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Abstracts

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cmfi Cluster of Excellence
Controlling Microbes to Fight Infections

Invited lectures

IT 03

Gut microbiome in the development and type 2 diabetes

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The microbial ecosystem, microbiota, of the human gut consists of trillions of bacteria and other microorganisms and recent data have demonstrated that an altered gut microbiota can be associated with a number of diseases, ranging from obesity and inflammatory diseases to behavioral abnormalities. We and others have analyzed the gut microbiome in different cardiometabolic diseases and observed that they often are associated with reduced bacterial diversity. In addition, we observed that type 2 diabetes is associated with reduced abundance of butyrate producing bacteria. However, it has become clear that common medications affect the composition of the microbiota and in an effort to further investigate the association between the gut microbiota and type 2 diabetes we recruited a large cohort of diabetes treatment naïve individuals and assessed the microbiota of individuals with prediabetes and screen-detected type 2 diabetes. We observed that the microbiota starts to change in prediabetes, independent of medications, and developed a model to identify individuals with type 2 diabetes based on the gut microbiota. To move beyond associations, we also address if the gut microbiota contributes to disease by transplanting the gut microbiota to germ-free mice using a new model for studying type 2 diabetes.

Next, we set out to isolate bacteria associated with normal glucose metabolism, which are reduced in type 2 diabetes. We observed that *Faeclibacterium prausnitzii*, which is a butyrate producing bacteria with anti-inflammatory properties, was co-isolated with *Desulfovibrio piger* that increased the biomass and butyrate production from *F. prausnitzii*. Thereafter, we developed a process to obtain oxygen-tolerant *F. prausnitzii* without compromising butyrate production and anti-inflammatory properties. Finally, we confirmed that the bacteria are safe for human consumption and thus may constitute a next generation probiotic to improve glucose metabolism in individuals with impaired glucose metabolism.

IT 10

Transkingdom signalling *via Legionella pneumophila* small regulatory RNAs and extracellular vesicles

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Legionella pneumophila is an intracellular, bacterial pathogen that can cause a severe form of pneumonia in humans, a phenotype evolved through interactions with aquatic protozoa, in which it replicates in the environment. It has been shown that many of the over 300 *L. pneumophila* protein effectors secreted by the Dot/Icm type IV secretion system mimic eukaryotic functions allowing the pathogen to modulate different host cell functions. Here, we reveal that *L. pneumophila* can also mimic eukaryotic microRNAs (miRNAs) that are key in regulating eukaryotic gene expression. We show that *L. pneumophila* uses extracellular vesicles to translocate at least two bacterial small RNAs (sRNAs) into host cells that mimic specific eukaryotic miRNAs. We reveal that these bacterial sRNAs are bacterial trans-kingdom regulatory RNAs acting in a miRNA-like manner with multiple functions. This direct miRNA-like regulation of host cell signalling pathways is a remarkable but previously unrecognised feature of *L. pneumophila* host-pathogen communication and likely represents a general mechanism employed by bacteria that interact with eukaryotic hosts.

IT 04

The consequences of drugging the gut microbiome – from the individual microbe to the host

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Over the past two decades, a fundamental role of the gut microbiome in host physiology and pathology has been demonstrated. Alterations in the microbiome signature have been associated with increased risk of a variety of different diseases, ranging from infections to diverse non-communicable diseases. Recently, drugs have emerged as one of the most effective modulators of gut microbiome composition. For the vast majority of these drugs that affect the composition of the microbiome, the corresponding targets in these microbes (if any) are still unknown. It is also unclear whether their effects on microbial growth are part of their mode of action and whether they are responsible for the side effects observed in humans. A detailed understanding of these interactions will lead to an improvement in the efficacy of current therapies and the development of new drugs for targeted interventions in the gut microbiome.

In my talk, I will present our latest findings on drug-host-microbiome interactions. Specifically, I will highlight examples of how we have incorporated advanced high-throughput screening platforms for anaerobic bacteria, bacterial genetics and gnotobiotic animal models to explore the effects of drugs on microbial communities. Furthermore, I will delve into the implications of such interactions on the host.

IT 02

A probiotic to prevent *Staphylococcus aureus* infection?

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Probiotic nutrition is frequently claimed to improve human health. However, underlying effects remain poorly understood. Furthermore, probiotic bacteria are often proposed for pathogen control in so-called "microbiome editing" approaches, but these strategies – often based on bacteriocin production – are rarely specific enough to target particular pathogens without undesired effects on the human microbiome. Infections by the important human pathogen *Staphylococcus aureus* stem from asymptomatic colonization and, given increasing problems to treat infections due to antibiotic resistance, decolonization is often proposed to prevent *S. aureus* infections. However, traditional *S. aureus* decolonization protocols, usually targeted at the nose, had mixed results. They are prone to recolonization from other body sites and, being achieved with general antiseptics or antibiotics, lead to total microbiome eradication and pronounced side effects.

We observed that intestinal colonization by *Bacillus* bacteria in a rural Thai population, which is transient and likely stems from repeated oral intake of unwashed vegetables, was negatively correlated with intestinal presence of *Staphylococcus aureus*. As underlying mechanism, we identified a quorum-quenching effect of a widespread class of *Bacillus* lipopeptides, the fengycins, on the *S. aureus* Agr quorum-sensing system. We demonstrated that fengycin and fengycin-producing *Bacillus*, but not isogenic fengycin biosynthesis mutants, inhibited Agr, which we also discovered is essential for *S. aureus* intestinal colonization, resulting in virtual elimination of *S. aureus* from the intestine in a mouse model. Furthermore, in a placebo-controlled human trial, oral administration of probiotic *Bacillus* spores resulted in significant reduction of *S. aureus* in stool (96·8%; $p<0.0001$) and nose (65·4%; $p=0.0002$). There were no differences in adverse effects or significant microbiome changes between the intervention and placebo groups.

Our studies present a pathogen-specific microbiome editing approach to decolonize the human body from *S. aureus* without side effects with an estimated efficiency of > 95% reduction of total *S. aureus* in the human body. Furthermore, our findings provide evidence that supports the biological significance of probiotic bacterial interference in humans. Moreover, our results indicate a hitherto overlooked pivotal role of the intestinal site for *S. aureus* colonization of the human body that should be considered in future *S. aureus* decolonization strategies.

IT 01

Resurrecting the intestinal microbiota to combat antibiotic-resistant pathogens

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A functional intestinal microbiota is composed of diverse obligate and facultative anaerobic bacterial populations that provide colonization resistance against antibiotic resistant pathobionts causing infections in hospitalized patients. Loss of microbiota diversity is common in patients hospitalized for liver disease, sepsis/respiratory failure or organ transplantation and is associated with systemic infections caused by expansion of pathobionts residing in the intestine. Targeted fecal metabolite measurements, including quantitation of short chain fatty acids and secondary bile acid variants, complement metagenomic sequencing approaches to identify compositional dysbiosis by providing a more nuanced view of the microbiota"s metabolic activities. Reduced fecal concentrations of microbially derived or modified metabolites are associated with a high risk of infection in hospitalized patients. Whether reconstitution of the intestinal microbiota with selected commensal bacterial strains can re-establish beneficial fecal metabolite concentrations, reduce the incidence of infection and improve clinical outcomes remains unknown. With the goal of reconstituting the microbiota of patients with profound dysbiosis, we have identified and isolated over 1,600 commensal bacterial strains from healthy human donors that produce metabolites that modulate host immune defenses or strengthen the mucosal epithelial barrier. We have tested commensal bacterial strains in pre-clinical models for their ability to colonize the gut and correct metabolite deficiencies associated with increased risk of infection. Consortia of commensal bacterial strains are being assembled and tested in gnotobiotic mouse models for cooperativity and metabolite production in preparation for clinical trials in patients with profound compositional and metabolic microbiota deficiencies.

IT 07

Sugar-coating mucosal vaccines

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How can we induce intestinal immune responses against bacterial surface glycans? And why would we want to? Many pathogenic *E. coli* produce two different surface glycolipids: a lipidA-linked O-antigen (Lipopolysaccharide or lipooligosaccharide) and a capsular polysaccharide. While O-antigens are highly immunogenic and their interactions with secretory IgA have been extensively studied, *E. coli* capsular polysaccharides have remained elusive. We will discuss the role of *E. coli* capsular polysaccharides in the gut, new tools for classifying and studying these structures and a novel class of glycoconjugate vaccines that provides hope in the battle against these increasingly multi-drug resistant pathogens.

IT 11

Dissecting the impact of malnutrition and dehydration on nosocomial infections

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Antimicrobial resistant (AMR) bacterial infections are one of the greatest threats to hospitalized patients. However, studying these infections in animal models is difficult as conventional animals are highly resistant to nosocomial pathogens. Dehydration and malnutrition are common and often underdiagnosed in hospital settings, but the effect of temporal dietary and water restriction (DWR) on susceptibility to nosocomial pathogens is unknown. I will present our new work exploring how DWR influences infection dynamics. We have discovered that DWR dramatically increased susceptibility to systemic infection by ESKAPE pathogens. Using a murine bloodstream model of methicillin resistant *Staphylococcus aureus* (MRSA) infection, we have found that DWR leads to significantly increased mortality and morbidity. DWR causes increased bacterial burden, severe pathology, and increased numbers of phagocytes in the kidney. Mechanistically, we found that DWR impairs the ability of macrophages to phagocytose MRSA and efferocytose apoptotic neutrophils. Together, this work starts to dissect the impact that diet and hydration play in protecting the host against infection.

Flash talks

FT 20

Radiolabeled antibody for translational specific PET imaging of invasive infections: the example of Aspergillosis.

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Introduction

Invasive pulmonary aspergillosis (IPA) is a deadly lung infection of immunocompromised patients resulting from inhalation of the opportunistic fungal pathogen *Aspergillus fumigatus.* Current diagnosis methods are inadequate for early diagnosis and often rely on invasive procedures. As a result, the administration of adequate antifungals is often delayed, worsening prognosis[1]. A non-invasive imaging approach allowing for unambiguous detection of the fungus and its response to treatment is thus needed.

Objectives

We aimed at improving the currently available *in vivo* IPA detection methods by capitalizing on the specificity of a *A. fumigatus* specific antibody which, after radiolabeling, could lead to the accurate monitoring of the progression of IPA using Positron Emission Tomography (PET).

Materials and Methods

The *A. fumigatus* specific antibody hJF5 was radiolabeled with ^{64}Cu (^{64}Cu-hJF5) and a fluorophore, and injected intra-venously (12.5 MBq) in neutropenic mice infected intra-tracheally with 4.10^6 *A. fumigatus* spores. Animals were imaged *in vivo* in a PET/MRI system 48h after injection and ^{64}Cu-hJF5 uptake quantified in percentage injected dose per cubic centimeter (%ID/cc). For therapy monitoring experiments, Voriconazole was used. *Ex vivo* validation of animal experiments was performed by *ex vivo* biodistribution, Light Sheet Fluorescence Microscopy (LSFM) and histology. Lastly, ^{64}Cu-hJF5 was evaluated in a limited group of patients with Leukemia and a fever of unknown origin with suspicion of IPA.

Results

In a murine model of IPA, we could clearly show ^{64}Cu-hJF5 preferential accumulation in the infected regions of the lungs by PET imaging, achieving a contrast ratio of ~2 between infected regions and healthy regions. Administration of Voriconazole early during infection was efficient in limiting disease progression, as reported by ^{64}Cu-hJF5. Moreover, LSFM evaluation showed that the antibody could unambiguously report on all infection sites, validating the diagnosis and therapy monitoring potential of the approach[2]. In first-in human experiments, ^{64}Cu-hJF5 accumulated in Leukemia patients presenting IPA confirmed by further *in vitro* assays[3].

Conclusion

Here, we show through the example of IPA, how the development of a radiolabeled antibody specific to a pathogen can enable, after radiolabeling, unambiguous diagnosis of an infectious disease *in vivo* without the need for invasive methods. Furthermore, as the radiolabeled antibody recognizes all infection sites, it also accurately reports on therapy efficiency in real time. First in human experiments showcased the potential of the approach in a clinical setting, although more work is required to further optimize the radiotracer.

Figure

PET/MR imaging of IPA using ^{64}Cu-hJF5 in mice (A) a humans (B).

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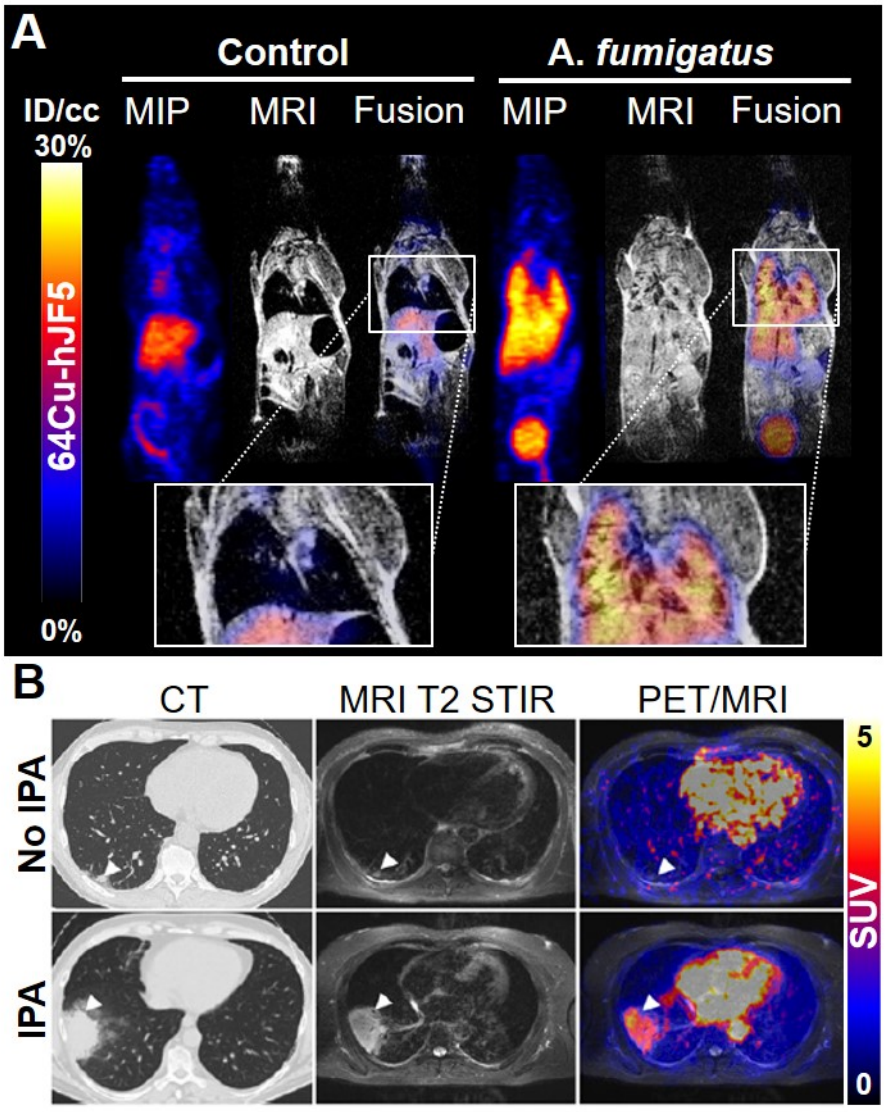
Fig. 1



FT 06

Exploiting microbial metabolites as narrow-spectrum antibiotics

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1. Introduction

Microbial communities offer a promising source of small molecule secondary metabolites. Some of these compounds mediate competitive interactions between microbes defending their niches and serve as antibiotics. Other metabolites are like swiss army knives with multiple functions for example signalling, defense, or interactions with their human hosts. Our research group investigates chemical interactions of human commensal and pathogenic microorganisms with the aim to exploit these molecules as customized antibiotics against infectious diseases and to develop strategies to selectively interfere with behaviors that contribute to bacterial virulence.

2. Materials & methods

Microbial metabolites were purified by activity-guided fractionation and their chemical structures were elucidated using 1D and 2D NMR spectroscopy in combination with high resolution mass spectrometry. Organic synthesis was used to generate known metabolites in larger quantities for bioassays and confirm hypothesis-driven potential congeners produced in microbial cultures. The chemical space was further exploited by synthesis of artifical derivatives to assess the structure-activity relationship and customize efficacy and species-selectivity.

3. Results

I here present our recent advances in the synthesis and quantification of antibiotic 2-alkyl-4(1*H*)-quinolone and 2-alkyl-4(1*H*)-quinolone *N*-oxide derivatives produced by *Pseudomonas aeruginosa* and *Burkholderia* species as well as their effects on other species of the human microbiome [1-2]. We demonstrate highly selective effects of 2-alkyl-4(1*H*)-quinolone *N*-oxides as bacterial weapons against *Staphylococcus aureus* and *Neisseria gonorrhoeae*. Their primary mechanism of action is based on inhibition of the electron transport chain and shows unexpected species-selectivity. In addition, we used the privileged structure of certain quinolones to develop species selective antibiotics against *Moraxella catarrhalis* [3]. I will also highlight our recent advances in generating chemical tools to modulate and control the biosynthesis of bacterial quinolones [4-5]. Finally, I will present ongoing work on small molecule control of the induction of latent bacterial viruses (prophages) [6].

4. Conclusion

Microbial interactions generate versatile metabolites with a high degree of functional diversification. One of their functions appears to be the selective inhibition of competing species of the human microbiome. Synthetic quinolone derivatives allowed to tune activity of these compounds and customize them for different human pathogens.

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FT 13

Studying the effects of gut microbiome dysbiosis on the outcomes of T-cell dependent cancer immunotherapies

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T lymphocytes and their capacity for antigen-directed cytotoxicity has become a key element for engaging the immune system in the fight against cancer. Curative intent therapy with genetically engineered T cells expressing chimeric antigen receptors (CAR-Ts) has been developed to boost antitumor abilities of host T cells and introduced into clinical medicine as CD19 targeted CAR-Ts for refractory and relapsed B cell malignancies. Preclinical and clinical studies have demonstrated that the intestinal microbiome and can regulate the mammalian immune system development and function and can regulate T cell immunity in various human diseases. Disruptions of the homeostatic host-microbiome relationship may contribute to reduced efficacy and development of toxicities of T cell driven immunotherapies against solid tumours and hematologic malignancies.

In this study, we performed co-incubation experiment with *Enterococcus spp*. isolated from patients stools together with gut microbiome ecologies exposed to various antibiotics as an in vitro model for dysbiosis that occurs in immunocompromised cancer patients receiving T cell immunotherapies. Furthermore, we used shotgun metagenome sequencing to show the abundance of different *Enterococcus* species at pre and post CAR-T therapy stages. We are currently studying the influence of antibiotic induced microbiome dysbiosis on the tumour growth and progression in tumour models receiving CD19-targeted CAR-T cells. Collectively, these *in vitro* and *in vivo* models will help us understand the influence of gut microbiome on the outcomes of T cell – based cancer immunotherapies.

FT 21

Adaptive evolution of virulence and persistence in carbapenem-resistant *Klebsiella pneumoniae* infections

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Within-patient evolution studies are limited by genome-centric investigations. In this study, phenotype-driven genomics and mechanistic investigations led to the discovery of global adaptive evolution of virulence and persistence in patients infected with carbapenem-resistant *Klebsiella pneumoniae*. The discovery of an intrinsic ability of *K.pneumoniae* to survive intracellularly in pathogen-containing vacuoles and tolerate lethal concentrations of last resort antibiotics highlighted the important role of intracellular infection stages in urinary tract infections (UTIs). Thus, targeting intracellular infection stages may be a promising antimicrobial strategy to treat persistent UTIs.

FT 19

Diversification of two-partner secretion systems in the bacterial plant pathogen Ralstonia solanacearum

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Introduction

Bacterial two-partner secretion (TPS) systems are involved in processes such as biofilm formation; adhesion and binding to host cells, and contact-dependent growth inhibition (CDI). TPS are described in many pathogenic bacteria, e.g. *Enterobacteria, Pseudomonas, Burkholderia* and *Xanthomonas* species. Previously characterized CDI locus in *Burkholderia*, encode a TpsB transporter and large exported TpsA proteins, and short downstream genes.

Objectives

For this research project I aim to characterize the CDI loci in *Ralstonia solanacearum* species complex (RSSC), an aggressive soil-borne plant pathogen that causes disease in diverse plant species. Especially I focus on a novel lineage of *R. solanacearum.* The novel lineage, called 4NPB, was first detected in the island of Martinique in early 2000s. It is characterized by a higher pathogenicity and an expanded host range.

Materials & methods

500 RSSC strains were sampled in Martinique and French Guiana during 4NPB emergence. Sequencing, genome assembly, phylogenetic and pangenomic analysis were done to detect genomic changes linked with the emergence of the new lineage. The dataset was supplemented with 400 genomes from NCBI database.

TPS proteins from *Burkholderia* were aligned against all the genomes of the dataset, in order to detect TPS homologs. Structures of the putative TPS proteins were predicted using AlphaFold2 and HMMER. The loci were clustered and assigned to groups by sequence and structure similarity. I will next measure how strains that share or vary in putative CDI loci perform in direct competition *in vitro* and *in planta* using a ddPCR assay.

Results

I have found an unusually large number of potential TpsA and TpsB homologs in RSSC strains. The putative TPS loci exhibit both conservation and patterns of recent recombination events typical for TPS systems. The loci can be divided into four groups which have diverse locus structures.

Analysis of the 4NPB population revealed recombination hotspots found between 4NPB and endemic strains. Recombination and gene gain/loss events were found in various toxin-antitoxin systems, including putative TPS loci.

Conclusion

This work will contribute to our understanding of how TPS systems mediate pathogenicity and interbacterial interactions in *Ralstonia solanacearum* and how TPS systems can potentially be involved into emergence of novel bacterial lineages.

FT 18

Understanding and fighting urinary tract infections (UTIs) in an innovative human bladder microtissue model

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Urinary tract infections (UTIs) are among the most common diseases worldwide and a major cause of antibiotic prescription. Their high recurrence rates show that the gold-standard treatments are frequently suboptimal, exacerbating the current antimicrobial resistance (AMR) crisis. The pathophysiological mechanisms underlying this ancient disease and its frequent recurrence remain largely understudied, hindering the development of effective therapeutics. Research in the UTI field has been dominated by studies using cancer cell lines or animal models that cannot fully recapitulate the ultrastructure, physiology and immunity found in the human urinary tract.

Here, we established a 3D microtissue urothelial model that successfully mimics the key features abovementioned. The model is currently being used under two configurations: upright and inverted, the latter facilitates high-resolution live cell imaging and assessment of urovirulence depending on particular factors (e.g. motility). These models recapitulate critical human urothelial features, distinguishing it from mice, namely 50-60 µm thickness stratified in ~7 cell layers, and embodying the three main urothelial subtypes (basal, intermediate and umbrella). Terminal differentiation was confirmed by UPIII and CK20 biomarkers, while tissue integrity and barrier function was shown by the presence of tight junctions and adequate transepithelial electrical resistance (TEER).

As this model tolerates urine, including under flow, we were able to assess uropathogenic *Escherichia coli* (UPEC) infection spatial-temporal dynamics for long time periods. Pathophysiological events from both host (e.g. cell exfoliation) and bacterial (morphological alterations, adherence and invasion) sides were monitored on the single-cell level in real-time, reflecting UPEC ability to tolerate and persist under shear stress. Infection with *Pseudomonas aeruginosa*, another common uropathogen, revealed that these bacteria rely on the formation of biofilm-like aggregates to persist in this environment, and compounds (alternative to antibiotics) are being used to target this strategy while in the model.

Overall, this model provides a relevant human set up to reveal new insights at tissue, cell and molecular levels of the host-uropathogen interaction. It emerges as a promising platform to study UTIs in a patient-like scenario and a testbed for novel therapeutic venues.

Fig. 1

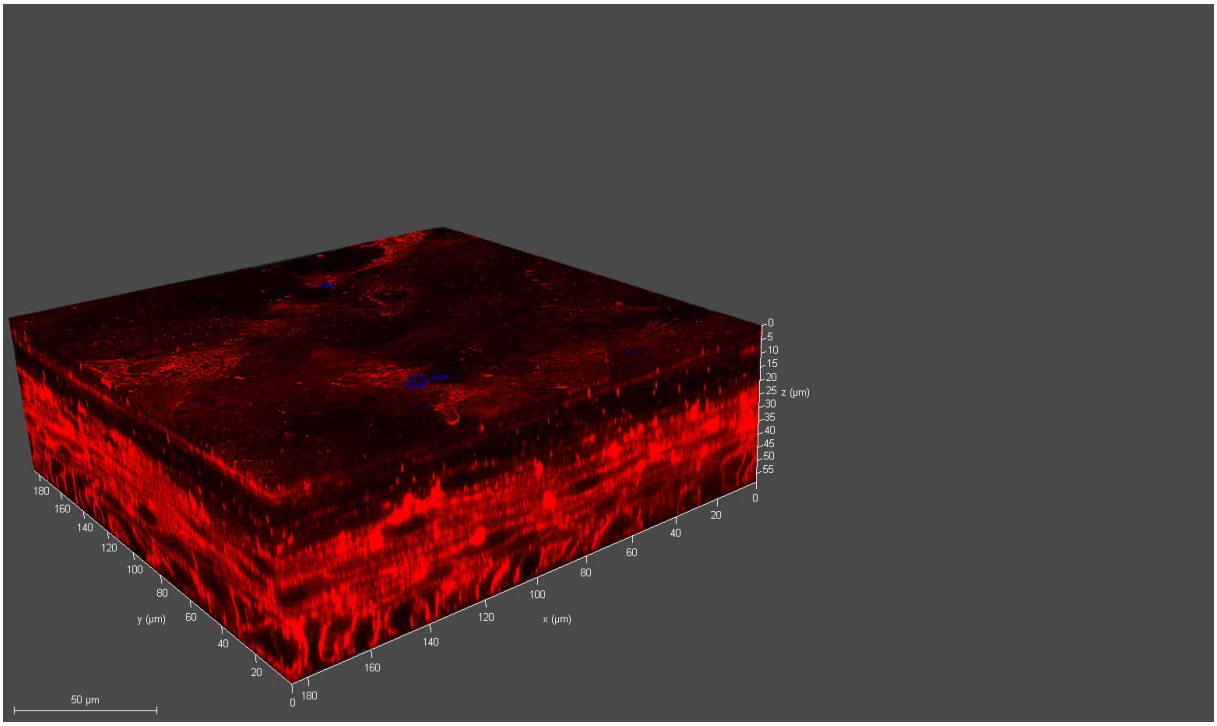
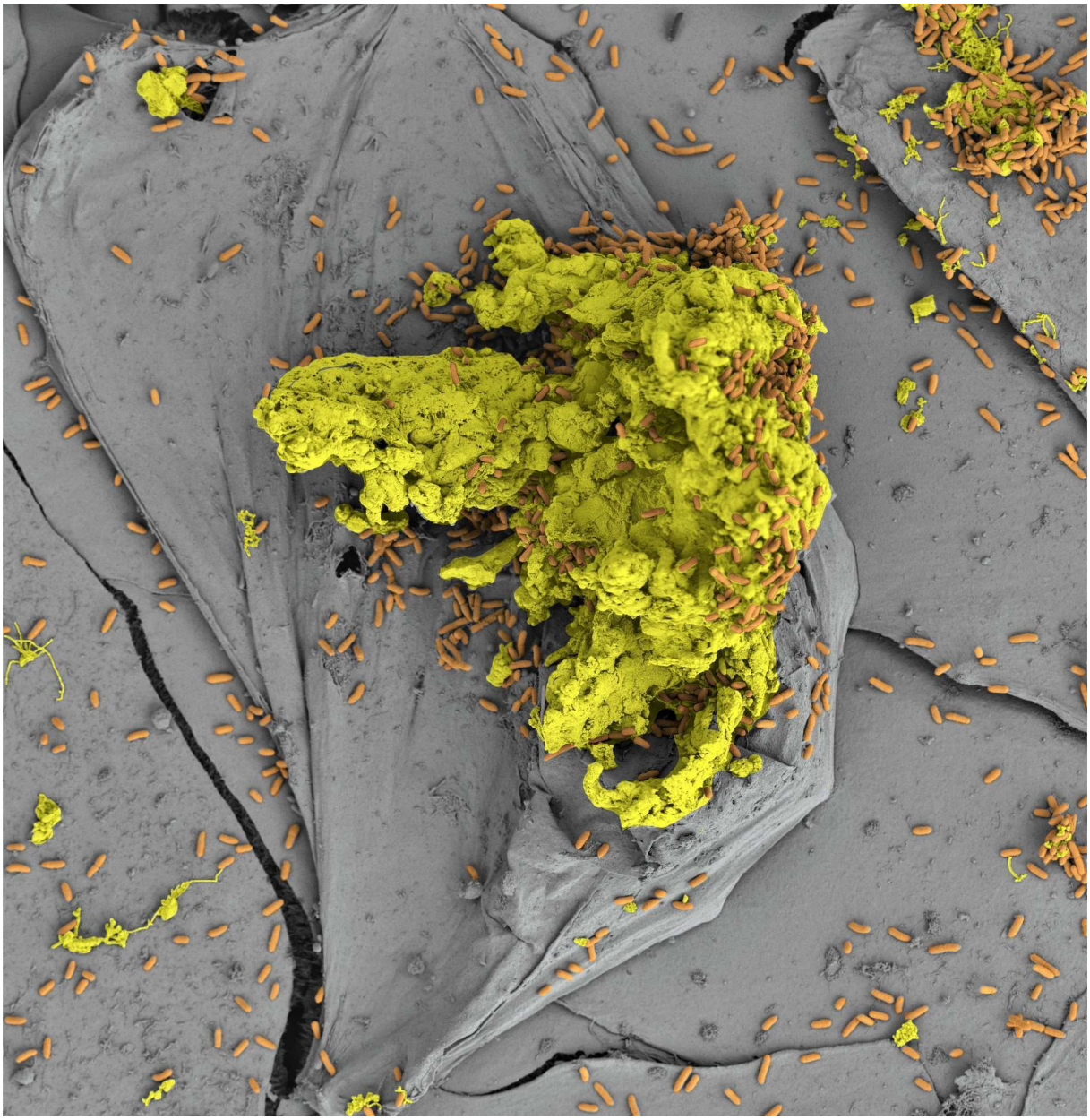


Fig. 2



FT 11

Differential host response to *Staphylococcus aureus* Panton-Valentine leucocidin in africans and europeans

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Introduction

The Panton-Valentine leukocidin (PVL) of *Staphylococcus aureus* is a bi-component pore forming toxin that targets the membrane of immune cells, resulting in their lysis. The two toxin subunits, LukS-PV and LukF-PV, bind to specific receptors, complement 5a receptor (C5aR) and CD45, on neutrophil granulocytes, macrophages, and monocytes. PVL is primarily associated with severe skin and soft tissue infections (e.g. pyomyositis), while the proportion of PVL-positive *S. aureus* is higher in Sub-Saharan Africa than Europe (approx. 50% vs 5%). However, the factors contributing to these differences remain unclear.

Objectives

In this study, we compared the cellular and humoral response to PVL in African and European volunteers.

Material & Methods

Between 2019 and 2023, we conducted a multi-center cross-sectional study in Sub-Saharan Africa (Gabon, Nigeria, Congo) and Germany (Münster, Jena). We collected blood, serum samples, and nasopharyngeal swabs from participants from both regions (Africa n=447, Germany n=325) with no signs or symptoms of an *S. aureus* infection. Subsequently, we examined the susceptibility of isolated granulocytes to different PVL concentrations using FACS. Additionally, we assesed inflammasome activation by quantifying secreted IL-1ß through ELISA after PVL stimulation of isolated monocytes. Furthermore, the levels of anti-PVL-antibodies in serum were quantified using ELISA, while we determined nasopharyngeal *S. aureus* colonization by bacteriological culture and molecular identification.

Results

Granulocytes from Africans were more susceptible to lysis by PVL compared to Germans, while the difference in damaged neutrophils was most significant at a concentration of 0.5 nM PVL (median, 51.2% vs. 24.1%, $p<0.0001$). Following stimulation with sub-cytotoxic concentrations of PVL (0.5 nM), we observed a 50-fold higher median induction of IL-1ß secretion from monocytes from African than from Germans (5201 pg/ml vs. 103 pg/ml, $p<0.0001$). Also, Africans exhibited notably higher median levels of anti-PVL-antibodies compared to Germans (9 AU vs. 3.8 AU, $p<0.0001$), while the rates of *S. aureus* nasopharyngeal colonization were similar in both groups (24% vs. 39%).

Conclusions

The enhanced inflammasome activation triggered by PVL in African monocytes, as opposed to German monocytes, may lead to a more effective recruitment of neutrophils to the infection site through the release of IL-1ß. Considering the higher susceptibility of granulocytes from Africans to PVL, this phenomenon could potentially result in amplified tissue damage, possibly contributing to the higher incidence of *S. aureus* skin and soft tissue infections (SSTI) in Africa in comparison to Europe. Additionally, the elevated levels of anti-PVL-antibodies found in African participants might be attributed to their increased exposure to PVL.

FT 17

Sensing host asparagine controls group A *Streptococcal* pathogenesis

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Group A *Streptococcus* (GAS) is the ninth leading infectious source of morbidity and mortality. GAS causes many infection types, ranging from uncomplicated pharyngitis and cellulitis to life-threatening diseases such as necrotizing fasciitis and streptococcal toxic shock syndrome. In addition, GAS causes autoimmune sequella, such as rheumatic fever, rheumatic heart disease, and glomerulonephritis. Although GAS remains sensitive to penicillin, its infection control is challenging. Since no effective vaccine exists, developing alternative treatments against GAS infection is crucial.

We have previously shown that GAS induces host cells to produce asparagine (Asn) during infection. GAS delivers streptolysin S (SLS) and O (SLO) to host cells causing endoplasmic reticulum (ER) stress and an unfolded protein response (UPR). As a result, host cells produce and release Asn utilized by GAS to increase virulence and growth. Recently we reported that abolishing Asn formation by inhibiting the PERK arm of UPR cures an invasive GAS disease in a murine soft tissue infection model. Thus, Asn plays a critical role in GAS-host interactions, and preventing the host Asn supply to the bacteria can be exploited for therapy.

Much less is known about how GAS senses Asn and adjusts its virulence accordingly. We performed RNAseq analyses and site-directed mutagenesis. We showed that the gene products required for Asn acquisition from the host, composed of SLO, SLS, the ABC transporter GlnPQ and Asn synthetase itself (AsnA), are upregulated in the absence and down-regulated in the presence of Asn. However, virulence genes controlled by the two-component master regulator CovR/S two-component system were upregulated in the presence of Asn. Both regulation circuits required intact AsnA, as deletion of AsnA or inactivation of its catalytic activity by a single amino acid replacement abolished gene regulation. The Asn-mediated increased virulence expression was concomitated with the CovR dephosphorylation, which was independent of the CovS sensor kinase. Thus, our study further deciphers the Host-Asn-GAS interface and facilitates the discovery of novel targets in the host and GAS for treatments.

FT 07

Naturally occurring variants of the antimicrobial lasso peptide Microcin J25

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Introduction

Lasso peptides are natural products with a unique threaded structure that are ribosomally synthesized and post-translationally modified peptides (RiPPs). As such, their biosynthesis starts with the ribosomal assembly of a precursor peptide that is then matured by the activity of specific processing enzymes.

Amongst lasso peptides, microcin J25 (MccJ25) takes a special place as it by far the most intensely studied member of this RiPP subfamily. It was discovered in 1992 by screening extracts of bacteria isolated from a human feces sample for antimicrobial activity. Produced by *E. coli* AY25, MccJ25 is active mainly against other *Enterobacteriaceae* (e.g., *Shigella*, *Salmonella*, and other *E. coli* spp.) and hence has the capacity to influence the intestinal microbiota with potential to provide protection against pathogen colonization.

MccJ25 enters target bacteria by high-jacking the TonB-dependent iron siderophore receptor FhuA and the innermembrane transporter SbmA. In the cytosol, MccJ25 inhibits the RNA polymerase by blocking the NTP uptake. Recently, a naturally occurring MccJ25 variant (MccY) was discovered and reported to have an altered activity spectrum. Hence, the search for naturally occurring variants of MccJ25 seems promising.

Objectives

A variety of gene clusters with the potential of producing novel MccJ25 variants was discovered via genome mining. The goal is to produce all of them and employ them in *in vitro* screens to see how the naturally occurring variations of their primary structures affects their activity profiles. In addition, we are interested in using strains bioengineered to produce MccJ25 to assess if the production of this antimicrobial lasso peptide could yield bacteria able to prevent *Salmonella* colonization in a model system.

Materials & Methods

The identified naturally occurring variants of MccJ25 are produced heterologously in *E. coli* by incorporating mutations in the precursor encoding gene in our established MccJ25 production plasmid or by generating new production plasmids based on the original cluster sequences of the newly discovered variants. Production is monitored via LC-MS and compounds are purified by preparative HPLC. Purified compounds are then employed in *in vitro* activity assays.

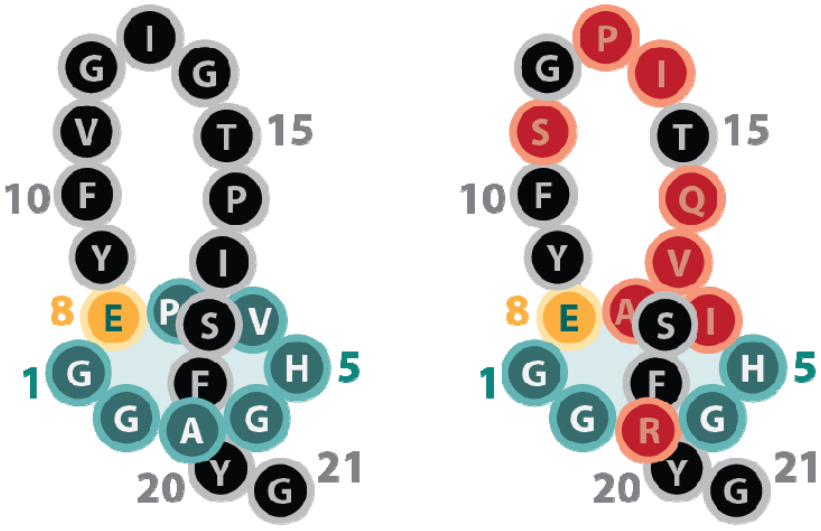
Results

Heterologous production of all novel MccJ25 variants has been accomplished and the purified compounds are currently used for activity screens. The results of these screens will be shown. It will also be demonstrated how we engineered strains to produce MccJ25 to employ them in screens for *Salmonella*-protective features.

Conclusion

Taken together, it will be shown how we identified novel naturally occurring variants of MccJ25, where they were found, and how they look like. Moreover, it will be demonstrated how the heterologous production of each of these variants was accomplished, how their production yields were optimized, and what was observed when using the purified compounds in activity screens.

Fig. 1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FT 14

Vaccine-induced fevers are associated with diet and the gut microbiome

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Introduction

The intestinal microbiota helps to modulate host immune responses, with consequences for susceptibility to infectious disease and responses to vaccination. However, data on microbiome-immune interactions in healthy humans remains limited. Recently, mass vaccination campaigns against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) provided an unprecedented opportunity to study interactions between the healthy human microbiota and a defined, sterile and predictable immune response.

Objectives

We aimed to understand (a) the influence of the microbiota on immune responsiveness to SARS-CoV-2 vaccination and (b) the impact of vaccine-induced immune activation on the microbiota.

Patients & methods

The µHEAT (Microbial-Human Ecology And Temperature) study recruited 179 healthy adults 18-40 years old being vaccinated against SARS-CoV-2 between December 2021 and May 2022. Oral body temperature was measured by participants three times per day as a read-out of the innate immune response (fever). Serum antibodies were measured before and after vaccination as a reflection of the adaptive immune response. Six longitudinal fecal samples per person were subjected to shotgun metagenomic sequencing (N=1046) and a subset were further selected for metatranscriptomic sequencing (N=246) to profile microbiome composition and activity before and after the vaccine.

Results

Fever responses to the vaccine were individualized and correlated with prior fever episodes, suggesting that certain people are more "fever-prone". Remarkably, the degree of fever was lower in participants who followed a plant-based diet. Furthermore, the baseline gut microbiome of individuals who experienced fever displayed a striking upregulation of flagellin gene expression, and an enrichment in flagellated *Lachnospiraceae* species. In contrast, anti-SARS-Cov2 antibody titres were not associated with diet or with flagellin expression, but correlated positively with abundance of the probiotic bacterium *Streptococcus thermophilus.*

Conclusions

Expression of flagellin in the gut microbiota was strongly associated with fever responses to vaccination. Although causality remains to be established, we speculate that flagellin - a known ligand of innate immune receptors - may act as a natural adjuvant to stimulate fever. Together, these data improve our understanding of human immune-microbiome interactions, with implications for vaccine development.

FT 03

Impact of the microbiome composition on the fitness of antibiotic resistant mutants

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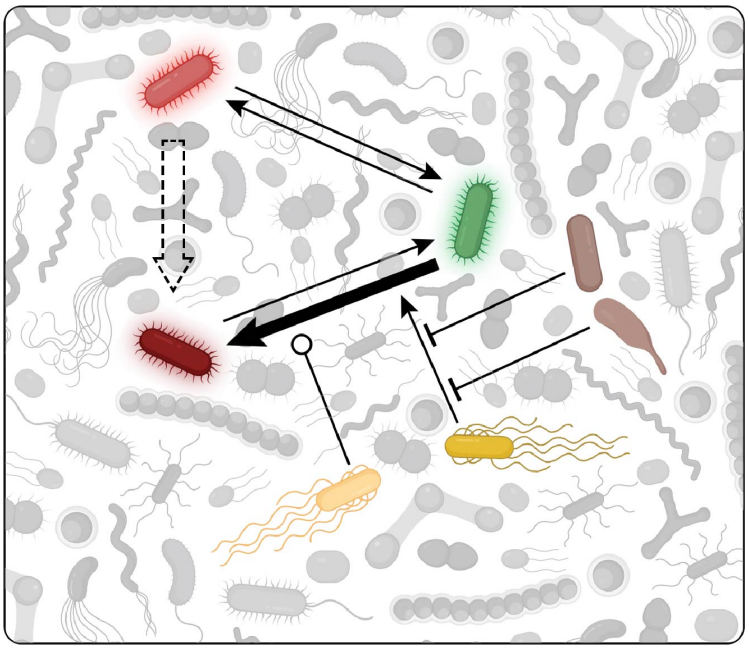
Antibiotic resistance can come with a fitness cost for the organism in the absence of selection. Identifying conditions in which the resistance element confers a cost for the organism offers a way to counter-select the resistant populations. Fitness costs are typically assessed in monocultures in lab conditions. Such conditions are far from the natural habitat of enteric pathogens, which have to compete with the surrounding microbiome. To determine the influence of the microbiota composition on the fitness cost (or advantage) of an antibiotic resistant strain, we have established a highly sensitive method that allows us to determine bacterial fitness in complex communities using a flow cytometry. Using this method, we identified a specific microbiome that selects for a carbapenem-resistant *K. pneumoniae* strain over the wildtype. We identified that the carbapenem-resistant *K. pneumoniae* can more easily acquire secondary mutations that lead to a specific upregulation of a carbohydrate catabolic operon. We further showed that the selective advantage was largely due to the presence of a specific *E. coli* strain, that it is contact independent, and that the effect size can be modulated by addition of further competitors in the microbiome. We hypothesize that this *E. coli* isolate is generating a metabolic niche that benefits the evolution of this specific carbohydrate utilization by the *K. pneumoniae*carbapenem-resistant mutant, which carries mutations in both major porins and hence has a different outer membrane permeability to small molecules. To further elucidate the underlying molecular mechanism, we are employing a multitude of methods, including metabolomics, catabolic profiling using carbon source arrays and *in vivo* studies using a germ-free mouse model. Our study describes a powerful and sensitive tool to study fitness of bacterial strains in complex environments, and our results highlight that the microbiome is an overlooked factor when characterizing the fitness of antibiotic resistant mutants, as it can strongly modulate their selective advantage.

Fig. 1



FT 15

Using complex human skin models to study the immunological response to colonization by *Staphylococcus species.*

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Staphylococcus aureus (S. aureus) is a highly reported antimicrobial resistant bacteria and poses a huge challenge to modern healthcare. The development of new antibiotics and treatments has been limited in part due to rapid resistance progression and the failure of vaccine candidates in clinical trials. To help overcome this crisis, our work aims to look beyond antibiotics and find new ways to prevent and treat *S. aureus* skin and soft tissue infections by understanding and augmenting the naive immune response of human skin.

We have developed a panel of human skin tissue models with different levels of complexity from mono cell cultures, advanced 3D tissue equivalents, clinical skin explant models to a human xenografted *in vivo* model. With our collection of model systems we will perform in-depth immunological profiling of human skin after colonization with commensal Staphylococci (*S. epidermidis/S. lugdunensis)* compared to potential pathogenic *S. aureus*, screening for protein as well as RNA expression at different time points.

Preliminary work has shown that the complexity levels of the model systems are important in interpreting results. The outermost layer of human skin, the stratum corneum, is a significant barrier to invasion of Staphylococci, and is often neglected particularly in mouse models. Corneocytes, which make up this layer, do not take up *Staphylococcus sp*. intracellularly and no IL-8 response was detected. In mono-cell cultures challenged with different *Staphylococcus sp*., intracellular bacteria in human keratinocytes and fibroblasts triggered an IL-8 response and destroyed the infected cells within 24h. In a bi-layer tissue equivalent model, we were able to detect IL-8 in response to *S. aureus*. Healthy human skin explants, topically colonized with Staphylococci confirmed the role of the corneocytes, with most bacteria adhering to the stratum corneum and not able to penetrate the tissue. No significantly increased IL-8 response could be detected in this experimental setup compared to controls. If we however disrupt this topmost corneocytes, bacterial CFUs and IL-8 production was increased in response to pathogenic but not commensal *S. aureus* strains. The most complex model, xenografted human skin, also showed that physically breaching the stratum corneum allows the bacteria to better attach to the underlying keratinocytes, opening the potential to enter host cells and deeper tissues.

Our model systems for human skin colonization show how tissue organization and the interplay of different cell types influence the outcome of encountering bacteria. With all this in place we can now step up to compare our dataset with already known responses in mouse skin and well as between commensal and pathogenic bacterial strains. The synergy of controlled tissue-equivalents with clinically relevant skin models provides a unique ability to address *Staphylococcus* pathogenesis in its natural environment, human skin.

FT 09

Metabolic bottlenecks decrease antibiotic efficiency in *Escherichia coli*

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Introduction:

Pathogenic bacteria can escape antibiotic treatments through various strategies. Among them is the selection of genomic mutations that lead to antibiotic resistance as observed in clinical isolates or strains obtained after laboratory evolution. Genomic mutations can occur in genes encoding the direct target of antibiotics and in genes encoding antibiotic efflux pumps and core metabolic enzymes. Although many resistance mechanisms are well-known, the role of metabolism in antibiotic resistance remains elusive. However, understanding mechanistic links between metabolism and antibiotic resistance can improve diagnosis and treatment of resistant bacteria.

Objectives: We aim at establishing a workflow to screen antibiotic resistant *Escherichia coli* strains and characterise their metabolic phenotypes using multi-omics. We intent to explore the relevance of our findings in clinical *E. coli*.

Material and methods:

We produced a mutant library of *E. coli* using genome engineering. Each strain of the library has a point mutation in an essential gene. We used Next Generation Sequencing on the library challenged with carbenicillin (inhibitor of peptidoglycan synthesis) or gentamicin (inhibitor of protein synthesis) to track resistant mutants. Resistant mutants were further characterised by using metabolomics and proteomics. Resistance and tolerance were tested using agar dilution and time-kill assays. Clinical isolates of uropathogenic *E. coli* (UPEC) were characterised in the same manner and their genome was sequenced.

Results:

Resistance and tolerance were confirmed for selected mutants. Gentamicin resistance mutations were mostly found in electron transport chain or TCA cycle. Mutations in purine pathway enzymes were enriched in the carbenicillin screen. We showed that the purine mutants have low intracellular ATP and a high expression of a transcription factor, which upregulates a transporter. We observed that the resistance and tolerance phenotypes of our mutants can be supressed by restoring ATP levels using adenine feeding, or by knock-out of the candidate transporter. We also showed that a multi-resistant UPEC strain harboured a mutation in the purine pathway that leads to a bottleneck. We demonstrated that feeding adenine to this strain increased its sensitivity toward carbenicillin.

Conclusion:

Our study demonstrates a mechanistic link between low ATP and carbenicillin efficiency in *E. coli*.

FT 12

Glycan-mediated immune selection of bacterial species at host barrier sites

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[2]*Institute de Biologie Structurale, Grenoble, France*
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Introduction: Bacterial microbiota located at host barrier sites harbor species essential for human health, but also species with pathogenic potential. Infection induces antibody responses to the causative pathogen, thereby establishing durable immunity. In addition, it has been demonstrated that select microbiota species induce local and systemic antibody responses that may contribute to host defense. However, the molecular mechanisms underlying selection and induction of immunity against microbiota species are currently unknown.

Antigen-presenting cells (APCs) are local immune cells that sample the environment by extending their dendrites across epithelial barriers. Interestingly, these dendrites are densely covered with glycan-binding C-type lectin receptors (CLRs). Thereby, APCs are ideally positioned and equipped to probe bacteria-expressed glycans and subsequently induce both local and systemic immunity.

Objective: We aimed to identify microbiota species that can be recognized by APC-expressed CLRs

Methods: We have developed CLR-sequencing, which uses soluble CLR receptors as probes to identify interacting microbiota species through bacterial cell sorting and subsequent metagenomic sequencing. We established and optimized our experimental pipeline using the fluorescently-labeled CLRs langerin and MGL, and fecal microbiota samples from healthy donors.

Results: Langerin and MGL bound on average 9% and 4% of the fecal microbiota, respectively, with substantial interindividual variation. Analysis revealed donor-specific enrichment of taxa in the CLR-sorted samples, but also shared enrichment of genera such as Ruminoclostridium_9, Coprococcus_1 and Butyricicoccus. Corynebacterium accolens was enriched in the langerin-sorted samples for all donors and langerin binding was validated using flow cytometry. Finally, we are currently expanding our glycan-probe panel with additional CLRs, as well as multiple glycan-specific antibodies.

Conclusion: With this experimental approach we will gain insight into bacterial microbiota members that produce immunologically-relevant glycans in the microbiome. Using these data as a blueprint, future research on clinically-relevant patient samples or CLRs with naturally-occurring mutations could reveal crucial disparities in glycan-induced immunity.

FT 16

Biomechanical alterations of endothelial cells exposed to bacterially-infected immune cells

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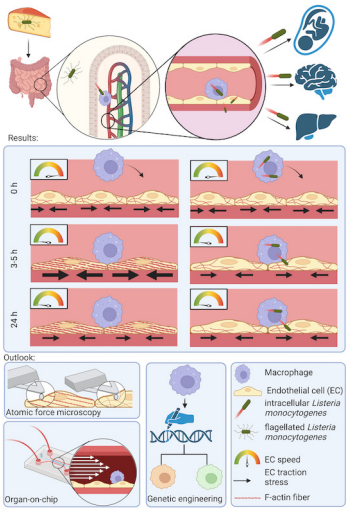
Infectious diseases pose a global health concern and a significant cause of mortality worldwide. This will be further exacerbated due to the emergence of antibiotic resistant strains. Yet, we still do not fully understand how many pathogens can infect and spread throughout the human body. In particular, intracellular bacterial pathogens, like food-borne *Listeria monocytogenes* (LM), have developed a multitude of strategies for manipulating their host cells, in a subtle way without destroying host cell integrity since they rely on it. One of the ways LM can achieve systemic spread, is by being carried within macrophages (MΦs) which are able to transverse the vasculature thus reaching distant organs such as the brain or the placenta, further spreading infection and causing fatalities. How do infected MΦs manage to successfully transverse vascular endothelia breaching their barrier integrity? Have they developed mechanisms of (in)directly manipulating endothelial cells (ECs)?

By performing immunostaining followed by microscopy imaging and image processing, I characterized in a highly quantitative manner the shape and cytoskeletal changes that ECs undergo, and found that when exposed to bacterially-infected or not MΦs they become significantly more polarized and collectively aligned as compared to non-exposed ECs. Prompted by these changes, I then sought to examine whether this impacts EC mechanics and how. By using traction force and monolayer stress microscopy, I found ECs increased both their traction and monolayer stresses (proxy of EC barrier integrity) when exposed to uninfected MΦs but not when the MΦs were infected or absent from the monolayer.

These alterations in EC biomechanics that I am currently investigating might be key in understanding how MΦs carrying bacteria manage to breach EC barriers like the blood brain barrier, by indirectly altering EC mechanotransduction of the endothelium. That in turn might be very important to understand how to obstruct LM spread in the brain, where it can cause deadly meningitis and encephalitis.

Fig. 1



FT 05

Mining antimicrobials in the human skin microbiome

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[2]*University of Wisconsin-Madison, Madison, WI, US, United States*

Introduction: The human skin microbiome plays a critical role in maintaining skin health, yet the complex communication and competitive interactions within this ecosystem remain largely uncharacterized. Certain skin colonizers have been discovered to produce bioactive specialized metabolites that exert colonization resistance, inhibiting the growth of competing species. Exploring the dynamics of these bioactive molecules represents a step towards unraveling the intricate interplay within the skin microbiome.

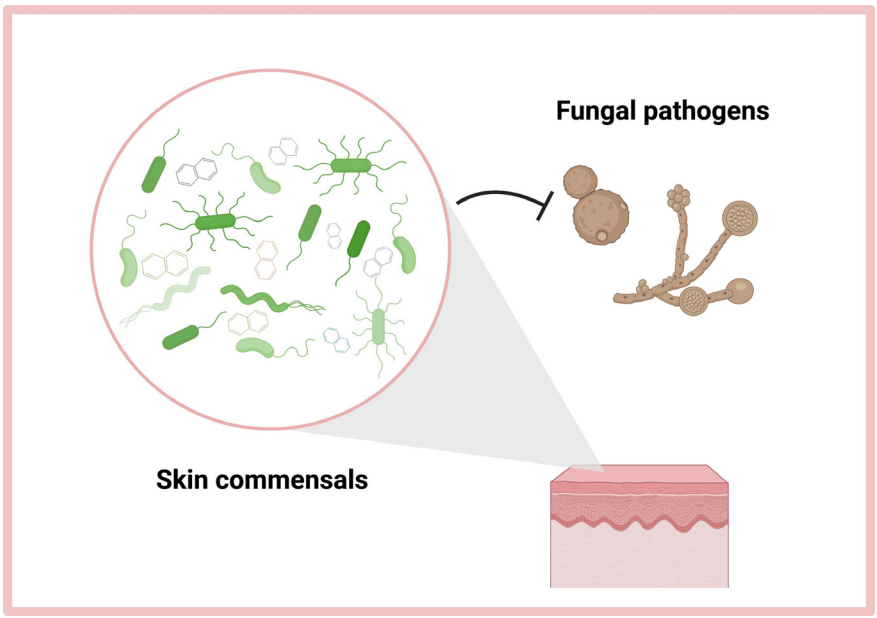
Objective: However, our understanding of the cross-kingdom interactions between bacteria and fungi on the skin, as well as the impact of metabolites on colonization resistance against fungi, remains limited. To tackle this question, this study aims to explore the hypothesis that bacterial metabolites stabilize the skin microbiome through inhibition of fungal colonization.

Methods: From our high-throughput screening, we have identified a skin-associated bacterium in the phylum Actinobacteria, that secretes metabolites inhibiting the growth of the fungal pathogens, *Candida albicans*. To determine whether the antimicrobial activity was indeed the result of a secreted metabolite, ethyl acetate extractions were conducted on pure cultures to select for secreted metabolites. RT-qPCR was employed to assess differences in antifungal production between varying growth conditions. Genomic analysis was performed to search for potential biosynthetic gene clusters predicted to encode in the genome, which may be responsible for metabolite synthesis.

Results: I observed potent antifungal activities with a dose-dependent response from the skin bacteria. Upon purification, I identified non-toxic yet active fractions against *C. albicans* and *C. auris* both *in vitro* and *in vivo*. To further explore biosynthetic capacity of the skin bacteria, I assessed different conditions (e.g., media types and growth stages) and found low and high production conditions of the antifungal molecules. Through genomic screening, three biosynthetic gene clusters were identified. Intriguingly, a polyketide gene was observed to be upregulated by 76-fold ($p < 0.001$) when the skin bacteria was fermented in the high production condition (solid media after five days of growth), suggesting a polyketide could be contributing to the antifungal activity.

Conclusion: This study demonstrates the significant role of bioactive specialized metabolites produced by skin colonizers in mediating colonization resistance within the human skin microbiome. Future directions would be elucidating the chemical identity of the bioactive molecules. Understanding colonization resistance in the skin microbiome could lead to finding novel antimicrobials that are safe and effective.

Fig. 1



FT 04

Systematic evaluation of long-term drug effects on the gut microbiome in the Estonian Microbiome Cohort

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Last decades of research have revealed an array of technical, biological, and environmental factors associated with the human gut microbiome composition. Usage of various pharmaceuticals is one of the most well-known contributors to the inter-individual microbiome variability. However, evidence is indicating that we might be underestimating the effect of drug usage due to the long-term drug usage effects. Recently, we showed that not only the usage of antibiotics and antidepressants at the time of sampling, but the history of the drug consumption, is associated with the gut microbiome composition1. Moreover, the effect can be "additive" - the more drug used in the past, the stronger the effect on the gut microbiome. Such signals of drug usage history haven"t been thoroughly investigated. Here, taking advantage of the electronic health records, we use the Estonian Microbiome cohort metagenomics dataset (n=2,500) to systematically evaluate the long-term effects of host-targeted medications. We show that the past usage of several drugs is associated with the gut microbiome and these effects can reflect drug dosage and adherence. Most notably, we show that the effects of antibiotics, psycholeptics, antidepressants, PPIs and beta-blockers can be detected several years after the previous usage. Furthermore, we show how the long-term drug effects can complicate the identification of disease-specific signals.

References:

1 Aasmets, O., Krigul, K.L., Lüll, K. et al. Gut metagenome associations with extensive digital health data in a volunteer-based Estonian microbiome cohort. Nat Commun 13, 869 (2022). https://doi.org/10.1038/s41467-022-28464-9

FT 08

From regulon to stimulus to metabolite: Zur – zinc – Transvalencins

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Natural products (NPs) have played a major role as biological probes, as inspiration for organic chemistry and as an important source for therapeutics. Over the last decades, however, we witnessed an innovation gap in discovering new NPs. One prerequisite to overcome this crisis is the development of methods facilitating today"s discovery and research projects.

Microbial NP production occurs in a growth-dependent manner as an adaptation to changing environmental conditions. As a consequence, it is not constitutive and requires the application of enabling strategies promoting NP expression under laboratory settings. Understanding cellular processes, especially the regulatory circuits controlling NP biosynthesis, is thus crucial to efficiently access the evolved NP diversity.

To obtain accessibility to yet hidden NPs, we implemented a bioinformatic-guided workflow in order to assign biosynthetic gene clusters (BGCs) to regulons of environmental signal sensing regulators (ESSRs). This allows a BGC-stimulus-pair prediction and in consequence a hypothesis-driven design of experiments to transcriptionally activate BGCs.

We initiated to sequence our >110.000 strain collection inherited from Sanofi. Scanning for BGCs predictably controlled by known ESSRs revealed a Zur (zinc uptake regulator) binding site in a yet unassigned thiazolidine-type NRPS-BGCs in several *Nocardia* genomes. As bioinformatically predicted, identified strains produce exclusively under low-zinc stress conditions a structural diversity of transvalencins. Matching the structural composition of BGC-sub-types we identified non-, mono- and di-halogenated as well as desmethoxy-transvalencins. Eventually, we showed a function of the transvalencins in the suppression of the phytopathogenic fungi *Zymoseptoria tritici* under zinc deficiency by comparative competition assays testing a transvalencin-producing WT and knock-out mutant.

FT 02

Microbiota context dependent dual protective role of Klebsiella oxytoca against Salmonella infection

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Question:

The composition of the intestinal microbiota and their secreted natural compounds have a significant impact on the outcome of enteric infections. The *Klebsiella oxytoca* species complex (KoSC) is a crucial component of the human microbiome and particularly prevalent during infancy and childhood. KoSC strains can produce two enterotoxic natural products, tilimycin and tilivalline, while also contributing to colonization resistance (CR) against pathogenic Enterobacteriaceae. The relationship between these seemingly contradictory roles has remained underexplored. Hence, our aim was to study how widespread the presence of toxin-producing *Klebsiella* strains is in the healthy community and whether tillivaline and tillimycin exert antibacterial activity on enteropathogens such as *Salmonella* Typhimurium.

Methods:

We characterized publicly available and de novo sequenced genomes of commensal and nosocomial KoSC strains for the presence of the gene cluster responsible for toxin production. To complement the bioinformatic analyses, an *ex vivo* screening assay to assess the capacity of toxin producing and non-producing strains to supress *Salmonella* growth *ex vivo* was utilized. Candidate strains with strong antibacterial effect were further characterized and the toxin cluster was inactivated using a CRISPR/Cas9-based gene editing system. Mouse models were utilized to identify whether toxin production contributes to protection against *Salmonella* strains in different microbiota settings *in vivo*.

Results:

In this study, we demonstrate that *K. oxytoca* provides CR against *Salmonella* Typhimurium infections. Genetic disruption of toxin production showed that tilimycin exhibits antimicrobial activity against various *Salmonella* strains *in vitro*. Notably, CR against *Salmonella* depended on toxin production in germfree mice, while it was largely toxin-independent in mice with a residual microbiota. Further, we found that availability of abundant carbohydrate availability induced toxin production, and that nutrient competition not only limited toxin production but was also critical for CR. Thus, mutual interactions occurring between KoSC members and the microbiota impact both gut community composition and function.

Conclusions:

KoSC strains are able to protect mice from lethal *Salmonella* infection via secretion of the natural product tillimycin in highly disturbed microbiota. On the other hand, these genotoxins have deleterious effects on the hosts. This study highlights the dual role of bacterial metabolites that can be both: beneficial and detrimental depending on the microbial microenvironment. Our finding might also be relevant for future intervention strategies to prevent the negative effects of acute or chronic toxin production and to design well-defined cocktails containing a "predator strain" like *K. oxytoca* and other broad substrate consumers that prevent potential toxicity.

FT 01

Evolutionary trajectories of gut bacteria determine resistance against pathogen colonization

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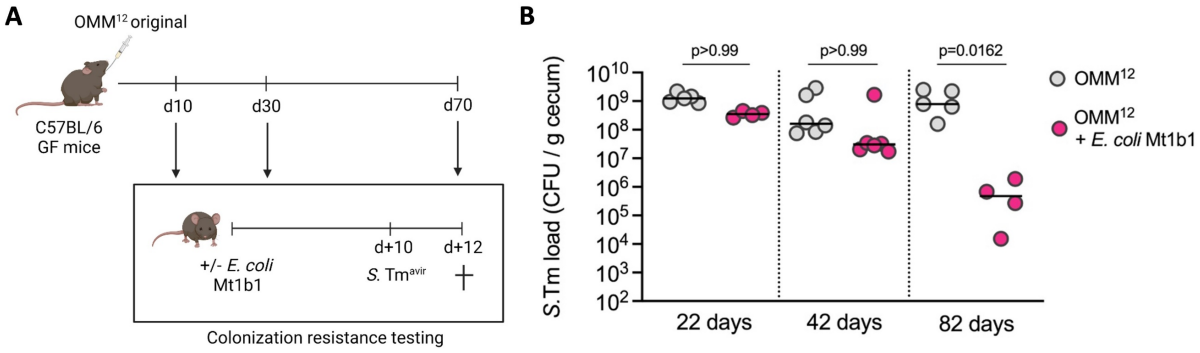
The human gut is a complex and highly dynamic microbial ecosystem with major impact on host physiology. Key microbiome functions, such as colonization resistance against enteric pathogens, are mediated by the metabolic potential of the intestinal microbiota, which is influenced by compositional, transcriptional and evolutionary changes. While microbiome composition and transcriptional adaptation have been extensively studied, the functional impact of within-host evolution remains largely unknown.

Here, we employ experimental evolution as a tool to study the adaptation of a bacterial synthetic community (Oligo-Mouse-Microbiota; OMM^{12}) to the mouse gut and explore its role in mediating colonization resistance against *Salmonella enterica* serovar Typhimurium (*S.* Tm.). We show that mice are protected from *S.* Tm. infection only after long-term colonization with the OMM^{12}, suggesting a role for bacterial evolution in mediating colonization resistance (figure 1). By analyzing the genomes of 122 bacterial re-isolates from OMM^{12} mouse lines housed in two different facilities, we identify numerous mutations which drive intra-species diversification and influence the metabolic potential of bacteria. By transplantation of a specific set of evolved strains we identify a minimal set of mutants which are required for the observed colonization resistance phenotype.

In summary, our work establishes bacterial within-host evolution as a mediator of colonization resistance against enteric pathogens. A mechanistic understanding of the forces driving the evolution of bacterial communities will aid the improvement of targeted microbiome manipulations towards a healthy state of the host.

Figure 1. Within-host evolution of OMM^{12} contributes to *E. coli*-mediated colonization resistance against *S.* Tm. (A) Experimental design: Germ-free mice were freshly colonized with OMM^{12} and gavaged with *E. coli* Mt1b1 or PBS 10, 30, and 70 days post-colonization. After 10 more days, mice were colonized with *S.* Tm before being sacrificed two days later. (B) *S.* Tm colony forming units (CFUs) per gram mouse cecum. Indicated times refer to the number of days mice were colonized with OMM^{12} before being sacrificed. Statistical analysis is based on non-parametric Mann-Whitney U test.

Fig. 1



FT 10

Transient intestinal colonisation of germ-free mice demonstrates long-lived microbiota-specific T_H cell memory

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1. Introduction

The mammalian gut microbiota induces specific T helper (T_H) cell responses including regulatory T cells and T_H17 cells. In contrast to well-characterized virus-induced T cell immunity, the dynamics of microbiota-specific responses have remained elusive. In particular, it is unknown whether the gut microbiota elicits true T cell memory characterized by long-term maintenance in the absence of cognate antigen. The obstacle has been the inability of uncoupling T_H cell induction from antigen persistence in the models of permanent colonization that have been deployed.

2. Objectives

We aimed to elucidate the dynamics of gut microbiota-induced T_H cell responses including their induction, immunological memory formation, reactivation, and function using a novel approach of transient intestinal colonization by non-pathogenic gut bacteria.

3. Material and Methods

We have genetically engineered a mutant strain of the gut commensal *Limosilactobacillus reuteri* that reversibly colonises germ-free mice. Through cell wall auxotrophies, the mutant can be supplemented to grow in vitro but gets cleared from the germ-free gut following a period of transient colonization by viable bacteria. This intestinal exposure induces specific T_H17 cells that can be followed beyond bacterial clearance thus uncoupling T_H cell induction from antigen persistence. To trace antigen-specific T_H cells, we have identified specific T cell receptors and devised retrogenic mice for adoptive transfer experiments.

4. Results

The inability of the auxotrophic *L. reuteri* mutant to replicate in vivo uniquely allowed for a dose assessment for microbiota-induced T_H cell responses. The induction of specific T_H cells was regulated by antigen dose at various levels with increasing amounts facilitating T cell expansion in mesenteric lymph nodes, homing to small intestine, and optimal T_H17 differentiation, respectively. Two weeks of transient colonization by *L. reuteri* induced memory T_H cells that were long-lived until at least week 12, tissue-resident in the small intestine, and persisted in the absence of cognate antigen. These memory T_H cells could be reactivated by intestinal challenge with *L. reuteri* which mediated the T cell's cell cycle entry and cytokine expression, as well as recruitment and activation of neutrophils. While *L. reuteri*-specific T_H cell memory was largely quiescent in mice that returned to germ-free state, monocolonisation with replication-competent *L. reuteri* led to continuous T_H cell activation and recirculation.

5. Conclusions

The gut microbiota induces long-lived, tissue-resident memory T_H cells that persist independently of cognate antigen and that are modulated by microbial abundance to promote intestinal immunity and homeostasis. These findings may help to understand disease flares during inflammatory bowel disease but also be leveraged for mucosal vaccine design.

Posters

Bacterial infections

P 01

Molecular cross-talk between Sa3int phages and their *Staphylococcus aureus* host

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As a major opportunistic pathogen of human and animals *Staphylococcus aureus* asymptomatically colonizes the nasal cavity, but is also a leading cause of life-threatening acute and chronic infections. *S. aureus* strains can carry up to four temperate phages, many of which possess accessory genes coding for staphylococcal virulence factors. More than 90% of the human nasal isolates of *S. aureus* were found to carry Sa3int phages, which integrate as prophages into the bacterial *hlb* gene thus disrupting the expression of the sphingomyelinase Hlb, an important virulence factor under certain infection conditions. The virulence factor-encoding genes carried by the Sa3-phages are all highly human-specific and probably essential for bacterial survival in the human host. Thus, both insertion of the prophages into and excision from the bacterial genome have the potential to confer a fitness advantage to *S. aureus*. However, how the *S. aureus* host modulates the life cycle of its temperate phages remains largely unknown (1). Our data suggest that the bacterial factors supposedly involved in the interaction of the bacterial host with its phages are strain specific, with certain *S. aureus* strains being more prone than others to support either a lysogenic or a lytic life cycle (2). We constructed and integrated Sa3int phages into different phage-cured *S. aureus* strains and found significant differences in phage transfer rates between different strains. Based on this finding, strains were grouped into low and high transfer strains. Indicating that in low transfer strains, the phages are more directed towards lysogeny. To get a more precise picture of the regulatory circuits we constructed replication deficient mutants, performed differential RNAseq to determine the transcriptional units and analysed a set of mutant strains. By transcriptional start site prediction we identified promoter-regions within the structural module of prophage Φ13 that are differentially active in high and low transfer strains and are a tool to identify regulators by measuring promoter-fusion constructs in different mutant backgrounds.

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P 02

Cell death during nutrient starvation in *Staphylococcus aureus* cells lacking (p)ppGpp is linked to disturbed GTP homeostasis

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In *S. aureus,* the alarmones ppGpp and pppGpp are synthesized upon amino acid limitation or in response to cell-wall stress by the alarmone synthetases Rel*Sau*, or RelP and RelQ, respectively. (p)ppGpp is important for bacterial survival, virulence and persistence (1-3). Upon synthesis of (p)ppGpp, GTP levels decrease sharply via consumption of GTP and inhibition of enzymes involved in GTP synthesis (1, 5). *S. aureus* wildtype and isogenic (p)ppGpp0 mutants show similar growth rates and final yields throughout growth. However, in the stationary phase (p)ppGpp0 mutants show a significantly decreased ability to form colonies indicating that stringent response induction either prevents cell death and/or supports escape from a "viable but non-culturable" state. Accordingly, we observed a stringent response-like transcription profile (4) or *rsaD* expression. *rsaD* is indirectly regulated by GTP levels through derepression by the GTP-responsive transcriptional factor CodY. However, (p)ppGpp dependent survival is independent of *codY*. Metabolome analysis further confirm dysregulation of GTP metabolism in the (p)ppGpp0 strain during starvation. When we compared the growth of guanine-auxotrophic mutants (*guaAB*) in wildtype and (p)ppGpp0 strains, no difference in survival in late stationary phase was observed. This indicates that the uncontrolled increase of GTP in the (p)ppGpp0 strain is sufficient to promote cell death under starving conditions. Cell membrane staining and analysis of membrane potential with the voltage-sensitive probe DiOC2(3) reveals alterations in membrane architecture and function in the (p)ppGpp0 strain. Further, we employed RNAseq to reveal global metabolic changes upon starvation allowing survival by regulating GTP levels.

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P 03

Differential survival of Staphylococcal species in macrophages

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The human pathogen *Staphylococcus aureus* is considered mainly an extracellular, opportunistic pathogen, yet the bacterium is able to survive within and escape from host cells, including macrophages. An *agr*/*sae* mutant of strain USA300 is unable to escape from human macrophages but can replicate and survive within macrophages. We questioned whether such "non-toxic" *S. aureus* resembles the less pathogenic coagulase-negative Staphylococcal species (CoNS) like *S. carnosus*, *S. lugdunensis*, *S. capitis*, *S. warneri* or *S. pettenkoferi.* We show that in contrast to the "non-toxic" *S. aureus* strains, the CoNS species are efficiently killed within 24 h post-infection in the macrophage-like THP-1 cells or in human primary macrophages. Bacterial persistence of "non-toxic" S*. aureus* or CoNS induced IL-1ß release but no cell-death. Mutations in genes coding for katalase, copper transport or the regulatory system GraRS or SigB did not impact bacterial survival in THP-1 cells. Deletion of the superoxide dismutases *sodA* and *sodM* impaired *S. aureus* survival in human primary macrophages but not in THP-1 cells. However, expression of the *S. aureus* specific *sodM* in *S. epidermidis* was not sufficient to protect this species from being killed in THP-1 cells. Thus, at least in those cells better bacterial survival of *S. aureus* could not be linked to higher protection from ROS. However, "non-toxic" *S. aureus* was found to be insensitive to pH, whereas *S. epidermidis* was protected when phagosomal acidification was inhibited. Thus, species differences seem to be linked to different sensitivity to acidification.

P 04

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

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Question: *Staphylococcus aureus* can cause life-threatening infections that are often resistant to multiple antibiotics due to horizontal gene transfer of antibiotic resistance genes from other staphylococci. One way to combat these antibiotic resistant infections are bacteriophages. The host range of *S. aureus* phages is determined by the species-specific structure of wall teichoic acids (WTA), which is currently the only known *S. aureus* phage receptor. While other Staphylococci such as *S. epidermidis* carry a glycerol-phosphate WTA, nearly all *S. aureus* strains possess WTA consisting of ribitol-phosphate repeats. This study aims to investigate the binding capabilities of *S. aureus* ribitol-phosphate binding phages to their host cells through the identification of receptor-binding proteins (RBPs). Through this, we want to understand the underlying mechanism leading to adsorption and thus infection of the phage, which might ultimately enable us to predict the host range of *S. aureus* phages.

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

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

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

P 05

ERK activation waves are key drivers in the mechanical cell competition that leads to collective onslaught of bacterially-infected cells

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Bacterial infections are a serious health concern and a significant cause of death worldwide. However, it is still not understood completely how intracellular bacteria can infect and spread throughout the human body. Epithelial cells infected with the food-borne facultative intracellular pathogen *Listeria monocytogenes* (*L.m.*), undergo a mechanical competition at late infection whereby stiffer and stretched uninfected surrounder cells squeeze, extrude and drive the collective onslaught of softer and less contractile *L.m.*-infected cells, that eventually undergo cell death. Although activation of nuclear factor kappa B (NF-κB) drives this mechanical battle, its inhibition does not abolish collective infected cell extrusion. Thus, we investigate whether extracellular signal-regulated kinase (ERK) plays a role given its involvement in other infections and its activation upon cellular stretching. We found that ERK inhibition in epithelial cell monolayers enhanced bacterial spread and decreased infected cell extrusion. Using Förster resonance energy transfer (FRET)-imaging, we discovered enhanced ERK activity waves propagating during infection which were absent in quiescent uninfected cells or cells treated with the ERK inhibitor. To elucidate the changes in cell biomechanics that occur during infection in ERK-inhibited monolayers and why those limit infected cell extrusion, we analyzed the spatiotemporal kinematics and dynamics of infected cells treated or not with the inhibitor. In the former case, we discovered that surrounder uninfected cells did not polarize in the normal direction to the tangent of the infection focus and all host cells exhibited less coordinated migration compared to untreated infected monolayers. Moreover, unlike control infected monolayers where cells pertaining in the infection focus appeared squeezed together, in ERK-inhibited infected monolayers we witnessed exactly the opposite. That is, infected cells pertaining in the foci were more spread out compared to surrounder uninfected cells. Triggered by this unexpected finding, we performed traction force microscopy, and found that infected cells exerted weaker traction stresses compared to uninfected surrounder cells in both ERK inhibited or not monolayers. This result suggests that upon ERK inhibition, surrounder uninfected cells are still more contractile compared to infected cells however they are not polarized or migrate in a coordinated way against the infected cells. Therefore, our findings most insinuate on an intricate link between ERK activity wave propagation and collective surrounder cell protrusion which in the context of infection is crucial for limiting bacterial spread.

P 06

Type III secretion chaperones and transmembrane proteins: elucidating the mechanisms underlying correct targeting to secretion

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Introduction

Type III secretion (T3S) systems are needle-like molecular machines that allow the transport of proteins across gram-negative bacteria membranes directly into the host cell to ultimately promote bacterial survival. Among the delivered proteins are those containing transmembrane domains (TMD). The presence of more hydrophobic TMD in T3S substrates requires the binding of a cognate T3S chaperone (T3SC) that allows correct targeting to T3S and avoids incorrect targeting to bacterial membranes by outcompeting from recognition by the components of the Sec-pathway. Thus, a precise orchestration of these processes is crucial for the efficient interaction of the chaperone/TMD-substrate pairs. Here, we aim to unveil the key features and processes that underlie T3S of TMD-substrates using as a model the *Salmonella*"s T3S chaperone/TMD-effector, SscB/SseF.

Methods

For the biophysical characterization of SscB and SscB/SseF, the purified proteins were analyzed by CD, Nano-DSF and SEC-MALS. To determine the key residues for complex formation was used alanine scanning mutagenesis. Furthermore, protein interactions were analyzed by in vivo photocrosslinking and protein stability in *Salmonella* using a chloramphenicol-based assay. To assess T3S, a Nanoluc luciferase-based assay was developed for SPI-2 (*Salmonella*"s pathogenicity island 2) inducing conditions.

Results

Previously, it was observed that *Salmonella*"s TMD-effector SseF required its cognate T3SC SscB to avoid erroneous insertion into bacterial membranes and to allow correct targeting to T3S. These proteins are encoded adjacently in SPI-2 (sscB-sseF), and when this order is changed a decrease in the T3S of SseF was observed, both in SPI-2 inducing conditions and during infection. Moreover, SscB was stabilized by SseF in *Salmonella*, and the presence of the chaperone binding domain (CBD) of SseF was sufficient for stabilizing purified SscB. The same was observed in SseF which requires SccB to be stabilized. Furthermore, SscB interacts with SseF"s CBD domain and first TMD, but not with the second TMD, in a stoichiometry of 1:1. Interestingly, SscB structural features resemble those of T3SC that bind translocators, also TMD-proteins. Furthermore, by performing alanine scanning mutagenesis, it was shown that SseF bore a "P/VXLXXP" consensus amino acid sequence in the CBD, which is conserved in the translocators of *Salmonella* and other bacterial species. Additionally, other *Salmonella*"s T3SC of translocators, although not able to promote SseF secretion, could stabilize SseF.

Discussion

Overall, these observations suggest that formation of SscB/SseF may occur rapidly since a specific gene organization is required and the proteins co-stabilized. This may be to allow for rapid protection of TMD and thus avoid mistargeting to the Sec-dependent pathway. Additionally, these results have shown that T3SC may have evolved to accommodate the structural characteristics of their respective interacting partners.

P 07

Molecular characterization of the Ser/Thr kinase HipA in *Klebsiella pneumoniae*

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Bacterial persisters are a subpopulation of antibiotic-tolerant bacteria produced by isogenic populations of antibiotic-sensitive bacteria. During antibiotic treatment, they can switch to slow growth with low metabolic activity (dormant-like state), resuming a normal growing state upon withdrawal of treatment. Persistent pathogens are a significant contributor to relapse in many chronic infections and frequently result in antibiotic overuse and the development of antibiotic resistance. One of the best-studied drivers of persistence is the eukaryotic-type Ser/Thr kinase, HipA from *E. coli* and a recent study has identified homologs of the *hipA* in many bacterial species.

Here, we aimed to characterize the putative *hipA* gene in the human pathogen *Klebsiella pneumoniae*, which shares 70.2% sequence identity with the highly conserved kinase domain of *hipA* in *E. coli*. *K. pneumoniae* belongs to the ESKAPE group of pathogens that are showing increasing resistance to antibiotics and the formation of multi-drug tolerant persister cells; however, mechanisms underlying persister formation in *Klebsiella* are poorly understood. To characterize *hipA* in *K. pneumoniae* (*hipAkp*), we first performed a shotgun phosphoproteomics study and analyzed the impact of *hipAkp* overexpression in WT and *delta-hipBA* mutant of *E. coli.* These experiments were then repeated in *K. pneumoniae*.

Our phosphoproteomics results showed that *HipAkp*, in both *E. coli* and *K. pneumoniae*, auto-phosphorylates at Serine 150. This residue belongs to a conserved loop in *E. coli* and is reported to be important for the regulation of kinase activity. Multiple putative substrates were detected in *hipAkp* over-expressing cells, including the GltX (glutamate tRNA synthetase), which was phosphorylated at Serine 239 in both WT and *dhipA* background. In *E. coli*, this phosphorylation leads to the accumulation of uncharged glutamate tRNA and activates the stringent response in cells, ultimately leading to antibiotic tolerance and persistence. Importantly, *HipAkp* over-expression showed increased protection against certain antibiotic classes as compared to WT *K. pneumoniae,* as also seen in *hipA* from *E. coli* over-expression. We, therefore, postulate that *hipA* plays a role in the regulation of antibiotic tolerance and persistence in *K. pneumoniae.*

P 08

Klebsiella pneumoniae brain abscess caused by dissemination of a virulent subpopulation from a urinary tract infection

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Simple point mutations that are acquired during infection can have significant consequences on the pathogenicity of bacterial and viral pathogens, as recently highlighted by the evolution of SARS-CoV-2 in an immunocompromised patient (Choi et al., N Engl J Med 2020). Here we present a case in which acquisition of simple mutations in the bacterial pathogen *Klebsiella pneumoniae* enabled dissemination from the urinary tract to the brain of a patient. Phenotype-driven genomics led to the identification of a subpopulation of hypercapsule producing mutants in urine which occured homogenously in a brain abscess. Hypercapsule producing isolates displayed phagocytosis resistance and were lethal in a mouse model of urinary tract infection. The combination of Illumina and Oxford Nanopore sequencing technologies established that the hyper and normal capsule producing populations were closely related and excluded the possibility of acquisition of virulence factors that could explain the virulence phenotype. Instead, single nucleotide polymorphisms in capsule biosynthesis genes were identified in the hypercapsule mutants, including point mutations in *wbaP*. Integration of a single *wbaP* point mutation in the parent isolate led to increased capsule production and virulence in the mouse model of urinary tract infection. This case illustrates that as whole genome sequencing of clinical isolates becomes increasingly accessible, we have the opportunity to understand the evolution of virulence within patients, with the potential to integrate such information into more sophisticated management of patients.

P 09

A hybrid computational approach to characterize the dynamics of epithelial cell monolayers during intracellular bacterial infection

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Epithelial cell monolayers are intricate and dynamic systems that exhibit temporal variations in their behaviour. The distribution of cells within the monolayer, as well as the coordination of cell migration, can impact critically the organization of the monolayer and in turn the function of a tissue. Despite this, the mechanisms underlying how cell monolayers respond to various (extra)cellular stimuli, such as mechanical or chemical signals, remain incompletely understood. Analysing *in vitro* and *in silico* the morphology of cells in a monolayer, alongside with the cell kinematics and dynamics, can provide valuable insights relative to the physical state of the cells. This in turn can enhance our understanding of how cells behave when encountered in confluent conditions but also when they experience insults such as bacterial infections.

In this study, we introduce a novel hybrid two-dimensional computational model to investigate how cell-generated forces are modulated during intracellular bacterial infection. Our model integrates an Agent-Based Model, which considers the individual properties of cells within the epithelial cell monolayer, with a Finite Element Method, which captures the collective behaviour of cells. By combining both models in a feedback loop, we overcome the inherent limitations of each individual approach and we can propose physical laws not only at the level of individual cells but also at the continuum level. This combinatorial approach offers a unique opportunity to gain insights into the complex interplay between cellular and collective phenomena.

Our model successfully reproduces the kinematics, dynamics, and the evolution of shapes and sizes of both uninfected and bacterially-infected epithelial cells in monolayer, as confirmed by experimental validation. In this study, we analyse the intra- and inter-cellular stresses exerted within the cell monolayer and compare our findings with experimental results obtained via Monolayer Stress Microscopy. Our analysis reveals that cell-cell interactions have a significant impact on the topography of cell-cell junctions, which in turn plays a critical role in cellular communication and force transduction and can determine the outcome of the cell competitions such as those that arises during bacterial infection.

P 10

Leveraging microphysiological technology to study the effects of fluid shear stresses apically imposed on endothelial cells interacting with bacterial pathogens

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Introduction

Bacterial infections are on the rise worldwide while treatment options are dwindling due to the emergence of pan antibiotic resistant strains and the low success in development of novel antibiotics. Thus, it is imperative to better understand the mechanisms that pathogenic bacteria employ to infect and spread throughout the human body to open the path towards novel treatment options. Microphysiological systems are a powerful tool to tackle such challenges, as they allow for the use of human-sourced biological material and the perfusion necessary to recreate the dynamic nature of the tissue environment. In this work we aim at using a microphysiological vessel-on chip model to decipher the role of shear flow in modulating interactions between vascular endothelia cells (ECs) and immune cells infected with bacterial pathogen *Listeria monocytogenes (Lm).*

Objectives

By leveraging microfluidic technology, we aim to create a device that allows us to recapitulate the human EC layer, account for the fluid shear stresses applied at the apex of ECs, and measure EC biomechanics during interactions with *Lm*-infected macrophages (MΦs). In particular, we will examine how shear stresses impact: (1) infected MΦ adhesion and transmigration through ECs; and (2) *Lm* transfer from MΦs into ECs.

Materials & methods

The microfluidic device is comprised of a PDMS layer, patterned through soft-lithography and bonded to a glass substrate *via* oxygen plasma surface functionalization. The inner surfaces of the microchannels are then coated with a mixture of fibronectin and collagen I. Primary HUVEC (ECs) are seeded on the base of the coated channel and let to adhere overnight. Once they are attached, the pumping system is connected, and the perfusion flow rate is ramped up to the working flow rate over several hours. Infected (or not) PMA-activated U937 are used as model MΦs and introduced into the system after 24 h of perfusion. The effect of varying magnitude shear stresses on EC biomechanics and interactions with MΦs are then assessed through a combination of live cell imaging and immunofluorescence staining, followed by image processing.

Results

Initial results using the system focus on the establishment of the healthy model. It is observed that ECs distinctively react to increasing magnitude fluid shear stresses by enhancing quasi-monotonically the VEC aspect ratio and orientation and also form a 3D vessel-like structure in the microchannel. Addition of MΦs to the system induces additional morphological changes to the EC layer even under static conditions, while also increasing the amount of F-actin stress fibers in ECs.

Conclusion

Here we show the establishment of a workflow for a microphysiological model for future research on intercellular interactions between circulating MΦs and ECs. This model will be further refined by using primary MΦs sourced from blood donors and will serve as a platform for the study of infection processes and beyond.

P 11

Role of the alternative sigma factor SigB on antibiotic tolerance in *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic pathogen that colonizes up to 30% of the human population asymptomatically, thereby increasing the risk of chronic and relapsing infections. The emergence of antibiotic-resistant *S. aureus* strains such as MRSA and VRSA, as well as its ability to form drug-tolerant persister cells are often implicated in treatment failure of such infections which are further associated with high morbidity, mortality, and healthcare costs.

Persistence, unlike resistance, refers to the ability of a sub-population of the bacteria to survive lethal doses of bactericidal antibiotics without being genetically distinct from the susceptible remnant population. The mechanism leading to such persister phenotypes /intrapopulation heterogeneity, however, are still not fully understood.

Previous research has shown that *S. aureus* cells in the stationary phase exhibit antibiotic tolerance similar to persisters. Thus, it was postulated that persistence in earlier growth phases of the bacteria might be due to cells that had (in response to ATP depletion) already entered the stationary state or remained within it [1, 2]. This was shown by the detection of stationary-phase reporters/markers, like the promotor of the capsular polysaccharide (P*cap*), in a growing culture using promotor fluorescence-protein fusion constructs. Activity of P*cap* was shown by our group to be regulated mainly on the transcriptional level by the alternative sigma factor B (SigB), resulting in a heterogenous expression pattern in the stationary phase [3].

We aimed to investigate the potential involvement of SigB in the regulation of persister cell formation in *S. aureus*. Therefore, we performed cooperative analysis experiments of *S. aureus* wildtype and SigB mutant strains after treatment with various bactericidal antibiotics, which revealed a reduced antibiotic tolerance of the SigB mutant.

Besides P*cap* as stationary phase marker we introduced a P*asp23* promotor-reporter construct, which is often used as a proxy for sigB activity, to directly observe the involvement of sigB on persister formation.

Further, we plan to connect the observation of these stationary-phase markers within a growing culture with individual metabolic activities of cells using different single-cell fluorescent based methods. We hope that these approaches will provide valuable insights into the formation of persister cells, enabling a more comprehensive understanding of the underlying mechanisms.

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P 12

Comparative genomics of disease associated Clostridium perfringens isolates

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1. Introduction: *Clostridium perfringens* is the etiological agent for numerous significant animal diseases, such as porcine necrotic enteritis (NE) and small ruminant enterotoxemia. Key to their pathogenicity is their capability to produce a wide array of virulence factors, including enzymes and pore-forming toxins (PFTs). However, our understanding of the virulence machinery associated with *C. perfringens* in different diseases is still limited.

2. Objectives: To address this issue, we built up a biobank containing *C. perfringens* isolates derived from diagnostic cases with typical signs for clostridial infections. We aimed to gain better understanding of genomic diversity of pathogenic clostridial strains.

3. Material & methods: We performed PacBio long- and Illumina short read whole-genome sequencing of 136 *C. perfringens* isolates derived from 89 animals, including pigs, horses, dogs, sheep, goats, alpacas, calves, and lorikeets. Fully assembled and annotated genomes were compared bioinformatically, and we performed comparative genomics, with a particular focus on pore-forming toxins.

4. Results: *C. perfringens* isolates derived from similar diseases are more closely related to each other and display similar toxin and virulence factor profiles. The recently described PFT homolog LdpC was found in five isolates. We provide first sequences of intestinal *C. perfringes* isolates derived from an alpaca and coconut lorikeets.

5. Conclusion: Our data expands the spectrum *C. perfringens* genomic data with a focus on disease association. Knowing the toxin and virulence repertoire of this important pathogen might improve our future attempts to prevent and treat C. perfringens induced diseases.

P 13

BV200: an anti-virulence agent to prevent/treat *S. aureus* infections and/or *S. aureus*-induced disease exacerbation.

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Background: BV200 is a novel series of anti-virulent small molecules designed to block *S. aureus* Agr quorum-sensing system. The Agr quorum-sensing system regulates the expression of many virulence factors that are required for *S. aureus* pathogenesis in skin and lung infections as well as for *S. aureus* mediated exacerbation of atopic dermatitis.

Objectives: We aim to characterize the *in vitro* activity and *in vivo* efficacy of the BV200 series against *S. aureus* virulence.

Materials & methods: The *in vitro* activity of the BV200 molecules was determined on a panel of 72 *S. aureus* clinical strains isolated from geographically diverse (12 different countries) skin and soft tissue infections. The *S. aureus* strains of the panel were characterized for their Agr type and AgrA mutations using genotyping and sequencing, respectively. The *in vitro* activity of the BV200 molecules was determined using HPLC-mediated determination of the expression of δ-toxin, the major Agr-regulated toxin. We assessed BV200 *in vivo* efficacy in a *S. aureus* lung infection mouse model and in *S. aureus* intradermal and superficial skin infection mouse models.

Results: The majority of the strains belonged to the Agr type I (55.6%) and only 3 strains (4.2%) carried an AgrA mutation, excluding the phylogenetic and functional K136R mutation. Strains from the Agr type I significantly expressed more δ-toxin than strains from the other Agr types, while δ-toxin expression was not significantly different between MRSA (n=34) and MSSA (n=38) strains. BV200 compounds at 16 µg/mL reduced δ-toxin expression in all the strains from the panel with a δ-toxin median population expression reduced by 20-fold. These data demonstrate the potent Agr inhibition *in vitro* activity of the BV200 compounds regardless of the Agr types and suggest the absence of pre-existing resistance to BV200. In addition, mice administered orally with BV200 compounds at 150 mg/kg showed improved survival in an *S. aureus* mouse lung infection model compared to untreated control (90% vs. 20%). Furthermore, BV200 compounds administered orally at 25 mg/kg or topically at 0.06% in murine intradermal or superficial skin infection models, respectively, significantly reduced the size of the skin lesions compared to vehicle controls (34 mm2 vs. 84 mm2 and 27 mm2 vs. 84 mm2). Altogether, these data confirm that the potent activity observed *in vitro* translates into efficacy in animal models.

Conclusions: Compounds from the BV200 series are able to inhibit the Agr quorum-sensing system leading to a reduction of disease-specific *S. aureus* virulence factors that are essential for pathogenesis. Together, these data highlight the potential of BV200 compound series to prevent *S. aureus*-mediated damages during infection or colonization.

P 14

Genome-scale metabolic modeling of *Rothia mucilaginosa* reveals insights into metabolic capabilities and antimicrobial strategies for cystic fibrosis

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Cystic fibrosis (CF) is an inherited genetic disorder caused by the mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene, resulting in the production of sticky and thick mucosal fluids[1]. This facilitates the colonization of opportunistic microorganisms, causing progressive acute and chronic lung infections. *Rothia mucilaginosa,* an oral commensal, is relatively abundant in the lungs of CF patients[2]. Recent studies have unveiled the anti-inflammatory effects of *R. mucilaginosa* using in vitro 3D lung epithelial cell culture and in vivo mouse models[3]. However, its metabolic capabilities and genotype-phenotype relationships remain largely unknown. To gain insights into *R. mucilaginosa"s* bacterial metabolism and genetic alterations, we developed the first manually curated genome-scale metabolic model, *i*RMUC23NL. Through growth kinetic experiments and high-throughput phenotypic microarray assays, we validated *i*RMUC23NL to accurately predict the bacterium"s growth patterns and substrate utilization. We refined the network reconstruction by comparing the *in vitro* results with *in silico* simulations, incorporating over 90 metabolic reactions and 60 genes into *i*RMUC23NL. Furthermore, we employed constraint-based modeling and flux balance analysis to generate predictions that could expedite the development of antiviral strategies. We identified putative essential and assessed their potential vulnerability under varying nutritional conditions. These predictions offer promising antimicrobial targets without the need for laborious large-scale screening of knock-outs and mutant transposon libraries. Additionally, *R. mucilaginosa* produces the most Fe3+-binding siderophore known, called enterobactin. This attribute contributes to its high virulence against cariogenic and multidrug-resistant pathogens[4]. Using our model, we examined the production levels of enterobactin under varying nutritional conditions. Overall, *i*RMUC23NL demonstrates a solid capability to predict cellular phenotypes and holds immense potential as a knowledge base for reliable predictions in antimicrobial therapy development. Moreover, it can guide metabolic engineering strategies to tailor *R. mucilaginosa"s* metabolism for desired performance. Figure: Network reconstruction process for *i*RMUC23NL. The workflow consists of eight steps starting from the annotated genome sequence until the validation using experimental data.

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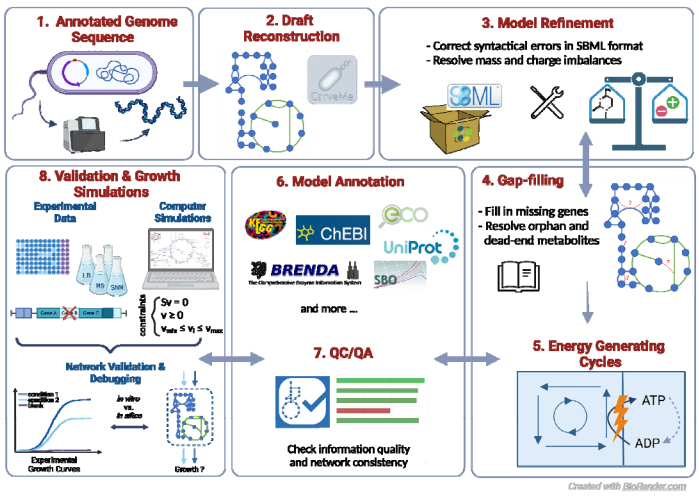
4. Uranga, C. C., et al. (2020). Commensal oral *Rothia mucilaginosa* produces enterobactin, a metal-chelating siderophore. *Msystems*

Fig. 1



P 15

Identifying the cellular receptor for *Clostridium perfringens* NetF.

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Introduction*: Clostridium perfringens* causes enteric diseases in different animal species and humans. Its pathogenicity relies on the secretion of a large arsenal of virulence factors, many of them belonging to the family of hemolysin-like β-pore forming toxins (β-PFTs). Knowledge about role and action of many of these toxins is still limited. To understand their role in the pathogenesis of bacterial infections we need to determine the molecular basis of their cell, tissue and species specificity.

Objectives: We aimed to determine the cellular receptor of Necrotizing Enteritis Toxin F (NetF), a hemolysin-like β-PFT associated with fatal hemorrhagic enteritis in dogs and foals caused by *C. perfringens* type A strains.

Materials and Methods: Using recombinantly expressed NetF we performed cell viability assays on 29 different mammalian cell lines and comparative RNA seq analyses on selected cell lines. Receptor candidate confirmation was performed using CRISPR/Cas9 single gene knockout and ectopic overexpression studies. Mutated receptor proteins were expressed in HAP1 cells to determine the receptor specificity of NetF.

Results: Using a comparative RNAseq approach on susceptible and resistant cells, we identified Capillary Morphogenesis Protein 2 (CMG2 or ANTXR2) to be important in NetF-mediated cytotoxicity. CMG2 is also one of the two known receptors for protective antigen (PA) of *Bacillus anthracis* anthrax toxin. We demonstrated that CMG2 expression on target cells is essential for NetF toxicity and that PA competitively inhibits NetF cytotoxicity. To further investigate the interaction of NetF with its putative membrane protein receptor, we engineered mutant versions of CMG2 lacking parts of its extracellular domain.

Conclusions: We determined the cellular receptor for a central virulence factor of C. perfringens strains causing enteric disease in horses and dogs. Furthermore, our results highlight molecular mechanisms and structures that confer receptor-, cell-type and species specificity for clostridial hemolysin-like βPFTs.

P 16

Resensitizing multiresistant strains with plasmid-delivered CRISPR systems

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The spread of antibiotic resistance among pathogens poses a significant threat to human health. Plasmids, small, circular DNA molecules found in bacteria, often harbor genes encoding antibiotic resistance traits and foster the spread and emergence of multiresistant strains. Traditional approaches to combating antibiotic resistance through the development of new antibiotics have slowed in recent years; however, the precision of the revolutionary CRISPR-Cas9 system offers a promising new approach to combating antibiotic resistance. By introducing CRISPR-possessing plasmids that specifically target the antibiotic resistance genes on the resistant ABR plasmids, the susceptibility of the bacteria to antibiotics gets restored and in addition the uptake of the resistance gene is prevented.

However, genetic variations within bacterial populations can hinder the effectiveness of CRISPR-mediated cleavage. Using mathematical modeling and simulations, we investigate the interplay between CRISPR-based plasmid cleavage and genetic variations. We compute the success probabilities of resensitizing a bacterial population with standing genetic variations that impede CRISPR-mediated cleavage of resistance plasmids. Our analysis predicts the success rate of resensitizing treatments and how it depends on the copy number of the plasmid, the compatibility of the CRISPR plasmid and the ABR plasmid, the effect of escape mutations, and the number of spacers in the CRISPR array.

We find that the usage of incompatible and fast-replicating CRISPR plasmids substantially enhances the efficacy of the treatment. In addition, we investigate how utilizing multiple spacers targeting critical regions of resistance plasmids increases the success probability. Our predictions emphasize the significance of taking these factors into account during the development and design of CRISPR possessing plasmids for targeting resistance plasmids. These results are a first step towards the design of novel strategies based on plasmid-delivered CRISPR systems to combat antibiotic-resistant strains and hinder the spread of antibiotic resistance without disrupting the microbiome.

P 17

Skin microbiome dynamics as predictor and pathogenesis mechanism for severe radiodermatitis in breast cancer patients

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Radiodermatits is commonly observed during radiotherapy in post-operative breast cancer patients, severly impacting the quality of life of the patients. So far, the inter-individual differences regarding radiodermatitis severity and the pathomechanism are not sufficiently understood. To elucidate the role of the skin microbiome and skin physiology in the development of radiodermatitis, we conducted a longitudinal pilot study with 20 female breast cancer patients undergoing radiotherapy.

From each patient, the skin pH and skin microbiome were assessed via next-generation sequencing of the V1-V3 region of the 16S rRNA and quantitative PCR on both the affected and non-affected bodysides before, during and after radiotherapy on a weekly basis (360 samples). Additionally, radiodermatitis severity was determined.

After five to seven weeks, n=4 patients developed severe radiodermatitis. Strikingly, low (<5%) colonization with skin commensals (*Staphylococcus epidermidis, Staphylococcus hominis, Cutibacterium acne*s) at baseline was highly predictive for the development of severe radiodermatitis. Instead, different *Corynebacteriaceae* species were more abundant in severe cases. The microbial composition was associated with the skin pH, where *Corynebacteriaceae* were positively, and commensals were negatively correlated with skin pH. Prior to the onset of severe symptoms, only severe patients showed an increase in total bacterial cell numbers estimated via qPCR of the 16S rRNA copies in contrast to stable bacterial colonization in mild and moderate radiodermatitis cases.

In summary, we have observed two logically linked phenomena exclusively in severe patients: a low baseline level of commensals, and an early increase in total bacterial load. Thus, our findings potentially show for the first time that microbes have a direct effect on the pathogenesis of radiodermatitis.

P 18

Invasive *Staphylococcus capitis* isolates from neonatal sepsis specified to oppose colonizers and resist antibiotics of last resort and human intestinal immunity

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Neonatal Late-Onset-Sepsis (LOS) is a major cause of neonatal morbidity and mortality, especially in preterm infants with very low birth weight (VLBW). Preterm infants possess a highly vulnerable gut immune barrier subjected to a premature microbial colonization. Intestinal dysbiosis is major risk factor and route of infection in LOS. In contrast to adult sepsis, LOS is most commonly (>50%) caused by coagulase-negative staphylococci (CoNS) with high rates of *Staphylococcus capitis* isolates. A multi-resistant S. capitis clone called NRCS-A has recently caused endemic outbreaks of bloodstream infections in neonatal intensive care units (NICU) in various countries worldwide. Our goal was to phenotypically characterize the NRCS-A linage to explain why this clone so successfully causes neonatal sepsis.

We established a collection of CoNS (*S. capitis*, *S. epidermidis* and *S. haemolyticus*) isolated from LOS in our NICU to assess genetical and phenotypical characteristics of such strains allowing to specify for the neonatal (intestinal) niche. Whole genome sequencing assigns our *S. capitis* isolates to the NRCS-A clone and reveals the exclusive presence of the nisin resistance gene *nsr* and a *tarIJL* gene cluster crucial for synthesis of specific Wall Teichoic Acids. The *S. capitis* isolates showed an increased cell wall thickness in transmission electron microscopy and a higher cell surface charge compared to non-invasive strains. *S. capitis* sepsis isolates where multi-resistant to antibiotics, including daptomycin, and accordingly possessed multiple genetic variations in genes associated to daptomycin resistance in S. aureus. Consistent with the expression of *nsr*, our isolates revealed an increased minimal inhibitory concentration for the bacteriocin nisin, likely to support overgrowth against nisin-producing intestinal colonizers such as Lactobacillales. We further analyzed resistance to intestinal antimicrobial peptides and bacterial binding to immunoglobulins. Our isolates show a decreased susceptibility to human alpha-defensin 5 (HD5), the major antimicrobial peptide controlling microbial homeostasis in the human gut, but not to HD6 and LL-37. FACS binding analysis to IgA, IgG and MBL binding revealed an altered binding of *S. capitis* and *S. haemolyticus* bloodstream isolates compared to non-invasive strains. While serum and colostrum IgA deposition on those strains was decreased, MBL and IgG deposition was increased in bloodstream isolates. Evasion from IgA binding could serve to support intestinal overgrowth of such strains to subsequently invade through a leaky gut barrier.

In conclusion, the *S. capitis* NRCS-A linage specified to colonize the neonatal intestinal niche by resisting co-colonizers´ bacteriocins (e.g., nisin) and antibiotics frequently used in NICUs which could facilitate intestinal overgrowth. NRCS-A clones further evade from intestinal immunity by decreased IgA binding and increased resistance to intestinal antimicrobial peptides.

P 19

Frequency of Hepatitis C virus in diagnosed Tuberculosis cases

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Background: The frequency of hepatitis C virus infection along with tuberculosis has not been widely investigated and very low statistics on rates of hepatitis C virus co-infection in tuberculosis patients. Hepatotoxicity is the major side effect of anti-tuberculosis therapy hepatitis HCVliver disease elevates the chances of hepatotoxicity up-to five folds Objectives & Aim: To see the frequency of Hepatitis Cvirus infection amongst people with diagnosed Tuberculosis using gene X-pert technique. To evaluate the factors associated with HCVinfection in patients with MTBtuberculosis and to determine sensitivity and specificity of the tests. Study design: Comparative analytical study. Methodology: Three hundred and thirteen patients of tuberculosis diagnosed by Genexpert included while testing hepatitis C virus using immunochromotography rapid test technique, enzyme linked immunosorbent assay method and polymerase chain reaction test for confirmation. Results:Higher frequency of tuberculosis infection in males 57.8%, 42.5% between 20-39 years and 22% of hepatitis C virus infection in tuberculosis patients.Sensitivity of rapid test and enzyme-linked immunosorbent assay was 79% and 96% respectively while the specificity of rapid test and enzyme-linked immunosorbent assay was 91% and 99% respectively.

Discussion:- The frequency of hepatitis C (HCV) positive cases were 22%. In male 44(24.4%) as compared to female 25 (18.9%) $p<0.05$. This study is with accordance to another study conducted by Kuniholm et al20 and Richard et al,21 in Georgia found 22% HCV although various international studies showed frequency rate of HCV in tuberculosis were 7% which documented by Reis et al19 that is very low compare with our study.While on the contrary in Thailand a study expressed very high frequency for HCV in tuberculosis which is 31%22. The study shows specificity and sensitivity of Rapid test (ICT) 91% and 79% respectively somehow matches with the another study conduct at king Edward medical university Lahore that compare the rapid test with ELISA showed specificity and sensitivity 97% and 95% respectively in our study PCR was the gold standard.On ELISA test the detection of HCV showed 99% and 96% specificity and sensitivity respectively which will be accordance with most of the international study.This study recommend and suggest that more work to be done on patient taken anti-tuberculosis therapy and infected to HCV get more hepatotoxicity than non-infected HCV.

CONCLUSION The high frequency of HCV co-infection was detected amongst TB cases in MirpurKhas division Sindh. This suggested to pay more attention on continuous surveillance by the authority to minimize the transmission. Gene Xpert technique is accurate and time saving test for tuberculosis, ELISA method is more accurate, reliable as compared to rapid ICT test for HCV and PCR is still gold standard.

Keywords: TB, Hepatitis C virus, Mycobacterium tuberculosis, PCR, Genexpert, Rapid test.

Fig. 1

Table 1: Frequency of diagnosed tuberculosis with gender (n=313)

Diagnosed cases	No.	%
Negative	244	78.0
Positive	69	22.0

Table 2: Comparison of positive cases among gender (n=69)

Gender	No.	%	P value
Male	44	63.7	0.002
Female	25	36.3	

Table 2: General characteristics of diagnose tuberculosis patients by gene x pert (MTB) (n=313)

Myobacterium tuberculosis	No.	%
Gender		
Male	181	57.8
Female	132	42.2
Age (years)		
< 20	32	10.2
20-39	133	42.5
40-59	108	34.5
>60	40	12.8
Socioeconomic status		
Fair	100	31.9
Good	29	9.3
Poor	184	58.8
Sputum AFB		
Positive	263	84.0
Negative	34	10.9
Contaminated	2	0.6
Not done	14	4.5

Table 3: Frequency and comparison of Hepatitis C (HCV) on rapid test device (ICT) and ELISA, with PCR in Mycobacterium tuberculosis (MTB) patients (n=313)

Method	No.	PCR	
		Positive	Negative
ICT			
Positive	91 (29.1%)	69 (75.8%)	22 (24.2%)
Negative	222 (70.9%	-	222 (100%)
ELISA			
Positive	72 (23%)	69 (95.8%)	3 (4.2%)
Negative	241 (77%)	-	241 (100%)

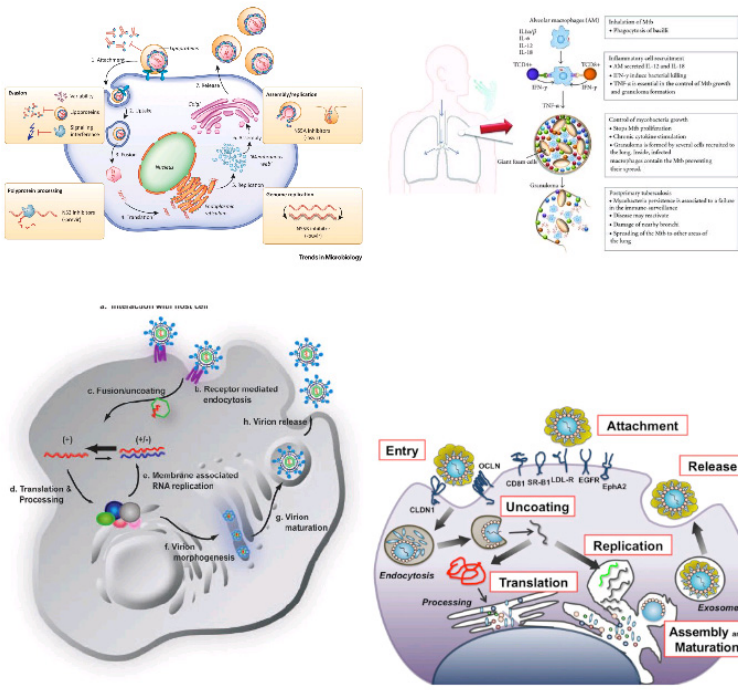
Sensitivity of ICT = 69/91X100 = 78.8%

Specificity of ICT = 222/244X100 = 90.9%

Sensitivity of ELISA = 69/72X100 = 95.8%

Specificity of ELISA = 24/244X100 = 98.7%

Fig. 2



P 20

Towards real-time imaging of the gut microbiome

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Intro

Disruption of the gut microbiota has been linked to various life-threatening diseases. Modulation of the microbiome is thought to be a promising complementary approach to classical antibiotic treatment. However, our understanding of the protective mechanism of the microbiome is still largely superficial. Current available monitoring tools (faecal sampling, sequencing, *ex vivo* strategies) provide little real-time information and require high animal numbers for longitudinal studies. Recently, metabolic engineering approaches have enabled species-specific labelling techniques free of genetic manipulation, notably leading to fluorescence imaging of the commensal *Bacteroides fragilis in vivo* (1), albeit with shallow imaging depth and low resolution. Further work capitalizing on positron emission tomography (PET) advantages showed promise but was ultimately limited by the time constraints resulting from the *in vitro* labelling strategy combined with radioisotopes half-life (2).

Aims

Quantitative, on-demand species-specific *in vivo* imaging could provide a detailed view of bacterial dissemination inside the gut, in real-time, non-invasively and with minimal animal sacrifice. This study aims to establish an *in vivo* PET imaging method based on metabolic engineering followed by *in vivo* click radiolabeling.

Methods

Supplementation of culture medium with azide-modified monosaccharides led to functionalization of *Escherichia coli* Nissle 1917 (*Ec*N)s membrane. Various azidosugars and incubation conditions were tested for protocol optimization. Verification of the labelling efficiency was performed by "clicking" Dibenzocyclooctyne (DBCO) conjugated fluorescent probes followed by fluorescence-activated cell sorting (FACS) analysis and *in vitro* fluorescence imaging. Radiolabelling was performed with ^{64}Cu-NODAGA-DBCO and quantified by *in vitro* Gamma Counter measurements. Finally, expansion of this toolbox for the imaging of *Salmonella enterica* Serovar Typhimurium (*S*. Tm) has begun to demonstrate translatability to other bacterial species.

Results

Azido-mannose provided the best performance for future *in vivo* studies due to the highest labelling efficiency being achieved within the shortest time frame. Results were confirmed by Gamma Counter readings, showing translatability to radiolabeling. Initial results for *S.* Tm decorated with azido-galactose illustrate efficient labelling, with testing of additional sugars still underway.

Conclusion

With high labelling efficiency being achieved, we are now starting to investigate the interaction between *Ec*N and *S.* Tm at a macroscopic level *in vivo* using PET. We aim to uncover the colonization of both bacteria in presence or absence of the other, and further apply this new toolbox to other microbiome modulation techniques.

Ref.

(1) Geva-Zatorsky et al. Nat Med. 2015; 21(9): 1091-1100.

(2) Wang et al. Eur J Nucl Med Mol Imaging. 2020; 47(4): 991-1002.

Fig. 1: Comparison of mean fluorescent intensity for labelled *Escherichia coli* Nissle

Fig. 2: IVIS panel of MOE-BCC labelled *Escherichia coli* Nissle

Fig. 1

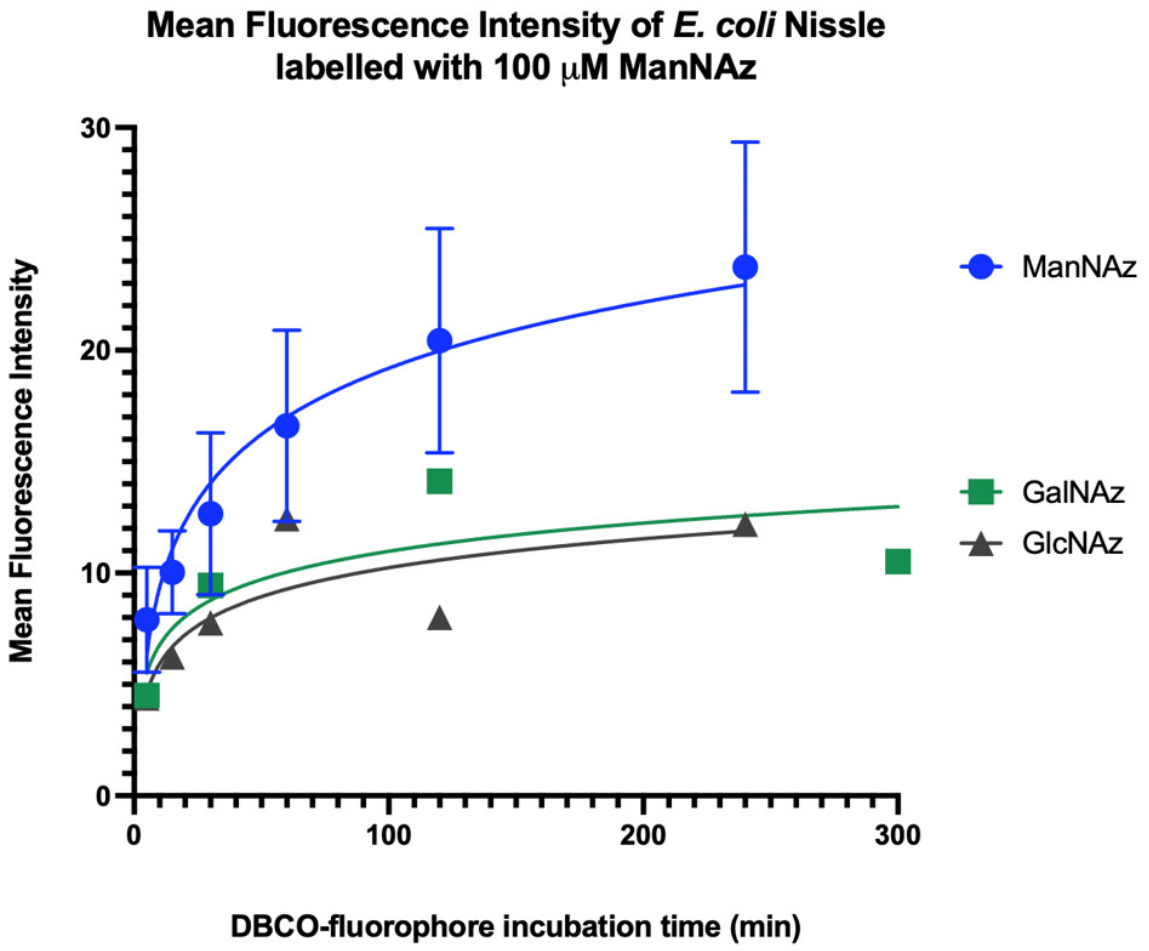
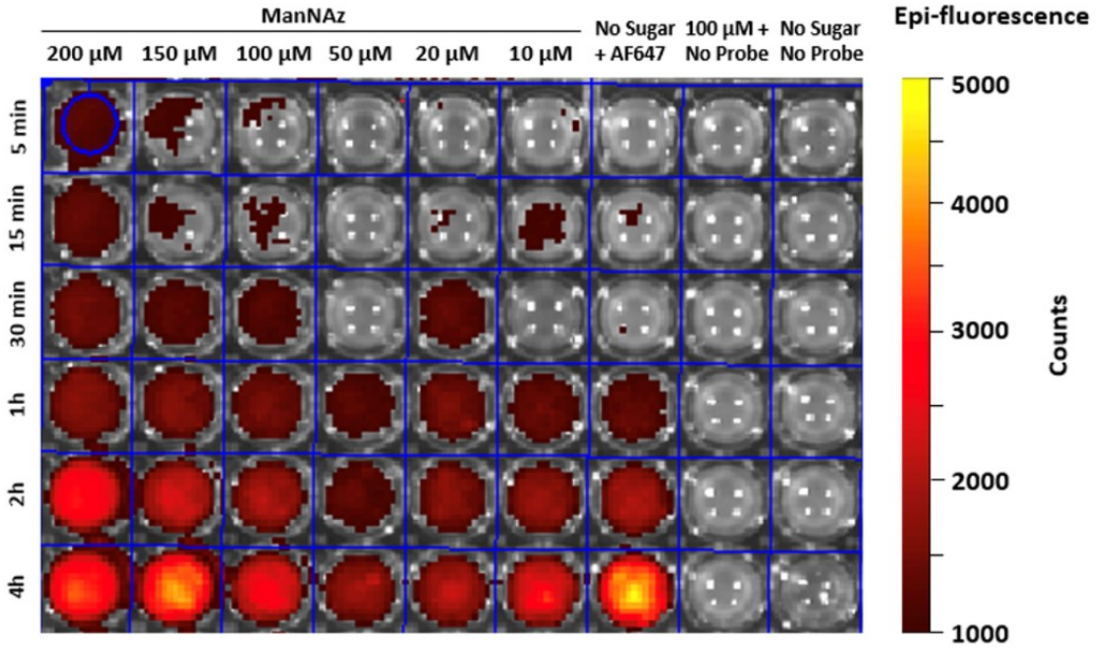

Mean Fluorescence Intensity of E. coli Nissle labelled with 100 μM ManNAz
Mean Fluorescence Intensity
30
20
10
0
0
100
200
300
DBCO-fluorophore incubation time (min)
ManNAz
GalNAz
GlcNAz

Fig. 2


ManNAz
200 μM
150 μM
100 μM
50 μM
20 μM
10 μM
No Sugar + AF647
100 μM + No Probe
No Sugar No Probe
5 min
15 min
30 min
1h
2h
4h
Epi-fluorescence
5000
4000
3000
2000
1000
Counts

P 21

Dynamics of antibiotic resistance in *Staphylococcus aureus* during persistent airway infection in people with cystic fibrosis

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Introduction: Cystic fibrosis is a hereditary disease which especially affects the airways causing chronic recurrent bacterial infections leading to lung insufficiency and decreased life expectancy. *Staphylococcus aureus* is one of the most cultured pathogens with increasing prevalence from early childhood until a peak in adolescence with a decline in adults. One of the pillars of treatment for pwCF is antibiotic therapy to treat exacerbations of bacterial airway infections or to suppress persistent infections. Recently, we conducted a study to determine the diversity of *S. aureus* during persistent airway infection. During a one-year study, from every sputum of 14 patients 40 *S. aureus* isolated were picked and characterized in terms of pheno- and genotypic characteristics, clinical parameters such as lung function, antibiotic therapy and co-infection with *Pseudomonas aeruginosa*.

Objectives: To investigate the dynamics of antibiotic resistance of *S. aureus* during our diversity study with analyses of pheno- and genotypic susceptibility.

Materials and Methods: From our study, 2319 *S. aureus* isolates collected from sputa of 14 pwCF during 58 visits, were phenotypically analyzed for their susceptibility to 8 important staphylococcal-active antibiotics: penicillin, flucloxacillin, erythromycin, clindamycin, gentamicin, levofloxacin, trimethoprim-sulfamethoxazole and rifampin. Resistance was genotypically tested for the presence of resistance genes or known mutations in target genes. Selected isolates were subjected to whole genome sequencing to assess clonal relatedness and to identify resistance genes and mutations.

Results: Forty-nine different resistance types (RT) could be distinguished with one to 425 isolates per RT. Nine RTs were most common and represented 70% of isolates. 11.5% of isolates were penicillin-susceptible (PSSA), 67.6 % methicillin-susceptible (MSSA) and 20.9% methicillin-resistant (MRSA). In single specimens, up to 13 RTs could occur (mean 5 RTs), which could vary from visit to visit. There was an incongruency of phenotypic and genotypic investigation of resistance. Interestingly, in some pwCF, antibiotic therapy selected resistant isolates, which diluted again at later visits. Also, resistant isolates could be detected, if pwCF were treated with anti-*Pseudomonas* antibiotics such as ciprofloxacin and tobramycin.

Conclusions: In the airways of pwCF, a variety of *S. aureus* with different RTs are present in various density, which can overtake the entire *S. aureus* population once they are selected by antibiotic therapy but are diluted again once antibiotic therapy is halted. Therefore, it is questionable if all isolates of interest are cultured if only one or two colonies are tested for resistance during routine microbiological diagnostic. Also, collateral damage of increased resistance towards aminoglycosides and fluoroquinolones could be observed, if pwCF were treated with these antibiotics to target *P. aeruginosa*.

P 22

The efficacy of the MicroScan WalkAway40 Plus system in reporting antibiotic resistance pattern in patients with urinary tract infection, North India

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Multi-drug resistance (MDR) and extensively drug-resistant (XDR) organisms pose significant challenges to healthcare systems worldwide. This study aims to analyze the prevalence, patterns, and associated factors of MDR and XDR UPEC at a tertiary care referral center in North India. Methods:A retrospective analysis was conducted on data collected from clinical samples obtained from OPD patients and admitted to the hospital in year 2021. The study included 200 urine samples from routine clinical laboratory. Microbiological cultures were performed using standard techniques, and antibiotic susceptibility testing was done using Microscan and carried out according to Clinical and Laboratory Standards Institute guidelines.

Results:The results revealed a high prevalence of MDR and XDR Uropathogenic *E.coli* in the study population. 73% of the tested isolates demonstrated resistance to multiple antimicrobial agents, highlighting the complexity of treatment options. Furthermore, 60% of the MDR isolates exhibited XDR profiles, indicating resistance to even higher classes of antimicrobials.The study identified several risk factors associated with the development of MDR and XDR organisms, including previous exposure to antibiotics, prolonged hospitalization, and healthcare-associated infections. Additionally, the emergence of MDR and XDR organisms was noted in various clinical settings, including intensive care units, surgical wards, and outpatient departments.

Conclusion: This study emphasizes the urgent need for effective infection control measures, rational antibiotic prescribing practices, and antimicrobial stewardship programs to combat the spread of MDR and XDR organisms. Understanding the local prevalence, patterns, and associated factors of drug resistance is crucial for tailoring appropriate strategies to mitigate the impact of these organisms on patient outcomes and public health.In conclusion, the findings of this study underscore the alarming prevalence of MDR and XDR organisms at a tertiary care referral center in North India. The study highlights the importance of continuous surveillance, prompt detection, and targeted interventions to prevent the further dissemination of drug-resistant pathogens and preserve the effectiveness of antimicrobial agents.

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Machine learning and phylogenetic analysis allow for predicting antibiotic resistance in *M. tuberculosis*

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Antimicrobial resistance (AMR) poses a significant global health threat, and an accurate prediction of bacterial resistance patterns is critical for effective treatment and control strategies. In recent years, machine learning (ML) approaches have emerged as powerful tools for analyzing large-scale bacterial AMR data. However, ML methods often ignore evolutionary relationships among bacterial strains, which can greatly impact performance of the ML methods, especially if resistance-associated features are attempted to be detected. Genome-wide association studies (GWAS) methods developed for bacteria, which may employ linearing mixed models, account for evolutionary relationships, but they uncover only highly significant variants which usually have already been reported in literature.

In this work, we introduce a novel phylogeny-related parallelism score (PRPS), which measures whether a certain feature is correlated with the population structure of a set of samples. We demonstrate that PRPS can be used, in combination with SVM and random forest-based models, to reduce the number of features in the analysis, while simultaneously increasing models" performance. Applying the pipeline to a publicly available set of *Mycobacterium tuberculosis* genomes with phenotypic data on resistance against six common antibiotics from the PATRIC database, followed by a feature importance analysis, we re-discovered known resistance-associated mutations as well as new previously not reported candidates.

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2-deoxy-2-[^{18}F]-fluoro-D-sorbitol: towards PET imaging of bacterial replacement strategies and bacterial infection

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Enterobacteriaceae, a family of Gram-negative bacteria, ranges from strains with notable probiotic properties [1] to infectious pathogens responsible for multiple diseases [2]. However, the current imaging of colonization, bacteria-bacteria, and host-bacteria interactions focuses on *ex vivo* and *in vitro* techniques, with biopsy as the actual gold standard [2]. Sorbitol is a sugar alcohol selectively metabolized by Gram-negative bacteria but not by mammalian cells, which makes the radiolabeled analog 2-deoxy-2-[^{18}F]-fluoro-D-sorbitol (^{18}F-FDS) a good candidate for targeted bacterial PET imaging, a specific, whole-body and no invasive *in vivo* technique [3]. In this study, we tested the uptake of ^{18}F-FDS by *Escherichia Coli* Nissle 1917 (*Ec*N), under different conditions. Additionally, we documented its selectivity towards Gram-negative bacteria over eukaryotes and the increased specificity of FDS for Gram-negative bacteria compared to the PET gold standard, 2-deoxy-2[^{18}F]-fluoro-D-glucose (^{18}F-FDG).

^{18}F-FDS was synthetized by reducing ^{18}F-FDG using $NaBH_4$, and two comparative uptake assays were performed *in vitro*. In a first assay, *Ec*N was incubated with ^{18}F-FDS. Radioactivity was measured in a gamma counter, and relative cell uptake was calculated as a percentage, dividing the counts in pellet by the applied dose. A second assay was conducted similarly, comparing the uptake of ^{18}F-FDS or ^{18}F-FDG by *Ec*N, *Salmonella* Typhimurium SL1344 (*S*Tm), U87MG cells and HeLa cells.

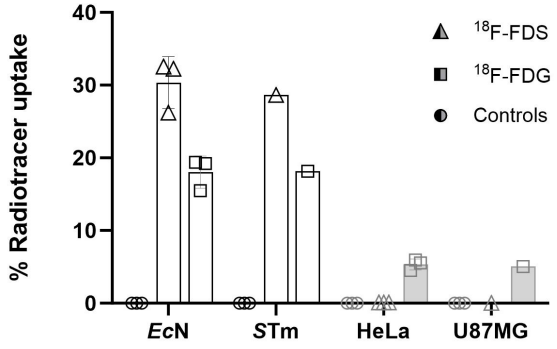
All *Ec*N samples incubated with ^{18}F-FDS showed a significant ^{18}F-FDS bacterial uptake with heat-killed bacteria and washing controls close to 0%. Increased ^{18}F-FDS relative uptake positively correlated with an increase in both bacteria amount (39.84% to 21.77%) and incubation time (34.69% to 26.91%). Increasing the radioactivity amount used did not influence the relative uptake. Furthermore, radiotracer relative uptake was significantly higher in *Ec*N and *S*Tm samples incubated with ^{18}F-FDS compared to those incubated with ^{18}F-FDG (32.72% to 19.52%, 28.66% to 18.14% respectively). In contrast, HeLa and U87MG cells showed minimal ^{18}F-FDS relative uptake (0.11%, 0.06% respectively).

An accurate imaging technology is needed to fully understand bacterial protection mechanisms, optimize bacterial replacement strategies, and localize and identify bacterial infections. Together, these data support ^{18}F-FDS as a candidate PET imaging probe with potential to image Gram-negative bacteria in vivo with high specificity and sensitivity, and therefore help in the prevention, diagnosis, monitoring, and treatment of multiple diseases.

[1] Haojie Chen, et al., Mater. Today Bio, 2023.
[2] Ordonez, A.A., et al., Sci. Transl. Med., 2021.
[3] Braams, L., et al., Int. J. Med. Microbiol., 2023.

Figure 1: Comparative *in vitro* uptake assay. Uptake of ^{18}F-FDS (triangle) and ^{18}F-FDG (square) in Gram-negative bacteria *Ec*N and *S*Tm (black), mammalian cell lines HeLa and U87MG (grey), and controls (circle).

Fig. 1



P 25

Assembly of the type III secretion system export apparatus is a fine-tuned process

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The type III secretion system (T3SS) is essential for virulence of many gram-negative bacteria and is used to translocate a wide diversity of effector proteins into host cells. This megadalton-sized complex is composed of more than 200 subunits, made up by ~20 different proteins. The successful assembly of such a complex system presents a challenge to pathogens and it requires highly coordinated orchestration of assembly. T3SS assembly begins with the formation of the export apparatus, which consists of the five highly conserved subunits SctRSTUV. In order to better understand export apparatus assembly, we performed a global assessment of the possible regulatory factors in the assembly process. Despite the highly conserved gene order of the export apparatus, the assembly sequence was found to be different. To assess the importance of this conserved order, SctT was expressed alone from a plasmid and this resulted in an overexpression phenotype and reduced secretion function. Scrambling the gene order abrogated secretion and disrupted formation of export apparatus assembly intermediates, as assessed in secretion assays and by blue native PAGE. This phenotype was found to be a result of *sctT* translation regulation by an element in the upstream *sctS* gene. This contains an mRNA stem-loop that conceals the ribosome binding site of *sctT*. We found that *de novo* translation of SctT is inhibited by the presence of this stem-loop and this is melted by ribosome translation of *sctS*. Without this regulation, SctT self-associates and aggregates, thereby preventing efficient assembly of the export apparatus. The data identify a regulatory mechanism for assembly of the export apparatus and this possibly contributes to achieving a correct 5:4:1:1 stoichiometry of the SctRSTU complex despite equal gene dosage.

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Increased temperature benefits *Staphylococcus aureus* competition against upper respiratory commensals

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Staphylococcus aureus is a common pathobiont of the upper respiratory tract (URT). Chronic rhinosinusitis (CRS) is an inflammatory disease affecting the URT that has a significant economic impact. *S. aureus* nasal carriage can be as high as 87% in CRS patients compared to ~20-30% in healthy populations, and *S. aureus* overabundance in the sinuses correlates with worse disease outcomes in CRS patients. Corynebacteria are commensal species that inhabit the URT and are abundant in heathy populations but decreased in chronic URT diseases such as CRS. Previous studies have identified an antagonistic relationship between *S. aureus* and some species of *Corynebacterium*. We hypothesized that different temperatures in the URT representing the healthy nares and the inflamed sinuses can alter commensal-pathogen interactions that occur between *Corynebacterium* and *S. aureus* and affect abundance and pathogenic outcomes. Using an air-liquid interface human nasal epithelial cell (HNEC) model of the URT and in vitro growth assays, we tested the effects of higher (37C) and lower (30C) physiologic URT temperatures on the growth kinetics of two *Corynebacterium* CRS clinical isolates and methicillin-resistant *S. aureus*. Assessing bacterial burden by counting viable colony-forming units and using confocal microscopy revealed lower temperatures benefitted *Corynebacterium* and significantly slowed *S. aureus* growth. Additionally, lower temperatures correlated with significantly lower levels of bacteria-induced cytotoxicity of HNECs. Furthermore, *S. aureus* secretions were found to inhibit *Corynebacterium* aggregation and adhesion to HNECs. Using an in vitro aggregation assay, a *S. aureus* secreted, agr quorum sensing-regulated protein has been identified to be pivotal for inhibiting *Corynebacterium* aggregation, and *S. aureus* production of this factor is increased at higher temperatures. Ongoing studies are evaluating the behavioral changes of both commensal and pathogenic species at different temperatures and their impact on cytotoxicity to host cells. Investigating how temperature differences play a role in the dynamics of URT microbiome constituents in CRS will provide insight into the pathology of the disease and provide possible avenues for therapeutic interventions.

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Microbial competition on human skin: *Staphylococcus aureus* versus *Staphylococcus lugdunensis*

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The skin microbiome is a crucial factor in our defence against invasive bacteria such as *Staphylococcus aureus*, with the microbiome showing to be a valuable source of antimicrobial compounds. These compounds could be highly advantageous as treatment options, amid the ever-growing antibiotic resistance problem. Previous work in our lab has shown that a commensal strain of *Staphylococcus lugdunensis,* isolated from human skin, can inhibit the growth of the methicillin resistant *S. aureus* USA300 strain (MRSA) under a set of specific experimental conditions. We found that pre-colonization of *ex vivo* human skin with *S. lugdunensis* was shown to restrict the growth of USA300. Supernatant of *S. lugdunensis* alone was able to inhibit the growth of USA300, but only when grown in specific media conditions. This raises a lot of questions regarding the identify of this inhibitory factor, how is secreted and regulated. When does *S. lugdunensis* deem it necessary to supress *S. aureus*? In this project, we have potentially discovered a novel inhibitory compound produced by *S. lugdunensis* which supresses the growth of *S. aureus.* This compound could be used as a means to protect from *S.aureus* infection without the need for antibiotics. However, more work needs to be done to further understand this compound.

P 28

Suppression of *Staphylococcus aureus* on human skin models by resident skin microbiota

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Human skin forms the first defensive line. Beyond the physical and immune barrier of the skin, skin microbiota plays an active role in this defense. *Staphylococcus aureus*, particularly methicillin resistant *S. aureus* (MRSA), is a leading cause of skin and soft tissue infections yet asymptomatically colonizes up to 30% of the population. My lab focusses on the interaction between skin microbiota and MRSA on healthy human skin. We use explant, xenograft, and bioequivalent human skin models to study co-colonization of MRSA with skin microbiota. We have identified strains of coagulase negative *S. lugdunensis* which effectively suppresses MRSA growth on our human skin models. This suppression only occurs under specific growth conditions, which we are currently working to define, and then correlate to the human skin microenvironment. By understanding the environmental trigger for this CoNS to suppress MRSA we aim to be able to induce the resident skin microbiota to become more defensive.

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Microbiome modulators for environmental enteric dysfunction

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Environmental enteric dysfunction (EED) is an incompletely defined inflammatory syndrome of the small intestine that is common among children in low-income countries. It is associated with a decreased absorptive capacity, reduced vaccine effectiveness, increased risk of infection and stunted growth.

Recent evidence suggests a microbial contribution to EED caused by an imbalance in the microbiome. Both, duodenal fluids and fecal samples from stunted children harbor a distinctive microbial signature dominated by bacteria that usually inhabit the oropharyngeal cavity but diminished in butyrate-producing Clostridia.

Using systematic *in vitro* screening approaches, we have identified several agents that selectively inhibit EED-associated taxa while sparing commensals. The most promising candidates were tested for selective inhibition in clinical isolates from stunted children and in synthetic and patient stool-derived bacterial communities.

Restoring a healthy microbiome balance is supposed to have anti- inflammatory effects, which consequently could alleviate EED symptoms and positively impact child health and development.

P 30

Protecting *Bacteroidaceae* from macrolide antibiotics by antagonizing drug interactions

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Antibiotic therapies not only target pathogens, but also affect health-promoting bacteria in the gut microbiome. Antagonistic drug interactions can be used to protect specific gut bacteria from broad-spectrum antibiotics. However, how these so-called antidotes work remains unclear.

We found that the antidote dicumarol protects *P. vulgatus* not only from erythromycin but also from other macrolides, such as azithromycin. Warfarin also acts as an antidote, indicating that the observed antagonism appears to be conserved across diverse 4-hydroxycoumarins. As protection by antidotes is strain-specific, comparisons of protected and unprotected isolates provide valuable mechanistic insights. For instance, in the presence of efflux pump inhibitors, dicumarol no longer antagonizes erythromycin in protected strains. These results suggest that the antidote affects efflux processes, which may lead to a reduction in intracellular macrolide concentrations.

Ultimately, understanding the molecular mechanisms underlying the protective effects will allow to selectively protect the gut microbiome under antibiotic therapy.

P 31

Autoinducer 3: a one-step construction of the DPO ring system and the formation of both DPO isomers.

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

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Functional analysis on the iron-intake mechanisms of Corynebacteria species

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Iron is vital for bacterial processes, but it is often found in its insoluble form [1]. For this, bacteria synthesize and secrete siderophores (SIDs) – secondary metabolites that bind and solubilize iron for its intake. Secreting these metabolites allows other organisms to profit from the ferric-siderophore compound without having to produce the SIDs themselves, especially in iron-limited environments, such as the nasal microbiome. Hence, the availability of this resource seems to play an important role in the shaping of bacterial communities [1]. To investigate the microbiome in the nasal environment, eight organisms were isolated from nasal swaps, sequenced, and assembled. Co-culturing experiments were also conducted to identify the influence of the bacteria on the growth of Staphylococcus aureus. From the eight organisms, two organisms, identified as Corynebacteria aurimucosum (CA) and C. simulans (CS) seem to reduce the growth of S. aureus. Interestingly, their iron-intake mechanisms differ from each other. While CA showed SID-producing potential on a CAS assay, CS did not. To identify functional differences within iron-related environments between both organisms beyond genome annotation, RNA-seq was used to analyze the expression profile of these two organisms in two different environments: one with EDDHA as a chelating agent and without any chelator, with three biological replicates each. The RNA-seq data was quantified with the assembled genomes as references and a computationally predicted annotation. A differential expression (DE) analysis identified 614 genes for CA and 549 genes for CS as DE genes To analyze and compare the functional differences, the DE genes were further associated with gene ontology (GO) terms and analyzed using GO-compass [2]. An enrichment analysis revealed 71 significant GO terms across all three ontologies (9 unique for CA, 26 unique for CS). Moreover, 27 of the shared terms were associated to iron-intake and to up-regulated genes in both species. Especially, both species showed up-regulation in genes related to SID-intake mechanism. Exploring the upstream regions of these genes could provide insights into transcription regulation mechanisms shared not only by these organisms but also by other nasal microbes. However, no major functional difference between species were found wrt. their iron-intake mechanisms, such as their SID-synthesis potential, as expected from the CAS assay. Hence, further computational analyzes would focus on identifying genomic differences between these organisms, such as orthologs or mutated protein sequences. We also aim to extend these combined genomic and transcriptomic analyzes to model a SID-based shaping of nasal microbiomes.

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[2] Harbig, T., et al. (2023). GO-Compass: Visual Navigation of Multiple Lists of GO terms. Computer Graphics Forum, 42(3).

P 33

Characterization and bioactivities of Exopolysaccharides from potential probiotics *Enterococcus faecium* MW725386 and *Streptococcus thermophilus* MW725391 and their Impact on bovine milk rheology and gut microbiome composition

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Exopolysaccharides (EPSs) are carbohydrate polymers from beneficial probiotic bacteria. This study characterized the EPSs from *Enterococcus faecium* (LB13) and *Streptococcus thermophilus* (MLB10) and evaluated their biological activities, prebiotic potentials, and effects on milk rheology and gut microbiome. Fourier transform infrared (FTIR) spectrum of EPS_LB13 and EPS_MLB10 were of typical EPS, both had a high molecular weight (EPS_LB13: 19.75×105 Da and EPS_MLB10: 15.53×105 Da) and were heteropolysaccharides with monosaccharides composition (Galactose: Lactose: Glucose: Mannose: Xylose) and (Glucose: Ribose: Mannose: Xylose) for EPS_LB13 and EPS_MLB10, respectively. EPS_LB13 and EPS_MLB10 at 250 mg/L showed scavenging rates for 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), Superoxide dismutase, Superoxide anion, Hydrogen peroxide, Hydroxyl radical, Metal chelation, and Lipid oxide peroxidation from 10% to 88.8%, and antioxidant capacity for Ferric ion reducing antioxidant power (FRAP), Total antioxidant capacity, and reducing power from 1219 to 1553 µg/mL, 2592 to 2848 µg/mL, and 714 to 792 µg/mL respectively. EPS_LB13 and EPS_MLB10 inhibited growth and biofilm formation of *E. coli* 0157:H7 1934, *S. typhimurium* 02-8423, *S. aureus* ATCC 25923, and *L. monocytogenes* DSM 20649 with 53% to 74%. Inhibition of amylase, glucosidase, cholesterol, and ACE were 2% to 66% and inhibition of Caco-2 and MCF_7 cancer cells were 14% to 83%. Both EPSs supported growth of beneficial gut bacteria from Proteobacteria, Bacteroidetes, Firmicutes, and Acinetobacter in fecal fermentation with total SCFA production from 5548 to 6023 PPM. Moreover, both EPSs improved gelation time of bovine skimmed milk fermented with the EPS-producing bacteria and starter culture. These results suggest that the EPSs from LB13 and MLB10 have promising applications in dairy and pharmaceutical industries.

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Investigation of the function of the ECF transporter LgbSTA of *Staphylococcus lugdunensis*

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Introduction

During host infection bacteria are exposed to nutritional limitation which is triggered by the host in a process called "nutritional immunity". Pathogenic bacteria use several mechanisms to ensure acquisition of essential nutrients during infection. These include the expression of high affinity metallophores (e.g., siderophores) and transporters (e.g., ECF-type transporters).

In *S. lugdunensis* an operon of 13 genes is upregulated under metal-limited conditions. The genes encode a non-ribosomal peptide synthetase (NRPS) system. The product (lugdubactin) was identified as a small peptide with structural similarity to ulbactin F. Ulbactin F has been described as an Fe3+ -binding siderophore. However, we observed Cu2+-binding for lugdubactin. Downstream of the NRPS system, genes encoding for an ECF- type transporter named LgbSTA was identified. Also, two additional genes *lgbK* and *lgbL* with homology to a multidrug exporter are present downstream of the ECF transporter genes.

Objectives

We hypothesize that lugdubactin represents a Cu-binding metallophore that is exported by LgbKL while its metal saturated form is imported by LgbSTA. Together the Proteins form an unconventional Cu-acquisition system. The aim of this project is to proof these hypotheses and to test the biological relevance of the system for *S. lugdunensis*.

Methods

We measured lugdubactin concentrations in BuOH extracts of cell culture supernatants of *S. lugdunensis* WT and mutant strains to assess the ability of strains to secrete or to take up lugdubactin. Additionally, growth was assayed in metal-limited medium in microplates.

Results

Compared to the WT strain, lugdubactin accumulated in the supernatant of the Δ*lgbSTA* strain suggesting that LgbSTA represents the lugdubactin importer. Genomic complementation reverted the phenotype. In contrast, the Δ*lgbKL* mutant failed to accumulate lugdubactin in the culture supernatant, supporting the idea of LgbKL being the metallophore exporter.

We found that medium supplementation with cysteine and salicylic acid improved production of lugdubactin. Interestingly, mutagenesis of *lgbKL* entailed a growth defect under these conditions, suggesting that a failure to secrete the compound entails toxic effects. The relevance of the system for bacterial proliferation under metal limiting conditions is under current investigation.

Conclusion

Our data suggests the export of lugdubactin by LgbKL and the import of lugdubactin by LgbSTA.

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"Hot" BGCs from "Cool" microbes

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Microbial resistance to antimicrobial compounds has become one of the biggest threats to global health, new antibiotics are urgently needed. Most antibiotics are isolated as secondary metabolites or so-called natural products from microbes. Microbes living in the harsh condition of Antarctica represent a promising new source that is worth exploring for new antibiotics. Recent studies showed a significant genetic divergence of Antarctic microbes from other microbes worldwide and suggested the enormous potential of these microbes to produce novel bioactive metabolites. Within our study, the main goal is the characterization of the biosynthetic potential hidden within different Antarctic microbial communities dominated by cyanobacteria. We investigated two main types of microbial communities from James Ross Island (maritime Antarctica), vertically stratified microbial mats and non-structured "Nostoc-like" mats. Our ultra-deep sequencing allowed the identification of more than 6000 highly diverse biosynthetic gene clusters (BGCs) encoding secondary metabolites. Additionally, we were able to assemble more than 1000 so-called metagenome assembled genomes (MAGs) and identify extensive taxonomic novelty from phylum to genus level. All identified BGCs were taxonomically classified and linked to respective MAGs, if possible. Moreover, we also analyzed which taxa, BGCs, and MAGs are shared across all studied mats, and which are unique for individual mat types. The integration of these analyses enabled the examination of the distribution of BGCs across various taxa/MAGs within diverse Antarctic microbial communities. It also facilitated the discovery of new BGCs and gene cluster families (GCFs) that hold significant potential as promising candidates for the investigation and development of novel antimicrobial compounds.

P 36

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

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

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

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

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

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

P 37

Unveiling the untapped potential: Exploring secondary metabolites in the genus Pedobacter

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Through bioinformatic analysis of genomic data, it has become evident that the bacterial kingdom's potential for discovering secondary metabolites (SMs) remains largely untapped. To uncover new SMs, this project investigates the biosynthetic capabilities of the Bacteroidetes genus Pedobacter. Pedobacter has been acknowledged as a reservoir for antimicrobial resistance genes, but their capacity for SM production has received limited attention. To this end, our bioinformatic analysis has identified an unevenly distributed biosynthetic gene cluster (BGC) load and a Pedobacter clade with a remarkable accumulation in its predicted biosynthetic potential. Utilising genetic tools, we successfully assigned an orphan BGC to the biosynthesis of new linear lipopeptides, expanding the repertoire of compounds discovered from the Pedobacter genus.

143 genomes from Pedobacter were curated and bioinformatically analysed. An NRPS cluster (crpA-C) from P. cryoconitis was chosen as a target for gene disruption. Comparative metabolomics (molecular networking) of the P. cryoconitis WT and crpA deletion mutant revealed the absence of distinctive ion clusters in the deletion mutant. CrpA-dependent SMs were isolated and structure elucidated, yielding 14 new lipopeptides called cryopeptins (A-N). Cryopeptins are linear lipopeptides that include the non-proteinogenic amino acid dehydrovaline. The predicted NRPS assembly line, encoded in crpB and crpC, aligns with the lipo-pentapeptidic structures found in the cryopeptins.

Our computational analysis further unveiled that the P. cryoconitis clade contains a diverse array of unique BGCs, setting it apart from the entries in the MiBiG database. To shed light on this putative yet uncharted SM space, we chose P. cryoconitis PAMC 27485 and P. cryoconitis DSM 14825 and examined their metabolome similarity.

Upon comparing the metabolomic profiles of both strains, 34% of the metabolites (n=683) were shared. The remaining metabolites were exclusive to either P. cryoconitis DSM 14825 (30%, n=589) or P. cryoconitis PAMC 27485 (36%, n=702). Notably, our metabolomic analysis revealed a considerable number of metabolites that do not correspond to known SMs. Although closely related, they exhibited differences in their BGC composition, with a multimodular NRPS similarity score range of 0.33 and an overall BGC similarity score of 0.51. Alongside the observed divergence in metabolic composition between the two strains, this highlights an unexplored metabolic space within the Pedobacter genus.

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Functional dissection of the transport process during glycopeptide antibiotic secretion

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Glycopeptide antibiotics (GPA) - e.g., vancomycin – are natural products that have been used as a last resort for many years and are still an important group of agents to fight bacterial infections by inhibiting cell wall biosynthesis. Numerous GPA producers have been found in the genera *Amycolatopsis* or *Streptomyces* within the phylum of actinobacteria.

GPAs are modified heptapeptides consisting of proteinogenic and non-proteinogenic amino acids. However, glycosylation and modification of the peptide scaffold leads to a large variety of different structures. The peptide is synthesized at non-ribosomal peptide synthetases (NRPS). Interestingly, in addition to all biosynthetic proteins, we identified, that a transport related protein is always co-encoded in the associated biosynthetic gene cluster (BGC). Interestingly, in all 89 clusters we looked at, this is always an ABC transporter, which is sometimes essential for the secretion of GPAs[1]. However, without functional analysis, we cannot conclusively determine whether they are specific for the export of their cognate GPA. Furthermore, its function in terms of effective production and thus interaction with the NRPS is still poorly understood. Understanding the transport mechanisms of glycopeptides holds great biotechnological potential to increase yields and implement modified production routes for next-generation antibiotics. Therefore, we aim to gain a better understanding of the specificity and interaction partners of GPA associated ABC exporters.

On the one hand we try to overexpress, solubilize and purify the transporter of ristomycin (Tri) of *A. japonicum*[2] and the transporter of balhimycin (Tba) of *A. balhimycina*[1] in order to biophysically characterize the proteins and perform *in vitro* transport assays. On the other hand, we are analyzing the specificity of GPA exporters *in vivo*, using *A: balhimycina* and the amount of exported balhimycina as model system. The strain is complemented with several other glycopeptide ABC exporters, and the culture supernatant is analyzed for the presence of balhimycin by HPLC-MS. Our results indicate that even highly similar proteins e.g. Tri of *A. japonicum* (88% sequence identity) are not able to fully complement the function of the native transporter Tba, and that they are specific for their substrates. Additionally, we are investigating the putative substrate binding site of GPA associated ABC transporters using fold structure models[3] and 3D structures of glycopeptides by MD-simulations. This led us suggest that specificity to cognate substrates is determined by the peptide backbone rather than the modification pattern. In order to assess protein-protein interactions of the GPA exporters, different mutants of producer strains are complemented with transporter-3XFLAG fusions and BioID variants to perform interaction studies.

[1] Menges et al., Appl Microbiol Biotechnol. (2007) [2] Spohn et al., Antimicrob Agents Chemother. (2014) [3] Jumper et al., Nature (2021)

P 39

Metabologenomic workflow development for strain prioritization

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Introduction. Global climatic changes lead to extreme weather events, as droughts and heavy rainfalls and in consequence to a weakening of eco systems. In this tense situation crop pests gain ground. At the same time, the use and misuse of chemical fungicides promotes the rise and spread of resistances, leading to failures in the control of plant diseases.

To counteract these developments, a transformation of agricultural systems towards a bio economical and sustainable food production is inevitable. To support this conversion functional solutions for the management of diseases and infection events must be provided. Soil living microorganisms open up an approach to naturally evolved solutions in the battle against fungal crop diseases.

Objectives. The goal of the here introduced project is the identification and characterization of bacterial metabolic profiles and their naturally evolved potential as sustainable control agent for phytopathogenic fungi.

The project is based on the former Sanofi strain collection consisting of >100.000 microbial strains, and therefore a huge variety of spore forming, soil inhabiting bacteria. Selected strains are rated using a metabologenomic profiling pipeline. The prioritized strains are then tested *in vitro* and *in vivo* as potential antagonists against *S. trici* and *C. coccodes.*

Materials & Methods. Organic extracts of ~300 Bacilliaceae were screened for antifungal activity and rated on account of MS-based metabolomic data. Combined with the genome sequencing of >100 of these strains this forms the basis for our metabologenomic-guided strain prioritization workflow.

The combined consideration of activity and diversity data enabled a strategic strain selection for further studies, as the determination of the production of plant growth promoting compounds.

Results. A prioritized group of 15 of 300 bacterial Strains was selected by taking the extensive metabolomic and genomic datasets in account. Structure elucidation of unknown compounds is running.

Conclusion. The project outlines the development of a workflow, on how to process huge datasets to identify the most promising candidates for costly and elaborate laboratory experiments in the future.

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Characterization of the potentially microbiome-shaping epilancin A37 reveals a unique mechanism of action

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Introduction: Many bacteria produce antimicrobial compounds such as lantipieptides to gain an advantage in the competitive natural environments of microbiomes. Epilancins constitute a comparably underexplored group of lantibiotics produced by staphylococci, with only two known specimens produced by *Staphylococcus epidermidis* and a currently unknown mechanism of action. However, due to the key role of *S. epidermidis* in human nasal and skin microbiota, epilancins were suggested to have microbiome-shaping impact.

Objective: We discovered production of a new member of the epilancin family named A37 by a nasal isolate of *S. epidermidis* and set out to characterize the potential ecological role as well as its mechanism of action.

Materials & Methods: We applied bioinformatic analysis to publicly available data to investigate the distribution of epilancins within staphylococci. We assessed the potential of A37 to confer competitional advantage to the producer strain using classical microbiological and microscopic approaches. The mechanism of action of A37 was investigated with microbiological methods and state-of-the-art fluorescence and Cryo-EM microscopy.

Results: Our bioinformatic analysis shows that the epilancin scaffold is widely distributed within the staphylococci family, highlighting the ecological role of this group of lantibiotics. We demonstrate that A37 production confers major competitive advantage to *S. epidermidis* against the natural competitor family of corynebacteria. A detailed investigation of the epilancins mechanism of action using *Corynebacterium glutamicum* as model organism revealed that A37 enters the cytoplasm without impairing cytoplasmic membrane function. Upon intracellular aggregation, A37 induces formation of intracellular membrane vesicles heavily loaded with the compound, which are closely linked to the antibacterial activity of the epilancin.

Conclusion: Our work shines light the ecological role of epilancins for staphylococci and contributes to understanding the unique mechanism of action of epilancins against a natural target family.

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Empowering microbial studies: Next-generation sequencing in microbiology and epidemiology

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Next-generation sequencing (NGS) has revolutionized the field of microbiology and epidemiology, enabling unprecedented insights into the genetic diversity and dynamics of microbial communities.

NCCT-Mibi, as a part of the CMFI excellence cluster, has contributed to numerous projects by offering an array of NGS tools (Illumina and Nanopore platforms) for comprehensive exploration of microbial diversity. Our services include RNA sequencing for transcriptome analysis, amplicon sequencing to unravel microbial community structures, and whole genome sequencing for comprehensive genetic insights. Our more recent workflows – metagenomic and metatranscriptomic sequencing – offer a powerful approach to understand the functional roles of microbial communities in various ecosystems and disease states.

In the past year, we contributed to the improvement of bacterial transcriptomics workflows by optimizing RNAseq for Prof. Dr. Kay Nieselt and developing a tailored protocol for strains with high ribosomal RNA content. Overall, in 2022 our facility successfully conducted more than 40 projects with almost 3500 samples sequenced, highlighting the significant demand in NGS for advancing microbiological research.

Our poster presentation will highlight our contributions to microbiological research, aiming to promote exchange and foster collaborations to further unravel the complexities of microbial ecosystems and their implications on human health and the environment.

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Towards a structure-based understanding of silent flagellin-TLR5 interactions

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Toll-like receptor 5 (TLR5) is a Pattern Recognition Receptor which targets and responds to a conserved site on bacterial flagellin, the protein subunit of bacterial flagella, which confers motility. TLR5-flagellin has widely been used as a proxy for immune activity.

However, we recently identified a class of flagellins termed "silent", which contain the TLR5 epitope allowing binding at the canonical binding site, but elicit a weak immune response. To better characterise the relationship between binding at the TLR5 epitope and signalling, we determined the relative binding and activity profiles of 116 flagellins with the TLR5 epitope against a truncated human TLR5 construct, containing epitope binding site.

Our results decoupled binding and subsequent immune activity. We found a continuum in binding strengths within TLR5 epitope-containing flagellins, with individual binding profiles correlating only weakly to immune activity. Further structural, kinetic, and biochemical studies on individual flagellins indicated that interactions with separate allosteric TLR5-binding regions are essential in triggering a strong response. This discovery represents a new mechanism for evasion of innate immune activation, potentially leading to new avenues for developing TLR5-targetted treatments.

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Development of a fungal heterologous host for enhanced production of Astin C

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Introduction: Activation of the STING signalling pathway by dsDNAs plays an important role in antiviral and antibacterial responses. Astin C has been shown to act as a STING inhibitor, making this molecule a promising drug candidate for the treatment of intestinal infections.

Objectives: Astin C represents a non-ribosomal peptide which is characterized by the unique amino acid (3S,4R)-dichloroproline. Although its chemical synthesis is theoretically possible, its industrial production fails due to environmental and economic constraints, making classical peptide synthesis an impractical approach. Consequently, microbial fermentation remains the only alternative. However, processing of axenic cultures of *C. asteris* is time-consuming and labor-intensive. In addition, an epigenetic adaption of the secondary metabolism leads to a strong reduction in Astin C product yield. As its biosynthesis is a complex intercompartmental process, heterologous expression in a heterologous fungal host offers a promising approach. Therefore, the objective is to develop an *Aspergillus nidulans* strain allowing enhanced production of Astin C for a thorough investigation of its STING-inhibitory properties.

Materials & Methods: For the development of an Astin C-producing *A. nidulans* strain, whole-genome sequencing was previously performed allowing the identification of the genes for all key enzymes. Standard methods were used to generate expression plasmids. These include the isolation of DNA, PCR, agarose gel electrophoresis, Gibson cloning and chemical transformation of *E. coli*. Genetic manipulation of *A. nidulans* was achieved by PEG-mediated transformation of protoplasts. Genetically modified *Aspergillus* strains are subjected to production assays. Astin C production is analyzed by HPLC-MS.

Results: In order to generate an Astin C-producing *A. nidulans* strain all biosynthetic genes were cloned into three expression plasmid: The first plasmid harbors the NRPS genes. The second plasmid carries all genes required for amino acids precursor supply and post-NRPS modifications. The third plasmid harbors genes encoding transport proteins. The generation of all plasmids followed the same strategy: Each gene was fused to a constitutively active promoter and terminator using OE-PCR. These expression cassettes were assembled by Gibson cloning. Moreover, a protocol for the preparation of *A. nidulans* protoplasts and their PEG-mediated transformation was successfully established. Finally, a convenient HPLC-MS based high throughput screening was developed.

Conclusion: With the successful construction of a fungal expression system we have set the stage to now optimize the heterologous production of Astin C to provide substantial amounts of this STING inhibitor. Its testing will be performed in cooperation with colleagues from the CMFI cluster.

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Invasive *Staphylococcus epidermidis* use a unique processive wall teichoic acid glycosyltransferase to evade immune recognition

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Staphylococcus epidermidis expresses glycerol-phosphate wall teichoic acid (WTA), but some healthcare-associated methicillin-resistant *S. epidermidis* (HA-MRSE) clones produce a second, ribitol-phosphate (RboP) WTA, resembling that of the aggressive pathogen *Staphylococcus aureus*. RboP-WTA promotes HA-MRSE persistence and virulence in bloodstream infections. We report here that the TarM enzyme of HA-MRSE (TarM(Se)) glycosylates RboP-WTA with glucose, instead of N-acetylglucosamine (GlcNAc) by TarM(Sa) in *S. aureus*. Replacement of GlcNAc with glucose in RboP-WTA impairs HA-MRSE detection by human IgG, which may contribute to the immune-evasion capacities of many invasive *S. epidermidis*. Crystal structures of complexes with UDP-glucose, and with UDP and glycosylated poly(RboP) reveal the binding mode and glycosylation mechanism of this enzyme and explain why TarM(Se) and TarM(Sa) link different sugars to poly(RboP). These structural data provide evidence that TarM(Se) is a processive WTA glycosyltransferase. Our study will support the targeted inhibition of TarM enzymes, and the development of RboP-WTA targeting vaccines and phage therapies.

P 45

Non-antibiotic drugs break colonization resistance against enterobacterial pathogens

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Drugs have been shown to be a critical determinant of interindividual differences in the composition of the human gut microbiome, which is true not only for antibiotics but also for non-antibiotic drugs. We hypothesized that by shifting the microbiome composition, non-antibiotic drugs open niches and facilitate pathogen colonization in a similar way to antibiotics. We selected drugs that inhibit a broad spectrum of commensals while sparing enteropathogens. Next, we developed an *in vitro* high-throughput assay to quantify the growth of *Salmonella* Typhimurium (*S*. Tm) in synthetic or stool-derived communities. For drug candidates with strong phenotypes, we tested their ability to disrupt colonization resistance in defined-colonized and SPF mice. In both models, non-antibiotic drugs from different therapeutic classes significantly increased *S*. Tm infection risk. Our work enables a systematic evaluation and identification of non-antibiotic drugs that break colonization resistance and increase the risk of infection with enteropathogenic bacteria.

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Microbial competition determines the requirement for a putative lipid transport operon in the human gut bacterium *Phocaeicola vulgatus*

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Introduction: The mammalian gut harbors complex communities of microbial species that interact and compete with each other while also influencing the host's physiology. Discerning the mechanisms by which major members of these gut microbial communities colonize the gut is a fundamentally important research goal. *Phocaeicola vulgatus* (*Pvu*) is a gram-negative anaerobe and one of the most abundant bacterial species found in the gut of humans and other mammals. *Pvu* has been associated with several metabolic and inflammatory disorders such as inflammatory bowel disease and type 2 diabetes. Objectives: Despite having an unusual capability to colonize established gut microbial communities following a single introduction, there is still limited information about the tools that *Pvu* deploys to colonize and compete within the gut. Using a variety of *in vivo* and *in vitro* techniques, we seek to identify potential genes that play a role in *Pvu* colonization thus allowing it to be such an efficient colonizer of the mammalian gastrointestinal tract. Materials and Methods: We utilized an *in vivo* transposon insertion sequencing (INSeq) approach to identify genes that were differentially required for fitness in germ-free and conventionally-raised mice. Combinatorial pooling was also combined with INSeq to create an arrayed library of thousands of *Pvu* transposon mutants. These mutants were then gavaged into germ-free and conventionally-raised mice to observe the enrichment and depletion of the mutants in the different environmental conditions. To further define the function of this gene and the rest of its operon, clean deletions of individual genes were made using homologous recombination. Then, selected mutant strains were subjected to liquid chromatography coupled with mass spectrometry (LCMS) and RNA sequencing (RNA-seq) grown *in vitro* to identify potential differences in metabolite production and gene expression. Results: We identified a gene that appeared to be required for *in vivo* fitness in mice colonized with a complex microbiota but dispensable in germ-free mice. Then, using untargeted liquid chromatography and mass spectrometry, we found that *Pvu* strains with deletions of this gene were associated with deficiencies in several lipid species. Homology prediction and transcriptomic data suggests that this gene is a potential secreted protein involved in outer membrane function and structure. Furthermore, this gene is part of an operon that appears to be unique to the *Phocaeicola* genus, including a DNA-binding protein and a putative lipid transport protein. Conclusion: Ongoing studies seek to elucidate the lipid transport mechanism of this operon and its impact on cell development and fitness. The outcomes of this work are expected to increase our understanding of the mechanisms that *Pvu* employs to invade and persist within the gut. Once identified, those mechanisms could be targeted to control gut microbiome structure and function to promote health.

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*In vivo p*henotypic response of *Escherichia coli* to Epinephrine exposure in albino rats

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Introduction: Epinephrine belongs to the catecholamines family, a naturally occurring stress hormone, administered to patients in intensive care units to maintain normal body functions.

Objectives: To assess the *in vivo* virulence factors produced by *Escherichia coli* and the alterations in biochemical, and histopathological variables in epinephrine-treated albino rats exposed to *E. coli*.

Methods: A pilot study determined the infectious dose of *E. coli* ATCC700728 as $1.0x10^3$ CFU/ml. The epinephrine dosage used was 2 µg/kg. The experimental design used four groups: R (rat with no treatment), Ec (rat + *E. coli* infection), E (rat + epinephrine treatment), and Ec+E (rat + *E. coli* infection + epinephrine). After 72 hr exposure, the *E. coli* was recovered from rats and subjected to viable bacterial count and quantification of the surface-adherent biofilm using the crystal violet method. Biochemical parameters of the liver and kidney were analysed. Histopathology of the kidney was further assessed.

Results: Bacterial growth analysis showed Ec+E ($260.5±27.1x10^5$ CFU/ml) was significantly higher (p=0.0003) than Ec ($136.5±20.4x10^5$ CFU/ml). The biofilm levels measured using the optical densities of crystal violet at 560 nm wavelength were: Ec (144.3±23.8), Ec+E (360.3±17.2), *E. coli* control (133.3±16.5) with a significant comparison between Ec+E and Ec (p=<0.0001) and Ec+E and *E. coli* control (p=<0.0001). The levels of biochemical parameters were aspartate aminotransferase (AST) Ec (13.5±1.3), Ec+E (20.0±2.2), E (11.9±3.0), and R (10.3±1.9) IU/l; alanine aminotransferase (ALT) Ec (13.0±2.8), Ec+E (23.5±0.6), E (13.0±2.2), and R (11.1±3.0) IU/l; alkaline phosphatase (ALP) Ec (61.0±4.6), Ec+E (62.5±4.0), E (54.0±6.9), and R (48.5±4.4) IU/l; total protein (TP) Ec (71.5±1.3), Ec+E (78.8±1.0), E (67.3±2.6), and R (67.0±1.6) g/l; albumin (ALB) Ec (33.5±1.7), Ec+E (44.3±2.5), E (37.3±1.5), and R (38.5±3.9) g/l; and total bilirubin (T. BIL) Ec (9.3±0.5), Ec+E (11.0±0.8), E (8.0±0.8), and R (6.3±0.5) µmol/l. More importantly, Ec+E was significantly higher than Ec in AST (p=0.0053), ALT (p=0.0002), TP (p=0.0004), BIL (p=0.0151), and ALB (p=0.0038). The kidney function parameters Na^+, Cl^-, HCO^-, urea, and creatinine in Table 1 showed significant (p=<0.0001) ANOVA analyses. Some kidney function parameters were significantly lowered in Ec+E than Ec for Na^+ (114±4.3; 139.0±4.3, p=<0.0001) mmol/L, Cl^- (79.8±6.3; 95.8±1.5, p=0.0099) mmol/l, and HCO^- (16.3±1.7; 19.5±1.3, p=0.0022) mEq/l while higher in creatinine (93.3±7.7; 78.8±4.3, p=0.0088) µmol/l and urea (8.8±1.0; 4.5±0.4, p=<0.0001) mmol/l. In Figure 1 the histology of the kidney in Ec+E treatment showed the most pathological damage with myxoid degeneration of the kidney.

Conclusion: Epinephrine potentiated the virulence of *E. coli in vivo* through growth and biofilm resulting in alterations in biochemical response and histopathological damage to the kidney.

Fig. 1

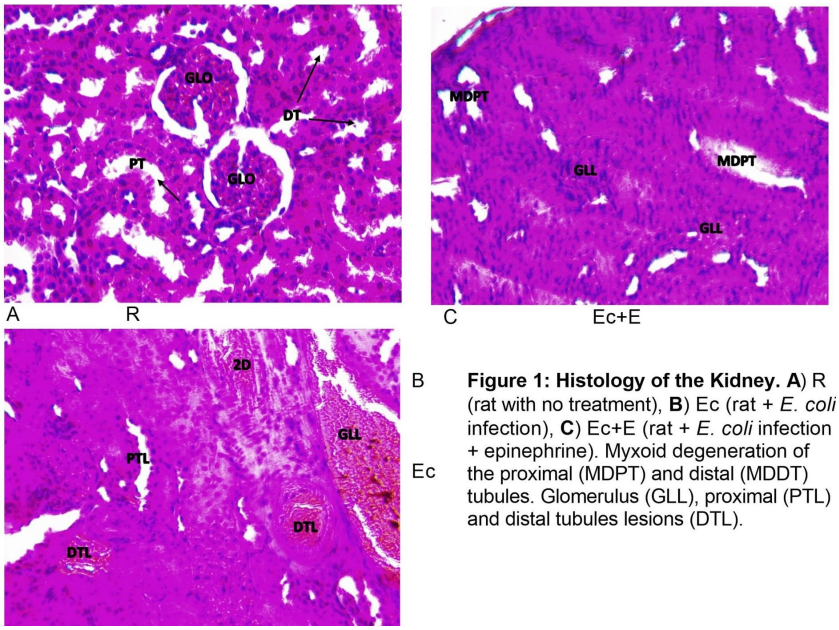

Figure 1: Histology of the Kidney. A) R (rat with no treatment), **B**) Ec (rat + *E. coli* infection), **C**) Ec+E (rat + *E. coli* infection + epinephrine). Myxoid degeneration of the proximal (MDPT) and distal (MDDT) tubules. Glomerulus (GLL), proximal (PTL) and distal tubules lesions (DTL).

Fig. 2

Table 1: Modulatory Effect of Epinephrine on Kidney Function Assessment

Groups	**Na^+**	**K^+**	**Cl^-**	**HCO^-**	**U**	**Cr**
Ec	139.0±4.3	3.5±0.2	95.8±1.5	19.5±1.3	4.5±0.4	78.8±4.3
R	142.8±4.0	4.4±0.5	105.3±7.2	22.3±1.0	2.3±0.2	59.0±4.1
E	145.8±2.8	4.4±0.2	107.0±6.3	24.5±1.3	3.1±0.3	70.0±3.6
Ec+E	114.3±4.3	2.5±0.4	79.8±6.3	16.3±1.7	8.8±1.0	93.3±7.7
p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Tukey's Intra-Group's Comparisons						
Ec vs. R	0.5492	**0.0156**	0.1490	0.0558	**0.0006**	**0.0008**
Ec vs. E	0.1226	**0.0189**	0.0738	**0.0010**	**0.0155**	0.1320
Ec vs. Ec+E	**<0.0001**	**0.0051**	**0.0099**	**0.0222**	**<0.0001**	**0.0088**
R vs. E	0.7061	0.9995	0.9728	0.1349	0.2722	**0.0469**
R vs. Ec+E	**<0.0001**	**<0.0001**	**0.0002**	**0.0002**	**<0.0001**	**<0.0001**
E vs. Ec+E	**<0.0001**	**<0.0001**	**0.0001**	**<0.0001**	**<0.0001**	**0.0002**

Key: Na^+ - Sodium, K^+ - Potassium, Cl^- - Chloride, HCO^- - Bicarbonate, U - Urea, Cr – Creatinine.

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Turning the good against the bad: machine learning to maximize disease-suppressing microbial interactions by optimizing nutrient conditions

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Antibiotics have served humanity with great success for almost a century, mitigating numerous infectious diseases. However, this outstanding success story is currently threatened as pathogens increasingly evolve resistances against antibiotics. The central goal of this work is to develop an alternative infection therapy that is based on the manipulation of microbial interactions; The outcome of microbial interactions is highly dependent on the nutritional environment. Thus, there must be such an environment which maximizes the suppression of an invading pathogen by a given commensal community.

Here, we developed a high-throughput in vitro assay and combined it with machine learning to predict the suppression of the model pathogen Pseudomonas aeruginosa, based only on a given commensal genome and the provided carbon sources. Despite the high complexity of genomics data, we show that our model can accurately predict the outcome of interactions across hundreds of strains and multiple carbon source conditions.

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Defininig microbial functions in chronic infection with metatranscriptomics

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Objectives:

Microbial communities are ubiquitous and interactions between microbes are critical drivers of overall community function. Thus, a fundamental question in human health and disease is to understand how microbes interact in infection sites. Our overall hypothesis is that specific interactions occur between members of the microbiota, that ultimately lead to synergy in polymicrobial infections. Although the microbes present in chronic infections have been established through culture-dependent and -independent methods, how community functions are conserved or differ across chronic infections remains an open question. To address this knowledge gap, we analyzed 102 published metatranscriptomes from CF sputum and chronic wound to identify the microbial constituents and key community functions in these infections.

Methods & Results:

Community composition analysis with MetaPhlAn4 and SAMSA2 revealed CF sputum was significantly more diverse than chronic wounds with a mean of 11.8 and 6.7 species identified (Shannon diversity index 5.9 vs. 5.1; Simpson diversity index 0.96 vs. 0.86), respectively. Both infection types were composed of a mix of traditional pathogens and members of the microbiota, with *Staphylococcus*, *Porphyromonas*, and *Anaerococcus* dominating the CW samples and *Rothia*, *Pseudomonas*, and *Prevotella* the most prevalent in the CF samples. Interestingly, anaerobic bacteria comprised a significant portion of the genera identified in both communities (46.7%), indicating these infections are likely hypoxic. Further, these strict anaerobes had negative correlations with traditional pathogens (Pearson correlation co-efficient = -0.43 and -0.27 in CW and CF, respectively), indicative of competitive interactions. Functional analyses with HUMANn3 and SAMSA2, found that while functions involved in in bacterial competition, response to reactive oxygen species, and virulence were conserved in both infection types, over 40% of the identified level 4 enzyme classes (EC) were differentially expressed. Interestingly, ECs involved in antibiotic resistance and biofilm formation were enriched in CF samples, while ECs involved in tissue degradation and tolerance of host defense mechanisms, were highly expressed in CWs.

Conclusions:

Collectively, our results suggest that anaerobic members of the microbiota likely play a significant role in the progression of CF and CW. Further, we show that microbial communities have distinct functions in each chronic infection site, indicating the infection environment strongly influences bacterial physiology and that the community structure influences function. Defining the rules of how microbes interact is critical for understanding bacterial physiology in polymicrobial infections and the development of improved treatment strategies.

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Biotic resistance of native microbes determines pathogen invasion dynamics

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A key to controlling infection is to predict whether a pathogen would fail or succeed in invading our bodies. We know intuitively that slowing down pathogen dispersal - the rate pathogen moves into our bodies - could slow down pathogen invasion/infection, but **how much must we slow down the pathogen dispersal to stop its invasion completely**? Moreover, our bodies have their native microbes, which interact with the incoming pathogen and could potentially hinder invasion – an ability called biotic resistance. **How does biotic resistance change the answers?**

Combining in vitro pathogen invasion experiments and math models, we found:

1. As ecological resistance of native microbes increases, consistent pathogen invasion transitions into a pulsed (intermittent) state, and finally becomes pinned (stopped).
2. **Without resistance, we need absolutely 0 pathogen dispersal** to stop the invasion completely.
3. Whereas sufficient **resistance creates a positive "critical dispersal rate"** that, if we control pathogen dispersal below it, the invasion stops. This is much easier than stopping pathogen dispersal entirely.
4. Measuring the interaction between a small amount of invader and the native community is sufficient to predict experimental invasion speeds, via a universally applicable model.

Our conclusions underscore **quantitatively** the crucial role of biotic resistance in controlling pathogen invasion. Similar principles can extend far beyond the interplay between pathogens and native microbes, applicable wherever the spatial arrangements are stable and discrete: such as disease transmission among plants, infections spreading across cities, or invasive species marching through habitats.

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Neutrophil Extracellular Traps enhance *Staphylococcus aureus* skin colonization by the induction of oxidative stress in the skin and downregulation of epidermal barrier gene expression

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Background

Staphylococcus aureus is the most common cause of bacterial skin infections in humans including in atopic dermatitis (AD) patients. We previously showed that on inflamed skin *S. aureus* colonization is enhanced by interaction of keratinocytes with neutrophil extracellular traps (NETs) released by infiltrating neutrophils [1]. However, the underlying mechanism is not yet understood.

Objectives

In this work we studied the role of neutrophils and NETs in *S. aureus* skin colonization and persistence.

Methods

Using an *in vitro* co-culture model, we investigated the interaction of NETs and keratinocytes by studying inflammatory responses via ELISA and activated signaling pathways by western blot. We analyzed neutrophil recruitment and *S. aureus* colonization in the skin of different knock-out mice using an epicutaneous colonization model. The role of neutrophils in persistence of *S. aureus* on inflamed and not inflamed skin was investigated by neutrophil depletion *in vivo*. We analyzed presence of neutrophils, NETs and damage-associated molecular pattern (DAMPs) in skin of AD patients by CODEX analysis.

Results

We show that neutrophils actively contribute not only to initial colonization but also to enhanced persistence of *S. aureus* on inflamed skin. A crosstalk between infiltrating neutrophils and keratinocytes primes neutrophils for enhanced NET formation which correlates with *S. aureus* skin colonization. Further experiments revealed that NETs induce oxidative stress in the skin leading to secretion of DAMPs and epidermal barrier gene downregulation. Interestingly, AD patients exhibit enhanced presence of PMNs, NETs and DAMPs in the skin which correlated with enhanced *S. aureus* colonization demonstrating the clinical relevance of our finding.

Conclusion

Our data indicate a functional role of neutrophils in shaping *S. aureus* skin colonization and persistence. We propose that in inflamed skin, neutrophils are primed for NET formation which induces oxidative stress and downregulation of epidermal barrier genes in the skin thus promoting skin barrier dysfunctions.

[1] Bitschar K et al, Staphylococcus aureus Skin Colonization Is Enhanced by the Interaction of Neutrophil Extracellular Traps with Keratinocytes. J Invest Dermatol. 2020 May;140(5):1054-1065.e4.

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Identification of *in vitro* and *in vivo* nutritional interactions between nasal commensals and *Staphylococcus aureus*

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Introduction

The asymptomatic nasal carriage of *Staphylococcus aureus* increases the risk of infection, urging the identification of the factors that enable *S. aureus* to colonize the nasal cavities. In this context, the role of the resident microbiota in creating a favourable or hostile nutritional milieu to *S. aureus* colonization has long been neglected.

Objectives

This project aims to identify the main nutritional interactions between nasal commensals and their impact on *S. aureus* physiology *in vitro* and *in vivo*, with the long-term goal to develop nutritional exclusion strategies.

Materials & methods

We first studied the nasal community structures and the metabolic potential of nasal species from 11 healthy subjects. *In vitro*, we could isolate a total of 291 bacterial strains from the anterior nostrils of the volunteers, and assess their lipolysis, proteolysis, haemolysis, mucinase and DNAse activities on solid media. Interaction experiments were performed in a synthetic nasal medium to elucidate interactions networks between commensals within each community. *In silico*, the genomes of the bacterial isolates as well as the nasal metagenome of each volunteer were analysed using three sequencing techniques to infer the composition of the nasal communities and their metabolic potential. Finally, an *in vivo* model of nasal colonization was set up in gnotobiotic mice.

Results

The *in vitro* and *in silico* approaches consistently identified communities of similar structure and the presence of *S. aureus* in three of them. Based on the catabolic abilities of individual isolates, we observed lipolysis activity to be particularly frequent in *Staphylococcus epidermidis* and other staphylococci. In contrast, the ability to degrade host proteins appeared more variable and species specific. Particularly, *Cutibacteria* showed high mucinase activity and carried numerous homologs of proteins involved in mucin degradation. *In vitro* interaction experiments suggest that *S. epidermidis* and *Cutibacteria* support the survival of *S. aureus*, potentially through their ability to degrade lipids and mucins, respectively. To assess the impact of these interactions *in vivo*, a gnotobiotic mouse model of stable colonization with *S. aureus* was established and co-colonization experiments are in progress.

Conclusion

Our results indicate that nasal commensals present diversified and specialized metabolic activities, from which *S. aureus* can benefit in co-culture. The investigation of these interactions at the molecular level *in vitro* and *in vivo* will inform strategies to prevent nasal colonization by *S. aureus*.

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Co-stimulation of formyl-peptide receptor 1 and 2 leads to synergistic activation of neutrophils

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As innate immune cells, neutrophils play a critical role in defending the human body against invading pathogens. To detect foreign organisms and initiate an immune response, neutrophils use a set of pattern-recognition receptors (PRRs) that are activated by pathogen-associated molecular patterns (PAMPs). These PRRs include the formyl-peptide receptors (FPRs). While FPR1 senses short formylated peptides of bacteria in general, FPR2 is activated by specific bacterial molecules including phenol-soluble modulins (PSMs). PSMs are small toxic peptides produced in high amounts by the pathogenic bacterium *Staphylococcus aureus*. Both FPR1 and FPR2 ligands stimulate neutrophils to release cytokines like IL-8 and produce reactive oxygen species (ROS) or guide neutrophil chemotaxis.

Since both FPR1 ligands and PSMs as FPR2 ligands are present at the site of infection with *S. aureus*, the aim of this work is to analyse the impact of co-stimulation of FPR1 and FPR2 on neutrophils. Preliminary data indicate that the activation of both receptors synergistically enhances the release of the chemokine IL-8 and the antimicrobial peptide LL-37 by neutrophils. Likewise, cell migration and ROS production are increased, resulting in an overall higher inflammatory immune response.

Activation of FPRs plays an important role during an immune response. Therefore, deciphering the interaction between FPR1 and FPR2 promises to shed light on the regulation of inflammation and pave the way for the development of new therapeutic approaches.

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Understanding the role of formyl-peptide receptors in skin colonization and inflammation

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Keratinocytes form a multilayer barrier that protects the skin from invaders or injuries. The barrier function of keratinocytes is in part mediated by the production of inflammatory modulators that promote immune responses and wound healing. Skin commensals and pathogens such as *Staphylococcus aureus* secrete high amounts of phenol-soluble modulin (PSM) peptides, agonists of Formyl-peptide receptor 2 (FPR2). FPR2 is crucial for the recruitment of neutrophils to the sites of infection, and it can influence inflammation. FPR1 and FPR2 are also expressed by keratinocytes but the consequences of FPR activation in skin cells have remained unknown. Since an inflammatory environment influences *S. aureus* colonization, e. g., in patients with atopic dermatitis (AD), we hypothesized that interference with FPRs may alter keratinocyte-induced inflammation, proliferation, and bacterial colonization of the skin.

To assess this hypothesis, we investigated the effects of FPR activation and inhibition in keratinocytes by measuring cytokines via ELISA and multiplex assay, performing microscopical wound healing and using an AD-simulating mouse model. We observed that FPR activation induces the release of IL-8, IL-1α and promotes keratinocyte proliferation in a FPR-dependent manner. To elucidate the consequence of FPR modulation on skin colonization, we used an AD-simulating *S. aureus* skin colonization mouse model using wild-type (WT) or Fpr2-/- mice and demonstrate that inflammation enhances the eradication of *S. aureus* from the skin in a FPR2-dependent way. Consistently, inhibition of FPR2 in the mouse model or in human keratinocytes as well as human skin explants promoted *S. aureus* colonization.

Our data indicate that FPR2 ligands promote inflammation and keratinocyte proliferation in a FPR2-dependent manner, which is necessary for eliminating *S. aureus* during skin colonization.

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The microvirome: Understanding phage-bacterial dynamics in the plant holobiont

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Eukaryotic organisms harbor large communities of microorganisms forming an holobiont, considered to be a single ecological and evolutionary unit. In recent years, bacterial community dynamics and their effect on the plant holobiont have been the subject of many studies. In spite of this, little is known regarding the role that bacteriophages play in shaping those bacterial communities. In my work I intend to set the basis for understanding the role of the microvirome in plant colonization and development, by studying *Arabidopsis thaliana* associated bacteria and phages, in laboratory and natural settings. Following a multilevel approach from isolates, to synthetic communities, to wild plants, I expect to gain a mechanistic understanding of the way phages affect plant-associated bacterial communities, deepening our basic understanding of the plant holobiont, and phage-host interactions in an oligotrophic environment. These findings can be projected to other significant plant-microbes systems, and be the foundation to design phage-based solutions to pest management in agriculture.

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A novel parallel pathway for inositol sphingolipid synthesis in gut Bacteroidota

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Inositol lipid production is phylogenetically restricted among bacteria but prevalent in host-associated Bacteroidetes. The inositol lipid metabolic pathway in the human symbiont *Bacteroides thetaiotaomicron* (BT) is similar to in mycobacteria and proceeds through a phosphatidylinositol-phosphate (PIP) intermediate. However, some Bacteroidota *spp.* lacking homology to the BT-like pathway for inositol lipid synthesis nevertheless produce inositol sphingolipids through a pathway we predicted to lack the PIP intermediate, instead generating CDP-inositol. Here, we characterize this alternative inositol lipid gene cluster via heterologous expression in BT and gene knockout in *Phocaeicola dorei*. We determine the activity of key enzymes in the gene cluster and characterize inositol lipid structural diversity in a panel of Bacteroidota, including novel inositol lipid structures present in abundant human symbionts. As inositol lipids are potent bioactive signaling molecules in humans, the inositol lipid contribution from gut-associated Bacteroidetes, via one of two metabolic pathways, offers future potential mechanisms for host-microbe interactions.

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Spatially resolved host-bacteria-fungi interactomes via spatial metatranscriptomics

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All multicellular organisms are closely associated with microbes, which have a major impact on the health of their host. The interactions of microbes among themselves and with the host take place at the microscale, forming complex networks and spatial patterns that are rarely well understood due to the lack of suitable analytical methods. The importance of high-resolution spatial molecular information has become widely appreciated with the recent advent of spatially resolved transcriptomics. Here, we present Spatial metaTranscriptomics (SmT), a sequencing-based approach that leverages 16S/18S/ITS/poly-d(T) multimodal arrays for simultaneous host transcriptome- and microbiome-wide characterization of tissues at 55-µm resolution. We showcase SmT in outdoor-grown Arabidopsis thaliana leaves as a model system, and found tissue-scale bacterial and fungal hotspots. By network analysis, we study inter- and intra-kingdom spatial interactions among microbes, as well as the host response to microbial hotspots. SmT is a powerful new strategy that will be pivotal to answering fundamental questions on host-microbiome interplay.

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Insights into the diversity and fitness of the potential nasal probiotic *Corynebacterium accolens*

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Introduction

The nasal microbiome plays a crucial role in safeguarding the respiratory tract from invasive pathogens. One of the most abundant nasal microbiome members is *Corynebacterium accolens*, a Gram-positive rod-like bacterium that was first isolated in 1943 and was found to grow near *S. aureus,* therefore named after the Latin word "*accola*" (neighbour). In 2016, Bomar *et al.,* have shown that *C. accolens* can degrade lipids in the nose, thereby releasing anti-*pneumococcal* oleic acid. It has also been suggested the ability of *C. accolens* to protect the mucosal barrier from *S. aureus*-induced damage. The closest relative of *C. accolens* is *Corynebacterium macginleyi,* which is known to be an ocular pathogen, and its presence in the nose is hardly reported. Despite the potential benefits of *C. accolens*, its nasal dominance remains poorly understood.

Objectives

This study aims to investigate the genetic divergence of the two species and to identify putative fitness factors of *C. accolens* by comparing the two closely related species.

Materials & methods

In total, 30 *C. accolens* and 25 *C. macginleyi* genomes were retrieved from public databases and in-house sequencing. From these, 12 *C. accolens* and 13 *C. macginleyi* isolates, collected from different countries and years were available for experimental analyses. After phylogenetic and comparative genomics analysis, wet-lab experiments were performed to confirm metabolic differences between the two species.

Results

Initial results highlighted that *C. accolens* is a more diverse species with a smaller mobilome and a bigger pangenome than C. *macginleyi*. Interestingly, adaptation of *C. accolens* to the nasal habitat seems to be facilitated due to modification of lipid metabolism and iron acquisition systems. The latter was of special interest, as it shows that *C. accolens*, over reductive evolution, lost its siderophore production and depends on other siderophore-producing nasal commensals for their establishment.

Conclusion

C. accolens is a diverse nasal bacterial species and a highly effective cheater for siderophores.

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Revealing membrane insertion mechanisms of *Legionella*'s integral membrane effector proteins in host cells

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1. Introduction: A virulence strategy used by the intracellular pathogen *Legionella pneumophila* is to manipulate host cellular processes in order to survive within phagocytic host cells. Hence, more than 300 virulent effector proteins are secreted into the host cells by the specialized Dot/Icm type IV secretion system (T4SS). Many effector proteins harbor hydrophobic transmembrane-domains (TMDs) to fulfil their function in host cell membranes. However, the mechanisms T4-secreted TMD-effectors (TMEs) use to target and insert into the correct membranes of eukaryotic hosts remain to be elucidated.

2. Material/Methods: To understand the relevance of host cell targeting factors and receptors involved in membrane insertion of bacterial TMEs, we will compare their localization after T4SS-assisted injection or in-host ribosomal translation either by live-cell fluorescence microscopy using the nanobody technology or self-labeling enzyme tags, or by subcellular fractionation of infected or transfected RAW264.7 macrophages. Moreover, to reveal membrane targeting and insertion pathways of bacterial TMEs, interactions with host proteins will be investigated by proximity biotinylation using the TurboID biotin ligase, followed by subsequent mass spectrometry-based protein correlation profiling.

3. Results: In order to co-localize T4SS-injected or plasmid-expressed TMEs with different host cell membranes by Western blotting, a protocol for subcellular fractionation of macrophages was established. This method combines differential centrifugation with a sucrose density gradient centrifugation, allowing the separation of most organelles based on their individual abundance distribution profiles in the gradient. In addition, the subcellular localization of in-host expressed TMEs was assessed by immunofluorescence microscopy, which revealed a similar membrane localization to that reported for T4SS-injected TMEs. To identify potential TME interaction partners involved in membrane targeting and insertion, a protocol for TurboID-mediated proximity dependent biotinylation was evaluated. The biotin ligase TurboID was fused to the TMEs, allowing the labelling of proximal or interacting proteins. Biotinylated proteins were then successfully captured on streptavidin beads and identified by mass spectrometry.

4. Discussion: The methods established here will allow us to investigate the mechanisms by which bacterial TMEs target and insert into host cell membranes. In doing so, we aim to unravel the details of the intricate interplay between bacterial pathogens and eukaryotic host cells, which is crucial for the virulence of many pathogens. In addition, these findings may reveal new general principles for the targeting and delivery of integral membrane proteins directly to their site of action.

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LukAB is essential for *Staphylococcus aureus* exit from within macrophages

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Staphylococcus aureus is a notorious facultative pathogen that causes a diverse range of illnesses world-wide. *S. aureus* has different strategies to avoid the host immune response, e.g. hiding inside of the phagocytic cells (here referred as "PersistStaph"). However, "ExitStaph" *S. aureus* can escape from and kill macrophages by the induction of a so far unknown type of cell death. The pore-forming Leukocidin A/B (LukAB) induced from within macrophages is considered the main factor required for exit and host cell death. LukAB when added exogenously potently triggers the activation of the NLRP3 inflammasome, promotes IL-1ß secretion and eventually kills primary human monocytes. Conversely, the role of LukAB, when expressed intracellularly by *S. aureus*, and its effects on the cell death pathways and NLRP3 inflammasome are not well understood. To study these questions we employed different *S. aureus* strains lacking LukAB and/or with inducible LukAB expression. We found a surprising decoupling of NLRP3 inflammasome activation and cell death via a non-pyroptotic route. Moreover, classical apoptosis and necroptosis were not involved despite the activation of known signaling mediators. We conclude that *S. aureus* LukAB employs a non-conventional type of cell death in human macrophages.

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Escherichia coli Nissle 1917 flagellin outer sheath domains are dispensable for TLR5 recognition but modulate bacterial motility

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The onset and course of different diseases like type I diabetes, rheumatoid arthritis and inflammatory bowel disease (IBD) can be influenced by the composition of the intestinal microbiota. In case of IBD, microbiome interventions via the probiotic *Escherichia coli* Nissle 1917 (EcN; Mutaflor®) prolongs the remission phases in IBD patients by strengthening the epithelial barrier. In a dextran sodium sulfate (DSS)-induced intestinal inflammation model in mice disease amelioration depended on the unique EcN flagellin (FliC). Flagellin is a microbe associated molecular pattern (MAMP) recognized by Toll-like receptor 5 (TLR5) on immune and epithelial cells. FliC is the structural subunit of the bacterial flagellum and crucial for bacterial motility. Here, the conserved D0 and D1 domains form the filament core and a hypervariable region (HVR) forms a so-called an outer sheath. However, the structure of the EcN HVR and its role in TLR5 recognition or motility are not known. Here, we report the structure of the EcN flagellin to 1.7 Å resolution, showing the HVR to adopt two canonical (D2 and D3) and one additional D4 domain. Using both recombinant proteins and gene-edited EcN strains expressing mutant flagellins, we found that human and mouse TLR5 recognition was unaffected by removal of the D4 or a unique D1-D2 linker. However, they affected bacterial motility. Additionally, bacterial fitness, colonization and symbiotic effects will be studied in the DSS-induced colitis mouse model. Collectively, these data suggest that the HVR of EcN is not important for Flagellin-TLR5 complex formation but for flagella stability and motility .

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Apically applied shear stresses impact the rheotactic behavior, physical forces and transcriptomic profile of three different endothelial cell types

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1. Introduction

Endothelial cells (ECs) inside blood vessels form a single cell monolayer that acts as a protective barrier against different harms, including pathogens. The integrity of this barrier is conserved through an interplay of several elements at the cell surface including focal adhesions, adherens junctions and surface receptors. In ECs, these components also play a fundamental role in the response to flow shears stresses (SS) and shear stress gradients (SSG) that vary in space, time and (patho)physiological conditions (e.g., arteriosclerosis, hypertension).

2. Objetives

Although EC responses to variations in SS magnitude have been studied to some extent, how SSGs modulate EC barrier function by impacting EC kinematics (i.e., speed, collective migration) and dynamics (i.e., traction and monolayer forces) is relatively less understood. Whether such effects are universal or cell type specific is also unclear.

3. Materials & methods

Live-cell imaging-compatible impinging flow device which exert a profile of shear stresses to three different EC types. Traction force (TFM) and monolayer stress microscopy (MSM) to quantify cell-to-matrix and cell-to-cell forces. RNA sequencing in all EC types exposed or not to flow for 20 h to identify the concurrent changes in biochemical signaling.

4. Results

Exposure of three different EC types to flow led to an immediate, sustained and reversible 2-fold decrease in EC migration speed. Two out of the three EC types exposed to flow also increased directional collective movement against the flow direction. TFM and MSM analysis revealed a 50% increase in the traction forces ECs exert on their matrix and a 20% decrease in monolayer stresses – a proxy for the EC barrier integrity – after 20h of flow exposure. This is consistent with the idea that enhanced actomyosin contractility and focal adhesion organization power the movement of ECs against the flow gradient, which we confirmed by pharmacological treatment. RNA sequencing revealed that all three cell types upregulated TGF-b1 or genes related to it. We are currently investigating whether this is the common denominator regulating the increase in EC traction forces and the corresponding decrease in monolayer stresses during flow.

5. Conclusions

Collectively, our work suggests that flow exposure drives a dramatic reprograming in EC signaling and biomechanics, which is partly conserved across different types of ECs.

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Surviving unknown waters: The journey of *Salmonella*`s integral membrane effector proteins SseF and SseG into eukaryotic membranes Sophie Schminke, Sarah Trenz, Samuel Wagner

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Introduction: *Salmonella* Typhimurium invades and replicates inside eukaryotic cells by injecting virulent effector proteins through two different type III secretion system (T3SS) into its host. While T3SS-1 is important for host cell invasion, the T3SS-2 is necessary for the replication of the bacteria inside the eukaryotic cell. Among the T3SS-2 injected effectors are the integral membrane proteins SseF and SseG. Both possess two trans-membrane domains and are essential for the survival of the bacterium inside the cell 1. After their injection into the hosts aquatic cytosol, SseF and SseG are integrated into the membrane of the *Salmonella*-containing vacuole by an unknown mechanism.

During my PhD, I want to investigate the membrane insertion mechanism of SseF and SseG into eukaryotic membranes.

Methods: To unravel the highly dynamic process of SseF and SseG"s membrane insertion, I intend to compare their microenvironment after T3SS-2 injection and in-host translation. Therefore, I will use microscopy, biotin proximity labeling and proteomics.

To observe possible effector-cell organelle co-localizations, I will apply live cell microscopy using a turnover-accelerated chromobody (PepCb) conjugated to RFP 2. Once the host cell expressed PepCb-RFP binds its antigen (Pep), membrane localization of SseF- and SseG-Pep constructs inside HeLa cells can be observed.

Further, *in vivo* biotin labelling of host cell proteins through the activity of biotin ligases will be used to detect also only transient protein interactions of SseF and SseG with proteins within a radius of 10 nm.

Results: First, I optimized the protocol for proximity biotin protein labeling using the biotin ligase TurboID (TID) 3, which was fused to the C-terminus of SseF and SseG.

So far, I showed that SseF and SseG were still injected via the T3SS-2 into host cell and then integrated into membranes after fusion with TID. After addition of biotin to infected HeLa cells, biotinylated proteins could be detected after their enrichment with Streptavidin-conjugated beads. Hence, protein biotin labelling by the effector-TID constructs was successful and will be further analyzed by mass spectrometry. Additionally, I am creating a stable cell line expressing PepCb-RFP to observe effector-organelle co-localization in live cells.

Discussion: The analysis of proteins in close proximity to SseF and SseG and their localization in the host membranes allow an insight into their microenvironment and indicate possible paths for SseF and SseG"s membrane insertion. This will help to further understand the role of SseF and SseG in the pathogenicity of *Salmonella*. Additionally, investigation of a mechanism allowing the pathogen to survive inside host cells could help the development of highly specific anti-infectives in the future.

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

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Introduction

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

Objectives

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

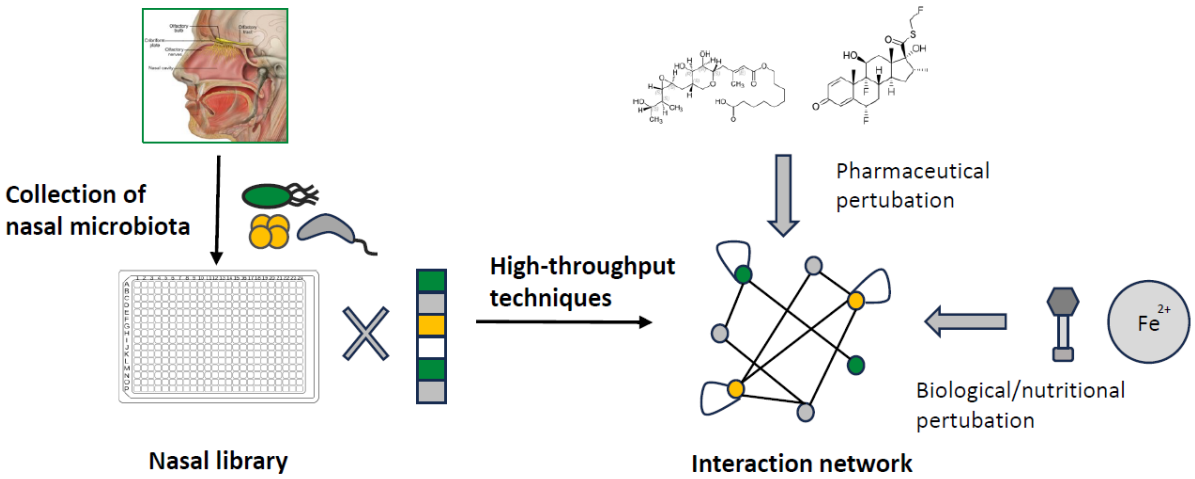
Material & Methods

Liquid and agar-based high-throughput methods supported by robotic systems.

Results & Conclusion

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

Fig. 1



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Role of *Staphylococcus aureus* Nuc1 in immune stimulation

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Staphylococcus aureus is an opportunistic pathogen, causing a wide spectrum of acute and chronic infections. To better understand the success of this pathogen, it is necessary to know more about its interaction with the immune system. In previous work, it has been shown that when stimulated with large undigested DNA from *Staphylococcus aureus,* mouse macrophages produced TNF-α. By contrast, small DNA fragments had no effect. Other immortalized cell lines such as MM6, HaCaT, and HT29 responded neither to large nor small DNA-fragments. In this study, we investigated whether the secreted *S. aureus* nuclease Nuc1 (also called thermonuclease or NucA) contributes to immune stimulation and virulence. We compared *S. aureus* USA300-JE2 parental strain with its Nuc1-deficient JE2D*nuc*1 strain. Stimulation of RAW264.7 murine macrophages, MM6, and PBMCs with live *S. aureus* JE2 and JE2D*nuc*1 cells led to the production of TNF-α or IL-6, with minimal differences between the two strains. In order to identify the staphylococcal factor that exhibited immune-stimulatory activity, we compared the cytokine release in response to JE2, JE2D*nuc*1, JE2D*lgt* and the double mutant JE2D*lgt*/D*nuc*1. The D*lgt* mutant strains are deficient in lipidation of lipoproteins, which are well characterized microbe-associate molecular patterns (MAMPs). In all the strains lacking *lgt* the production of TNF-α or IL-6 was drastically decreased. This was observed in both murine macrophages and MM6 cell. This result suggests that *nuc1* does not play a major role in immune stimulation in this setting and that lipopeptide sensing is dominant over DNA sensing. However, when we investigated the internalization of *S. aureus* strains by HaCaT and murine macrophages, we observed that the D*nuc*1 mutant was significantly affected in internalization, and this effect was independent of *lgt*. Since bacterial internalization plays a role in virulence, we compared the behavior of the *S. aureus* Newman strains with its D*nuc*1 mutant in a mouse model of infection. We observed that the mutant caused significantly lower weight loss and abscess score than its parent strain in infected mice. These findings confirm that Nuc1 is an important virulence factor in *S. aureus*.

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Exploration of the regulatory networks responding to environmental microbiota in the nematode *Pristionchus pacificus*

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Introduction: The development and metabolism of nematodes can be significantly impacted by the microbes in their environment. Adaptation to environmental stimuli may also be facilitated by the creation of new genes. About one third of genes in the nematode *Pristionchus pacificus* are characterized as taxonomically-restricted orphan genes, i.e. genes whose function can't be inferred from homology.

Objectives: Our main goal is to gain insight on the interactions between the nematode *P. pacificus* and bacterial food sources by answering two questions: do novel genes contribute to environmental adaptation and which are the bacterial metabolites that modulate the environmentally responsive networks containing those novel genes.

Materials & methods: To determine the functionality of novel genes, we generated transcriptome profiles of *P. pacificus worms* that were grown on 24 different bacteria isolated from Pristionchus-associated environments[1] and clustered the genes in co-expression modules. In order to characterize the bacterial metabolic potential, we performed whole genome sequencing of 93 bacterial strains and computationally identified the metabolic pathways present or absent. This allowed us to correlate the metabolic potential of bacteria with various nematode traits including survival, chemoattraction and the response of the previously identified co-expression modules.

Results: Based on the co-expression analysis, we identified 28 large modules that harbor 3,727 diplogastrid-specific orphan genes and that respond dynamically to different bacteria. These modules showed distinct regulatory architecture and differential expression patterns across development. Integrative analysis associated most coexpression modules with biological processes or tissues, which resulted in the first functional annotation for thousands of orphan genes. In addition, the presence of bacterial metabolic pathways was associated with higher indexes of chemotaxis and survival. For example, the ability of bacterial diets to supply spermidine and its derivative, spermine, vitamins of the B complex and molybdenum cofactor correlated with higher chemoattraction and survival compared to bacteria lacking those biosynthesis pathways. The only case of lower chemoattraction was associated with the production of propionic acid from the bacteria.

Conclusion: We showed that new genes aid environmental adaptation in the nematode *P. pacificus* by exploring the transcriptomic changes after exposure to different bacteria[2]. This allowed us to better understand the plastic response to diverse environmental microbiota. We are currently extending this work to include transcriptomic data on a larger number of bacterial food sources in order to improve the resolution of the co-expression network and explore how metabolic pathways in the worm are regulated by the presence/absence of metabolites.

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P 67

The community remembers: Role of collective memory in shaping complex microbial interactions

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Information storage and memory are generally discussed in higher organisms with complex nervous systems. Challenging the conventional definitions of memory, Slime mold *Physarum polycephalum* utilizes slime as externalized memory for spatial navigation. The presence of cellular memory has also been described in microbes influencing decisions such as sporulation or prolonged persistence to antibiotics. Depending on the environment, microbes also secrete different metabolites, ranging from metabolic by-products to antimicrobial compounds, which alter the immediate environmental conditions. Microbes acting in such collective behaviors result in 'externalized biochemical memory,' which we call Collective memory.

We show how collective memory impacts microbial interactions in deciding competition outcomes and shaping complex bacterial communities. In a pairwise competition assay, we employ an established 2-species model system involving *Lactobacillus plantarum* and *Corynebacterium ammoniagenes*. Different environmental stressors such as temperature, oxygen, and antibiotics result in distinct collective memories, imparting a competitive advantage to the bacteria. On the contrary, when exposed to antibiotics, memories can become "stronger," potentially bolstering bacterial survival in future encounters.

Furthermore, we demonstrate collective memory's role in assembling complex bacterial communities using eight isolates from *C. elegans* natural gut microbiome. Our results show that depending on collective memory, similar initial 8-member bacterial assemblies end up in different bacterial community compositions, which cluster into two distinct states. Interestingly, Collective memory from certain bacteria can influence diverse initial assemblies, leading them to converge into similar final states.

Further research would be required to explore whether collective memory plays a role in the dynamics of gut microbial communities and whether their ability to maintain stability against invaders could contribute to unraveling the intricate interactions within microbial ecosystems.

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Albugo – plant pathogen with detrimental influence

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Introduction: While microbial communities inhabiting the human host are thoroughly studied, research on the plant microbiome lags behind. However, as plants provide the largest living habitat for microbial colonization and ensure the working of our planet"s ecosystem, studying plant systems is equally urgent. Particularly important in this context is the study of plant pathogens, as they can have detrimental effects on crop plants. One of these pathogens is *Albugo candida* which, upon infection, causes white rust disease in a number of Brassicaceae plants. As an obligate biotroph, it is tightly adapted to its host and has lost most of its primary and secondary metabolism. It relies on released proteins to perform critical functions. One of these functions is proposed to be the shaping of the plant microbiome, especially through negatively influencing other community members.

Objectives: We studied the negative interactions of *A. candida* with the aim to understand its role in the microbiome and to identify released antimicrobial peptides.

Material and methods: The first stage consisted of a network analysis of the microbiome of wild *Arabidopsis thaliana*, focusing on correlations involving *A. candida*. Subsequently, we performed a proteomics analysis to find proteins released by *A. candida* during infection and developed bioinformatic pipelines to identify their potential antimicrobial activity. We expressed and purified protein candidates heterologously. We used these proteins first in pairwise assays to determine their activity against common plant-inhabiting bacteria by measuring growth curves. Afterwards, we tested their activity in a microbial community context and finally in the native plant system using Nanopore sequencing and qPCR.

Results: Our correlation network showed that *Albugo* was one of the microbes with the most negative interactions. We were able to single out proteins potentially taking part in these interactions as antimicrobials, during proteomics analysis, since we saw an enrichment of predicted antimicrobial propensity in the subset of released proteins. We chose candidates based on our predictions and found two proteins which inhibited the growth of specific bacterial strains commonly occurring in the *A. thaliana* microbiome. Notably, the proteins had high intrinsic disorder and had a high positive net charge, which we hypothesize may be responsible for the observed inhibition. We could additionally provide evidence showing that these proteins retained their selective activity in a microbial community as well as in the plant system.

Conclusion: With the release of antimicrobial proteins *Albugo* takes an active role in selectively shaping the microbial community of the host plant. This suggests that the plant microbiome should not be neglected as a resource for discovering novel microbial interaction mechanisms.

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Bacterial exometabolites function additively to regulate the growth of plant-pathogenic fungi

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Healthy plants interact with a staggering diversity of microorganisms including bacteria, fungi, and oomycetes. Recent investigations revealed a protective activity of bacteria against fungal dysbiosis in *Arabidopsis* roots highlighting the impact of microbe-microbe interactions on general plant performance [1,2]. Our objective was to decipher the molecular mechanisms underlying antagonistic bacterial-fungal interactions in the context of plant protection. We first performed an *in vitro* bacterial-fungal interaction assay using 185 bacteria and 40 fungi (7400 interactions) isolated from the roots of healthy *Arabidopsis* plants. Thereafter, we selected the most antifungal bacterial strains for genetic manipulation of bacterial biosynthetic gene clusters to identify various bacteria-encoded mechanisms that act independently and/or in concert to regulate fungal growth.

Results showed that there is a widespread production of antifungal exometabolite across all the bacterial taxa tested. Additionally, we found consistent phylogenetic signals in bacteria-mediated fungi inhibition for several families including Pseudomonadaceae, Commamonadaceae and Alcaligenaceae. Contrastingly, fungal sensitivity to bacteria-produced antibiotic metabolite is rather strain specific and not clade-conserved. Our results further revealed that naturally prevalent fungal strains are the most resistant to bacteria-produced antifungal exometabolites. To identify genetic mechanisms underlying this bacteria-mediated fungal inhibition, we screened *Pseudomonas brassicacearum* R401, one of the strong broad-spectrum fungi inhibitors, and 7 of its mutants which are deficient in siderophore, antibiotic and/or virulence factor biosynthesis. We found that 2,4-diacetyl phloroglucinol, pyoverdine and syringopeptin contribute additively to the fungal inhibition by this strain.

In conclusion, our results suggest that, although fungi inhibition might be widespread across several bacterial taxa, multiple mechanism with diverse mode of actions might be involved in the regulation of fungal-host homeostasis at the root interface. The elucidation of these mechanisms preventing fungal dysbiosis could pave the way towards the design of beneficial microbial synthetic communities that promote plant health and productivity.

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P 70

Uptake of pyrroloquinoline-quinone (PQQ) by *Escherichia coli*

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Glucose is taken up by *Escherichia coli* through the phosphotransferase system (PTS). PTS mutants grow with glucose as carbon source only in the presence of **pyrroloquinoline quinone (PQQ)**, which is needed as a **redox cofactor for the glucose dehydrogenase Gcd**. The **Gcd enzyme oxidizes glucose to gluconolactone in the periplasm**. External supply of PQQ is required as *E. coli* is unable to produce PQQ *de novo*.

Growth experiments show that **PqqU (YncD) is the TonB-ExbBD dependent transporter for PQQ through the outer membrane**. PqqU allows *E. coli* to activate Gcd even at surrounding PQQ concentrations of about 1 nmol/l. At 30 fold higher PQQ concentrations the activation of Gcd gets PqqU independent since the small Pqq may also pass the outer membrane through porins. PQQ protected the cells from the **PqqU dependent phage Bas10** by competition for the receptor protein. The **PQQ dependent production of gluconate is found** in many plant growth promoting bacteria that **solubilise phosphate minerals** in the soil by secreting this acid. Under Pi limiting conditions also *E. coli* induces the glucose dehydrogenase and secretes gluconate, even in absence of PTS, that is, even when the bacterium is unable to grow on glucose without PQQ.

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The role of secondary metabolites in plant associated microbial communities

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The progressive climate change is threatening plant growth all over the planet. Since plants represent the most important nutrient source for humans, their health is of great interest for our food supply. The plant microbiome is essential for healthy plants and represents a promising target for plant promotive treatments. [1]

The microorganisms within plant microbiomes not only interact with the plant but furthermore strongly affect each other. Recent studies found that the leaf microbiome of *Arabidopsis thaliana* shows a huge potential to produce secondary metabolites. [2] Since these compounds are fundamental units for microbes to sense and respond to their environment, we hypothesized, that they play a major role in mediating interactions in the leaf microbiome.

To investigate secondary metabolite-based interactions within plant microbiomes, we use a beneficial synthetic community (SynCom) assembled from *Arabidopsis thaliana* leaves. We used genome mining to analyze the potential of each Syncom member to produce secondary metabolites. Additionally, by using single strain non-targeted-metabolomics, we identified secondary metabolites and compared the metabolome of single strains to the metabolome of the whole Syncom.

In addition to metabolomics, we aimed to identify and isolate secondary metabolites produced by Syncom members to investigate their effect on the community. In further experiments, we investigate whether the presence or absence of certain compounds change the community composition on the plant leaf and therefor influences plant infection prevention.

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SynTracker differentiates evolution via mutation or recombination on a per-species basis in complex microbiomes

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Microbial species diversify into separate strains through mutation, recombination, and gene loss/acquisition. Elucidating mechanisms driving the genomic diversity of species residing within complex microbiomes remains biased towards mutation, because current strain tracking methods have low sensitivity to genomic structural differences. To overcome this and to complement existing strain tracking tools we developed SynTracker, a tool that compares strains using synteny, i.e., the conservation of the order of genomic markers in homologous regions in pairs of metagenomic assemblies or genomes. SynTracker"s attributes include low SNP sensitivity, no database requirement, and a high comparative performance. The combined use of SynTracker and SNP-based tools in metagenome analysis allows the identification of species undergoing high rates of recombination with low rates of mutation, or conversely, high rates of structural change with low mutation rates. When used as a standalone, SynTracker can accurately track strains using a fraction of the total genome length, allowing strain tracking in low abundance taxa, plasmids and phages. Taken together, SynTracker, when used alone or combined with other existing tools, provides a novel window into different modes of evolution on a per species-basis in complex microbiomes.

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Unraveling bacterial-archaeal interactions within the human oral microbiome – insights into the etiology of periodontal disease

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Methanogenic archaea are known for their ability to produce methane as a metabolic end product for energy conservation. Among other methanogenic pathways, the ability to produce methane from CO_2 and H_2 in hydrogenotrophic methanogenesis is the most widespread.

Methanobrevibacter oralis is the predominant methanogen residing in human subgingival plaque. Its prevalence has recently been linked to periodontitis, indicating a pivotal role in oral health and disease. The unique microenvironment within human periodontal pockets provides favorable conditions for the growth of strict anaerobes. Previous studies yielded evidence of close interactions between *M. oralis* and various bacterial species, as they synergistically participate in the anaerobic degradation of organic compounds. Importantly, this syntrophic cross-feeding between methanogens and hydrogen-producing bacteria, referred to as interspecies hydrogen transfer, is suggested to indirectly promote pathological conditions because it facilitates the growth and colonization of secondary fermenting pathogens.

Yet, much is still unknown about the complex microbial interplay between *M. oralis* and oral community members, contributing to dysbiosis and, ultimately, to the pathogenesis of periodontitis. To gain deeper insight into the etiology and manifestation of this polymicrobial disease, a more profound knowledge of the role of *M. oralis* within the oral microbial community is required.

In this context, we aim to study the relationships (mutualism and competitiveness) between *M. oralis* and bacterial interaction partners. We will employ co-culturing methods and quantitative PCR techniques to achieve this objective. Additionally, *M. oralis* and community members will be grown in batch and continuous reactor cultures to obtain fermentation data. Further, genetic approaches as well as proteome and metabolome analyses will be applied to gain information on the molecular mechanisms underlying these complex microbial interactions.

Ultimately, these findings will contribute to a greater understanding of the dynamic interplay between *M. oralis* and other oral microbes, offering valuable perspectives for developing potential therapeutic strategies against periodontal infections.

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Bacterial sensing regulates metabolism in the *Drosophila* intestine

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1. Introduction

The gut microbiome is essential for health, contributing to crucial functions in their host. Conversely, dysbiosis has been associated with immune and metabolic dysfunction, and is suggested to play causative roles in severe disorders like inflammatory bowel diseases.

2. Objectives

The mechanisms involved in the relationships between dysbiosis, immune disorders and metabolic dysfunction remain poorly understood. *Drosophila melanogaster* is a powerful model to address these gaps in knowledge: their microbiome can easily be manipulated in the lab and the basic mechanisms of immune and metabolic control are conserved between invertebrates and vertebrates.

3. Material and Methods

By using pathogenic bacterial infection as well as genetic modifications of immune signalling in *Drosophila*, we were able to untangle parts of how bacterial sensing regulates host metabolism.

4. Results

Drosophila infected with the intestinal pathogen *Pseudomonas entomophila* mount a strong immune response in their guts. We observed that they also suffer from severe maldigestion, with a global suppression of transcripts encoding digestive enzymes. Since previous studies in our lab identified a role for Pattern Recognition Receptors in the regulation of protein digestion, we further investigated the connections between bacterial sensing and intestinal metabolism. We discovered that PGRP-LE, the functional analog of NOD receptors in *Drosophila*, suppresses intestinal carbohydrase and lipase activities, resulting in systemic depletion of lipid stores. The effects of PGRP-LE are not limited to intestinal digestion, as this receptor has broad, negative impacts on central carbon metabolism and cellular energy levels in the intestine. Surprisingly, our genetic studies show that PGRP-LE can act independently of NF-kB signaling to regulate metabolism. Pattern Recognition Receptors could therefore employ distinct pathways to control immunity and metabolism, and our transcriptomic studies suggest that PGRP-LE regulates nuclear receptor signaling to adjust the latter. Additionally, our data provide evidence that PGRP-LE acts via the formation of signaling amyloids to control metabolism in response to bacterial cues.

5. Conclusion

Small intestinal bacterial overgrowth suppresses brush border disaccharidase activity and carbohydrate absorption in patients. This suggests that the crosstalk between symbionts, host bacterial sensing, and enterocyte metabolism that we discovered in *Drosophila* is conserved in vertebrates. Our studies in *Drosophila* could contribute to a better understanding of the molecular mechanisms involved in these relationships.

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How to get rid of *S. aureus* - Siderophore-based interactions between *S. aureus* and the nasal microbiota

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Staphylococcus aureus can be found as a commensal in the nasal microbiota of approximately one third of the human population. The presence of *S. aureus* in the nasal microbiota is significant due to the potential risk of infection. Therefore the understanding of why some individuals are colonized by *S. aureus* and others not is of particular relevance. In the nasal microbiome cooperative and antagonistic interactions among species were already shown. Among these, competition for iron is suggested to shape the composition of nasal bacterial communities. Iron plays a fundamental role in various metabolic processes in bacteria, and its availability is restricted in nasal secretions due to high amounts of the host-secreted iron-chelating molecule lactoferrin. Iron acquisition in bacteria relies on the secretion of iron-binding siderophores. *S. aureus* produces two distinct siderophores, staphyloferrin A and staphyloferrin B, and is also able to use xenosiderophores that are produced by other bacterial species.

We tested whether siderophore-based interactions may be responsible for the presence/absence of *S. aureus* within the nasal microbiome.

To understand the interactions we screened nasal bacterial isolates regarding their ability to produce (chrome azurol S overlay assay) / use (agar based *in vitro* co-culture) siderophores. Liquid based *in vitro* co-cultivation was used to assess the effect of siderophore stealing on *S. aureus*. Using a cotton rat nasal colonization model, we investigated whether siderophore acquisition is relevant during *in vivo* colonization.

In vitro, three siderophore-based interactions were identified: (i) Commensals consume staphyloferrins without producing siderophores themselves. (ii) Others consume staphyloferrins while producing siderophores that cannot be utilized by *S. aureus*. (iii) And some species consume staphyloferrins, while producing siderophores that also support *S. aureus* proliferation. All the interactions that resulted in siderophore stealing by commensals reduced *S. aureus* proliferation. Additionally, we observed reduced levels of colonization of an *S. aureus* strain lacking an ATPase energizing siderophore uptake systems, which led to the finding that siderophore uptake is important during *in vivo* nasal colonization. Furthermore, we identified a siderophore uptake system in *Corynebacterium aurimucosum* by the knock out of an ortholog of a known *S. aureus* siderophore uptake system, resulting in its loss of siderophores uptake ability.

Our data indicate that siderophore production and acquisition is an important trait during nasal colonization and is involved in bacterial interactions. Suggesting, that several commensals may hinder *S. aureus* proliferation by decreasing iron-availability and that these commensals might be used as nasal probiotics to reduce *S. aureus* colonization in humans.

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Neurotransmitter-producing commensal bacteria at the interface of host metabolism

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Introduction. Trace amines (TAs) are a subclass of neuro-monoamines produced by bacteria and humans mainly by decarboxylation of aromatic amino acids (AAAs). Four different bacterial AAA decarboxylases (AADCs) have been reported so far of which 3 enzymes are very specific in substrate preference. Staphylococcal AADC (SadA) is, however, non-specific and can decarboxylate tryptophan, tyrosine and phenylalanine to tryptamine, tyramine, and phenethylamine, respectively. It is also able to convert dihydroxy phenylalanine and 5-hydroxytryptophan to the neurotransmitters dopamine and serotonin. The wide distribution of TA-associated receptors (TAARs) in human tissues supports TAs" role as neurotransmitters able to trigger host cellular events directly. **Objectives.** What are the potential interactions between TA-producing commensal bacteria and the host cells? **Materials & methods.** The skin microbiome of healthy subjects was examined using shotgun metagenomics. At protein sequence level, a phylogenetic analysis of SadA homologs was performed on four different taxonomic levels: within the species *S. epidermidis*, the genus *Staphylococcus*, the phylum Firmicutes and within the whole Bacteria domain. The investigation of bacterial adherence and internalization was carried out using the HT-29 cell line. The *in vitro* and *in vivo* effects of TAs and TA-producing bacteria on wound healing assays were evaluated in HaCaT cells and in murine model. **Results.** Metagenomic analysis of the skin microbiota revealed a wide distribution of SadA homologs among classical skin and intestinal bacteria. These homologs were found in at least 7 different phyla, remarkably within the Firmicutes phylum. The potential interaction of TA-producing bacteria with the host was studied using *Staphylococcus epidermidis* and *S. pseudintermedius*. They secrete TAs which in turn trigger their adherence and internalization into human cells by activation of the α2-AR. In addition, TAs could accelerate wound healing by antagonizing the b2-adrenergic receptor in keratinocytes. This finding was confirmed *in vivo* by an application on the wound bed of TAs or a TA-producing *S. epidermidis* strain. Despite their rapid turnover rate, TAs were found at high concentrations in feces (20–600 µg/g) and skin (2.4-10.5 µg/100 cm2) samples from healthy subjects. As TAs have been shown to activate TAARs at nanomolar affinities, it is not unexpected to have their wide-range presynaptic "amphetamine-like" effects all over body and brain. Currently, the AAA decarboxylation activity is investigated in bacterial genera other than *Staphylococcus*. Afterwards, the affinity and catalytic efficiency of AADCs from different bacteria in the presence or absence of possible inhibitors or stimulators will be compared. **Conclusion.** TA-producing bacteria are ubiquitous in human microbiota which interact with host metabolism through direct stimulation of TAARs. Further studies are required to address more aspects of this interaction.

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Quorum sensing autoinducer-3 in *Salmonella* Typhimurium: from its biosynthesis to its impact on cell physiology

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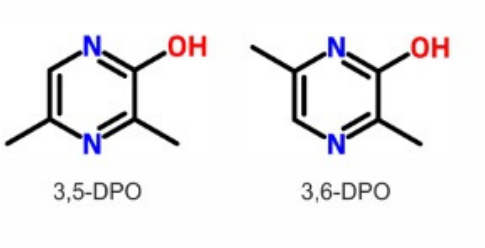
Quorum sensing (QS) is a bacterial intercellular communication system using small, secreted molecules (auto-inducers, AIs) to coordinate population wide-behaviors in a cell-density dependent manner. AI-3 has been reported to play roles in virulence and biofilm formation, and recently, in *Escherichia coli* and *Vibrio cholerae*, the structure of AI-3 was proposed to be either 3,5- or 3,6-dimethylpyrazin-2-ol (DPO) (Figure 1). However, AI-3 synthesis and its role in the major human pathogen *Salmonella* Typhimurium (STM) remains largely unknown. In this study, we first developed a method for measuring the production of DPO in complex biological samples using UHPLC-MS/MS. Our method also, for the first time, enables distinction between the two isomers 3,5- and 3,6 DPO. Using this method, we were able to monitor the biosynthesis of 3,5/3,6 DPO in *V. cholerae*, *E. coli* and STM and explore the genetics of its biosynthesis. In all species, we find that 3,5-DPO is present at higher concentrations than 3,6-DPO. In STM, we find that biosynthesis of both isomers is dependent on the presence of L-threonine and the enzyme L-threonine dehydrogenase encoded by the *tdh* gene. With that knowledge, we explored the STM transcriptional response to DPO biosynthesis, and showed that virulence behavior in a mice infection model was promoted by biosynthesis of this proposed AI-3. Through future experiments, we hope to reveal the potential receptor for DPO in STM and unfold the molecular response to this newly found AI-3.

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

Fig. 1



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Fecal microbiota transplant from EstMB participants repeatedly using antibiotics results in gut barrier malfunction in mice

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It has been shown that the gut microbiota plays a crucial role in the digestion of food, the pathogenesis of different diseases, and drug metabolism and can thereby greatly affect our health. We have established the Estonian Microbiome Project (EstMB) which includes gut metagenomic sequencing data from 2509 biobank participants supplemented with multi-omic measurements, questionnaires, and regular linkages to national electronic health records (EHRs), adding a new data layer to the Estonian Biobank. Our dataset enables us to follow the participants' medication history for more than 15 years, allowing us to, for example, study the accumulative effect of drug consumption on the microbiome. Our recent study showed that the long-term usage history of antibiotics is dose-dependently associated with lower diversity and changed the composition of the gut microbiome, independent of the recent 6 months of usage[1]. In order to see if these changes in the microbiome result in physiological differences, we carried out fecal microbiota transplantation (FMT) study transferring the microbiome from humans into a mouse model to unravel the effects of the microbiome on the intestinal mucosal barrier function. The FMT samples originated from biobank participants who had repeatedly but not recently taken antibiotics (i.e. > 5 courses of antibiotics in the last 5 years, but none in the last 6 months) and matched healthy controls. Our results indicate that the intestinal mucosal barrier is dysfunctional in mice who received microbiota from donors repeatedly using antibiotics, with significantly reduced mucus growth rate and increased penetrability detected in the intestinal mucus barrier. Metagenomic analysis results indicate significant changes in the microbiome between the two groups. As the mucosal barrier acts as an important defense mechanism against the microbes from entering our body, these results provide a novel knowledge mechanism linking repeated antibiotic use to changes in the microbiome and intestinal mucus function.

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S. aureus strains derived from healthy and atopic hosts differ in metabolite responses to AE-like environmental stress

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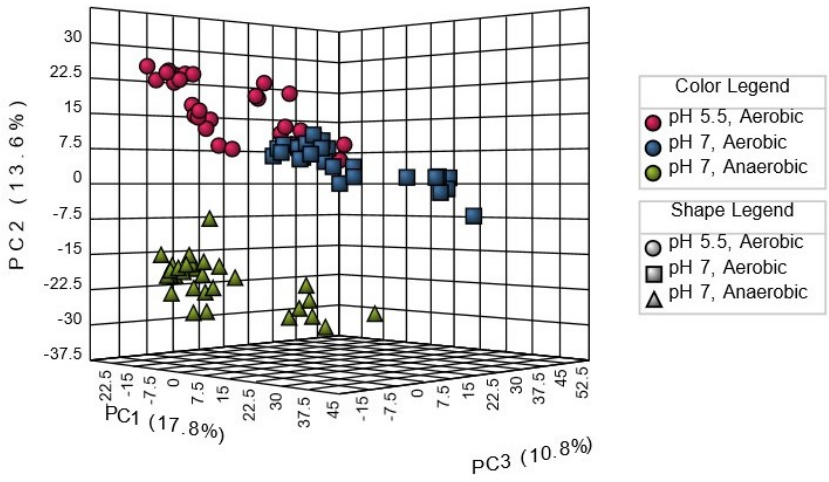
Introduction: Atopic eczema (AE) is an inflammatory skin disorder affecting approximately 20% of children worldwide with early onset leading to later development of asthma and allergies. The microenvironment changes in AE, where the pH is elevated and the micro-wounds in AE change the availability of oxygen within the skin from anoxic to normoxic. In addition, there is the overgrowth of *Staphylococcus aureus*, where *S. aureus* is a predictive factor for severity. Although *S. aureus* is known as an opportunistic pathogen – present in AE and chronic wound infections – this species has also been shown to be present on healthy individuals. As the environment of the skin is drastically different in AE as compared to healthy (HE), could a change in the environment result in *S. aureus* changing from a commensal to a pathogen?

Method: Here, we challenged 30 *S. aureus* isolated from 15 HE and from 15 AE participants to different environmental conditions (anaerobic pH 7.0 [HE-like], aerobic pH 5.5 [HE-like], and aerobic pH 7.0 [AE-like]) relevant to the environmental changes seen in AE and observed changes in growth pattern and bacterial secretions (the secretome). The bacterial secretions were measured for small molecules < 1.5 kDa by LC-MS^2 with a HILIC column, and to grasp all the potential biomarkers the study was an untargeted study.

Results: All three environments impact the growth potential of *S. aureus* with the anoxic environment inhibiting the growth the most. Based the unsupervised principle component analysis, the environment has a large impact on the secretions of *S. aureus*. The environments that more closely resemble the healthy environment (lower pH and anoxic) were more similar in secretions according to the dendrogram. Of the metabolomic patterns that were significantly different according to the environmental changes (pH or oxygen-changes), there were unique metabolites linked to the host's health status, healthy or AE. In addition, pH was found to be the more influential factor in distinguishing the secretions from healthy or atopic strains. Tentative annotation of metabolites specifically expressed in healthy or atopic strains, such as 1'-Acetoxychavicol acetate and 2'-Deoxycytidine, hint towards why AE strains are capable of evading the skin"s immune system in AE environmental conditions and *S. aureus'* capability to outcompete the host's microbiome.

Conclusion: The environment plays a role on both AE and HE isolates' growth and their secretions. In addition, the differential environmental response suggests a fundamental difference between isolates derived from healthy or atopic hosts. Further study on the metabolite markers of interest is still needed to confirm its impact in a in-vivo environment, but the research here provides the first step towards better understanding *S. aureus* infection in atopic eczema.

Fig. 1


PC2 (13.6%)
PC1 (17.8%)
PC3 (10.8%)
Color Legend
pH 5.5, Aerobic
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pH 7, Anaerobic
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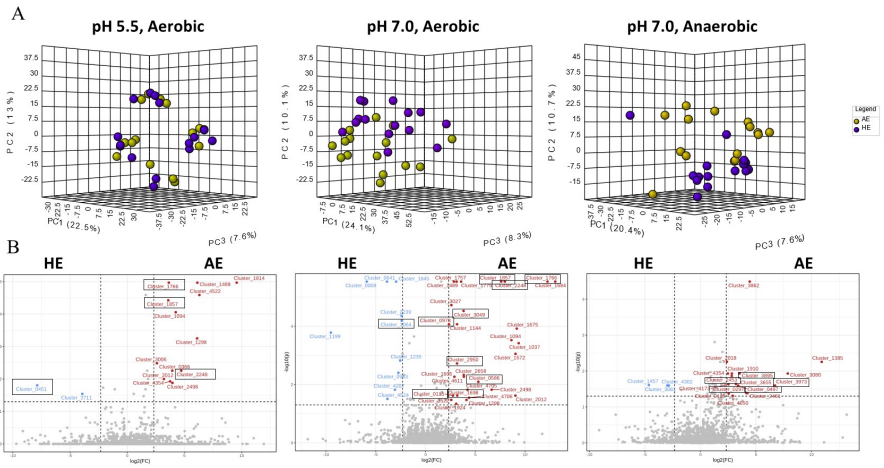
pH 5.5, Aerobic
pH 7, Aerobic
pH 7, Anaerobic

Fig. 2


A
pH 5.5, Aerobic
pH 7.0, Aerobic
pH 7.0, Anaerobic
B
HE
AE
HE
AE
HE
AE

P 80

Predictive value of the gut microbiome in immunotherapy responses of hepatocellular carcinoma patients

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Introduction

Hepatocellular carcinoma (HCC) is a type of cancer that typically arises from excessive inflammation in the liver. Since 2020, a new checkpoint inhibition therapy (ICI), namely Atezolizumab in combination with the VEGFR-blocker Bevacizumab (A/B), has been approved for HCC. The intestinal microbiome is important in modulating host immune responses and has been shown to influence ICI effectiveness in different cancer types, e.g. melanoma and lung cancer.

Despite the therapeutic improvements in ICI over the past years, therapy response is often heterogenic and sometimes not durable. Hence, the microbiome"s predictive role on ICI efficacy for treatment of HCC requires further scrutiny.

Objectives

We aimed to establish a combined predictive biomarker for short-term response to therapy with A/B in hepatocellular carcinoma.

Materials & methods

We collected stool, saliva and serum samples from 50 patients with multifocal or advanced hepatocellular carcinoma whose recommended treatment is A/B. This was done before start of treatment as well as weekly (microbiome sample collection) and three-weekly (serum collection) at the time points of antibody application until the fourth antibody application after 9 weeks.

Microbiome samples were subjected to whole-genome shotgun sequencing for microbiome profiling and serum samples are being subjected to targeted and untargeted metabolomics. For short-term therapy response, patients were divided into non-responders (progressive disease) whose therapy was discontinued and responders (response, stable disease) who continued A/B therapy at their first staging after 3 months.

Results

In a first pilot experiment with baseline samples of 26 patients, we observed that patients who responded to therapy had higher gut microbial alpha-diversity, increased abundances of Bifidobacteriaceae and Oscillospiraceae, and reduced Enterobacteriaceae, compared to non-responders. Furthermore, a random forest model trained on a training set of pre-treatment samples was capable of correctly predicting therapy response in an independent test set of pre-treatment samples.

Conclusion

Taken together, our results indicate that the baseline gut microbiome is associated with subsequent responses to immunotherapy in hepatocellular carcinoma patients, suggesting it could be useful as predictive biomarker to inform treatment choice.

P 81

An approach to investigate interbacterial interactions in a community of human gut microbes

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The efficacy of antibiotics against specific bacteria may differ in diverse microbial communities compared to monocultures. This suggests that microbial interactions are essential within a microbiome. Investigating the molecular pathways of cross-species interactions will allow us to modulate the microbiota to benefit the host"s health and to find new possibilities to fight infections. The goal of this project is to investigate how the pathobiont *Clostridium perfringens* can modulate the human gut microbiota and influence the abundance of common commensal strains. In a first step, we identified interaction partners of *C. perfringens* in a 17-member community. Every community strain was cultured alone and in co-culture with *C. perfringens,* and the growth under the two conditions was compared. We used qPCR with species-specific primers to monitor the growth of the strains of interest in monocultures and co-cultures. The molecular mechanisms underlying these interactions are investigated through proteome and metabolome analysis and genetic approaches. This approach enabled us to identify beneficial and adverse effects on the growth rate of the strains of interest. Most of the identified interactions were found to be inhibitory under these growth conditions. The observed phenotypes will be further explored in a bioreactor system combining the knowledge gained from the metaproteomic and metametabolomic study. Taken together, this knowledge will be valuable in modulating the human microbiota for therapeutic purposes.

P 82

Codiversification of salivary microbiota with humans

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The oral microbiome of human populations worldwide includes commonly-shared core species whose dominant strains differ between populations. The origin of population-specific oral strains is unclear. To address this question, we analysed paired saliva metagenomes and human genomes for 538 adult women living in Germany, Vietnam and Gabon. Between countries, a parallel evolutionary history was evident for humans and over 88% of the assessed common saliva microbes (23/26 taxa). A subset of the common saliva species displayed codiversification even within countries. In addition, species that exhibited the most pronounced codiversification have independently developed characteristics linked to host dependency, including reduced genomes sizes, sensitivity to oxygen and antibiotics, and genome reduction of genes associated with replication, recombination, repair and cell motility. Our findings suggest a shared evolutionary history between humans and oral microbiota.

Fig. 1

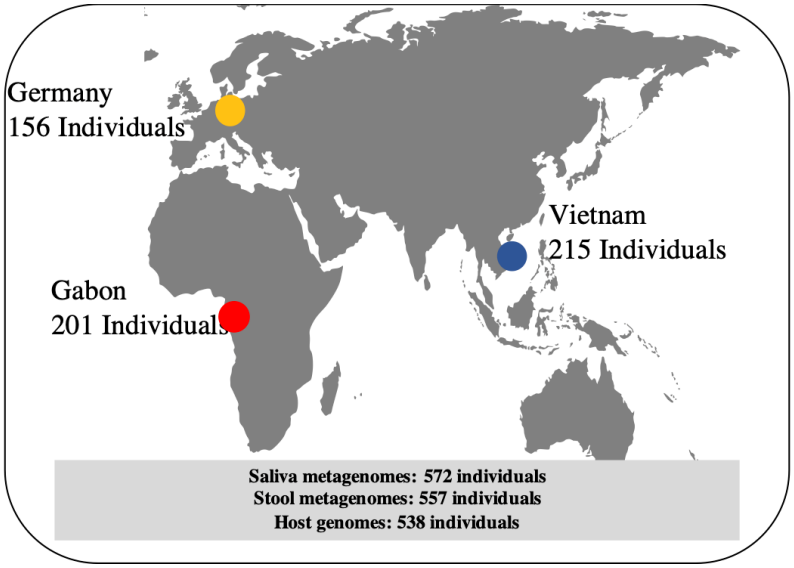
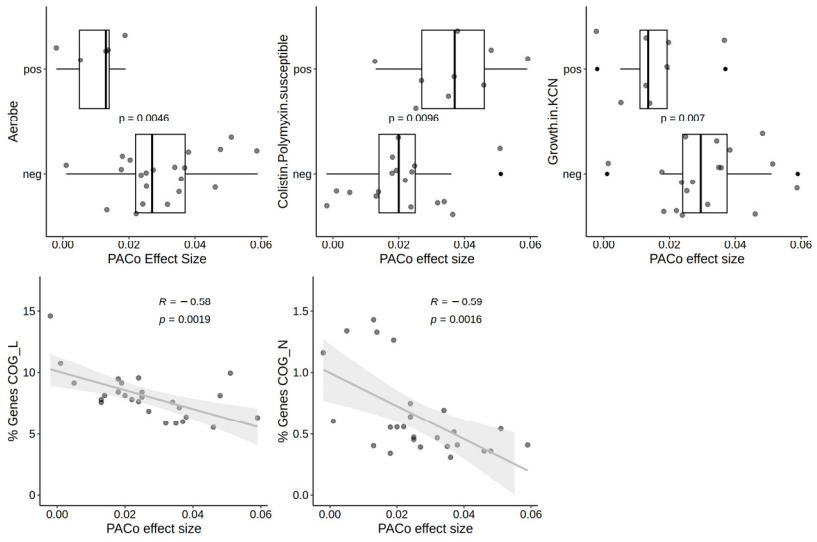


Fig. 2



P 83

Getting to know the neighborhood: using proximity dependent biotinylation to identify protein interactions

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The organization of the membrane is important for the correct function of several proteins involved in cellular processes like signal transduction, protein sorting, membrane trafficking, or pathogen invasion. Recent studies in bacteria show that proteins involved in these processes are organized in functional membrane microdomains (FMMs) that promote the efficient interaction of protein partners. Understanding the organization of proteins in the membrane might lead to a better understanding of infection mechanisms.

Hence, the aim of this project was to establish proximity dependent biotinylation as a method to identify protein interactions in FMMs to gain further insight into membrane organization. Therefore, the previous identified FMM associated proteins YqiK, YbbK, AcrA, AcrB and PrgH were C-terminally fused to the biotin ligase TurboID. For protocol optimization, and analysis of expression, the membrane insertion and biotinylation efficiency of the protein-TurboID fusion constructs was evaluated in *Salmonella* Typhimurium.

Most of the C-terminal fusion proteins were detected in crude membranes indicating membrane insertion of the fusion constructs. When evaluating the biotinylation efficiency of the protein-TurboID fusion constructs, diverse biotinylation patterns for the different proteins of interest (POI) fused to the TurboID were detected.

After optimizing the biotinylation the next steps include the purification of labeled proteins using streptavidin-conjugated beads and their identification by mass spectrometry.

This study could provide the foundation for the mapping of membrane proteins, to gain a better understanding of their organization in the membrane.

P 84

Maternal microbiota-derived metabolites drive embryonic epidermal formation and set neonatal skin barrier

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Introduction

Neonates need a competent skin barrier at birth to deal with environmental challenges such as microbial colonization, chemical exposure, and physical stress. The impact of the skin microbiota on postnatal barrier maturation has been largely studied. Our previous investigation showed that embryonic exposure to maternal microbiota metabolites set intestinal innate barrier (*Gomez de Agüero et al. Science, 2016*). Maternal microbiota-derived products, but not alive bacteria, reach not only the sterile embryonic intestine but also the skin. It remains unknown whether these maternal microbial metabolites can shape embryonic skin ontogeny and train early-life skin barrier function.

Objectives

The goal of this study is to investigate the contribution of maternal microbiota metabolites in the embryonic skin ontogeny and preparation for early-life skin barrier function independently of postnatal colonization.

Our hypothesis is that keratinocyte stemness is regulated by maternal microbial metabolites which control transcriptional regulators required for the shift between proliferation and differentiation, important for the development of a proper epidermal structure.

Materials & methods

The experimental approaches are carried out using our established model of gestational colonisation of germ-free pregnant mouse transient colonized with auxotrophic Escherichia coli HA107 with sterile offspring and an organoids 3D epidermal model. Single cell sequencing, flow cytometry and histological analysis allow us to analyse epidermal differentiation.

Results

Our data show that metabolites involved in epidermal differentiation and skin barrier development accumulate in embryonic skin of the offspring of gestational colonization dams. A large amount of them is maternal microbiota-derived, including major components of tryptophan metabolic pathways and therefore, ligands of aryl hydrocarbon receptor (AhR). Using 3D epidermal organoid model, we have shown that AhR ligands coming from maternal microbiota enhance epidermal maturation. The permeability barrier function, as determined by trans-epidermal water loss and dye penetration, is enhanced after gestational colonization. Moreover, hair follicle restoration after epidermal barrier disruption is impaired in absence of maternal microbiota contribution.

Conclusions

In summary, our results show that the maternal microbiota metabolites shape embryonic epidermal ontogeny and sets skin barrier function by the time of birth.

These findings would allow to develop targeted strategies for enhancing skin development in the neonatal period, critical to reduce morbidities and mortalities in preterm infants.

P 85

Peptidoglycan salvage enables the periodontal pathogen *Tannerella forsythia* to survive within the oral microbial community

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Tannerella forsythia is an anaerobic Gram-negative oral pathogen strongly associated with periodontitis, an inflammatory disease that leads to the destruction of the teeth-supporting tissue, ultimately causing tooth loss [1]. It *depends on* co-habiting bacteria in the oral biofilm for the provision of nutrients and it is strongly associated with periodontal pathogens of the red complex consortium during the late stages of periodontitis progression. Earlier axenic culturing studies revealed that the organism is auxotroph for *N*-acetylmuramic acid (MurNAc), which is an essential component of the bacterial peptidoglycan (PGN) cell walls. Later, whole genome sequencing revealed that the bacterium lacks the generally essential genes encoding the canonical MurA/MurB enzymes, required for the *de novo* synthesis of PGN precursors, thus rationalizing MurNAc auxotrophy. *T. forsythia belongs to the Bacteroidetes phylum and* possess the typical PGN structure of Gram-negative bacteria [2]. We showed that *T. forsythia* accepts PGN-derived fragments and polymeric PGN as sources of exogenous MurNAc [3]. We discovered two, presumably periplasmic, unique exo-β-*N*-acetylmuramidases NamZ1 and NamZ2, which cleave exogenous PGN glycan sugar strands at the non-reducing ends, generating *N*-acetylglucosamine (GlcNAc)-MurNAc disaccharides and MurNAc, respectively [4]. Further, we identified two inner membrane transporters: AmpG, imports the disaccharides generated by NamZ1 [3] and MurT, imports MurNAc generated by NamZ2 [5]. Recently, two intracellular MurNAc kinases were characterized and their crystal structures solved [6, 7]. Our studies allow a better understanding of PGN salvage metabolism of the oral pathogen *T. forsythia*, which is crucial for the bacterium to survive within the oral microbial community.

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P 86

Distinct colonisation niche and immune system interaction between commensal and pathogenic *Staphylococcus epidermidis* strains

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Introduction: *Staphylococcus epidermidis* is one of pioneer skin colonizers of the mammalian skin at birth. Permanent component of the commensal skin microbiota, *S. epidermidis* plays a key role in the host-microbial interactions in early life setting up tissue baseline and preventing pathogen accumulation. Recently, *S. epidermidis* has emerged as main pathogenic agent causing neonatal sepsis, especially in preterm infants. Pathogenic *S. epidermidis* strains form biofilms and express multidrug resistance genes to evade therapeutic strategies. Dysfunctional body barriers would be associated with accumulation of pathogenic strains of *S. epidermidis* leading to sepsis in case of host penetration. Moreover, secondary colonization of intestine in infants is a major risk for necrotic enterocolitis.

Objectives: In this study, we aimed to investigate the unique functional features of pathogenic skin strains of *S. epidermidis* such as spatial colonization patterns, interaction with the immune system and ability to evade immunesurveillance.

Materials and methods: *S.epidermidis* strains were isolated from human skin. The S. epidermidis strains were charachterized phenotypically and genotypically. Human 3D-skin models as well as to gnotobiotic murine models were used to study the study the colonization pattern and cutaneous immune responses.

Results: *H*uman 3D-skin and gnotobiotic murine models showed that commensal strains isolated from the skin were more adapted to colonize the skin, while pathogenic ones prefered the intestine. Commensals housed in deep layers of the stratum corneum and swiftly induced alterations in the skin myeloid compartment. On the contrary, pathogenic strains silently formed biofilms at the surface of the skin. Furthermore, long-term exposure to pathogenic strains triggered tissue inflammation illustrated by activation of Langerhans cells and influx of neutrophils. Moreover, pathogenic strain escaped the liver firewall and invaded the rest of the organs upon blood infection.

Conclusion: Our results illustrated that pathogenic strains of *S. epidermidis* are covert to skin immunosurveillance and therefore, their accumulation represents a threat to the host.

P 87

Gut colonization with a branched chain amino acid synthesis mutant of *Phocaeicola* (*Bacteroides*) *vulgatus* affects host organ metabolism

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INTRODUCTION: The branched-chain amino acids (valine, leucine, and isoleucine, or BCAA) are essential amino acids in animals and synthesized by plants, fungi, and some gut microbes. Circulating levels of BCAA are associated with increased weight and insulin resistance in humans and rodents. Colonization with gut bacteria that synthesize BCAA, like *P. vulgatus*, is associated with weight gain and increased levels of circulating BCAA in mice. However, the mechanistic contribution of gut microbes to BCAA metabolism in host tissues has not been established.

OBJECTIVES: The objective of this work was to test whether gut microbial production of BCAA influences circulating and tissue levels of BCAA and related metabolites.

MATERIALS AND METHODS: We made a clean deletion in the *P. vulgatus ilvC* gene, which encodes an enzyme required for BCAA synthesis. We then colonized germ free mice with either the wild-type (WT) or Δ*ilvC P. vulgatus* deletion strain for 4 weeks, then examined BCAA levels and related metabolites in serum, liver and adipose tissue. We also sequenced RNA from bacteria recovered from mouse ceca colonized with either the WT or Δ*ilvC* strains of *P. vulgatus*.

RESULTS: The Δ*ilvC* mutant required exogenous BCAA for growth in minimal medium but colonized germ-free mice to a similar level as WT *P. vulgatus*. Mice colonized with the Δ*ilvC* mutant gained less weight and had smaller adipose depots than WT-colonized mice. Liver tissue from Δ*ilvC P. vulgatus* colonized mice had lower BCAA levels than that of WT colonized mice. Further, adipose tissue from Δ*ilvC* colonized mice had lower levels of the branched chain keto acids (BCKA) than WT controls. Microbial transcripts for peptidoglycan, folate, and tRNA synthesis, fatty acid synthesis and metabolism, and RNA degradation pathways were significantly upregulated in the cecal *ilvC* mutant bacteria when compared to WT *P. vulgatus*.

CONCLUSIONS: Gut colonization with an Δ*ilvC P. vulgatus* mutant strain had effects on BCAA metabolite levels in tissues throughout the body. The lower BCKA levels in adipose tissue suggested that transamination of BCAA to BCKA in the host may be regulated by gut microbial BCAA production. Cecal Δ*ilvC P. vulgatus* transcriptomics suggested that the mutant strain had altered biosynthetic capacity to compensate for the loss of BCAA synthesis. We are continuing to investigate intestinal, liver, and adipose transcriptional response to *P. vulgatus* colonization with and without BCAA synthesis. This work will help to clarify the roles of gut microbial amino acid production on host BCAA metabolism and underlying molecular mechanisms.

P 88

Measurement of proteome dynamics in bacterial co-cultures

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Natural habitats host complex bacterial communities, in which the relationship between different species can range from synergism to antagonism. In the competition for limited nutrients, each bacterium interacts with and affects its microbial neighbors by an array of mechanisms. Known mechanisms range from secretion of small effector molecules in quorum sensing to contact-dependent exchange of larger biomolecules by secretion systems and bacterial nanotubes. In order to characterize the overall impact of mixed-species interactions for two bacterial model organisms, *Escherichia coli* and *Bacillus subtilis*, we aimed to establish a bottom-up proteomics workflow in which individual changes of the proteome can be compared between mixed and pure cultures of each species. To this end, bacterial cultures were propagated in minimal medium with low agitation to facilitate cellular contact in a sediment layer. Subsequently, we compared the proteome composition of both species after short (30 min), intermediate (60) and prolonged (120 min) incubation times relative to pure cultures of each species by label-free protein quantification. Based on measured protein intensities, *E. coli* cells had constantly increasing protein abundances in mixed cultures compared to pure cultures, whereas *B. subtilis* cells revealed opposed trends after prolonged incubation times.

Among individual proteins with significantly altered abundances upon mixed cultivation, proteins involved in protein synthesis and transport, phosphate import, lipopolysaccharide biosynthesis and biotin metabolism were up-regulated in *E. coli* already after 30 min. In *B. subtilis*, significant protein regulation was observed mainly at later time points (60 and 120 min) on proteins involved in amino acid synthesis and cellular metabolism. Interestingly, proteins involved in the incorporation of nitrogen compounds (nitrate/nitrite) reveled increasing abundances in *B. subtilis* throughout the time course, whereas homologous proteins in *E. coli* showed reducing abundance levels. Overall, our experimental data indicates that both species react to their mixed cultivation in a controlled manner dominated by adaption to limiting nutrient availability. The established proteomics workflow can be easily applied to multiple cultured species and alternating cultivation conditions, which enables the characterization of bacterial interspecies interactions on the proteome level.

P 89

Harnessing the CRISPR Immunological memory of bacteria to track the spread and transmission of resistant pathogens in microbiomes

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The CRISPR-Cas adaptive immune system is found in many bacteria and enables them to defend against viral infections. The CRISPR system can acquire small fragments of foreign DNA known as spacers that are inserted into the CRISPR array. As new spacers are always inserted from one direction, the CRISPR array not only serves as an immunological memory for the bacterium, but also represents a partial timeline of ancestral encounters of phages and plasmids that tried to invade the bacterial cell. Notably, compared to genetic mutations the evolution of CRISPR arrays through acquisitions and deletions occurs at a much faster rate. This allows us to to follow the evolution of pathogens and antibiotic resistance conferring plasmids at a much finer scale by leveraging the information within CRISPR arrays. Firstly, we are able to track closely related bacterial strains themselves, through the quick evolution of CRISPR arrays, e.g. in a clinical outbreak of a pathogenic strain. Secondly, through the correspondence between spacers and phages, it is possible to identify ancestral phage invasions from the CRISPR arrays within a microbiome. Moreover, we are able to track the spread of antibiotic resistance genes on conjugative plasmids through spacers in related organisms that target these plasmids. To investigate CRISPR array evolution, we developed SpacerPlacer, a tool that is able to reconstruct ancestral states of a group of closely related CRISPR arrays by reconstructing spacer insertion and deletion events along a phylogenetic tree. Alternatively SpacerPlacer can estimate a phylogenetic tree based on the CRISPR arrays. The core concept behind SpacerPlacer is to leverage the polarized insertion of spacers into CRISPR arrays. SpacerPlacer initially generates a straightforward preliminary maximum likelihood reconstruction and refines this reconstruction based on the partial spacer insertion order. One limitation is that we are reliant on complete genomes or long-read sequencing data as we require the precise order of spacers in the CRISPR arrays. For bacterial strain tracking in clinical settings based on CRISPR arrays, we obtain phylogenetic trees of higher resolution compared to conventional methods such as MLST. Through spacer-phage correspondence, we are able to construct timelines of past phage infection attempts and track the evolution of the microbiome. Similarly, we are able to track the history of mobile plasmids carrying antibiotic resistance genes by following plasmid-targeting spacers through their evolution. Reconstruction of CRISPR array evolution is a new and promising avenue for small-timescale strain tracking and analysis of the interactions in the microbiome, including clinical and pandemic settings. Thus allowing us to track the transmission of pathogenic strains and their antibiotic resistance genes to achieve a better understanding of their evolution and improve our tools in the fight against the spread of antibiotic resistance.

P 90

Bacteriophages influence the *Arabidopsis thaliana* phyllosphere microbial community

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The plant phyllosphere is inhabited by a variety of different microorganisms, including bacteria, fungi and protists, as well as viruses. Over the past two decades, there has been an increasing interest in the influence of the viral group of bacteriophages on the plant microbiome (1). Especially studies in marine and soil environments revealed a significant impact of phages on in microbial communities (2,3) In order to understand the role of phages in the plant phyllosphere microbial community we use the model organism *Arabidopsis thaliana*. We isolated various phages from environmental *A. thaliana* leafs and probed them with a pathogen-protective synthetic community (SynCom) composed of core members of the *A. thaliana* microbiome. To understand the influence of those phages we focused on changes in the pathogen protective effect of the SynCom. This was examined via CFU determination of two pathogenic *Pseudomonas* sp. *Pseudomonas syringae* and *Pseudomonas viridiflava* in vitro and in planta. Results revealed potential beneficial effects by some of the isolated phages with respect to disease suppression. This supports the idea of phages as active participants in the phyllosphere microbiome. Compared to the mechanisms described in literature, direct interactions such as horizontal gene transfer with the pathogenic and commensal bacteria can be considered as an explanation. In addition, the lysis of or predation on host bacteria from the SynCom could change the composition of the microbial community. For example, pathogen and commensal bacterial molecules might act as Microbial Associated Molecular Patterns (MAMPs) that could trigger the plant immune system. The aim of our project is to shed light towards the currently unknown influences of phages in the plant phyllosphere. Understanding the mechanisms behind and results of phage-bacteria interactions in these communities increases the knowledge we have about the complex network of microbes on their plant host.

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P 91

Receptor identification and structure function analysis of *Clostridium perfringens* beta-toxin.

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Introduction*:* Clostridial infections are a leading cause of morbidity and mortality in animals and humans. They frequently utilize pore-forming toxins (PFTs) as virulence factors. PFTs are secreted from the bacteria as water-soluble monomers, bind to cell membrane receptors and damage cells by inserting oligomeric membrane-spanning pore complexes. Because of the essential role of PFTs in the virulence of many *Clostridium perfringens* strains, they are attractive targets for the development of novel anti-virulence strategies. *Clostridium perfringens* produces many different toxins belonging to the family of hemolysin-like β-PFTs. Beta-toxin (CPB), the prototype of these clostridial toxins, is the main virulence factor for type C strains, causing fatal necrotizing enteritis mainly in newborn animals but occasionally also humans. CPB is highly potent and specific to endothelial cells (ECs), platelets, and leukocytic cell lines across different species.

Objectives: We aimed to unravel the molecular and structural basis of CPB cell type specificity.

Material and methods: To identify host factors involved in CPB toxicity, we performed genome-wide CRISPR-Cas9 loss-of-function screens in ECs. Receptor candidate confirmation was performed using CRISPR-Cas9 single gene knockout and ectopic overexpression studies. Mutated receptor proteins were expressed in receptor deficient cells to determine the CPB binding domain. Antibody blocking assays were used to validate our findings. Finally, Cryo-Electron Microscopy (Cryo-EM) was used to determine the pore structure of CPB.

Results: We identified Platelet Endothelial Cell Adhesion Molecule-1 (PECAM1 or CD31) as the cell surface receptor for CPB. We also determined the membrane proximal Ig6 domain of CD31 as the toxin binding domain. The oligomeric pore structure of CPB was resolved as symmetrical eightfold protomers, consisting of an N-terminal β-barrel protrusion site (NBP), a cap, a rim, and a stem domain. The NBP site represents a novel conformation, most likely influencing pore conductivity and stability. The rim domain contains unique loops with surface-exposed charged and aromatic residues that most likely define the receptor specificity of CPB. Structural predictions using alpha fold show that these loops would build an ideal binding pocket for the CD31 Ig6 domain.

Conclusions: We determined the cellular receptor for a central virulence factor of *C. perfringens* type C causing fatal enteric disease in animals and humans. Our results highlight molecular mechanisms and structures that confer receptor-, cell-type and species specificity for clostridial hemolysin-like β-PFTs.

P 92

Cervix-infection-on-chip: A microphysiological model of Chlamydia and Neisseria gonorrhoeae infection for mechanist studies and development of therapeutic interventions

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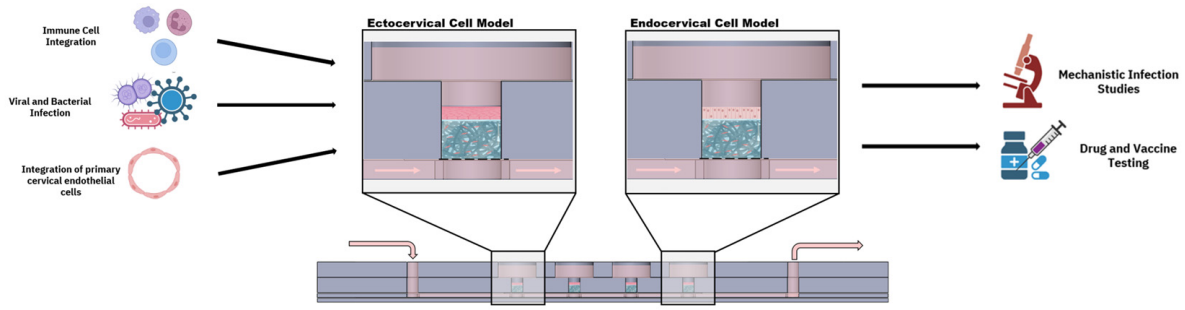
Introduction: Cervix infections caused by Chlamydia and Neisseria gonorrhoeae pose a significant health burden among women worldwide. Traditional in vitro models lack the complexity and physiological relevance of the cervix microenvironment. In this study, we introduce a novel approach utilizing a cervix-on-chip model to gain valuable insights into bacterial infections, host responses, and explore potential therapeutic interventions. Objectives: Our study aims to establish Organ-on-chip models of ecto- and endocervical tissue and infect them with Chlamydia and Neisseria gonorrhoeae. To simulate host-pathogen interaction, we aim at incorporating immune cells into the models. By monitoring the infection process in real-time and studying host responses, our objectives include understanding pathogenesis, deciphering host-pathogen interactions, and host immune responses. Materials and Methods: Ectocervix and endocervix models were constructed in tailored microfluidic chips using tissue-engineering and microfabrication techniques. The models were infected with Chlamydia and Neisseria gonorrhoeae, and the infection process was monitored using live-cell imaging. The microfluidic chips facilitated precise control over the microenvironment, including nutrient availability, fluid flow, and immune cell perfusion. Analysis of infection progression and host response was performed using image processing and cytokine assays. Results: Our findings demonstrate successful infection of the ectocervix and endocervix models with Chlamydia and Neisseria gonorrhoeae. Real-time imaging revealed distinct infection patterns, showcasing variations in pathogen tropism across different parts of the cervix, as well as host immune responses. We observed active replication, cellular damage, and the release of pro-inflammatory cytokines in response to infection. Furthermore, we observed immune cells being recruited into infected tissues. Conclusion: Our cervix-on-chip platform provides a valuable tool for studying the dynamic nature of Chlamydia and Neisseria gonorrhoeae infections. The microfluidic system enables precise control over the microenvironment and real-time observation of infection processes and immune cell responses. Our findings shed light on the pathogenesis of cervix infections and offer a platform for developing therapeutic strategies. The developed models hold significant promise for accelerating drug discovery and advancing vaccinations in the context of cervix infections.

Figure1: Schematic of the cervix-on-chip for mechanistic studies and therapeutic interventions

Fig. 1



P 93

Unveiling reproducible gut microbiome alterations in anxiety: enhanced aromatic amino acid metabolism across cohorts

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Anxiety disorders have a significant impact on global population health and have been found to influence the composition of the microbiome. However, the specific taxonomic and functional features of the microbiome associated with anxiety scores vary across studies, potentially due to limitations in taxonomic resolution, methodological differences, and confounding factors. We aimed to identify universal and cohort-specific species/strain-level taxonomic and functional features of the gut microbiome linked to anxiety.

To achieve this, we collected stool samples from two large cohorts: Tulsa1000 (n=270, USA) and TwinsUK (n=359, United Kingdom), along with clinical information. The microbiome profiles were analyzed using shotgun metagenomics, and the pool of metabolites in the blood was additionally assessed through untargeted LC/MS metabolomics. We employed a compositionality-aware Nearest Balance method to examine the associations between taxonomic composition with anxiety and other factors.

Our results revealed consistent taxonomic enrichments in high- or low-anxiety individuals across the cohorts, using both k-mer based metagenome profiling and metagenome-assembled genomes (MAGs). Among the highlights, several members of Enterocloster genus were positively linked with anxiety. Notably, enrichment often varied within genera and species, emphasizing the importance of fine taxonomic resolution to identify true taxon-anxiety associations. We further refined the correlates of anxiety by examining balances of microbial species. While we observed a significant association between alpha diversity and anxiety, the effect size was modest. Additionally, specific genomic features were significantly linked to anxiety, indicating that the microbiome of individuals with high anxiety may be conducive to pathogen growth and influenced by inflammation.

In terms of functional alterations, we found that the gut microbiome in individuals with anxiety demonstrated an increased propensity for tryptophan synthesis, along with other aromatic amino acids. From a community structure standpoint, this manifested as a reduction in taxa predicted auxotrophic for tryptophan synthesis. However, our comparison with serum levels of tryptophan and related compounds did not reveal direct associations with the microbiome composition or anxiety.

In conclusion, our study revealed taxonomic features consistently linked to anxiety across diverse cohorts, highlighting the significance of specific taxa that necessitate targeted experimental validation to elucidate their causal roles. Differences related to tryptophan metabolism suggest possible alterations of the pool of neuroactive compounds and their precursors in the gut (including kynurenine and indole). Further exploration of these mechanisms will enhance our understanding of how the microbiome contributes to the pathogenesis of anxiety and compensatory processes.

P 94

Bifidobacteria confer microbiome-acquired lactose tolerance in genetically lactase-non persistent humans

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Baby mammals produce the enzyme lactase, that cleaves the milk sugar lactose, until production stops with the cessation of lactation. A subset of humans have evolved lactase persistence (LP) into adulthood. LP confers lactose tolerance, allowing milk digestion in adults. One key aspect of lactose tolerance is low levels of H_2 gas production from lactose ingestion. Intriguingly, many lactase non-persistent (LNP) individuals also exhibit low levels of H_2 production from lactose (termed "acquired lactose tolerance", ALT). Although the microbiome is suspected to play a role in ALT, studies to date have failed to associate specific microbiota with ALT. Here, to identify microbiota that confer ALT, we assessed lactose tolerance (blood glucose and breath H_2 monitored after lactose dose), LP/LNP genotype, and microbiome metagenomic diversity in adult volunteers (Gabon n=152, 0% LP; Vietnam n=190, 0.5% LP; Germany n=141, 77% LP). In each country, ~20% of LNP individuals exhibited ALT (breath H_2 rise below 30 ppm). In accord with previous studies, no metagenome differences were detected between microbiomes of lactose intolerant and ALT individuals. To better understand how lactose is metabolized in the gut of lactose intolerant versus ALT individuals, we added lactose to the stool in vitro and measured metabolite production. We found that stool could be sorted into 4 groups based on response to lactose: (1) inactive - lactose hydrolysis only; (2) weak - lactose hydrolysis with little fermentation; (3) gassy - lactose hydrolysis, fermentation and high H_2 production; and (4) tolerant - lactose hydrolysis, fermentation and low H_2. This result implies that ALT results from two distinct processes: low metabolic activity, or high metabolic activity resulting in low gas production. Using this classification for the metagenomes, we observed that Bifidobacteria were enriched in the tolerant group compared to the others. The tolerant group was also enriched in lactate and acetate, metabolic byproducts of the Bifid shunt. Our results indicate that ALT is prevalent across populations, including where the LP genotype is rare, and can result from two distinct processes. For metabolically-active microbiomes, the Bifidobacteria are the key microbiota conferring ALT. These findings indicate that Bifidobacterial-conferred ALT is widespread, and that by facilitating the digestion of lactose, may have enabled the incorporation of milk into the adult diet and the evolution of LP.

P 95

Unraveling the role of biofilm forming microbes in complex leaf microbiome: Insights from a synthetic community study

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Plants leaves are associated with a diverse microbes consisting of bacteria, fungi, and protists forming a microbial community contributing to the health of their host under atypical environmental stresses. (Agler et al. 2016; Chaudhry et al. 2021; Almario et al. 2022). Mechanistic insights into community assembly are crucial to better understand the functioning of complex microbial community. In this study, we aimed understand the role of biofilm forming microbes in shaping functional microbial communities. We assemble co-occurring and abundance based microbes to design a complex Synthetic Community (SynCom). We performed *in-silico* mining of genes/gene clusters responsible for leaf adaptive, protective, and probiotic traits. We resolved microbe-microbe interactions in SynCom by dropout approach and investigate their protective outcome under pathogen perturbation. We found that SynCom member *Pseudomonas koreensis*, has a fitness advantage and a key protective role in the SynCom against the model phytopathogen *Pseudomonas syringae* under *in-vitro* and *in-planta* conditions. Mutagenesis of one of the genes responsible for biofilm formation in commensal *Pseudomonas* showed weaker colonization on leaves indicating the role of biofilm in leaf adaptation and protection. Insights gained from this study aid in understanding the role of biofilm forming trait in key player of the community that help in designing functionally stable SynComs for plant protection.

P 96

A rewired human probiotic prevents pathogen colonization by signal interference and antibiotic production

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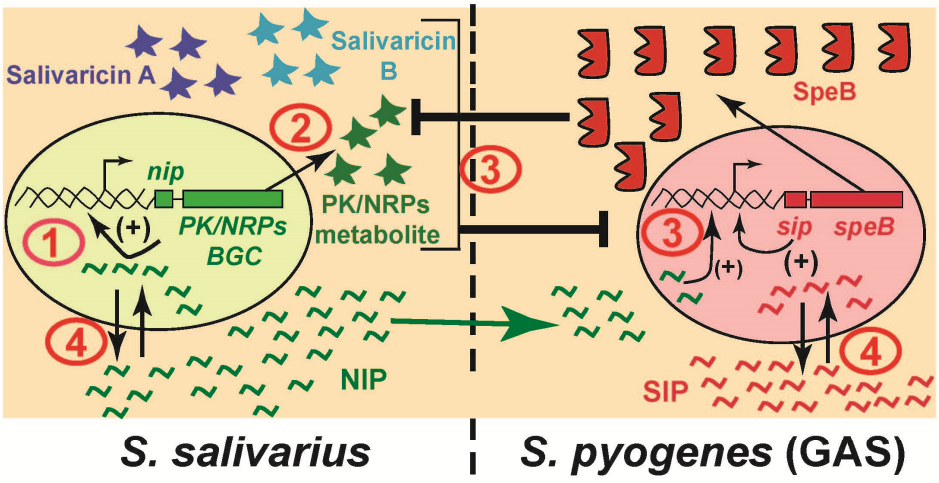
Probiotic dietary supplements are suggested to promote human health by preventing pathogen colonization. However, the mechanistic basis for their health benefits, interference with pathogen colonization, and prophylactic efficacy ***in vivo*** remain poorly characterized. Here we report that a human oral probiotic ***Streptococcus salivarius*** K12 (SAL) produces a previously unknown antibiotic with a novel chemical scaffold, salivabactin. We further show that salivabactin is effective in inhibiting human pathogen ***S. pyogenes*** (GAS) ***in vitro*** and ***in vivo***. However, contrary to the expected inhibitory effect, prophylactic dosing with antimicrobial producing probiotic SAL enhanced GAS colonization ***ex vivo*** in human saliva and in multiple mouse models of infection. We further uncover that GAS employs an innovative strategy by hijacking a SAL intercellular peptide signal that controls salivabactin production to activate a secreted protease production and enhance its survival. The secreted protease degrades SAL-derived antimicrobials and confers survival advantage to the pathogen during dual species growth ***ex vivo*** and ***in vivo***. Based on this knowledge, we re-engineered the probiotic bacterium to disarm GAS defenses by inactivating the intercellular peptide siganal and potentiate SAL antimicrobials by coupling salivabactin BGC expression with a high level constitutive promoter. We further show that the engineered probiotic has superior efficacy in preventing GAS colonization ***in vivo***. Collectively, our findings identify novel antibiotic- and probiotic-based strategies to combat human infections. Importantly, the reengineered SAL made the widely used human probiotic more effective in preventing streptococcal infections.

Fig. 1



P 97

The two-component system ArlRS is essential for β1,4-N-acetylglucosamine glycosylation of *Staphylococcus aureus* wall teichoic acid

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Introduction *Staphylococcus aureus* is an important opportunistic pathogen and among the leading causes of hospital-acquired infections. Wall teichoic acids (WTA) are critical components of the *S. aureus* cell wall. Approximately one-third of *S. aureus* isolates express WTA molecules that are decorated with α1,4- and β1,4-N-acetylglucosamine (GlcNAc) by the glycosyltransferases TarM and TarS, respectively. These different glycoforms of *S. aureus* influence a range of biological traits, such as innate sensing by skin Langerhans cells, antibody recognition and β-lactam resistance.

Two-component systems (TCSs) allow the sensing of specific external triggers by the sensor kinase and downstream activation of the response regulator, which alters expression of specific genes. *S. aureus* can adapt its WTA GlcNAc phenotype upon response to differently environmental conditions, such as a shift to higher salt conditions. However, the underlying regulatory processes are currently unknown.

Objective We screened the Nebraska Transposon Mutant Library (NTML) to determine the regulatory mechanisms underlying differential expression of GlcNAc phenotypes.

Material & Methods The NTML contains 1,920 arrayed mutants and to detect the presence of α1,4- and β1,4-GlcNAc we used monoclonal fragment antigen-binding (fab) clones 4461 (α-GlcNAc specific) and 4497 (β-GlcNAc specific). Fab binding and intensity of the transposon mutants were analyzed by colony blotting and using mutants *S. aureus* Δ*tarM* and Δ*tarS*, and the double mutant Δ*tarMS* as positive and negative controls, respectively. Visualization through an alkaline phosphatase linked secondary fab correlated to the amount of α- or β-GlcNAc present on the WTA. Validation of relevant hits were analyzed in flow cytometry using 4461 and 4497 clones and an Alexa Fluor 647 linked secondary fab.

Results The library screen identified 150 potential hits for regulation of *tarM* and 128 potential hits for regulation of *tarS*. Among the identified genes, the TCSs ArlRS and AgrCA were found to be involved in the regulation of WTA glycosylation. Further analysis of a deletion mutant of *arlRS* in a MW2 background showed complete lack of β1,4-GlcNAc decoration, whereas complementation with an *arlRS*-expressing plasmid restored these levels to wild type level. In addition, β1,4-GlcNAc levels were restored to wild type in a strain lacking all 15 non-essential TCSs when transformed with an *arlRS*-expressing plasmid. Analysis of *S. aureus* in high salt conditions confirmed the switch in WTA glycosylation from α1,4-GlcNAc to β1,4-GlcNAc compared to growth in regular medium. Salt-induced regulation of WTA glycosylation was abrogated in the *arlRS* mutant.

Conclusion These data suggest that the two-component system ArlRS is essential for β1,4-GlcNAc glycosylation of *S. aureus* WTAs, which is normally triggered in high salt environments.

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Studying low-biomass microbiome in tumors and tissues

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Tumor microbiome, microorganism identified in tumor tissue, has great potential in treatment of tumors because they play a role in the formation of tumor microenvironment. Characterization of tumor microbiome would not only help in better understanding of clinical features of tumor, tumorigenesis and progression but also treatment of tumors. However, tumor microbiome remains challenging to investigate due to its low biomass. In addition, contamination of tumor microbiome from confounding bacterial DNA found in DNA extraction kits and environment can masks or alter the microbiota signal that may lead to erroneous results. Therefore, an extensive and reliable method for DNA extraction of tumor microbiome needs to be established. Our study aims to characterize the microbial community of tumor microbiome. We also aim to use tumor microbiome to identify important biomarker and regulatory factor of cancer progression. Our study will gain more insights into the role of tumor microbiome in cancer development. In addition, better understating of tumor microbiome as indicator pathological types drug response and prognosis involves in tumor progression. Here, we employed multiple extensive methods to characterize tumor microbiome given the technical challenge due to its low biomass. Total genomic DNA from mice and human tissues were extracted using different commercial DNA extraction kits and reagent. We used negative controls and positive controls to detect contamination in the process of DNA extraction. We used real-time quantitative PCR (qPCR) to quantify bacterial DNA. Genomic DNA were then used for library construction and sent for shotgun metagenome and 16S rRNA gene sequencing. Overall, it is essential to establish a reliable method to characterize microbial community of tumor microbiome for clinical outcomes.

P 99

From niche to niche: Investigating microbial communities and their specialised metabolite gene clusters in human microbiomes

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The human microbiomes comprise a variety of microorganisms, encompassing bacteria, archaea, fungi, protozoa, and viruses, all of which contribute to complex interactions between microbes, and between microbes and their host. Understanding the biological activities of microbiota natural products (Nps) is paramount, given their pivotal roles in functions like cell signaling, defense mechanisms, and the evolution of microbial communities.

This study aimed to explore the significance of commensal diversity within the human body and its impact on social communication among different microbiomes. It also aimed to identify popular taxa and present the repertoire of biosynthesis gene clusters (BGCs). Through a comprehensive analysis of 1191 metagenomic samples from seven human microbiomes, we identified over 25,668 BGCs belonging to diverse classes of metabolites.

Alpha and Beta diversities varied across the niches, with each habitat being associated with distinct bacterial communities. The BGC families were indicative of ecology at each site. For instance, certain BGCs like colibactin, ruminococcin A, colicin V, aerobactin, and staphyloferrin A were enriched in the Large intestine, while others like macedocin, streptococcin A-FF22, and suicin 65 were found in the Oral-cavity. Pyrrolnitrin, gallidermin, and cutimycin BGCs were observed on the skin surface, and ε-Poly-L-lysine, staphyloferrin A, and staphyloferrin B BGCs were enriched in the Nasal cavity. Aryl polyene and lacticin 3147A1 were prevalent in the Tonsil, and enterobactin, aryl polyene, resorcinol, and kolossin BGCs were found in the Pharynx microbiome. Enterobactin, aryl polyene, and frederiksenibactin BGCs were abundant in the Biliary-tract.

Remarkably, around 97.5% of the total BGCs showed no similarity to known clusters in the MIBiG repository, indicating a vast potential for discovering novel functions within the human microbiome. Further research is necessary to elucidate the molecular mechanisms of communication between the microbiome and the human host, discern the impact of these microorganisms on human health, and potentially identify new therapeutic targets.

P 100

Establishing workflows for isolation and identification of tissue bacteria in order to build a cancer microbiome biobank.

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The gut microbiome has been implicated in human health and diseases. Increasing evidence has suggested that intestinal anaerobic bacteria, notably *Fusobacterium nucleatum*, plays an important role in colorectal cancer. However, the specific mechanisms between anaerobic bacteria and tumor development and metastasis remain unknown. Isolating anaerobic bacteria from tumors can help to better understand their role in cancer. Here, we aim to establish a workflow for effectively isolation and characterization of anaerobic bacteria from murine and human tissues including colorectal cancers. This workflow requires several steps ranging from tissue processing, culturing to species characterization. In this process, we analyzed various tissue homogenization methods (e.g., enzymatic or mechanical disruptions or combinations thereof). We used several different nonselective or selective broths and agars to culture different bacterial species in an anaerobic chamber. We characterized tissue-derived bacteria according to their morphology and sent single bacterial colonies to Sanger sequencing to confirm the exact bacterial species. Our preliminary result showed that using grinder homogenization combined with enzymatic digestion can achieve a diverse yield of anaerobic bacteria from tissue samples. In summary, several steps with QC are necessary to accurately and efficiently isolate bacterial species from tumor tissues, which is essential for establishing a cancer microbe biobank to allow future mechanistic experiments on microbe – cancer interactions.

P 102

Molecular basis of methanogen-virus interactions

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Methanogens are a group of archaea that produce methane gas as a byproduct of their metabolism. Like other archaea, they can be infected by viruses, and there is growing evidence to suggest that viral infection plays an important role in regulating their population dynamics and gene transfer. Despite their scientific importance, methanogenic viruses are still poorly understood compared to viruses that infect bacteria and eukaryotes, especially their infection cycles and host-range specificity. Therefore, further research is needed to understand better the factors that contribute to the host-range specificity of methanogenic viruses and to explore their potential biotechnological applications. We aim to understand the resistance of *Methanothermobacter thermautotrophicus* DH *(M.t.)* to virus ΨM2, in spite of its infectivity to the closely related *Methanothermobacter marburgensis* Marburg *(M.m.)*. *In-silico* analyses were performed to understand potential mechanisms for host-range specificity. Differences in the CRISPR loci in *M.t.* and *M.m.* suggest that *M.t.* has a stronger antiviral defense. For example, we found 7 spacers in the largest CRISPR array in the *M.t.* genome that align to the ΨM2 genome. We further follow the hypothesis that packaging multimers of the cryptic plasmid pME2001 from *M.m.* in the ΨM2 viral particles has an impact on host-range specificity. Wet-lab experiments, including interference assays with ΨM2 genome sequences based on our genetic tools for *M.t.*, as well as infection assays with plasmid-cured *M.m.* strains will provide insight into the hypotheses about host specificity and a potential function of the cryptic plasmid pME2001.

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Secretory IgA impacts the microbiota density in the human nose

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Introduction: Respiratory mucosal host defense relies on production of secretory IgA (sIgA) antibodies. sIgA is critical to protect from infection and maintain homeostasis of the microbiome, which may host opportunistic pathogens and affects the predisposition to respiratory disorders. However, we currently lack fundamental understanding of how sIgA is induced by contact with microbes and how such immune responses vary between individuals.

Objectives: We aimed to identify which human nasal microbiota members are targeted by the hosts" own nasal sIgA antibodies, and determine whether sIgA abundance and specificity affects the microbiota density and composition.

Materials & methods: We applied IgA-seq analysis to human nasal microbiota samples from healthy individuals to identify which bacterial genera and species are targeted by sIgA on the level of the individual host. Furthermore, we used nasal sIgA from the same individuals in sIgA deposition experiments to validate the IgA-seq outcomes.

Results: We observed that the abundance of sIgA secreted into the nasal mucosa by the host varied substantially and was negatively correlated with the bacterial density, suggesting that nasal sIgA limits the overall bacterial capacity to colonize. The interaction between mucosal sIgA antibodies and the nasal microbiota was highly individual with no consistent differences between potentially invasive and non-invasive bacterial species. Instead, through hierarchical cluster analysis, we identified clusters of hosts that produce an sIgA repertoire that broadly covers their nasal microbiota, whereas other hosts are generally poorly reactive to their nasal microbiota or display varying levels of sIgA reactivity to the different members. Importantly, we show that for the clinically relevant opportunistic pathogen and frequent nasal resident *Staphylococcus aureus*, sIgA reactivity was in part the result of epitope-independent interaction of sIgA with the antibody-binding protein SpA through binding of sIgA Fab regions.

Conclusion: This study offers a first comprehensive insight into the targeting of the nasal microbiota by sIgA antibodies. It thereby helps to better understand the shaping and homeostasis of the nasal microbiome by the host and may guide the development of effective mucosal vaccines against bacterial pathogens.

P 104

PUL-dependent cross-feeding mechanisms between the gut commensal *Bacteroides thetaiotaomicron* and the enteric pathogen *Salmonella* Typhimurium

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Introduction: Commensal bacteria residing inside the human intestine provide colonization resistance against invading pathogens, primarily through nutrient competition and the production of bacterial metabolites. However, emerging evidence indicates that the indigenous microbiota may also provide nutritional resources to enteric pathogens, thereby creating a metabolic niche and promoting pathogen outgrowth. Polysaccharide utilization loci (PULs) enable *Bacteroides thetaiotaomicron* (*B. theta*), a prevalent member of the human intestine, to degrade a variety of dietary- and host-derived polysaccharides, eventually releasing monosaccharides as by-products to the intestinal lumen. It is hypothesized, that these liberated substrates serve as nutrient source for pathogens, enabling their persistence in the colon. This further suggests polysaccharide utilization by *B. theta* to actively influence pathogen invasion, which represents a potential therapeutic target to combat enteric infections.

Objectives: Here, we explore metabolic cross-feeding mechanisms between the commensal gut bacterium *B. theta* and the enteropathogenic model organism *Salmonella enterica* servovar Typhimurium (*S.* Tm). Specifically, we aim to identify PULs present in the genome of *B. theta*, which play a crucial role in providing nutrients to *S.* Tm. By modulating the activity of those PULs, we aim to indirectly affect the fitness and virulence of *S.* Tm.

Methods: We monitored the *in vitro* growth of *B. theta* in a defined medium supplemented with a panel of dietary and host-derived polysaccharides. We applied transcriptome analysis to identify genes involved in the enzymatic breakdown of these polysaccharides. Additionally, we assessed the capability of *S.* Tm to grow in the same polysaccharide panel and conducted spent media assays to investigate the effect of *B. theta*-derived metabolites on the growth of *S.* Tm.

Results: Overall, we identified 17 distinct polysaccharides from the group of pectins, starches, fructans, and host-derived glycans to permit growth of *B. theta* under *in vitro* conditions. Subsequent transcriptome analysis allowed the characterization of gene expression profiles and revealed specific PULs to be induced during growth in single polysaccharides. In contrast to *B. theta*, *S.* Tm was unable to thrive on most of the dietary polysaccharides as sole carbon source. Strikingly however, the pre-degradation of these polysaccharides by *B. theta* resulted in significantly increased pathogen outgrowth.

Conclusion: Our data suggest a metabolic interplay between *B. theta* and *S.* Tm, where *B. theta* facilitates the utilization of dietary-derived carbon sources by *S.* Tm. LC-MS-based metabolomics will further enable us to identify specific PUL-derived metabolites that constitute substrates for *S.* Tm. Furthermore, genetic modification will allow us to pinpoint PULs responsible for cross-feeding and the effect of PUL gene deletion will be evaluated in both *in vitro* and *in vivo* conditions.

P 105

Determining the ecological roles of *Methanobrevibacter smithii* adhesins-like-proteins (ALPs) in the gut

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Methanobrevibacter smithii is the dominant archaeon in human gut and promotes bacterial fermentation by consuming hydrogen, a major fermentation waste product. The aggregation of *M. smithii* with fermentative bacteria enhances the availability of hydrogen, yet the underlying mechanisms governing this aggregation process remain elusive. Bioinformatic analysis based on homology to bacterial adhesins annotated a high presence of adhesin-like proteins (ALPs) in the *Methanobrevibacter* pan-genome, potentially involved in cell-cell aggregation. However, to date, no experimental characterization of these ALPs has been conducted for *M. smithii*. Our study aims to address this knowledge gap by experimentally identifying the *M. smithii* protein adhesins responsible for mediating cell-cell interactions, thereby validating the adhesive function of annotated ALPs using phage display and binding assays. To find the adhesive protein with phage display, An M13 phage library has been constructed using *M. smithii* genome fragments, and multiple rounds of affinity screening with various bacterial baits have been conducted to discover the adhesive proteins. Genes enriched due to their binding to the baits were identified using Illumina sequencing. Up to date, the preliminary results suggested several annotated ALPs being enriched during the selection against *Christensenella spp.* Additionally, we are conducting binding assays of M. smithii against its bacterial counterparts, followed by cross-linking proteomics to detect the proteins involved in the binding process. In the future, we will further characterize the expression of ALPs and other genes in order to determine the conditions that mediate ALPs production and aggregation, utilizing multi-omics to access the regulatory profile of ALPs in varying coculture combinations within a bioreactor system.

The outcomes of this research will enhance our comprehension of how the dominant methanogen, *M. smithii*, directly mediates metabolic cooperation with bacterial fermenters within the human gut. The insights gained from this study may have broader implications for understanding gut microbiota interactions and their impact on host health.

P 106

High-throughput analysis of primary metabolites with mass spectrometry-based metabolomics

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The study of the composition of the cellular metabolome is an important task in various biological fields. For this purpose, we have developed two complementary metabolomics methods to measure hundreds of samples within a few days.

The first one, a targeted liquid chromatography-tandem mass spectrometry (LC-MSMS) method on a triple quadrupole can be used to measure and relatively quantify around 120 metabolites within less than 20 minutes per samples. The isotope ratio approach ensures a high confidence of the data by correcting for matrix effects and ion suppression and relative quantification. Besides common metabolites, like amino acids and nucleotides, we can measure several intermediates of the purine and MEP pathway for the first time.

In our second method we use flow injection mass spectrometry (FI-MS) for high throughput measurements on a high-resolution time-of-flight mass spectrometer. Metabolites are discriminated on a MS1 level by their mass-to-charge ratio. Due to the lack of a chromatographic separation the analysis time is very fast (1 min per sample). For annotation we have implemented different databases (e.g. *E.coli*, Microbiome Metabolite database, human metabolome database).

In the last years we gained experience in the measurement of different sample types, e.g. different bacterial species, (*Escherichia coli*, *Bacillus subtilis*, *Helicobacter pylori*, *Staphylococcus aureus*, *Burkholderia anthina*, *Amycolatopsis balhimycina*, cyanobacteria), but also human serum, plasma and tissue.

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Changes in oral and fecal microbiome of patients with pancreatic ductal adenocarcinoma undergoing chemotherapy and their correlations with clinical outcomes.

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a low survival rate. In the last decade, it has been shown that the gut microbiome plays a critical role in cancer development.

Objectives: Study the oral and gut microbiome from PDAC patients to identify and characterize species, genes, and metabolic pathways changes throughout the course of chemotherapy and investigate whether the composition of the microbiome over time can predict the response to therapy and development of toxicity.

Methods: One hundred and thirty-one patients diagnosed with PDAC from 4 different centers (University Clinic Tübingen, NCT Heidelberg, Westdeutsches Tumorzentrum at University Hospital Essen and Klinikum rechts der Isar, Techn. Univ. Munich) were included in this study. Saliva and fecal samples were collected before they receive their first chemotherapy infusion, and every 4 weeks thereafter until 9 months following the initiation of the oncological therapy. For microbiome analysis we are going to use 16S ribosomal RNA (16S rRNA) gene sequence and shotgun metagenome sequence. Clinical data, such as age, gender, diagnosis, performance status, histopathological and molecular tumor features, concurrent medication, and the type of chemotherapy regimen were also collected. Quality of life was assessed by SF-36 questionnaires, and the development of toxicities, infections, and response to chemotherapy were monitored through routine clinical labs, scans, and assessments.

Results: After conducting initial 16S rRNA gene sequencing of a set of fecal specimens (n = 34), we found that there was a gradual decrease in alpha diversity throughout the course of chemotherapy. Additionally, the composition of the gut microbiome was found to be impacted by the type of chemotherapy regimen given to the patients, leading to changes in the relative abundances of certain gut commensals, such as *Faecalibacterium* or *Bacteroides.*

Conclusions: After preliminary analyses, the results allow us to conclude that the diversity of the intestinal microbiota was affected by chemotherapy over time and that the type of chemotherapy directly impacts the decrease of certain populations of intestinal bacteria. Our next steps in this study will involve the investigation of microbial mechanisms in response vs. resistance to chemotherapy in PDAC, and the use of gnotobiotic mouse models to explore mechanistic analyses.

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Characterisation of the genetic determinants for *R. hominis* flagellin-host interactions

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Roseburia hominis is an abundant constituent of the human gut microbiome and a member of the *Lachnospiraceae* bacterial family. Its ability to produce short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate has been associated with the modulation of gut microbial ecology and host energy homeostasis. Significantly, we have recently shown that *R. hominis* produces "silent" flagellins that can bind to host toll-like receptor 5 (TLR5) without initiating a pro-inflammatory response. This suggests that these organisms can actively modulate their interaction with the host immune system, challenging our current understanding of flagellin-TLR5 interactions. Little is known about this flagellin type, or about the purpose of the four separate flagellins encoded in its genome; without a genetic system, it is unclear how much we can fully elucidate about role flagellin expression and production plays for the bacterium, or its host. In response, we developed a genetic system for *R. hominis* to unravel the specific molecular mechanisms driving these flagellin-host interactions. We showed that *R. hominis* has four restriction-modification systems and characterised the methyltransferases and their subunits responsible for differentiating self and non-self DNA. Next, we constructed a series of *E. coli-Lachnospiraceae* shuttle vectors and developed a strategy for both *in vivo* plasmid methylation according to *R. hominis*-specific nucleotide motifs and DNA transfer. We will then construct knock-out vectors to sequentially remove each flagellin gene from the chromosome and will characterise mutant impact on *R. hominis in vitro* growth kinetics, substrate utilisation, and motility.

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Studying the role of the microbiome in carcinogenesis of pancreatic cancer

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Pancreatic ductal adenocarcinoma (PDAC) is a highly lethal disease, characterized by poor prognosis and limited treatment options. This is because of late diagnosis, therapeutic resistance, high metastatic potential and rapid progression. Recent studies propose a potential interplay between the microbiome and PDAC. For instance, alterations of the microbiome are suggested to influence cancer development, progression as well as efficacy and toxicity of chemotherapy. Nonetheless, significant knowledge gaps persist regarding the specific roles of individual bacteria in the gut lumen, but also within the tumor. With our work we want to expand the knowledge on the contribution of the microbiome to PDAC development, to propose microbiome-based treatment strategies, ultimately enabling improved clinical outcomes of PDAC patients. To address these questions, we plan to carry out animal experiments to investigate whether antibiotic- or diet- induced microbiome modulations affect PDAC development and the immunological tumor environment by utilizing mouse orthotopic tumor implantation models. To analyse the treatment impact on stool, intestinal and tumor tissue microbiome composition as well as bacterial translocation, we are establishing a 16S rRNA gene sequencing pipeline. In addition, an In-Situ-Hybridization method is implemented for visualization of bacteria in tissue sections. For analysis of the tumor microenvironment, fluorescence-activated cell sorting (FACS) will be realized. In synopsis, with this project we aim to contribute to the understanding of the role of the microbiome in carcinogenesis in PDAC and contribute to new treatment approaches due to suggestions for microbiome manipulations.

P 110

Characterizing environmental adaption and virulence of *Enterococcus spp.* isolated from immunocompromised patients

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The gut microbiome is a major modulator of the host"s homeostasis, notably adaptive and innate immunity. Several clinical studies were able to show an association between gut microbiome dysbiosis and poor clinical outcomes of immunocompromised patients, e.g., allo-HCT patients with lower progression-free survival, lower overall survival, increased transplant-related and lethal GvHD. A hallmark of dysbiosis was identified in the intestinal expansion of facultative anaerobes such as *Enterococcus spp*.. Multiple experimental studies also found associations between the presence of specific carbohydrates (i.e. fructose or lactose) and the expansion of enterococci. Therefore, diet is also suspected to take part in aforementioned clinical observations.

In our work we aimed to isolate *Enterococcus* spp. from stool specimen of patients undergoing T-cell transfer therapies. These enterococci are then tested for their antibiotic resistance profiles, immunogenicity and biofilm-formation capacity. Nutrient-dependent growth-rates of *Enterococcus* spp. are also being assessed under different nutritional milieus. The patient cohort consists of 60 patients undergoing CAR-T cell therapies against lymphoma or leukemia recruited in two different centers in Germany (Munich & Heidelberg). In total 240 stool samples were longitudinally collected during therapy, including pre- and post-therapeutic timepoints, and are used for *Enterococcus* isolation. The study design also includes the collection of a huge array of clinical data including dietary data. *Enterococcus* spp. were isolated on *Enterococcus*-specific agar. Antibiotic resistances were assessed in vitro through disc diffusion tests and sequencing. To determine immunogenicity, we stimulated PBMCs from healthy donors with individual *Enterococcus* protein-lysates and recorded the cytokine-response through an immune-assay. Biofilm-formation capabilities were tested quantitively through a microplate assay including crystal violet staining. To assess the nutrient-dependent growth of the patient derived enterococci we cultured in a minimal medium while adding different carbohydrates and assessed growth rates over 48h. In preliminary analyses, we observed mainly *E. faecium* and *E. faecalis* in patient stools with predominant Ampicillin and Ciprofloxacin resistances. Enterococci can induce pro-inflammatory cytokine releases (e.g., IL6), but with considerable variance in immunogenicity. This variance is also observed on the level of carbohydrate-dependent growth-rates.

The scientific relevance of this project lies in revealing mechanisms that could explain the clinically overserved relation of *Enterococcus*-driven gut dysbiosis and poor clinical outcomes in T-cell therapies. Additionally, it will hopefully improve therapeutic regimes and minimize adverse events and outcomes.

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Updating microbial genetics tools for their application to the human gut microbiome

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The human gut microbiome consists of trillions of organisms from all three domains of life. This complex and interconnected system is driven by the biological interactions between its constituents (host-microbe, microbe-microbe) and plays a major role in human health and development. However, we know very little about the specific mechanisms driving these interactions. The application of microbial genetics to the gut microbiome represents a powerful opportunity to tease out the functional basis for community dynamics, yet the majority of gut-associated microbes are genetically intractable. This is primarily due to an incompatibility between traditional genetic tools (developed for model organisms like *E. coli*) and the phylogenetically diverse non-model organisms associated with the human gut. In response, we have developed a conjugation donor optimized for compatibility with diverse gut organisms and the anaerobic conditions in which they are cultured. The donor incorporates strategies for microbial defence system inactivation, a kill-switch for counter-selection following DNA transfer, and a conjugation system that can be "primed" for diverse recipients. This represents the first microbiome-specific genetic tool in this emerging era of microbiome genetics and engineering and will potentially enable a deeper and more mechanistic understanding of human gut microbiome interactions.

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Methanogen diversity and evolutionary dynamics across global human populations

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Methanogens, the most abundant archaea in the human gut, play a crucial role in hydrogen consumption derived from carbohydrate fermentation. Despite their association with various health conditions, the specific impact of methanogens on human health remains poorly understood. Existing studies on methanogen diversity in the gut have predominantly focused on Western populations, leaving a knowledge gap concerning less-represented human populations in regions such as Africa and Southeast Asia. Recent reports indicate the potential existence of a new species within the Methanobrevibacter genus, named *Candidatus Methanobrevibacter intestini*, but only one study has successfully isolated this proposed species from human feces.

In this study, I hypothesize that Methanobrevibacter species exhibit widespread distribution across diverse human populations, with the ability to develop distinct niche adaptation strategies based on available growth resources. To test this hypothesis, I collected human stool samples from three geographically distinct populations in Africa, Europe, and Asia, and subsequently isolated Methanobrevibacter species/strains. Using these isolates, I conducted a comprehensive assessment of genotype and phenotype differences among methanogens from the three populations. Moreover, I analyzed and characterized the pan-genomes of these isolates, identifying several gene loss and gain events, horizontal gene transfer occurrences, and potential morphological differences between *M. smithii* and *Candidatus M. intestini*.

Remarkably, this study successfully isolated and characterized both *M. smithii* and *Candidatus M. intestini* in populations from Europe, Asia, and Africa. Furthermore, the observed differences in pan-genomes and gene content underscore the evolutionary dynamics of these methanogens and their adaptation to distinct host environments. Additionally, the investigation into the co-diversification of *M. smithii* with its human host suggests a parallel evolutionary history between the two.

In conclusion, this research provides valuable insights into the evolutionary dynamics and adaptive strategies of methanogens across diverse human populations. Furthermore, the findings support the designation of *Candidatus M. intestini* as a new species and shed light on the complex interactions between methanogens and their human hosts in the context of human health.

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MMonitor: Tracking metagenomes using long read nanopore sequencing

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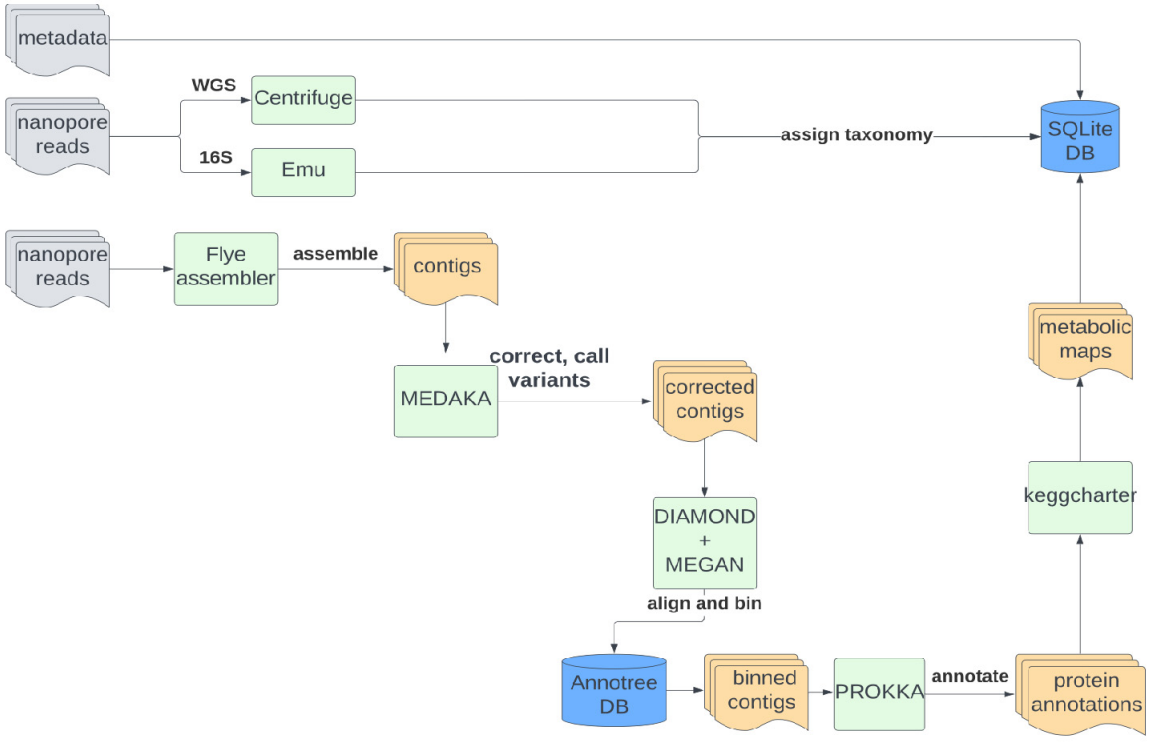
Nanopore sequencing can be used to track microbes in real-time. Its ability to swiftly generate high-quality de novo assemblies makes it especially useful for metagenomics. However, for those lacking a computational biology background, the selection and usage of appropriate software tools pose a challenge. We've developed MMonitor, an integrated software solution, combining bioinformatics pipelines with a visualization platform. It was initially designed for bioreactor monitoring, but can be used for all nanopore metagenome sequencing data. MMonitor allows scientists to conduct efficient metagenome analyses, such as taxonomic and functional gene analysis, with a user-friendly GUI or a command line interface. The results, viewable both locally or remotely via a web server, make it accessible for data exploration and metagenome tracking.

Fig. 1



P 114

The defence arsenal of *Methanobrevibacter smithii*

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One of the current goals of human gut microbiome research is to develop genetic systems for non-model organisms found in the human gastrointestinal tract to obtain a better understanding of community dynamics and function. Currently, prokaryotic defence systems represent one of the most significant barriers to the establishment such genetic systems for intractable microbes. The majority of these defence systems employ various strategies to recognise and eliminate foreign DNA, whether derived from phages/viruses, plasmids, or other mobile genetic elements. One such system is the **B**acte**r**iophage **Ex**clusion (BREX) system, which methylates the microbe"s own DNA at a non-palindromic 6 bp sequence and prevents viral replication via an undetermined mechanism. In addition to viral defense, BREX has also been shown to negatively impact plasmid uptake efficiency. We identified a novel BREX anti-defence systems in the major archaeon associated with the human gut, *Methanobrevibacter smithii* and have characterised its *in vitro* role as an anti-viral and anti-plasmid system. We show that the *M. smithii* type strain contains an atypical type I BREX system that methylates the chromosome at two distinct nucleotide motifs. This methylation activity is carried out by the pglX methyltransferase, but its activity is dependent on BREX genes pglX, brxA, brxB, brxC, and pglZ. Additionally, we report the role of *M. smithii* BREX in modulating viral infection in an *E. coli* host and its effect on plasmid transformation. This represents the first characterisation of a BREX system in an archaeal host and paves the way for future genetic manipulation of this non-model organism.

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Lipase-mediated detoxification of host-derived antimicrobial fatty acids by *Staphylococcus aureus*

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Skin lipids represent a formidable physical and chemical barrier against opportunistic pathogens like *Staphylococcus aureus*. Specifically, long-chain fatty acids are also referred to as antimicrobial fatty acids (AFAs) because of their ability to inhibit and/or kill bacteria. *S. aureus* has developed an armamentarium of resistance strategies against AFAs, which are not yet fully understood and may hold keys to new therapeutic approaches to prevent pathogen colonization. Here, we identify the *S. aureus* lipase Lip2 as a novel resistance factor against AFAs. Lip2 detoxifies AFAs via esterification with hydroxylated molecules such as cholesterol and fatty alcohol. This is reminiscent of the activity of the fatty acid-modifying enzyme (FAME), whose identity has remained elusive for over three decades. *In vitro*, Lip2-dependent AFA-detoxification was apparent during planktonic growth and biofilm formation. A lipase-deficient *S. aureus* mutant could be complemented with lipases from coagulase-negative staphylococci. Moreover, our genomic analysis revealed that prophage-mediated inactivation of Lip2 was more common in blood and nose isolates than in skin strains, suggesting a particularly important role of Lip2 for skin colonization. Accordingly, in a mouse model of *S. aureus* skin colonization, bacteria were protected from the human-specific AFA, sapienic acid, in a cholesterol- and lipase-dependent manner. Collectively, these results suggest that (i) Lip2 is the long-sought FAME of *S. aureus*, (ii) lipase-mediated FAME activity is common in coagulase-negative staphylococci, and (iii) lipases exquisitely manipulate environmental lipids to promote bacterial growth while changing the host lipid landscape. Considering their ability to both degrade lipids and detoxify AFAs, staphylococcal lipases may play an underappreciated role at the host-microbe interface with potential implications for the microbiome composition.

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Using transposon directed site insertion sequencing to identify genes involved in interspecies competition of *Pseudomonas aeruginosa*

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Introduction

Pseudomonas aeruginosa (PA) is a Gram-negative opportunistic pathogen that can cause a wide range of infections. It is the most common bacterium involved in lung infections in cyctic fibrosis (CF) patients[1]. The *Burkholderia cepacia* complex (Bcc) is a large cluster of *Burkholderia* species that are sorted into nine distinct genomovars based on genetic relatedness[2]. Like PA, Bcc species can cause lung infections in CF patients, but much more rarely[1]. Most of those infections are associated with reduced long-term survival of those patients, in some cases through rapidly progressing pneumonia and sepsis in the so-called "cepacia syndrome"[3]. A recent study suggests that host adaptation of PA in the CF lung over time leads to mutations that render it susceptible to attacks from Bcc species via the Type VI secretion system (T6SS)[4].

Aim

In this ongoing project, we want to employ a transposon insertion sequencing (TIS) assay in order to detect other genes besides the already described T6SS that allow PA to survive in co-culture with Bcc strains or outcompete them, be it contact-dependent or -independent.

Methods

We established an interspecies competition assay based on FACS analysis of a fluorescently labelled clinical PA isolate and Bcc strains (*B. cepacia* or *B. cenocepacia*) before and after co-incubation on solid media. A transposon insertion screening (TIS) assay will be employed to identify the prinicipal armament of PA required to withstand the attacks of Bcc species during growth on solid surfaces, and first results will be discussed.

Results and Discussion

So far, we could confirm Tse5, a pore-forming T6SS-dependent toxin, as an important player in PA survival in competition against Bcc species. First, incomplete results from the TIS assay suggest an important role of the GacS-GacA two-component system, the cognate immunity gene against Tse5 (*tsi5*) or general stress response factors. After obtaining the complete data set, we will employ our FACS-based competition assay and genomic KO mutants to confirm these preliminary results.

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Extracellular DNA plays a critically role in *Staphylococcus epidermidis* escape from human macrophages

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Background

Over the past decades, the human skin colonizing bacterium Staphylococcus epidermidis has been documented as a major cause of hospital-acquired infections. Despite S. epidermidis lacks dedicated programmes/factors for virulence and invasion it is the leading causative pathogen in central line associated blood stream infections and prosthetic joint infections, causing approximately 30% of these infections. Biofilm formation has been shown to be of key importance for immune evasion in this context.

Objectives

The main objectives of this study was to characterize the importance of specific biofilm matrix components for interactions between sessile S. epidermidis populations and primary human macrophages, and to unravel mechanisms supporting S. epidermidis evasion from phagocytic uptake.

Methods

Primary human macrophages isolated from buffy coats were infected with different S. epidermidis strains. Phagocytosis rates, macrophage polarization and TLR involvement were analyzed by confocal laser scanning microscopy, FACS and qPCR analysis. Furthermore transcription and protein levels of Il-1ß, TNF-a, Il-6 and Il-10 were assessed and a RNAseq experiment was carried out.

Results

The ability of professional phagocytes to uptake sessile S. epidermidis consortia is modulated by the biofilm matrix composition, with PIA and eDNA as key players. It could be shown that eDNA present in S. epidermidis biofilms can alter the macrophage polarization and pro- inflammatory activation via TLR9 recognition. After S. epidermidis infection TLR-mediated DNA sensing is critical for macrophage reprogramming and specific upregulation of anti-viral immune pathways.

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Microbiome Metabolome Integration Platform (MMIP): a web-based platform for microbiome and metabolome data integration and feature identification

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A microbial community maintains its ecological dynamics via metabolite crosstalk. Hence knowledge of the metabolome, alongside its populace, would help us understand the functionality of a community and also predict how it will change in atypical conditions. Methods that employ low-cost metagenomic sequencing data can predict the metabolic potential of a community, that is, its ability to produce or utilize specific metabolites. These, in turn, can potentially serve as markers of biochemical pathways that are associated with different communities. We developed MMIP (Microbiome Metabolome Integration Platform), a web-based analytical and predictive tool that can be used to compare the taxonomic content, diversity variation and the metabolic potential between two sets of microbial communities from targeted amplicon sequencing data. MMIP is capable of highlighting statistically significant taxonomic, enzymatic and metabolic attributes as well as learning-based features associated with one group in comparison with another. Further MMIP can predict linkages among species or groups of microbes in the community, specific enzyme profiles, compounds or metabolites associated with such a group of organisms. With MMIP, we aim to provide a user-friendly, online web-server for performing key microbiome-associated analyses of targeted amplicon sequencing data, predicting metabolite signature, and using learning-based linkage analysis, without the need for initial metabolomic analysis, and thereby helping in hypothesis generation.

Data and code availability: The web-server is available at https://github.com/websandip/mmip