2024 MIT Microbiome Symposium

Abstract Book

Sponsors:

1. **Alex Crits-Christoph**, *Identifying DNA methylation patterns and restriction systems in non-model microbes with nanopore sequencing*

A Crits-Christoph (1), SC Kang (1), CF Gilbert (1), HH Lee (1), N Ostrov (1)

1. Cultivarium

Diverse bacteria rely on restriction-modification (RM) systems to distinguish self and foreign DNA using unique genome methylation patterns. Identification of DNA methylation patterns in microbes across human and environmental microbiomes can improve our understanding of phage susceptibility and alleviate barriers for laboratory transformation. Here, we describe the MicrobeMod computational toolkit for the identification of methylated DNA and methylation sequence motifs in bacterial genomes from native DNA alone using long-read nanopore sequencing. We validated performance of this approach using E. coli strains expressing methyltransferases with known methylation specificities. Using nanopore sequencing of over 40 non-model bacteria and microbiome strains, we describe the methylation patterns of strains with previously unexplored epigenetics. Further, we identify and annotate restriction methylation operons to putatively link methylated motifs back to their underlying RM genes. This methodology can guide informed decisions for overcoming restriction modification barriers to genetic engineering in non-model microbes.

2. **Ari Machtinger**, *Protocol optimization for viral wastewater metagenomics of municipal and airplane waste*

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Metagenomic sequencing of viruses in wastewater can be used to monitor known viral pathogens in the community and to identify new pathogenic variants or species. However, there is currently no published comparison of methods that is both sufficient for deep sequencing and easy to use. We compared protocol options from the literature to find options that gave a high yield of DNA and RNA, yielded a diverse set of viruses, resulted in a high ratio of viral relative to bacterial nucleic acids, and that were also practical for daily use. This poster shows some of the key experiments we performed for comparing protocol options for the processing of raw influent and primary sludge from municipal wastewater, and aggregated airplane waste, and shows our final protocols. We developed methods of sample processing through experiments that compare input volumes and dilutions, dissociation treatments, filtration treatments, concentration methods, and nucleic acids extraction kits. Our final protocol gives high yield of DNA and RNA, a high yield of viral relative to bacterial nucleic acids, and a diverse set of viruses including human-infecting viruses. It is also practical to use for daily sample processing of multiple sample replicates. Our methods could be used to run daily testing using untargeted metagenomic sequencing for novel pathogen surveillance.

3. **Charlie Gilbert**, *Scalable approaches for high-throughput identification of functional genetic tools in non-model bacteria*

CF Gilbert (1), Z Martin-Moldes (1), A Crits-Christoph (1), SC Kang (1), E Ledieu-Dherbécourt (1), S. Brumwell (1), J Leung (1), AC Esmurria (1), WH Alsharif (1), HH Lee (1), N Ostrov (1)

1. Cultivarium

Microbiomes across a range of environments consist of an abundance of diverse and understudied microbes. To better understand and develop useful applications from the species present in microbiomes, tools and methods for genetic manipulation are required. However, establishing even a foothold of initial genetic tractability in a non-model microbe can be a major challenge. To support researchers working with non-model microbes, we are developing tools and resources to help overcome this obstacle. Here we describe experimental workflows which enable scalable identification of optimal culture methods and establish genetic tractability for diverse non-model microbes. By applying these workflows we have generated culture and genetic data for a collection of >100 aerobic and anaerobic strains from >50 diverse families, including species of interest to the human gut microbiome such as Lachnospiraceae, Ruminococcaceae, Erysipelotrichaceae, and Rikenellaceae. Our growth screen probes >20 different culture media and a dozen antibiotic selections. Our molecular screen is capable of testing >100 unique combinations of selection markers and plasmid origin of replication sequences through high-throughput conjugation. Efforts to improve these tools and apply them to microbiome strains will enable deeper studies and manipulation of these complex communities.

4. **Christine Tataru**, *Inferring human-microbiome interactions with a novel generative artificial intelligence method*

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Interactions between pathogenic bacteria and human immunity have been studied extensively, however, less is known about how commensal gut bacteria interact with our immune systems. Here, we introduce a new generative artificial intelligence (AI) method, based on Neural Topic Models, to jointly learn host and microbiome "topics" and a directed interaction network between them, from datasets with paired host and microbiome sequencing data. Our methodological contributions are: (a) modeling interactions between topics of the same or different data modalities, using a Bayesian Network (BN) to capture dependencies, (b) directly inferring model structure, including BN edges and temporal dependencies, using Bayesian Variable Selection-style priors, and (c) an efficient end-to-end Variational Inference approach that leverages relaxations of discrete distributions.

We apply our method to a time-series dataset that concurrently measures the gut microbiome (shotgun metagenomics sequencing) from stool samples and host gene expression (mRNA-seq) from blood samples. The dataset comprises 98 participants, followed for two years, with antibiotic-resistant tuberculosis infection undergoing treatment with a five-drug regimen. Our method learns interpretable human gene expression and microbiome topics significantly enriched for Gene Ontology categories, taxonomic clades, and microbial metabolic pathways, and

simultaneously learns interactions between these multi-modal topics. We demonstrate that learning the BN structure significantly improves topic quality, as assessed on known biological relationships. Notably, our method infers interactions between human gene expression topics significantly enriched for genes with specific innate and adaptive immune function, and microbiome topics, including a previously reported relationship that has been mechanistically elucidated in mouse models, but not yet demonstrated in humans. These findings highlight the ability of our model to automatically infer biologically relevant relationships and suggest its power as an analysis and discovery tool to facilitate illuminating relationships between the microbiome and the human immune system at scale.

5. **Dogus Dogru**, *Uncovering the Role of Microbiome-Derived Autoantigen Mimics in Type 1 Diabetes*

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Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β-cells. The development of islet autoantibodies is one of the earliest markers of T1D. One possible cause of autoimmunity is molecular mimicry, where a foreign antigen stimulates an immune response targeting a similar epitope on a host protein. Insulin autoantigen epitope insulin B:9-23 is one of the most predominant epitopes in T1D. We previously reported a 15 amino-acid peptide (hprt4:18) in a human gut commensal, Parabacteroides distasonis, similar to insB:9-23. We showed that hprt4:18 can stimulate human T cells specific to insB:9-23. Reanalyzing infant gut microbiome data, we showed that the presence of hprt4:18 in the gut microbiota during the first three years of life significantly increases the risk of developing autoantibodies (seroconversion). Here, we hypothesize that microbiota-derived islet autoantigen epitope mimics play a key role in T1D pathophysiology. To test this, we generated a list of 519 epitopes derived from 20 autoantigens in T1D. We will identify all microbiota-derived epitope mimics and determine the presence and distribution of these mimics in seroconverters and controls. To this end, we have analyzed gut microbiome data (DIABIMMUNE and TEDDY cohorts) obtained from children developing T1D compared to healthy controls. We will further use machine learning to model the recognition of these microbial-epitope mimics by the autoantigen-specific T-cell receptors. In a test case, we focused on glutamate decarboxylase 2 (GAD2) autoantigen epitopes, another major autoantigen in T1D. Using DIABIMMUNE data, we showed that a 9 amino-acid peptide RIP metalloprotease RseP from Ruminococcus bromii may act as a GAD2 epitope mimic (GAD2:114-123) and found seroconversion rates to be significantly higher in individuals carrying the RseP peptide encoding sequence. This study will provide new insights regarding T1D pathophysiology and serve as a proof-of-concept study linking microbiota-derived neo-epitopes to T1D etiology.

6. **Emily Bean**, *Development of the Reconstructed Human Epidermis S. aureus Activity (RHESA) Model to validate a microbial ensemble for treating atopic dermatitis*

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Microbes within our microbiomes work in concert to improve human health. For example, interactions among members of the skin microbiome drive robust inhibition of Staphylococcus aureus colonization and virulence; individuals with skin microbiome deficiencies are prone to S. aureus blooms and, by extension, inflammatory diseases like atopic dermatitis (AD). With a deep knowledge of interactions among skin microbes, we could in principle distill out a set of microbes that recreates this microbiome-endowed protection. To attain this knowledge, Concerto Biosciences used kChip, an ultra-high-throughput coculture platform first prototyped at MIT. By measuring the behavior of millions of defined combinations of skin-dwelling microbes, we were able to reconstruct a massive skin microbiome interaction network. This network revealed “Ensemble No.2” (ENS-002), a specific strain combination that drives the microbiome’s ability to arrest S. aureus proliferation and inhibit its virulence. To assess the performance of ENS-002 in a setting that more closely mimics that of human skin, we developed a specialized S. aureus-colonized in vitro skin model, the “Reconstructed Human Epidermis S. aureus Activity model” (RHESA model). Studies using the RHESA model corroborated ENS-002’s protective potential. We’re now developing ENS-002 as a topical microbiome therapeutic that treats or prevents AD flares, and we expect our first in-human trials later this year. Concerto has also initiated discovery projects in vaginal health, gut health, and agriculture, aiming to unleash a new era of microbial research and a treasure trove of microbe-based products.

7. **Gianfranco Yee**, *An in vitro strategy to elucidate commensal antigen trafficking in the gut*

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The gut microbiome consists of numerous commensal species that educate host immunity and maintain gut health. Segmented filamentous bacteria (SFB) are a notable group of commensals that induce protective Th17 cells in the gut. This non-invasive process depends on SFB adhesion to intestinal epithelial cells (IECs), endocytosis of SFB cell-wall proteins, and the presence of antigen presenting cells (APCs) in the lamina propria. However, mechanisms by which APCs acquire SFB antigen from IECs remain unclear. Elucidating this is particularly challenging because SFB is notoriously difficult to culture in vitro, which restricts experiments to low-throughput in vivo models. Here, we engineered a microfluidic chemostat to co-culture SFB and primary murine IECs. This reductionist approach allows us to directly monitor isolated SFB-IEC interactions in physiological hypoxia with live imaging. Upon successfully expanding SFB in vitro, we confirmed previously reported SFB-host dynamics. Furthermore, we uncovered that SFB induces the exocytosis of actin-rich extracellular vesicles (EVs) by IECs. We hypothesize that these EVs shuttle SFB antigen to APCs and thus will characterize the contents and fate of these EVs. Alternatively, we are investigating of role of apoptotic IECs in transferring SFB antigen to APCs. Our findings will extend knowledge of how commensal microbes non-invasely shape mucosal immunity.

8. **Guilherme Fahur Bottino**, *Gut-microbial age derived from globally sampled gut metagenomes*

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The gut microbiome plays an important role in early child development. As a consequence integrated models of child health should incorporate information derived from gut metagenomics. However, the gut microbiome changes rapidly during the first three years of life, posing challenges to that integration. We hypothesized that there is a latent universal generative pattern in the succession of taxa in the gut that can be learned, enabling microbial estimated ages that can act as indirect probes of delayed or hastened gut development for health modeling. Here, we used 3000 infant gut metagenome samples from over 1600 individuals collected from publicly available sources and new data from low-resource settings cohorts. After harmonizing the data, we built a classical machine learning regressor targeting a continuous age range from 2 to 18 months based on the relative abundance of microbial taxa identified in metagenomes. After repeated cross-validation, we achieved a RMSECV of 2.6 months and a validation-set correlation of 0.80. Our model shows that, although individual samples can contain a substantial number of location-specific taxa, around 30 taxa are responsible for reproducing over 90% of the model capability, regardless of geographic location. We intend to test the impact of adding this measure of predicted microbial age to improving models that utilize other multi-’omic and health data and increase our understanding of the interaction between microorganisms and pediatric health.

9. **Izzy Goodchild-Michelman**, *Probiotic-induced functional alterations in the gut microbiome of preterm infants*

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Preterm infants exhibit a distinct gut microbiomes characterized by a low microbial load and reduced microbial diversity. While probiotics have shown promise in transforming preterm infants' microbiomes to resemble those of full-term infants, their influence on microbiome functionality remains underexplored. This study aimed to investigate the impact of probiotics on the functional development of the gut microbiota in pre-term infants. Our population study includes 105 preterm infants (gestational age 23-36 weeks) from the observation longitudinal BLOOM study, 68 of whom received a multi-strain probiotic treatment for the first eight weeks post-birth. We developed GEnome-scale Models (GEMs) of metabolism for each probiotic strain and species-resolved GEMs for the infants' microbiota from 762 stool samples, incorporating known Human Milk Oligosaccharides (HMOs) degradation pathways. We simulated each GEM under a breastmilk diet. Species abundance analysis revealed that probiotics accelerated gut microbiota maturation in preterm infants. Metabolite profiling in feces using GEMs highlighted functional differences between control and probiotic groups, with notable increases in several metabolites in the latter. GEMs additionally pinpointed key microbial species and probiotic strains crucial for producing modulatory metabolites like short-chain fatty acids (SCFAs) and HMO degradants. Distinct microbial contributions to metabolite production were observed between the groups. Finally, shadow price analysis in GEMs indicated that the probiotic-treated microbiomes had enhanced capabilities for producing SCFAs and degrading HMOs, suggesting a more adaptable metabolic environment.
Our study offers novel insights into how probiotics fundamentally shape the functional landscape and dynamics of pre-term infants’ gut microbiota development.

10. **Kasturi Lele**, *Generalized Lotka-Volterra models are poor predictors of community assembly in sourdough microbial communities*

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To understand and manipulate microbial communities, it is crucial to be able to predict how they assemble. Using a combination of experimentally tractable model communities and theoretical and empirical approaches, we can develop a framework to predict and manipulate microbial community dynamics. Mathematical models widely used to understand growth and interactions in two-species communities (such as Lotka-Volterra models), have recently been extended to multi-species communities. However, recent studies question the suitability of pairwise models to understand the complex dynamics in a multi-species community. Thus, we wanted to understand whether a generalized Lotka-Volterra model would be able to capture the dynamics of assembly of a multi-species community. To do this, we used a microbial community derived from sourdough starters, consisting of five bacteria and four yeasts. We extracted growth parameters from microbes grown alone and pairwise to develop a multi-species generalized Lotka-Volterra model. To validate model predictions, we established stable replicate experimental communities, which showed little overlap with model predictions. We further explored the parameters underlying the model and observed that a smaller subset of parameters did not improve the accuracy of model predictions. We also tested this reduced parameter set by generating model predictions for 9 groups of communities, each lacking one species in the species pool. In both model predictions as well as observed communities, a few key species may have disproportionate impacts on community composition. Overall, our results indicate that pairwise interactions between sourdough microbes are insufficient to explain the patterns of multi-species community assembly.

11. **Kathryn Atherton**, *Predicting fungal community functional potential from ITS rRNA sequencing*

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The most common method to characterize fungal communities is to sequence the ITS rRNA genes. While these methods can tell us the taxonomic composition of a community, they do not directly tell us about the functional profile of the community. Metagenomic or metatranscriptomic datasets can provide this functional information but can be more than double the cost of amplicon sequencing. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2) was developed to predict prokaryotic metagenomic contents from 16S rRNA sequences, but a comparable tool for fungal communities has not yet been created due to a lack of high-quality, annotated genomes representing fungal phylogeny. Leveraging the Joint Genome Institute's rapidly-expanding MycoCosm database of fungal genomes, we have produced a tool to infer functional profiles of fungal communities from ITS rRNA sequences. Validation of the results of this tool against metatranscriptomic sequencing and functional data will highlight knowledge gaps in gene annotation and transcription. This tool will allow for the assessment of gene-based fungal community functional profiles with amplicon sequencing, improving whole microbial community functional characterizations when used in combination with PICRUSt2's bacterial methods.

12. **Kevin Bonham**, *Gut-resident microorganisms and their genes are associated with cognition and neuroanatomy in children*

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The gastrointestinal tract, its resident microorganisms, and the central nervous system are connected by biochemical signaling, also known as the "microbiome-gut-brain-axis." Emerging evidence implicates gut microorganisms and microbiota composition in cognitive outcomes and neurodevelopmental disorders (e.g., autism and anxiety), but the influence of gut microbial metabolism on typical neurodevelopment has not been explored in detail. We investigated the relationship of the microbiome with the neuroanatomy and cognitive function of 381 healthy children, demonstrating that differences in gut microbial taxa and gene functions are associated with overall cognitive function and with differences in the size of multiple brain regions. Using a combination of multivariate linear and machine learning (ML) models, we showed that many species, including Alistipes obesi and Blautia wexlerae, were associated with higher cognitive function, while some species such as Ruminococcus gnavus were more commonly found in children with low cognitive scores after controlling for sociodemographic factors. Microbial genes for enzymes involved in the metabolism of neuroactive compounds, particularly short-chain fatty acids, were also associated with cognitive function. In addition, ML models were able to use microbial taxa to predict the volume of brain regions, and many taxa that were identified as important in predicting cognitive function also dominated the feature importance metric for individual brain regions, and for specific subscales of cognitive function. Several species from the phylum Bacteroidetes, including GABA-producing B. ovatus, were important for predicting the size of the left accumbens area, but not the right. These findings provide potential biomarkers of neurocognition and brain development and may lead to the future development of targets for early detection and early intervention.

13. **Kristina Kelley**, *Exploring the Role of Taurodeoxycholic Acid in Pediatric Celiac Disease Pathophysiology*

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Celiac Disease (CD) is an autoimmune condition triggered by gluten ingestion and is defined by small intestine inflammation that leads to villous atrophy. Individuals with CD possess the predisposing HLA-DQ2 or HLA-DQ8 alleles. However, while 20-40% of the general population possess these alleles, only 1-2% of the population develops CD. This indicates that there are additional environmental factors at play in the pathogenesis of the disease.
We recently conducted a study using fecal and plasma samples obtained from children at ages 2.5 and 5 years in a prospective cohort study (n=16). These samples were collected months to years before the onset of CD (CD progressors). We found that children who develop CD have an altered gut microbiome composition, IgA response and cytokine profile years before diagnosis. Further, CD progressors have a distinct plasma metabolome profile. The most significantly altered metabolite increased in CD progressors was taurodeoxycholic acid (TDCA), a secondary bile acid produced by gut commensals. When we treated C57BL/B6 mice with TDCA (0.4%, n= ~8 /sex, 10 weeks), it induced villous atrophy in the duodenum as well as upregulated CD4+ T-cells and Natural Killer cells while decreasing T regulatory cells. Additionally, treatment with TDCA upregulated the expression of two important immunoregulatory proteins on T cells, Qa-1 and NKG2D. These two proteins are critical to the play a key role in destruction of epithelial cells in CD autoimmunity. To determine the effects of the TDCA on a relevant mouse model of CD, we used the DQ8-Dd-villin-IL-15tg mouse. These animals possess the predisposing HLA-DQ8 molecule and overexpresses IL-15 in the lamina propria, mimicking human disease. Treating these mice with TDCA (0.4%, n= ~10/sex, 4 weeks), caused villous atrophy and decreased the villi/crypt ratio in the ileum. Our results indicate that TDCA and TDCA-producing bacteria could potentially contribute to the pathogenesis of pediatric CD.

14. **Krishna Girish**, *Synthesizing the sensitivity of microbiota to external perturbations*

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The composition of microbial communities, in terms of the relative abundances of different constituent taxa, is constantly subjected to a wide variety of external perturbations, such as addition and removal of species, changes in parameters of the physical environment, and transitions between different dynamical regimes. Understanding the sensitivity of community composition to such perturbations is of paramount importance, given their role in maintenance of ecosystem function at scales from human health to global biogeochemical cycles. In this study, we unify statistical techniques from empirical dynamical modeling for nonlinear dynamical reconstruction with causal inference to evaluate the role of abundance and interactions on the sensitivity of microbial communities to perturbations in environmental parameters. Using long-term abundance data of taxon abundances in two human gut microbiota and one salivary microbiota, we identify four microbial community profiles with differing effects of both abundance and interactions on sensitivity. While profiles whose abundance and interactions have a negative effect on sensitivity are the most abundant, profiles with a positive effect respond with greater volatility during periods of perturbation. Additionally, we find that the randomness of fluctuations of sensitivity has contrasting responses to pulse (short-term illness) and press (long-term travel) perturbations across different subjects' time series. Despite the individualized nature of microbial compositions and the variety of possible perturbations and responses, our study shows the possibility to synthesize the nonlinear nature of microbiota dynamics. This approach can be relevant for personalized approaches in monitoring and designing optimal therapeutic treatments.

15. **Michael S DeMott**, *Proportions of Individual Phosphorothioate Sequence Contexts Remain Constant Despite High Chronological Variation in Levels of Human Gut Microbes with PT DNA Modifications*

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There are dozens of enzymatically-inserted DNA modifications, encompassing the epigenomes of all forms of life. While bacterial DNA modifications are best known from restriction-modification systems, they are also known to regulate gene expression. Phosphorothioation (PT) of DNA, in which a sulfur atom replaces a non-bridging oxygen in the sugar-phosphate backbone, is the only known naturally occurring DNA backbone modification. Here we show ongoing efforts to define the landscape of PT-possessing gut organisms using a combination of chromatography-coupled mass spectrometry (LC-MS), novel next-generation sequencing technologies (NGS), and metagenomic analysis. LC-MS analysis of nuclease P1 limit-digests of fecal DNA isolated from mice revealed 11 out of 16 possible PT dinucleotides, with all tested mice containing the same sequence contexts for PT. In contrast, PT analysis of 11 healthy human donors revealed highly variable combinations and levels of PTs. Time course analysis over many months revealed a constant proportion of individual PTs but a highly variable (≤18-fold) relative abundance of total PTs compared to ≤1.5-fold variation in a widely distributed epigenetic mark, m6dA. To identify longer sequence motifs for PT sites and specific organisms possessing PTs, fecal DNA was treated with iodine, which reacts with the sulfur atom to induce oxidative strand breaks, and the resulting breaks mapped to their parent bacteria using a unique NGS approach. Metagenomic analysis revealed a dominance of PT-containing microbes in Bacteroidota, Firmicutes, Actinobacteria, and Proteobacteria communities, which differed from the unbiased spectrum of gut bacteria. These results provide a backdrop for understanding the behavior of an important DNA modification in the human gut microbiome, with implications for

inflammatory conditions of the gut, given the acute sensitivity of PTs to oxidants such as hypochlorous acid from neutrophils. To explore this hypothesis, we quantified PT levels in 72 clinical fecal samples from patients with active and inactive Crohn's Disease and Ulcerative Colitis.

16. **Nick Quinn-Bohmann**, *Microbial community-scale metabolic modeling predicts personalized short chain fatty acid production profiles in the human gut.*

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Microbially-derived short chain fatty acids (SCFAs) in the human gut are tightly coupled to host metabolism, immune regulation, and integrity of the intestinal epithelium. However, the production of SCFAs can vary widely between individuals consuming the same diet, with lower levels often associated with disease. A systems-scale mechanistic understanding of this heterogeneity is lacking. We present a microbial community-scale metabolic modeling (MCMM) approach to predict individual-specific SCFA production profiles which show significant correlation with measured SCFA production profiles in ex vivo models. Predicted production profiles are also significantly associated with inflammation and blood-derived clinical chemistries in in vivo models. We then demonstrate how MCMMs can be leveraged for the pursuit of precision nutrition, to design personalized dietary, prebiotic, and probiotic interventions that optimize SCFA production in the gut. Finally, we integrate these patient-derived MCMMs with context-specific metabolic models of the colonic epithelium, to interrogate the host-microbiome interface and model metabolic transfer in response to microbiome-focused interventions. Our results represent an important advance in engineering gut microbiome functional outputs for precision health and nutrition.

17. **Natalie Culler**, *Pilot data from the first double-blind, placebo-controlled vaginal microbiota transplantation trial to treat recurrent bacterial vaginosis*

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Bacterial vaginosis (BV) frequently recurs after antibiotic treatment, likely because treatment does not promote colonization with the health-associated species, Lactobacillus crispatus. Vaginal microbiota transplantation (VMT), the transfer of vaginal fluid from a healthy donor to a recipient with BV, has been proposed as a means of establishing durable colonization. Here we present pilot data from the first placebo-controlled clinical trial using VMT to prevent recurrent BV. We aim to determine the safety and efficacy of VMT compared to placebo. The donor passed extensive screening tests using an FDA-approved protocol and provided 12 L. crispatus-dominant vaginal fluid donations. Recipients received either 2 doses of saline-placebo or VMT on non-consecutive days starting 24-48 hours after completing metronidazole treatment. At each follow-up visit, recipients completed questionnaires about symptoms and behavior, and vaginal fluid samples were collected. We determined the bacterial community composition using 16S rRNA amplicon sequencing and species-specific qPCR. We isolated strains of Lactobacillus from the donor and all recipients and used comparative genomics to detect strain-level colonization. Over 6 months, no recipients reported any serious adverse events. There were no Grade 2 or higher urogenital adverse events in the VMT arm. In three different placebo participants, three such events occurred. At baseline, 1/4 placebo and 0/4 VMT recipients had L. crispatus detected at >50% relative abundance. One month after intervention, this was 1/4 for placebo and 3/4 for VMT, and at 6 months 0/4 placebo and 2/4 VMT recipients had >50% L. crispatus. L. crispatus genomes from the donor and VMT recipients clustered into a monophyletic clade separately from the isolate genomes derived from the placebo recipients. These data demonstrate initial safety and proof-of-concept for VMT to promote durable vaginal L. crispatus colonization.

18. **Audrey Randall**, *A gut commensal, Parabacteroides distasonis, and molecular mimicry in type 1 diabetes.*

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Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of pancreatic, insulin-producing beta cells by autoreactive T-cells. The trigger(s) of T1D are unclear and genetics alone cannot explain the recent increase in T1D diagnoses. The gut microbiome is thought to play a key role in T1D pathogenesis. Insulin is a key autoantigen in T1D autoimmunity, and insulin B-chain amino acids 9-23 (insB:9-23) is an important, commonly recognized T-cell epitope. We previously identified a peptide mimic, amino acids 4-18 of hypoxanthine phosphoribosyl transferase (HPRT:4-18) of Parabacteroides distasonis (Pd). HPRT:4-18 cross-reacted with insB:9-23-specific human and non-obese diabetic (NOD) mice T-cells. We hypothesize Pd HPRT:4-18 is required to accelerate diabetes via molecular mimicry of insB:9-23. Molecular mimicry is an autoimmunity mechanism where host-like microbial proteins trigger immune responses. Orally gavaging NOD mice (n=40 mice/group) and germ-free NOD mice (n=6-8 mice/group) with P. distasonis (Pd) accelerated diabetes compared to the saline-treated mice. The Pd-colonized mice had significantly more splenic CD8 and naïve T-cells, dendritic cells and macrophages but fewer FOXP3 cells (n=3-6 mice/group). To test if Pd colonization disrupts intestinal barrier function which has been associated with diabetes, the expression of tight junction proteins, zonulin, claudin and occludin, in the duodenum, ileum and jejunum was quantified. Pd colonization did not alter gut permeability and among the intestinal intraepithelial lymphocytes (IELs), there were fewer naïve, central and effector T-cells and B-cells. The findings indicate Pd decreases intestinal inflammation and induces pancreatic autoimmunity.

19. **Saja A. Fakhraldeen**, *Metagenomics-based spatiotemporal profiling of bacterial and archaeal communities in the Northwest Arabian Gulf*

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Kuwait Bay is a unique aquatic environment located in the Northwest Arabian Gulf. Kuwait Bay serves as an important regional nursery ground for myriad marine biota and is a water body of vast cultural, economic, and environmental significance. Due to extreme temperatures, hypersalinity, and shallowness, the Bay is an ideal environment for development of novel genetic adaptations that are unique and necessary for survival of resident organisms. To begin the process of systematically identifying putative unique functional genetic adaptations, it is essential to identify resident organisms and understand the dynamic nature of their existence. Thus, seawater samples were collected from three locations in Kuwait Bay from both the surface and bottom of the water column monthly over a period of six months to construct community profiles and investigate their variability with respect to depth, location, time, and various physicochemical parameters of the seawater. Nucleic acid was extracted from the collected samples and subjected to shotgun metagenomic sequencing at an average depth of 15 million paired end reads per sample. Results showed an approximately even abundance of archaeal and bacterial communities, but significantly greater diversity among the bacterial population, which consisted of members of the Proteobacteria, Cyanobacteria, and Bacteroidetes phyla in decreasing order of abundance. Little to no significant variations were observed in the abundance of archaeal and bacterial populations with respect to depth down the water column. Measurements of species richness and evenness revealed negligible variation between the various locations. Archaeal abundance significantly increased while bacterial abundance significant decreased in the winter season. Overall, this study provides the first in-depth analysis of bacterial and archaeal community structures in Kuwait Bay developed using a shotgun metagenomics approach thus paving the way for future investigations of functional genetic adaptations developed by resident biota attempting to survive in the uniquely extreme surrounding conditions.

20. **Mani Sai Suryateja Jammalamadaka**, *An experimental method to perform spatio-temporal studies of bacterial communities on water-insoluble polymer surfaces*

Mani Sai Suryateja Jammalamadaka, Patrizia Stadler, Benedict Alois Borer, Otto X. Cordero

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InsolubleInsoluble biopolymers in ocean do not accumulate in the ocean sediments due to efficient bacterial degradation. The biodegradation triggers a range of microscale interactions that regulates these particulate organic matter turnovers. Understanding these microscale biodegradation interactions requires an experimental platform with transparent surfaces, continuous microscopy at single-cell resolution, and a continuous flow system. In the current work, a simple tape based microfluidic experimental platform with an intact insoluble biopolymer layer (chitin) was developed that allows us to characterize these microscale interactions of biodegradation.

21. **Utkarsh Sharma**, *New ultrasensitive assay to measure host immune profile from feces*

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Immune activity is often profiled using blood or tissue samples, which is invasive and difficult to scale. Dense, noninvasive access to the gut immune system is necessary to understand gut microbiome-immune crosstalk. Fecal matter has been used to profile gut immune status using a few high abundance markers like calprotectin and eosinophil derived neurotoxin. But, low abundance proteins (including cytokines) which offer a detailed view into immune activity have been difficult to detect in feces using existing methods, particularly in the absence of inflammation. We present a new ultrasensitive fecal assay that profiles immune proteins at femtomolar levels, providing an unprecedented window into gut immune activity. We are able to quantitate seven cytokines—TNFa, IL-17a, IL-10, IL-18, IL-22, IL-6, IL-1b—in healthy conventional and germ-free mice. We will present ongoing mouse experiments demonstrating the correlation between gut-tissue, blood and fecal levels of cytokines, as well as their dynamics with gut microbiota. Our assay will be used to generate data to train machine learning models of immune-microbiome dynamics.

22. **Yixiang Deng**, *AGE-RELATED DECLINE IN THE ANTIBODY-MEDIATED FUNCTIONAL RESPONSE TO CONJUGATE STREPTOCOCCUS PNEUMONIA VACCINATION*

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Antibodies represent the primary correlate of protection against Streptococcus pneumoniae infection. However, the induction of high titer antibodies following pneumococcal vaccination does not guarantee protection. Specifically, adults ≥65 yrs are at increased risk of pneumococcal infections and pneumococcal vaccines are less effective with increasing age, yet the mechanism(s) underlying both susceptibility to infection and reduced vaccine efficacy in the elderly remains unclear. Here we used a systems serology approach to deeply profile pneumococcal vaccine-induced humoral responses in a subset of adults aged 50-80 years who took part in a randomized controlled trial of a seven valent pneumococcal conjugate vaccine (PCV7) compared to a 23-valent polysaccharide vaccine (PPV23). One month after a single dose of PCV7 or PPV23 both vaccines generated various IgG subclass, IgA, and IgM responses against vaccine-containing capsular polysaccharides. Longitudinal analysis revealed that PCV7 vaccinees uniquely demonstrated an age-associated reduction in the ability of pneumococcal antibodies to bind Fc□- and □-receptors, most pronounced for the opsinophagocytic FcgR2A and FcgR2B receptors. This reduced Fc receptor binding profile was associated with compromised antibody-mediated effector cellular functions, specifically antibody-mediated neutrophil phagocytosis which declined with age for those receiving PCV7 but not PPV23. Given that glycol-conjugate vaccines, compared to polysaccharides, uniquely leverage T cell help, our data suggests that age-dependent T cell helper defects may drive functionally deficient IgG that may limit protection in those most vulnerable to disease.