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"Horizons in Metaorganism Research"

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"Microbiomes in agricultural systems and human nutrition"

Poster overview

1 Karlis Arturs Moors

Metabolic changes in the human gut microbiome due to proton-pump inhibitor and alcohol use

Proton-pump inhibitors (PPIs) are one of the most prescribed over-the-counter drugs in the world. PPIs are used to treat gastrointestinal reflux disease (GERD), heartburn, peptic ulcers, as well as to eliminate the gastric pathogen Helicobacter pylori. The use of PPIs has been linked with changes in the composition of the microbiome due to decreased gastric acid production. Usual counterindications for PPI use discourages alcohol use while taking PPIs, however, these are often disregarded. Alcohol use itself has been shown to affect the gut microbiome, however due to the lack of pharmacological interaction with PPIs, their combined effect on the microbiome has not been investigated. In this study, we employ multiple metabolic modelling and statistical approaches to investigate the functional changes that occur in the host and their microbiome, using multidimensional data from the German FoCus cohort.

2 Md Shahinur Islam

Bovine teat hygiene with disinfectants and selective effects on bacteria

Udder hygiene is the most cost-effective method to control bovine mastitis [1]. Post-milking teat disinfection is a widely practiced teat hygiene to prevent mastitis in dairy herds [2]. However, application alters the udder bacterial composition, susceptibility, and sensitivity towards the applied disinfectants and other antibiotics. Most recent studies found lactic acid altered the distribution of bovine udder bacteria. Where chlorhexidine and lactic acid reduces coagulase-negative staphylococcus (CoNS) from 64.3% to 51% and 58.6% to 39.6% but Corynebacterium spp. proportion occurred as 7.1% and 16.7%, respectively after a six day intervention [3]. Chlorhexidine reduced the sensitivity of qac-positive coagulase-negative staphylococcus to kanamycin [4]. In the ongoing study, we aim to assess the co-selection effect of teat disinfectants on the distribution and susceptibility of bacterial isolates from the bovine udder. The bacterial isolates will be tested towards chlorhexidine, lactic acid, and commonly used antibiotics in mastitis prevention and treatments. Quarter milk samples from 26 cows were collected before and after a week-long intervention following a split-udder design with chlorhexidine and lactic acid. Following National Mastitis Council Guidelines [5], 201 isolates were

subcultured and pure isolates were examined further using biochemical assays (catalase, oxidase, 3% potassium hydroxide) and Gram staining. 181 bacterial isolates of 16 bacterial genera were identified by MALDI-TOF MS on species level. The susceptibility of identified isolates is being investigated by microdilution and macrodilution for antibiotics and applied disinfectants, respectively. First results indicate that a selective effect of lactic acid towards the increased occurrence of Corynebacterium spp. occurred. Where, Corynebacterium spp. proportions were 23.7% and 32.3%, respectively.

3 Saskia Ertmann

The causality of bacterial infections in human cancer and other remote pathologies

It is widely accepted that disbalances in microbial communities contribute to an increasing number of human diseases. Yet, uncovering the foundations for such complex relationships is lagging behind. In order to shed light on the mechanistic basis of microbe-dependent pathogenesis in human tissues, our group has put focus on tangible pathogenic traits highly regulated by microbial communities; these traits may become activated under conditions of microbial disbalance. To unravel the long-term impact of microbiome imbalance and pathogenic infections and to understand the causal relationships between infections and latent human diseases, we have directed our interest into two avenues: (i) infection models clearly related to human carcinogenesis and (ii) the possible undesirable modulation of the gut-brain axis, which may result in neurodegenerative disease. Recent work from our laboratory has discerned the exceptional ability of Helicobacter pylori to chronically colonize the human gastric mucosa. We hypothesize that mutational and epigenetic changes provoked by the chronic presence of H. pylori trigger the development of intestinal metaplasia and the subsequent spontaneous clearance of this bacterium from the stomach. However, at the same time, this condition constitutes premalignancy. This casual biological phenomenon raises the question of whether the cell-autonomous antimicrobial defense could be a driver of premalignancy in mucosal tissues.

4 Mohammad Abukhalaf

We applied qualitative and quantitative LC-MS-based proteomics techniques to identify key pathways and interactions at the molecular level in multiple metaorganism systems. In plants, analysis of the wheat Triticum aestivum apoplastic proteome under infection with the fungus Zymoseptoria tritici revealed conserved and unique defence-related signatures between the susceptible (Obelisk) and the resistant (Chinese Spring) wheat cultivars and identified potential novel fungal effector proteins. In Nematostella vectensis, proteome analysis of the nematosomes of animals colonized with native and non-native vibrios demonstrated the phagosome pathway as a key player in response to non-native vibrios. In an interaction study of closely related Bacteroides of the murine intestinal microbiota, proteins with antibacterial properties, such as bacteroidetocin B, were found to be responsible for bacterial growth inhibition. These examples demonstrate the high potential of proteomics to contribute to the understanding of the molecular factors triggering and regulating interspecies interactions in metaorganisms, and their reaction on environmental factors.

5 Janna Wuelburn

Host fitness is closely correlated with a stable and species-rich microbial community. A shift towards dysbiosis might lead to the overrepresentation of certain (harmful) taxa. According to ecological theory, the structure of a population (Î±-diversity) is shaped by competition and predation from organisms of higher trophic levels. The understudied Bdellovibrio and like organisms (BALOs) are obligate predators of Gram-negative bacteria but are susceptible to ingestion by protists, thereby

representing an intermediate trophic level in microbial communities. BALOs have been shown to function as key predators, directly shaping microbial communities by driving susceptible strains to extinction and thus allowing less dominant strains to reach higher cell density. However, a direct relationship between the presence of BALOs and the increase in microbiota diversity can only be determined in controlled laboratory experiments using a suitable host, such as the nematode Caenorhabditis elegans. In this study, I am investigating the probiotic potential of B. bacteriovorus to promote microbial diversity and affect the community composition in the natural microbiota of C. elegans. Indeed, first results suggest higher Î±-diversity and microbiota community stability in the presence of BALOs compared to the control treatment, both in the nematodeâ€™s gut as well as a controlled viscous medium environment. Further, I assess the effect of BALO presence on C. elegansâ€™ life history traits. I hypothesize that the BALO-induced altered microbiota community composition may directly impact the nematode. In microbial communities treated with BALOs, I observed the enrichment of B12-producing taxa (e.g. Pseudomonas) which may benefit the nematodes. Interestingly, we found that BALO presence significantly increases the mean lifespan of C. elegans. This effect may correlate with prolonged fertility and higher offspring counts in nematodes exposed to BALOs, compared to the control group. During the course of this study, I aim to further improve our understanding of the functional consequences of microbial community dynamics in the presence of bacterial predators (BALOs) for both the microbiota and the nematode C. elegans.

6 Anna Czerwinski

Adaptation of commensal bacteria to persist in C. elegans is mediated by cyclic-di-GMP

Microbe-host associations are ubiquitous on Earth, and much work focuses on how the host is affected by the microbes. However, less is known about how commensal bacteria optimize their fitness during interaction with a host and adapt to host association, particularly at the molecular level. Pseudomonas lurida MYb11, an occasional commensal of natural C. elegans populations, was used to study bacterial adaptation to the host. An evolution experiment with P. lurida and C. elegans resulted in the emergence of host-specialist bacteria with a wrinkly colony morphology. The wrinkly morphology was caused by specific mutations in wspF and wspE of the wsp operon and in rph. The wsp operon encodes a two-component regulatory system responsible for intracellular c-di-GMP production, whereas rph encodes the ribonuclease PH, involved in t-RNA maturation. The bacterial messenger c-di-GMP is known influence biofilm formation, which promotes the persistence of pathogenic bacteria in an animal host, but its role in beneficial host-microbe interactions is less well understood. Our work now demonstrates that wrinkly bacteria with elevated intracellular c-di-GMP levels increase both host persistence and biofilm formation as part of their adaptation to a host-associated lifestyle. More specifically, I combine functional genetic analyses with transcriptomics and confocal microscopy to elucidate how exactly changes in the wsp operon and rph affect bacterial c-di-GMP levels and thereby promote bacterial association with the C. elegans host. Overall, our work contributes to an enhanced understanding of how bacteria adapt to a host organism, both at the molecular and evolutionary levels.

7 Marjan Ghotbi

Invasive metaorganism shapes its microbiome Microbiomes are important functional interfaces that play a key role in hosts physiology and ecology. The seaweed microbiome, in the form of an epiphytic multikingdom biofilm, influences the host development and acts as a second skin regulating physical and chemical defense against pathogens. Thus, seaweedsâ€™ ability to control the composition of their microbial biofilm can affect their health and resilience. To test whether the algaâ€™s potential to control its biofilm differs between native and invasive species, we experimentally studied microbial biofilms (prokaryotes and microalgae) formed on a porous proxy surface in the vicinity of three

seaweeds, including one invasive and two native species (Gracilairia vermiculophylla, Fucus serratus, Fucus vesiculosus) and compared them with mature epiphytic biofilms from the same seaweeds. Although mature biofilm on native seaweeds showed higher prokaryotic diversity, the invasive seaweed had a higher diversity in its core microbiome, i.e. ability to maintain a higher diversity of persistent microbes. Microalgae diversity exhibited no significant differences among seaweeds. Substrate type imposed a stronger force in shaping composition of microbial communities rather than algal treatments or time of exposure. At the community level, no significant compositional differences were found among biofilms on proxy surfaces adjacent to native vs. invasive holobionts and empty control bottles. However, at taxon level the highest similarity was seen between the invasive holobiont and its adjacent biofilm. We observed the highest contrast between control biofilms and invasive seaweeds adjacent biofilm. This may represent the higher capacity of invasive seaweed for control of their microbiome. Keywords : Holobiont, Microbiome, Biofilm, Seaweed

8 Jay Bathia

Bacterial quorum sensing molecule affects development of the metaorganism host

Hydra vulgaris AEP A distinct microbiome colonizes glycocalyx of the metaorganism Hydra with Curvibacter being the main colonizer. The complex inter-kingdom interactions result in host protection against fungal pathogens and influence on host behavior and development. Apart from the bacterial symbionts, temperature has a strong influence on the host development. Low temperature as well as bacterial symbionts elevate the transcript levels of taxonomically restricted peptides Eco1/2. These peptides are expressed in the foot region of Hydra and have an antagonistic effect on the Wnt pathway. These peptides can also be induced in germ-free animals on mono-colonization with Curvibacter as well as its quorum sensing molecule 3OHC12-homoserine lactone. With the help of comparative transcriptomics data and the single cell expression profiles of Hydra, we could identify the most affected cell types under various bacterial backgrounds. This analysis revealed that a number of cell types, including basal disc cells of the foot, were affected by bacteria and its signaling molecule 3OHC12. Tissue transplantation experiments revealed that germ-free animals had significantly lower foot inhibitory activity mediated by Eco peptides. Interestingly, the TCF inhibitor iCRT14 was able to rescue the foot inhibition mediated by Eco peptides, supporting the hypothesis that Eco peptides antagonize Wnt signaling at the level of TCF. Overall, our results suggest an intimate interkingdom communication via quorum-sensing molecules produced by bacteria that influence host development.

9 Ina Becker

Do ancient tir domain-containing receptors exert immune function?

Cnidaria, due to their status as the sister group to Bilateria, hold a prominent role as model organisms. Organisms experience continuous microbial exposure in their environment, including pathogens. They have evolved an immune system consisting of innate and adaptive components. Cnidarians rely exclusively on the innate immune system, which is equipped with pattern recognition receptors (PRRs), including receptors containing TIR domains. These TIR domain-containing receptors are also found in other invertebrates like sponges and in vertebrates. However, a comprehensive investigation into TIR domain-containing receptors within basal metazoa including Nematostella vectensis remains absent. This study aims to examine the ancestral function of TIR domain proteins, including the TLR. For this purpose, CRISPR/Cas9- generated knockout animals were created, targeting the sea anemone's five TIR domain receptors: NvTLR-1, IgTIR1 to IgTIR4. Knockout animals were successfully generated. Genotyping was performed using High-Resolution Melting (HRM) analysis and Sanger sequencing. The F1 generation has been established and will be expedited into the F2 generation promptly to manifest

the genetic alteration and initiate the infection experiments. Pathogen infection assays will validate their role as immune receptors. Vibrio coralliilyticus, a gram-negative bacterium known to induce coral diseases, will be chosen for the fitness assessment. Additionally, phylogenetic trees are constructed for the purpose of comparing TIR domain-containing proteins across taxonomic groups. The imminent experiments will illuminate the precise role of TIR domain proteins in the realm of innate immune responses.

10 Hanna Domin

Mechanisms controlling microbiome establishment of the starlet sea anemone Nematostella vectensis

Multicellular organisms possess a microbiota that is largely specific for their species, but the factors controlling community membership and establishment are poorly understood. Nematostella vectensis has a complex life cycle with a planula larva stage which undergoes metamorphosis before it develops into a juvenile polyp before it reaches the stage of a sexual mature adult polyp. These three life stages all exhibit a developmental-specific microbiome1. Here, we investigate the drivers of the development-specific microbiome of N. vectensis with by (1) tracking the recolonization process after transplantation of the hostâ€™s own developmental-specific microbiomes on germfree adult polyps, by (2) characterizing the hostâ€™s transcriptomic response upon recolonization, and by (3) analyzing the microbiomeâ€™s metabolic potential during recolonization. For this, we recolonized germ-free adult polyps with the ageâ€specific microbiota of all three life stages, respectively. The dynamic reconstruction of the microbiome on the host showed very similar patterns for all three inocula, with a convergence of all three inocula to the larval-specific microbiome during early recolonization. Subsequent recolonization recapitulated the colonization pattern occurring during ontogeny. The common host's transcriptomic response to the recolonization revealed a regulation of gene clusters belonging to the immune system, phagocytosis, cell regulation and migration, and to carbohydrate metabolism, especially via upregulation of chitin synthesis. The modeling of the bacterial metabolic capabilities reveals chitin degradation as the most promising mechanism controlling early recolonization, suggesting that chitin is an important driver of early recolonization. Our results indicate that while early recolonization is host-controlled, late recolonization is dependent on bacteria-bacteria interactions. We propose that the host-produced chitin is an important factor influencing the bacterial dynamics, initiating a dynamic interplay between the host and its microbiome: its production by Nematostella sets up a cross-feeding chain for the microbiome, in which early-colonizing bacteria degrade chitin, providing chitin metabolites to late-colonizing bacteria. Future experiments will investigate the cross-feeding abilities of the bacterial colonizers with just chitin as a carbon source, and the chemoattractive potential of chitin.

11 Tassneem Ebniof

The significance of nematosomes in the immune memory of Nematostella vectensis

The communication between the microbiome and the host immune system plays an essential role in the establishment of a healthy microbiome. Many invertebrate hosts that exclusively rely on their innate immune system can adapt their immune responses to the type of bacteria, demonstrating that innate immunity also has adaptive features. The so-called immune memory is a process by which a host can enhances its immune defenses after an initial contact, resulting in better protection upon a secondary exposure. Phagocytosis is one of the evolutionary conserved components of innate

immunity eliminating non- native microbes, with discrete receptors mediating the recognition of targets and activate immune signaling pathways and may participate in this immune memory. The starlet see anemone N. vectensis thrives in shallow estuarine habitats, forming beneficial symbioses with its colonizing bacteria. Previous results showed that nematosomes, multicellular, free floating bodies within the gastric cavity, can phagocytose bacteria and distinguish between native and non-native colonizer by selective phagocytosis. Therefore, we hypothesize that nematosomes play an important role in the regulation of the microbiome, in which they can distinguish native and non-native colonizers through an immune memory. Currently we are testing this hypothesis by performing phagocytosis experiments using nematosomes extracted from polyps with defined bacterial colonization status. We are comparing their phagocytic activity to test if animals that have already had contact with a certain bacteria build up a memory and subsequently increase or decrease the phagocytosis activity. Preliminary experiments already show that nematosomes phagocytose native isolates in lower amounts if the polyp was previously colonized with the same isolate. Thus, we conclude that the immune memory is a feature of the innate immune responses of early branching metazoans and plays an important part in the regulation of bacterial colonization.

12 Gabriela Maria Fuentes Reyes

Bacteria-bacteria interactions determine colonization succession in Nematostella vectensis microbiome establishment

Mostly all living organism are considered as a metaorganism, composed of a host and a diverse microbiome. Microbial communities play an important role in the development, function and fitness of the host. Thus, the host-microbe interactions have been of great importance for the host to evolve, diversify and adapt during time. The sea anemone Nematostella vectensis has emerged as a marine model organism for studies on host-microbe interactions. Living in estuarine ecosystems, highly variable aquatic environments, N. vectensis has evolved the capability of surviving in a wide range of temperatures and salinities (Darling et al., 2005). Its microbiota is specific for its three developmental life stages (Mortzfeld et al., 2016) and contributes to thermal resistance (Baldassarre et al., 2021). Interestingly, recolonization dynamics of adult polyps recapitulates ontogenetic colonization events, while early colonizing bacteria are capable to mono-associate with germ-free animals, in contrast to most late colonizers (Domin et al.,2022). However, most of the mechanisms underlying the complexity of the natural microbiome form and function remain unknown. Here, we intent to establish a minimal synthetic model microbiome to recapitulate ontogenetic colonization events. We made use of a collection of bacterial isolates (611 isolates), retrieved from different developmental stages (Domin et al., 2022) and characterized as early or late colonizers. Di-association experiments combining early and late colonizers, based on their ability to colonize Nematostella in mono-association, aim to identify positive interactions leading to microbiome establishment. This experiment shown different types of interactions, from commensalism to amensalism between early and late colonizers. A promising pair has been observed, a vibrionales early colonizer promoting hyphomicrobiales late colonizer during recolonization. Additionally, based on metabolic network reconstruction, other colonizers for di-association experiments might be identify for further experiments. Finally, we aim to design a synthetic microbiome recapitulating the complexity of ontogenetic colonization with simplicity.

13 Nida Hatice Kaya

The starlet sea anemone Nematostella vectensis and its associated microbiome offer a valuable platform for investigating the symbiotic relationship between a host and its microbiome. Gnotobiotic adult N. vectensis polyps were recolonized with bacterial consortia representing all life cycle stages. Interestingly, regardless of the bacterial inoculum used, the successions of recolonization followed the ontogenetic pattern. This suggests that factors other than ontogeny play a crucial role in shaping the microbiome, such as host-driven factors and interactions among bacteria. Among these host factors, we hypothesize that nematosomes shape microbiome composition. Nematosomes are free-floating

multicellular bodies inside the host's body cavity, possessing the ability of phagocytosis. Here, we demonstrate through phagocytosis assays and proteomic comparisons that nematosomes distinguish between native and non-native Vibrio strains. Phagocytosis assays and confocal imaging demonstrated higher phagocytic activity in nematosomes exposed to non-native Vibrio strains like Hal025 and Hal281 provided by Halichondira panicea, compared to treatments with early native colonizers like NJ1 and NJ33. Additional proteomic analysis revealed an increase in proteins related to the phagosome pathway in nematosomes following exposure to non-native colonizers compared to native colonizers or control group without any bacterial challenge. This selective phagocytosis is also reflected in the ability of native and non-native Vibrio strains to colonize host tissue. Nematosomes with CRISPR/Cas9-mediated deletion in the nematosomes specific cJUN gene (NVE21090) highlight a potential molecular mechanism responsible for selective phagocytosis in an early branching metazoan, revealing still integration of the bacteria but no lysosomal behavior after treatment with native and non-native strains. Therefore, we assume that selective phagocytosis by nematosomes is an important host mechanism for recognizing, regulating and maintaining microbiome composition and colonization events.

14 Alejandro Caro-Quintero

Domestication Effects on Diversity and Role of Seed-Borne Microbial Endophytes in Cacao Genotypes

Research into the interplay between plants and microbes is quickly evolving, with a particular focus on microbial endophytes found in seeds. These microorganisms can safeguard germinating seeds and seedlings from a range of stressors, such as fungal diseases and drought. Current findings imply that seed microbiota play a crucial role in defining the plant's microbial community, influencing its constitution and configuration, as well as impacting plant physiology and ecology. For Theobroma cacao L., the seed-associated microbiome has been examined indirectly through the fermentation process used for chocolate manufacturing. Yet, a comprehensive study on the diversity and makeup of vertically transferred microbes is pending. This research scrutinized the diversity and composition of endophytes in seeds of different cacao pod genotypes. Established commercial genotypes (ICS95 and IMC67), recently released genotypes from AGROSAVIA (TCS01 and TCS19), and new local genotypes from Tumaco, Colombia (AC09, ROS1, ROS2) were employed to investigate microbial vertical transmission and establishment across various plant tissues. It was noted that the local and AGROSAVIA genotypes were predominantly populated by Pseudomonas and Pantoea bacteria, whereas the commercial genotypes revealed a broader bacterial diversity. Interestingly, all genotypes and plant tissues showed similar fungal composition, primarily of the Penicillium genus. Additionally, we noted a higher bacterial abundance in local and newly released cacao genotypes compared to commercial ones, implying that cacao domestication boosts bacterial diversity but diminishes abundance. Seed-borne endophytes were also isolated to assess their potential influence on plant growth and revealed several bacteria, including Bacillus, Pantoea, and Pseudomonas strains, that exhibited strong growth-promoting characteristics. Our findings propose that reducing seed-borne bacteria in domesticated cacao genotypes might result in the loss of vital bacteria for seedling growth and development. This study enhances our comprehension of the interaction and relationship between tropical perennial plants and their seed-borne microbiota.

15 Shihan Wang

The bacterial genotoxin colibactin has been demonstrated to be a potent inducer of DNA damage in epithelial cells in vitro. Infection with E.coli harboring the pks island, which encodes the biosynthesis gene cluster of colibactin, has been linked to increased tumorigenesis in various mouse models (Dougherty & Jobin, 2021). Recent biochemical advances allowed characterization of this toxinâ€™s chemical structure and its plausible mode of action, mediated by two cyclopropane warheads towards DNA adenines. Two recent independent in vitro studies from our lab (DziubaÅ„ska-Kusibab et al., 2020) and Cleversâ€™ lab (Pleguezuelos-Manzano et al., 2020) have been able to reveal the

preferential attack sites by colibactin in the DNA, represented by the motif AAWWTT. Colibactinâ€™s damage at this motif gives rise to a mutational signature that can be identified in the genomes of both phenotypically normal colonic epithelial cells from a cohort of healthy individuals and in a subset of colorectal cancer patients. These observations indicate that colibactin causes DNA damage in human intestinal epithelial cells, leading to long-lasting mutations, including in known driver genes. However, only a fraction of tumors bears the footprint of colibactin, well below the estimated prevalence of pks+ E.coli in healthy individuals. This finding raises the question of whether colibactin-associated DNA changes are purely incidental or whether they are indeed active drivers of human colon carcinogenesis. Our recent work (Iftekhar et al., 2021), however, provides interesting clues on an additional pathway by which colibactin initiates carcinogenesis. In fact, short but intense colibactin action in vitro transforms primary colonic cells towards a Wnt-independent state without showing the colibactin-associated mutational signature. Rather, we depict strong chromosomal aberrations that likely constitute the origin of colibactin-induced cancer phenotype, reminiscent of the in vivo route of colon carcinogenesis.

16 Ana Schaan

Vesiculome: Origin, Diversity, and Evolution of Bacterial Extracellular Vesicles in the Human Gut Microbiome

The Global Microbiome Conservancy Gut microbiome research has provided invaluable insight into the impact of microbes in the functioning of human organisms. Despite the well-known role of host-microbe and microbe-microbe interactions, the role that bacterial extracellular vesicles (BEVs) play in shaping this ecosystem has remained largely underestimated and overlooked. These BEVs, which encompass a varied array off membrane-enclosed particles ranging from 10 to 400 nanometers, are released by bacterial cells. Their composition, size, and type vary across diverse environments, bacterial species, and strains. The significance of BEVs extends to potential functions such as nutrient sharing, stress response, horizontal gene transfer, and quorum sensing. Notably, they can influence intestinal epithelial barrier integrity and trigger anti-inflammatory responses in animal models of intestinal inflammation. However, our understanding of vesiculation across bacterial evolution, as well as their impact on the host gut ecosystem is extremely limited. In this project, we aim to address this gap by characterizing the origin, evolution, and roles of BEVs within the human microbiome system. Our pilot study involves the isolation BEVs from a selection of 22 bacterial strains of the Global Microbiome Conservancy collection. Our isolation approach combines ultrafiltration and density-gradients followed by ultracentrifugation, to ensure maximum BEV yield while maintaining integrity and purity. In adherence to EV-TRACK guidelines, the isolated vesicles are meticulously characterized and visualized using nanoparticle tracking analyses and electron transmission microscopy, providing invaluable data regarding the concentration, size distribution and ultrastructure of the BEVs. Our preliminary findings reveal that the characteristics and proteomic diversity of BEVs exhibit differences that correspond to their taxonomy. Our current efforts focus on expanding our collection of BEV isolates (from monocultures and fecal samples) and performing proteomics analyses to characterize BEV protein composition. We will leverage the collection of bacterial isolates and stool samples from the Global Microbiome Conservancy to characterize potential vesiculome signatures of human lifestyles and expand to in vitro and in vivo tests to understand the impact and measure the effects of BEVs on the gut microbiome.

17 Thomas Dost

Microbial Metabolic Biogeography of the Murine GI-tract in the Context of Aging

Microbial Metabolic Biogeography of the Murine GI-tract in the Context of Aging Thomas Dost, Lena Best, Stefano Flor, Daniela Esser, Johannes Zimmermann, Otto Witte, John Baines, Christiane Frahm, Christoph Kaleta Due to easier availability, most studies use fecal samples as a proxy for the gastrointestinal microbiome. Thus the taxonomic and functional biogeography of the intestinal tract is often not considered. Therefore we analyzed the taxonomic composition and functional capabilities of the microbiome of 52 mice at three different gastrointestinal (GI) sites, cecum, proximal, and distal colon, across five age groups (2, 9, 15, 24, and 30 months). To this end, we mapped 16S data of the intestinal sites to 249 metagenome assembled genomes (MAGs) which resulted in 161 matches. We were able to identify several MAGs whose abundances were age-specifically altered along the GI tract. We then used metabolic modeling (flux-variability analysis) and MAG abundance information to investigate the metabolic capabilities of each of the three niches. We found several site-specific differences in metabolic activity in the younger three age groups while the older two showed no such signal. Exemplarily, in two-month-old mice, we can observe an increase of tryptophan degradation to indole along the GI tract. Thus indicating that the metabolic differences in the niche-specific microbiota are spatial and temporal dependent. In summary, we set up a pipeline to link 16S with metagenomic and metabolic model information. We then employed this pipeline to show considerable differences in the aging of the microbiota along the gastrointestinal tract.

18 Andy Mercado Gamarra

Analyzing Gut Microbiome Conventionalization in Mice: Differential Gene Expression and Metabolic Flux Variability

Abstract The coevolution between host organisms and their microbiomes, vital for essential functions, is disrupted when microbiome diversity decreases, impacting species adaptability. Investigating this relationship's complexity in different microbiome compositions, we examined gene expression and metabolic processes in CONVD mice, categorizing them as CONVR (responsive), GF (non-responsive), or unique. We analyzed RNAseq data from various tissues in 24 female C57BL6 mice, utilizing PCA, DESeq2, and genome-scale metabolic modeling with flux variability analysis (FVA). Histone modification and catabolism regulation were enriched in the "non-responsive" gene set, while ribosomal processes and dendrite development were under-expressed. The "unique" CONVD gene set exhibited over-expressed terms in mRNA processing and immune regulation and under-expressed terms in RNA splicing and neurogenesis. In the "responsive" gene set, enriched terms included pyrimidine processing and immune regulation, while circadian rhythm, RNA splicing, and metabolism were under-expressed. FVA results indicated complex metabolic shifts, including down-regulation in heme synthesis and glyoxylate metabolism in the brain and up-regulation in tyrosine and phosphatidyl-inositol phosphate metabolism in the colon. These findings highlight intricate host metabolism dynamics in CONVD compared to GF and CONVR conditions.

19 Malte Rühlemann

A fine-scale knowledge of the changes in composition and function of human gut microbiome compared to our closest relatives is critical to understanding the evolutionary processes underlying its developmental trajectory. To infer taxonomic and functional changes in the gut microbiome across hominids at different timescales, we performed high-resolution metagenomic-based analyses of the fecal microbiome of over two hundred samples including diverse human populations, wild-living chimpanzees, bonobos, and gorillas. We find human-associated taxa depleted within non-human apes and vice versa, suggesting the widespread acquisition of novel microbial clades along the evolutionary divergence of hosts, but in contrast also reveal multiple lines of evidence for a pervasive loss of diversity in humans populations in correlation with high Human Development Index,

including otherwise evolutionarily conserved clades. Similarly, patterns of co-phylogeny are also found to be disrupted in humans. Together with identifying individual microbial taxa and functional adaptations that correlate to host phylogeny, these results offer new and important insight into specific candidates playing a role in the diverging trajectories of the gut microbiome of hominids, whereby repeated horizontal gene transfer and gene loss, as well as the adaptation to transient microaerobic conditions appear to be of particular importance.