia oxidizers can survive and be abundant in oxygen-limited ecosystems. Moreover, these observations are relevant for the better understanding of N_2O and NO production by ammonia oxidizers in oxygen-limitation.

[PB32] BACTERIA SWARMING AROUND CABLE BACTERIA IS COMMON AND CONSISTENT BEHAVIOR IN OUR FRESHWATER SEDIMENT ENRICHMENTS

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Cable bacteria are centimeters-long filamentous bacteria that oxidize sulfide in sulfidic sediment layers and reduce oxygen at the top, connecting these reactions via electron transport. In freshwater cable bacteria enrichments, we consistently observe swarming behavior around actively electron-conducting cable bacteria. We hypothesize that swarmers interact and possibly transfer electrons to cable bacteria. We aim to discover how the appearance of swarming is regulated.

For this, microsensor profiling, microscopy observations and sequencing were performed during 163 days on our in-house cultivated cores. Geochemical changes were determined by measuring pH, oxygen and sulfide over time, combined with electric potential for cable bacteria activity. Microscopy observations showed swarming behavior.

Swarming was surprisingly consistent and occurred only whenever cable bacteria were active (connected to oxygen and sulfide and conducting electrons). We also observed diverse cell morphology, demonstrating that swarming behavior was not restricted to a single bacterial clade.

In short, swarming is frequent and widespread behavior among several different groups of bacteria, suggesting a common mechanism for swarming. No measured geochemical parameter seemed to be responsible for swarming. The regulating key potentially lies in the shared mechanics, the swarmers' metabolism and interactions, rather than in the geochemistry that gets altered by cable bacteria activity.

[PB33] DO HIGH CO2 LEVELS HELP PROTECT TERMITOMYCES AGAINST ANTAGONISTS?

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Termites of the subfamily Macrotermitinae are extremely successful due to their symbiotic lifestyle with their *Termitomyces* fungal crop. The termites provide their fungus with nutrition in the form of plant material, protection as well as optimal growing conditions. In return, the fungal symbiont aids in digestion and provides nutrition to its host. The symbiotic *Termitomyces* fungus grows in monoculture despite being an excellent target for exploitation. Protecting these fungal gardens is essential for maintaining this symbiotic relationship. The termites built large and complex mound structures, which are built to enhance nest ventilation and maintain nest interior microclimates favourable for the growth of *Termitomyces*. When colonies mature, the fungal and termite metabolism increases, resulting in up to 6.4 % CO_2 concentrations within mounds. The termites themselves can tolerate high CO_2 conditions, it might negatively affect the growth of *Termitomyces* which would limit the termite's food supply and consequently the colonies growth. Using eight *Termitomyces* strains as well seven pathogenic fungal strains we compared the growth rate under ambient and high (5%) CO_2 conditions. Our results revealed that in contrast to other fungi, *Termitomyces* is generally not affected by high CO_2. This suggests that the high levels of CO_2 we see inside the nest is not an unfortunate trade-off between ventilation and humidity but instead could be an adaptive trait evolved through million years of coevolution between termites and *Termitomyces*. As CO_2 is a stressor to most fungal species, maintaining the nest at high CO_2 levels could not only provide optimal growth conditions, but also assist in defending the symbiosis against pathogens.

[PB34] ASCOM: AN ECOSYSTEM-SPECIFIC REFERENCE DATABASE FOR INCREASED TAXONOMIC RESOLUTION IN SOIL MICROBIAL PROFILING

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Intensive agricultural systems have paved the way for a growing human population, but chemical fertilizers and pesticides negatively affect nutrient cycles and biodiversity, respectively. A sustainable alternative is to harness symbiotic relationships between plants and plant growth-promoting rhizobacteria to increase nutrient uptake and provide pathogen resistance. Novel plant growth-promoting rhizobacteria can be identified using 16S rRNA gene amplicon sequencing. However, precise taxonomic classifications require a reference database with high-identity references and a comprehensive taxonomy for all sequences, including non-cultured taxa. These requirements are not satisfied in commonly used reference databases, highlighting the need for improved solutions like ecosystem-specific databases. We sequenced around 1,000,000 full-length 16S rRNA genes from Askov and Cologne reference soils and processed them using AutoTax to create an ecosystem-specific database for bacteria and archaea in agricultural soil. Here we: 1) increase classification rate of V5-V7 amplicons from Askov soil at genus- and species-level, 2) Use the full-length 16S rRNA dataset to evaluate the performance of amplicon primer pair, V5-V7, commonly used within the field, and 3) Investigate host selection of specific taxa for two legumes and two cereal species.

[PB35] UNRAVELING BIOTIC AND ABIOTIC FACTORS DETERMINING BACILLUS SUBTILIS ABUNDANCE

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Bacillus subtilis is a soil-dwelling organism that can be isolated from soil and plant roots and serves as a plant benefiting bacterium. However, the survival of *B. subtilis* and competitive success in the upper layers of field soil and plant rhizosphere in the long term is limited. Interestingly, *B. subtilis* is rarely isolated from forest soil suggesting that ecological factors determine their abundance. This difference in abundance might be caused by different plant species or due to root exudates having different influences on soil bacteria diversity, and therefore the interactions between community members also link to the abundance of *Bacillaceae*. Therefore, in this work, we aim to unravel the reasons why *B. subtilis* abundance differs between grass field and forest soil by determining the physical-chemical properties and microbial community structure of these environmental niches. Identified key abiotic factors and hub species affecting this biocontrol strain will have great potential to be translated into practical outcomes, e.g. increase *B. subtilis* abundance and improve crop production.

[PB36] MICROFLORA DANICA – THE MICROBIOME OF DENMARK

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Between 1761 and 1883 a comprehensive atlas of botany named Flora Danica was published. The atlas contains all wild plants native to Denmark. We feel the time is right to expand this atlas by addition of the Microflora Danica – a reference database of the microbes of Denmark. By sampling soil, sediment, and water across both natural and cultural habitats and by applying novel DNA-based sequencing technologies, we wish to generate an almost complete reference database of the microbiome of Denmark. Furthermore, 500 samples from the Earth Microbiome Project, has been included with the purpose of evaluation of the contribution towards illuminating the global microbial dark matter.

The purpose of the Flora Danica was to share the knowledge of botany and by doing so to obtain greater knowledge of both the useful and harmful properties of the various native plants. Similarly, the purpose of Microflora Danica is to establish a common reference database and to link the identified microbes to their functions and distributions across different habitats. Roughly 7.000 samples have been collected and 3.100 have had DNA extracted. Around 2.000 samples have been prepared for sequencing using a custom 10x shotgun miniaturization protocol and 600 has been sequenced on the NovaSeq 6000 platform. Furthermore, 130 samples have been chosen to serve as the backbone of a Denmark-specific reference database built on high-accuracy full-length 16S sequences generated on the Nanopore MinIon platform.

[PB37] MICROFLORA DANICA: SINGLE PRIMER ENRICHMENT OF BACTERIAL RRNA GENES

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The small subunit ribosomal rRNA genes (SSU rRNA) represent the gold standard in phylogenetic markers used for the characterization of microbial diversity. Conventionally, studies of these genes are subjected to primer biases and chimerism resulting in skewed and incomplete representations of the samples. Additionally, the taxonomic resolution is often limited by incomplete databases, erroneous sequences, or micro diversity. We have developed a method for enriching and sequencing the rRNA genes requiring only one target primer significantly lowering primer biases. The dependence on only a single primer sequence further allows for studies of flanking genomic regions with no prior knowledge. At the same time, our approach allows for sequencing of the complete rRNA operon and not just the 16S gene as demonstrated in a bacterial mock community from ZymoBIOMICS consisting of 8 different bacterial species with a total of 49 rRNA operons. We successfully enriched for rRNA genes with > 70 % of all reads representing different bacterial rRNA operons with an average read length of 4.5 kb. With full-length rRNA operons enabling higher taxonomic resolution and identification of rRNA copy numbers. Combing our approach with thorough UMI barcoding and long-read sequencing provides a basis for generating long and near-perfect rRNA operon sequences while drastically reducing the presence of chimeras. With a mean UMI consensus error rate below 0.01% and a chimera rate below 0.02%.

[PB38] SHAPING THE TRIPARTITE SYMBIOSIS: TERMITE MICROBIOME DIRECTED BY HORIZONTALLY ACQUIRED FUNGAL CULTIVAR

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Horizontally acquisition of coevolved symbionts presents a unique opportunity to investigate the adaptive functions. Fungus-growing termites engage in a 30 MYA obligate mutualism with a horizontally acquired fungal cultivar and gut microbes that collectively enable symbiotic digestion of plant material. The tripartite symbiosis exhibits considerable phylogenetic congruence and co-adaptation, yet it remains unclear precisely how horizontal transmission of the fungus shapes microbiome structure, function, and evolution. Here we examine the change in microbiome composition and metabolism before and after fungus acquisition the fungus-growing termite *Macrotermes natalensis*. Using 16S rRNA amplicon sequencing, we identify microbes that increase in abundance with fungus acquisition, some of which are vertically transmitted with the termite host, and transient microbes present only before that event. Further, we estimate putative differences in carbohydrate active-active enzymes with gut metagenomics and gut enzymatic activity using AZCL-polysaccharide assays. Our findings point to distinct gut microbiome composition and lack of enzymes for degradation of complex fungal carbohydrates prior to fungus comb acquisition. Thus, the horizontally acquired mutualist likely drives dynamic shifts in bacterial contributions over the ontogeny of the symbiosis. Ultimately, fungus acquisition shapes persistent and coevolved microbiome structure and function, and the contrast in horizontal and vertical transmission of the gut microbes potentiates further co-adaptation, cooperation, and conflict within the symbiosis.

[PB39] A DOMESTICATED FUNGAL CULTIVAR RECYCLES ITS CELLULAR CONTENTS AS NUTRITIONAL REWARDS FOR ITS LEAFCUTTER ANT FARMERS

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The rise of domesticated agriculture by humans ca. 10000 years ago was a turning point for our species in terms of physiological adaptations. Fascinatingly, humans are not the only farmers to have achieved domesticated farming systems. For instance, leafcutter ants evolved systems of fungus cultivation, with derived strategies of planting, provisioning, protection, and consumption. These ant farmers provide their fungal cultivar with fresh plant material, and the cultivar produces nutritional reward structures unique among all fungi—swollen hyphal tips called gongylidia. The molecular and cellular mechanisms of gongylidia formation are currently poorly understood. Microscopic imaging approaches of gongylidia ultrastructure and cellular makeup, suggested that gongylidia form by a process of autophagy whereby the growing gongylidia cells digests its own organelles and cytosol and repurposes them as food for the ant farmers. We next used an *in vitro* experiment to test whether autophagy mediates gongylidia formation. After 6 weeks of incubation, gongylidia production was lower in the presence of two autophagy inhibitors (chloroquine, 3-MA) compared to when it was provided an autophagy promotor (rapamycin) or grown in control conditions. These results suggest that an autophagic process was harnessed by ant farmers to produce a nutritionally optimized crop.

[PB40] THE KEY TO A GOOD RELATIONSHIP : INTER-KINGDOM COMMUNICATION IN A FUNGAL-ANT NUTRITIONAL SYMBIOSIS

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Communication is fundamental to a good relationship, regardless of the interacting partners. The fungal symbiont of the attine ants is provisioned with substrates of a range of nutritional qualities by its ant hosts. However, in order to maximise its performance, the fungus requires a very specific nutritional blend. For the fungus, communicating these nutritional needs to the superorganismal, decentralised, ant colony presents a challenge. However, the ants are able to forage and provision their fungus within very specific nutritional parameters. Exactly how the fungus can communicate its nutritional needs to the ant foragers, who do not interact directly with the fungus, is unclear. Here, we combine metabolomic and transcriptomic approaches to test the hypothesis that the expression profile of fungal signalling compounds, in particular surface hydrocarbons and fatty acids, changes with the nutritional composition of provisioned diets. We predict that these variable compounds from the surface of the fungus are transferred to the ants' cuticle, and that a suite of hydrocarbons and fatty acids can mediate subsequent provisioning behaviours in the ants. These results will assist in elucidating the pathways by which the fungus communicates its nutritional needs to its symbiotic partners.

[PB41] MICROFLORA DANICA - ESTABLISHING A PIPELINE FOR FUNCTIONAL CHARACTERISATION AND TAXONOMIC CLASSIFICATION OF 10 000 METAGENOMES

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Estimates suggest that more than 90% of microbes are yet to be discovered and constitute what is popularly called the microbial dark matter. Microflora Danica is an ambitious project that, amongst other things, seeks to generate 10,000 metagenomes from across Danish habitats and thus contribute to unraveling the microbial dark matter.

This sub-project assessed bioinformatic methods for taxonomic classification and antibiotic resistance gene mining for use in the microflora Danica project. Kraken2, GraftM and Core Kaiju were evaluated as possible tools for taxonomic classification while DeepARG and minimap2 mappings to CARD were evaluated as possible antibiotic resistance gene mining methods. Fourteen wastewater treatment plant metagenomes were used for preliminary investigations of the tools, and further 283 Microflora Danica metagenomes from various habitats, were used to assess all tools. GraftM, which utilizes metagenomics reads originating from 16S rRNA genes, was evaluated as the most optimal tool for taxonomic classification, while no tool for antibiotic resistance gene mining was evaluated as sufficient for use on 10 000 microflora Danica metagenomes. Both DeepARG and minimap2 mappings to CARD revealed spurious mappings, noticeably to the rpoB2 gene.

[PB42] DISCOVERY OF MUTATIONAL LANDSCAPES IN EVOLVING BACTERIAL POPULATIONS

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Experimental evolution provides a powerful tool to determine how living creatures adapt to certain conditions, while subsequent genome sequencing allows studying the genetic bases behind adaptation in these experiments. Microbial biofilms are tightly packed, heterogeneous structures that serve as arenas for social interactions, these spatially structured environments have been recognized as appropriate models to study microbial growth and interactions. In this project, we combined metagenome sequencing and bio-informatic methods to reveal the mutational trajectories of two Gram-positive bacteria, *Bacillus subtilis* and *Bacillus thuringiensis*, under different selection conditions, including evolution during pellicle biofilm, plant root colonization, plastic bead re-colonization, and in co-culture with or without fungi.

We have sequenced time series population samples at ultra-high depth (>200X) from parallel evolving linages and identified mutations in each population using a bioinformatic method. After filtering, the mutational spectrum of the different species and different selection conditions have been compared to reveal the significant differences among the distinct selection pressures. Genealogy and genotype frequencies analysis are also performed and the results will be used to reveal (1) what kind of mutation appeared and disappeared in each experimental condition; (2) how do different populations evolve; (3) what is the repeatability of mutations among the different parallel populations. The knowledge from our project would help us to understand the molecular basis of evolutionary adaptation in a more comprehensive, in-depth level in a higher temporal scale.

[PB43] MYCOVIRUSES: A HITHERTO UNKNOWN MEMBER OF THE SOIL FOOD WEB

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Viruses are found everywhere and infect all living organisms, including soil fungi. However, the leading view is that viruses infecting fungi (mycoviruses) are only transmitted intracellularly and consequently cause mild or no effects on the fungal host. This project proposes to challenge this view. Our hypothesis is that extracellular mycoviruses exist and are capable of killing the fungal host. As such, the project has two main objectives: to determine the abundance and genetic diversity of extracellular mycoviruses in a variety of soils (agricultural, grassland and forest), and to isolate, identify and characterize mycoviruses that can infect and kill soil fungi. Viral abundances in the different soils during the spring were found in the range between 1.9 and 2.9 $x10^{10}$ virus like particles per g^{-1} of soil, and over 140 fungal strains have already been isolated. At the end of the project, we expect to establish a new conceptual view of mycoviral infections in soils.

[PB44] TOTALRNA SOIL METATRANSCRIPTOMICS: SIMULTANEOUS ANALYSIS OF THE ENTIRE ACTIVE SOIL COMMUNITY AND ITS FUNCTIONS.

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The rhizosphere microbiome consists of prokaryotic and microeukaryotic (e.g., protozoa, fungi) organisms. The microbiome is involved in nutrient cycling, carbon sequestration, plant health and plant growth promotion. At the interface between plant root and bulk soil, the rhizosphere is influenced by a plethora of biotic and abiotic factors, resulting in a diverse and complex biome. Understanding how different factors affect the rhizosphere community is crucial to predict effects on plant phenotype and yield. Most soil microbiome studies rely on amplicon sequencing and very few on shotgun metagenomics. However, both methods show considerable weaknesses, either due to low resolution or very high costs, but most importantly they fail to distinguish between active and inactive cells. TotalRNA soil metatranscriptomics provides a solution by targeting only the active microbial community, while at the same time avoiding PCR biases, assembling complete rRNA genes, covering all Kingdoms simultaneously, and revealing functions (mRNA). We have developed an optimized protocol from sampling, RNA extraction to sequencing and analysis by using four commercial TotalRNA extraction kits and decreasing sequencing depths. Our results show that 200 mg of freeze-dried soil extracted with the NucleoBond RNA Soil Mini kit can yield high concentrations of relatively intact RNA (3.5<RIN<6) while most of the diversity in all Kingdoms can be captured already at 5% sequencing depth (20 Gb 2x150bp Illumina reads). These findings are particularly important when working with minute amounts of staring material, like rhizosphere samples from mesocosms. Our bioinformatics pipeline is modular and can accommodate better algorithms as they are being released.

[PB45] HOW WELL IS THE GLACIER ALGAE ADAPTED TO LIFE ON THE GREENLAND ICE SHEET?

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The surface of the Greenland Ice Sheet is a harsh environment for most life forms, because of the low temperatures and nutrient concentrations, high irradiation, and repeating freeze-thaw cycles. Nevertheless, it is home to the pigmented algae *Ancylonema alaskana* and *Ancylonema nordenskioldii*. These glacier algae contain a pigment called purpurogallin carboxylic acid-6-O-β-d-glucopyranoside, which has a deep purple/brown color and absorbs light at both visible and UV wavelengths. The glacier algae are significantly contributing to the darkening of the ice in Greenland due to this dark pigmentation, which lowers the albedo of the ice surface, increases the adsorption of solar radiation and increases melting. Basic knowledge about growth rates and growth conditions of the glacier algae is not known, in part due to the lack of stable laboratory cultures. We performed a range of *in situ* experiments during the summer 2021 in order to test the growth conditions of glacier algae under a range of physico-chemical conditions. Therefore, the natural microbial community on the ice surface (*i.e.*, glacier algae and their associated microbial community) was incubated under different conditions, including variations in pH, visible and UV light, salt concentration, and temperature. Samples for reactive oxygen species (ROS) and ROS scavenging enzymes were collected from each incubation experiment to study responses to the different conditions. Furthermore, pulse-amplitude-modulated fluorometry was performed, giving insight into the photosynthetic performance of glacier algae under each condition. This study provides insight in adaptation of the glacier algae and microbial community to life on the surface of the Greenland Ice Sheet.

[PB46] DEVELOPING NEW BIOINFORMATIC METHODS TO SUPERCHARGE GENOME-CENTRIC METAGENOMICS USING MACHINE LEARNING

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Microbes are everywhere and play important roles in most aspects of life and an important part of complex microbial community investigation is the extraction of single organism genomes. The maturation of metagenomic binning techniques has greatly increased the quality of metagenomic assembled genomes, by utilizing features such as sequence coverage and K-mer frequencies. However, challenges remain with these approaches. K-mer frequencies depend on long contigs for stabilisation and sequence coverage information can be biased by high copy number sequences. The nanopore sequencing platform, which is already an often integrated step in the metagenomic analysis, produces information rich data containing information on the possible methylation of DNA bases. Methylation represents a powerful feature, as the DNA modification depends on the state of the methylome of the organism. Here we explore incorporation of methylation modification as a feature into metagenomic binning using machine learning to complement challenges inherent in sequence centric binning features.

[PB47] HUNDREDS OF VIRAL FAMILIES IN THE HEALTHY INFANT GUT

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The gut microbiome (GM) is shaped through infancy and plays a major role in determining susceptibility to chronic inflammatory diseases later in life. Bacteriophages (phages) are known to modulate bacterial populations in numerous ecosystems, including the gut. However, virome data is difficult to analyse because it mostly consists of unknown viruses, i.e. viral dark matter. Here, we manually resolved the viral dark matter in the largest human virome study published to date. Fecal viromes from a cohort of 647 infants at 1 year of age were deeply sequenced and analysed through successive rounds of clustering and curation. We uncovered more than ten thousand viral species distributed over 248 viral families falling within 17 viral order-level clades. Most of the defined viral families and orders were novel and belonged to the Caudoviricetes viral class. Bacterial hosts were predicted for 79% of the viral species using CRISPR spacers, including those in metagenomes from the same fecal samples. While Bacteroides-infecting Crassphages were present, novel viral families were more predominant, including phages infecting Clostridiales and Bifidobacterium. Phage lifestyles were determined for more than three thousand caudoviral species. Lifestyles were homogeneous at the family level for 149 Caudoviricetes families, including 32 families that were found to be virulent, while 117 were temperate. Virulent phage families were more abundant but temperate ones were more diverse and widespread. Together, the viral families found in this study represent a major expansion of existing bacteriophage taxonomy.

[PB48] ACTIVATION AND IDENTIFICATION OF A GRISEUSIN CLUSTER IN STREPTOMYCES SP. CA-256286 BY EMPLOYING TRANSCRIPTIONAL REGULATORS AND MULTI-OMICS METHODS

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Activation and identification of a griseusin cluster in *Streptomyces* sp. CA-256286 by employing transcriptional regulators and multi-omics methods

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Streptomyces are well-known producers of antibiotics and other bioactive compounds, including secondary metabolites belonging to the type II polyketides. Recently, it has been demonstrated that “silent” biosynthetic gene clusters (BGCs) can be activated by overexpressing transcriptional regulators. Here, we have activated a silent BGC in *Streptomyces* sp. CA-256286 by overexpression of a set of SARP family transcriptional regulators. The structure of the produced compound was elucidated by NMR and found to be a *N*-acetyl cysteine adduct of 3´-O-α-D-forosaminyl-(+)-griseusin A, a new derivative of the known pyranonaphtoquinone polyketide. Employing a combination of multi-omics and metabolic engineering techniques, we identified the responsible BGC. These include genome mining, proteomics and transcriptomics analyses, in combination with CRISPR induced gene inactivations and expression of the BGC in a heterologous host strain. This work demonstrates an easy to implement workflow on how silent BGCs can be activated, followed by the identification and characterization of the produced compound, the responsible BGC and its biosynthetic pathway.

[PB49] VIRAL SIGNATURES ON THE GREENLAND ICE SHEET

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Dark pigmented glacier and snow algae present on the bare ice and snowpack of the Greenland Ice Sheet (GrIS) accelerates the ice melting. Glacier algal blooms are dominated by the species *Ancylonema alaskana* and *A. nordenskioeldii*, while red snow patches are dominated by *Chloromonas* spp. and *Chlamydomonas* spp. Very little is known about the biological controls on glacier algae and the potential for viruses to act as a top-down control for glacier algal blooms is not known. Giant viruses, belonging to the nucleocytoplasmatic large DNA viruses (NCLDV) supergroup, have recently become of great interest due to their large genome and virion size. Their presence and role in icy habitats still need to be verified, despite their global distribution and diversity.
To recover signatures of NCLDVs and assess their diversity on the GrIS, samples of dark ice and red snow were collected during the 2019 and 2020 summer seasons and sequenced using metagenomic and metatranscriptomic approaches. Genomes of six relevant snow and glacier algal culture species were also analyzed. The omic data highlighted that all the samples contained sequences of ten different NCLDVs hallmark genes (e.g. MCP, polB). The recovery of conserved core genes in the metagenomic data confirmed the distribution of giant viruses in both ice and snow habitats, while their presence in algal genomes suggested a process of integration or endogenization of such genes. Phylogenetic analyses uncovered that genomic, metagenomic and metatranscriptomic sequences clustered together with known NCLDV families. The recovery of transcribed viral genes, clustering with sequences from the corresponding metagenomes, is a possible indicator of the role of viruses in controlling glacier algal blooms.

[PB50] THE DIVERSITY AND ACTIVITY POTENTIAL OF THE AIR AND SNOW MICROBIOMES IN THE HIGH ARCTIC, AND HOW THEY ARE LINKED THROUGH BIOPRECIPITATION

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An important factor for the high warming rate of the Arctic is the type and level of cloud cover. An increasing number of studies points towards the involvement of microorganisms in the formation of cloud and precipitation, thus impacting the Arctic climate. We studied 18 air samples in a time series spanning a month and 10 snow samples collected in a transect of ~50 km after a snowstorm in the spring of 2015 together with 7 consecutive air samples collected in the summer of 2016, from Northeast Greenland. 16S rRNA amplicon sequencing of DNA and cDNA together with qPCR were utilized to process the samples. We found that the bacterial abundance is higher in the spring air (2015) compared to snow (2015) and summer air (2016). Alpha diversity metrics were similar across the two seasons, although more ASVs were observed in the snow samples. Beta-diversity analysis revealed a clear separation between the seasonal air microbiomes as well as the snow microbiome. The air sample collected just before the snowstorm occurred had a more similar community composition with the snow microbiome than with the other air samples. 134 ASVs (~35%) were shared between the air sample and the snow samples consisting of the 4 genera *Hymenobacter, Nocardioides, Gemmatimonas* and *Abditibacterium.* The Cyanobacteria had the highest activity potential, both in the air sample on the day of the snowstorm, as well as in the snow. After the snowstorm, we were not able to detect airborne bacteria for the next 3 weeks, which we suggest was due to a washout of the microbial community from the air into the snow.
These results show that the air and snow microbiome are dynamic, in abundance and community composition during the seasons and how succession of specific microbial taxa can occur in the high Arctic

[PB51] APPLICATION OF A NOVEL IN SITU CULTURING APPROACH ON THE GREENLAND ICE SHEET

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Only a very small fraction of microbial life has so far been cultured in the laboratory. Known as microbial dark matter, this problem limits the study of the diversity of microbial life. Novel culturing methods are being developed to tackle this problem. For instance, microbes can be grown in small culture chambers, separated from their natural environment by a semi-permeable membrane, allowing passage of growth factors and nutrients into the chamber. Compared to conventional methods, this *in situ* method can bring more isolates into culture. In this study, *in situ* incubation is employed during fieldwork on the Greenland Ice Sheet, targeting the microbial communities of ice surface habitats. The device developed for this purpose uses an array of miniature growth chambers, filled with a solid medium inoculated with samples taken from cryoconite sediment and surface ice, and sealed on both sides by a semi-permeable membrane. After assembly, they were placed on the ice surface and in a cryoconite hole, depending on inoculum. To our knowledge, while similar approaches have been used in soil and marine environments, this is the first time it is applied in a glacial setting. To evaluate the use of *in situ* culturing in a cold environment, a comparison to conventional culturing methods, like plating on solid medium, is made. For this purpose, a culture collection derived of isolates acquired through this method is currently being set up.

[PB52] FINDING A NEEDLE IN A WAXSTACK: TRACKING DOWN PAENIBACILLUS LARVAE IN MODERN AND ANCIENT BEESWAX SAMPLES

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Paenibacillus larvae is the causative agent of American Foulbrood, the most destructive bacterial disease in honeybees. The analysis of DNA from both modern and ancient beeswax samples provides an opportunity to untangle the evolutionary history of honeybee-associated microbes, including *P. larvae*. DNA extracted from ancient and modern beeswax samples was sequenced and the resulting datasets were screened for the presence of *P. larvae*. Contigs were assembled from positive datasets and contig binning was subsequently applied to obtain draft genomes of *P. larvae*. Finally, evolutionary relationships with reference modern *P. larvae* genomes were ascertained through the analysis of the species' persistent genome. *P. larvae* was detected in three of the modern samples and one of the ancient samples, with varying degrees of breadth and depth of genome coverage. High-quality draft genomes were recovered from two of the positive modern samples, and phylogenetic analysis of *P. larvae*'s persistent genome suggested that these genomes are more closely related to the subspecies *larvae* than the subspecies *pulvifaciens*. Our results demonstrated that the analysis of DNA extracted from beeswax is suitable for the recovery of high-quality microbial genomes. Therefore, DNA extracted from beeswax is a valuable source of information that could be exploited for evolutionary and epidemiological studies of relevant honeybee pathogens.

[PB53] VISUALIZATION OF THE INTERNAL PH IN BACTERIAL COLONIES USING A GENETICALLY ENCODED SENSOR PROTEIN AS NOVEL TOOL FOR HIGH-THROUGHPUT SCREENINGS

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Cytoplasmic pH is tightly regulated by diverse active mechanisms and interconnected regulatory processes in bacteria. Many processes and regulators underlying pH-homeostasis have been identified *via* phenotypic screening of strain libraries towards non-growth at low or high pH values. Direct screens with respect to changes of the internal pH in mutant strain collections are limited by laborious methods. Genetically encoded biosensors allow to equip strain libraries with an internal sensor molecule during the generation of the strain. We here used the pH-sensitive mCherry variant mCherryEA as biosensor and visualised the internal pH of *E. coli* colonies on agar plates in a Gel-Doc Imaging System. Combining this imaging technology with robot-assisted colony picking and spotting allowed us to screen and select mutants with altered internal pH values from a stransposon mutagenesis derived *E. coli* library. Identification of the TN insertion sites in strains with altered internal pH revealed that the transposon was inserted into *trkH* (encoding a transmembrane protein of the potassium uptake system) or the *rssB* gene (encoding the anti-adaptor protein RssB), two genes known to be associated with pH-homeostasis and pH stress adaptation. This successful screening approach demonstrates that sensor-based analysis of arrayed colonies on agar plates is a sensitive approach for the fast identification of genes involved in pH-homeostasis or pH stress adaption in *E. coli*.

[PB54] LARGE-SCALE IDENTIFICATION OF PHAGE-INDUCIBLE CHROMOSOMAL ISLANDS

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Phage-inducible Chromosomal Islands (PICI) are a type of hyperparasitic mobile genetic elements that hijack phages to propagate and horizontally transfer into new bacterial hosts. Their life cycle involves parazitising their helper phages, they are important players in phage evolution and ecology.These conserved elements, with a distinct genetic architecture, have been recently identified and characterized in both Gram-positive and Gram-negative bacteria. Here we developed a software pipeline to identify PICIs and type them via their marker gene contents.

We first benchmark the software by identifying experimentally-validated PICIs, after which we apply it to large data-sets to find PICIs in putative hosts in an attempt to expand the range of possible PICI hosts by leveraging the complete genomes from the RefSeq database. In doing so, we found 574 PICIs in E*. coli* and 381 in *Staphyloccocus*, a vast expansion of PICIs in known hosts. We have examined isolate databases and found PICIs in novel *Escherischia* hosts

This newly developed software can detect PICIs and substantially expand the range of known PICIs and hosts; we aim to refine this tool further to identify other types of phage satellites in bacteria.

[PB55] REWIRING METABOLIC FLUX FOR EFFICIENT PRODUCTION OF AROMATIC AMINO ACIDS AND DERIVATIVES FROM LIGNOCELLULOSIC BIOMASS.

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Lignocellulosic biorefineries is a powerful emerging manufacturing process that is expected to play a major role in the transition from a petrochemical-based to a sustainable society. However, valorization of lignocellulosic biomass is impeded by biomass recalcitrance and the presence of inhibitory compounds after physico-chemical pretreatment of the biomass. In this study we have combined cutting-edge processes for pretreatment of complex lignocellulosic biomass with advanced synthetic biology tools to facilitate efficient production of aromatic amino acids, and their derivatives from the cellulosic part (C6) of lignocellulosic biomass.

To speed up the generation of the multiple genomic alterations needed to increase the flux towards aromatic amino acid production, we first established an improved and rapid method that allows for the creation of multiple markerless gene deletions in various *E. coli* strains. Subsequent gene deletions, combined with adaptive laboratory evolution, and heterologous overexpression of selected pathway genes enabled us to create strains that produced aromatic amino acids from second generation biomass at similar titers, rates, and yields as compared to production from refined glucose. We are currently optimizing these chassis strains for the efficient and sustainable production of various higher value-added derivatives.

[PB56] ISOLATION, CHARACTERIZATION AND GENOMES OF NOVEL YEASTS FROM THE INTESTINAL TRACT OF TERMITES

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Bioconversion of hemicelluloses into simpler sugars leads to production of a significant amount of pentose sugars, such as D-xylose. However, efficient utilization of pentoses by conventional yeast production strains remains challenging, especially due to inhibition by hexose co-fermentation. Wild yeast strains isolated from guts of termites may produce degrative enzymes that target cellulose and hemicellulose in termites. Those yeasts can provide new industrially relevant characteristics to bypass these inhibitions and efficiently utilize pentose sugars. To explore this strategy, we isolated gut-residing yeasts from the termite *Macrotermes bellicosus* collected in Côte d´Ivoire. The yeasts were classified through their ITS and LSU gene, and genomes were sequenced and annotated. We identified a novel yeast species, which we name *Barnettozyma botsteinii* sp. nov. 1118^T and two new strains of *Kurtzmaniella quercitrusa*: var. nov. 1112 and 1120. The two *K. quercitrusa* strains grow 15% faster on synthetic glucose medium than *Saccharomyces cerevisiae* CEN.PK^T in acidic conditions and both strains grow on D-xylose as the sole carbon source at a rate of 0.35 h^{-1}. At neutral pH, the yeast form *of K. quercitrusa* 1120 switched to filamentous growth in a carbon source-dependent manner, revealing phenotypic diversity among strains within species. Our findings increase the understanding of microbial diversity within fungus-farming termite guts. The use of plant-derived sugars by *K. quercitrusa* shows a mutualistic relationship between this yeast and host termites. *K. quercitrusa* 1120 also has potential as a production organism for its capacity to grow at low pH and to undergo a dimorphic shift.

# These authors contributed equally to this work

[PB57] TRANSPORT OF MICROORGANISMS VIA WORK CLOTHES - DO WE TAKE MICROORGANISMS WITH US?

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The aim of the study is to obtain knowledge on whether work clothes can act as a vector for microorganisms and thus lead to secondary exposure. We have investigated if microorganisms accumulate on workers' clothes in environments with elevated microbial exposures, and whether these microorganisms are transported with clothes and subsequently resuspended to inhalable air. In order to study this, we investigated the potential transport of bacteria and fungi via waste workers' work clothes to the truck cabs of waste collection trucks (an indoor environment), and we compared the microbial communities within the air of the truck cabs, the workers' personal exposures and their clothes. The results show that microorganisms accumulate in large quantities on the workers' clothes (GM = 3.69×10^5 CFU/m^2/h for bacteria, GM = 8.29×10^4 CFU/m^2/h for fungi). The concentrations and species communities of airborne fungi in the air of truck cabs correlated significantly with that of the clothes, and these also correlated with the personal exposures (a trend). However, the same patterns were not found for bacteria, which indicates that work clothes to a lesser degree act as a vector for bacteria compared to fungi. We conclude that the large accumulation of microorganisms on work clothes combined with the overlap of fungal species between different sample types provides a basis for the encouragement of good clothing hygiene during as well as post working hours.