Symposium on
MICROBIOME RESEARCH
at the Interface of Environment, Health & Agriculture

Abstract Booklet

January 16, 2024

Brendan Iribe Center for Computer Science and Engineering
University of Maryland
College Park, MD 20742

CENTER OF EXCELLENCE IN
MICROBIOME SCIENCES

KEYNOTE PRESENTATIONS

Tuesday, January 16, 9:15-10:15am

Susannah Green Tringe. *Sequence-based Interrogation of Soil Microbiomes and Their Ecosystem Benefits* pg. 5

Tuesday, January 16, 4-5pm

Joff Silberg. *Using Synthetic Biology to Program Microbes and Environmental Consortia For Real-Time Sensing and Actuation* pg. 5

ORAL PRESENTATIONS

Tuesday, January 16. 10:45-11:45am

Steve Kutos. *What's brewing? Impacts of cultivation management on the Coffea arabica soil microbiome* pg. 14

Taylor K.S. Richter. *Long-term impacts of untreated dairy manure on the microbiome and pathogenic E. coli persistence in agricultural soil* pg. 19

Hannah Zierden. *Vaginal bacterial extracellular vesicles as mediators of gynecologic health* pg. 21

Glory Minabou Ndjite. *Fructose Epimer Utilization by Gut Bacteria* pg. 17

Jeba Mercy Gnanasekaran. *The Role of Periodontitis in Inflammatory Bowel Disease* pg. 10

Tuesday, January 16. 1:30-2:30pm

Julian Urrutia-Carter. *Antimicrobial Peptides in Appalachian Salamanders* pg. 23

Zeyang Shen. *A genome catalog of the infant skin microbiome indicates microbial diversity, function, and transmission in early life* pg. 20

Justin Grynovicki, Mangeet Kaur. *"Making the Invisible Visible" Decode the microbial universe*

Samuel P. Forry. *A Sensitivity Analysis of Methodological Variables for Metagenomic Sequencing* pg. 8

POSTER PRESENTATIONS

Tuesday, January 16. 12-1:30pm

* indicates presenter

1. Susan R. Leonard*, Taylor K.S. Richter, Mark K. Mammel, Ivan Simko, Maria T. Brandl. *Characterization of Soil and Lettuce Resistomes from Harvest Through Storage in Modified Atmosphere Packaging* pg. 14

2. Autumn Salcedo*, Qingyue Zeng, Kevin Lam, Derek Konsen, Ryan Blaustein*. *Antimicrobial Resistance in Urban Agriculture Environments* pg. 20

3. Dhiraj Chundru*, Mostafa Ghanem, David Erickson, Xinyang Huang, Jianghong Meng. *Optimizing Metagenomic Approaches for Enhanced Food Safety in Poultry Production Environment* pg. 7

4. Madhusudan Timilsina*, Dhiraj Chundru, Mostafa Ghanem. *Characterization of Upper Respiratory Tract Microbiome of Chickens following Avibacterium paragallinarum Infection* pg. 22

5. Diksha Klair*, Marissa Lee Sang, Shirley A. Micallef. *Exploring the Diversity of Culturable Endophytic Bacteria in leafy Brassica spp. intended for fresh consumption* pg. 13

6. Zhujun Gao*, Ryan A. Blaustein, Gail M. Bornhorst, Nitin Nitin, Rohan V. Tikekar. *Enrichment of apple microbiome and its survivability during simulated gastric digestion* pg. 9

7. Shane Cronin*, K. Hobert, A. Biddle, T. F. Gressley. I*n vitro effects of starch with or without buffers on dairy cattle fecal microbiome* pg. 7

8. Michael T. France, Issac Chaudry*, Madeline Alizadeh, Pawel Gajer, Jacques Ravel. *VIRGO2: A Remastered Gene Catalog for Vaginal Microbiome Omics Analysis* pg. 9

9. Bern Monari*, Hannah Wilcox, Pawel Gajer, Jason Hallarn, Greta Bauer, Jessica Prodger, Jacques Ravel. *Characterizing the Vaginal Microenvironment of Transmasculine Individuals Accessing Testosterone Therapy* pg. 15

10. Baris Ozdinc, Holly Hack, Dana Walsh, Kelly Moffat*. *The Vaginal Microbiome: Implications for Treating Vaginal Disease* pg. 17

11. Himanshi Tanwar*, Jeba Mercy Gnanasekaran, Devon Allison, Vivek Thumbigere-Math. *Role of Gut Microbiota in Osteoclast Regulation and Skeletal Homeostasis* pg. 21

12. Sally L. Bornbusch*, Hannah E. Shinner, Lindsey Gentry, Mia M. Keady, Virginia Glick, Carly R. Muletz-Wolz, Michael L. Power. *Local environment shapes milk microbiomes while evolutionary history constrains milk macronutrients in captive Cercopithecine primates* pg. 6

13. Nicholas Pirolli*, Zuzanna Mamczarz, Dan Levy, Talia Solomon, Emily Powsner, Raith Nowak, Chris Bridgeman, Sulayman Khan, Laura Reus, William Bentley, Steven Jay. P*robiotic bacterial extracellular vesicles treat mouse model of colitis after oral delivery* pg. 18

14. Carly R. Muletz-Wolz*, Jimenez, R.R., Osborne, O., Byrne, A.Q., Kutos, S.J., Cleland, T.P., Gratwicke, B., Ellison, A. *Host and environmental microbiomes in a disease system in Appalachian salamanders* pg.16

15. Malique Bowen*, Jason Rosales, Jennifer F. Biddle. *Investigating the gut microbiome of Skeleton Shrimp (Caprella sp.) in Delaware Bay* pg. 6

16. Guglielmo Raymo* Ali Ali, Ridwan Ahmed, Mohamed Salem. *Early-life fecal transplantation from high muscle yield rainbow trout to low muscle yield recipients accelerates somatic growth through respiratory and mitochondrial efficiency modulation* pg. 18

17. Kasey Hobert*, Destiny Mann*, Michael Crossley, Amy Biddle. *Changes in growth rate and gut microbiome of an important animal waste upcycler reared on horse manure* pg. 11

18. Nora D. Hamovit*, Stephanie Yarwood, Taniya Roy Chowdhury, Denise Akob, Gregory McCarty, Xuesong Zhang. *13C-DNA stable isotope probing reveals active methanogen are more numerous in a restored freshwater wetland* pg. 11

19. Yuzhu Mao*, Birthe Veno Kjellerup. *City and Neighborhood Scale Wastewater Based Epidemiology Monitoring and Modeling of SARS- CoV-2, Influenza A and RSV in Maryland* pg. 15

20. Johanna B. Holm*, Amaury Maros, Michael T. France, Pawel Gajer, Bing Ma, Rebecca M. Brotman, Michelle Shardell, Larry Forney, Jacques Ravel. *Integrating compositional and functional content to describe vaginal microbiomes in health and disease* pg. 12

INVITED KEYNOTES

Using Synthetic Biology to Program Microbes and Environmental Consortia For Real-Time Sensing and Actuation
Joff Silberg

The rapid diversification of synthetic biology tools holds promise in making some hard-to-solve environmental problems tractable. In this talk, I will discuss problems in the Earth and environmental sciences that could be addressed using engineered living microbes. Such synthetic cells have the potential to offer new perspectives on open questions, including understanding microbial behaviors in heterogeneous materials (e.g., soil and sludge), monitoring pollutants in real time, tracking cryptic elemental cycling, and detecting dynamic cell-environment interactions. First, I will describe our efforts to overcome biological component limitations by creating new classes of electrical protein switches. Second, I will describe our efforts using these switches in synthetic electron transport chains to program microbes to convert environmental information into electrical information in real time within riverine samples. Finally, I will describe our efforts to develop a new class of catalytic RNA for programming consortia-scale information storage, such as data about participation in gene transfer, as well as our efforts to use catalytic RNA to control cell-cell signaling, information storage, and cell fitness. These synthetic biology approaches are expected to be broadly useful for programming microbial consortia for fast sensing and actuation within hard-to-image environmental and built materials.

Sequence-based Interrogation of Soil Microbiomes and Their Ecosystem Benefits
Susannah Green Tringe

Plants roots and the soil they grow in are heavily colonized with microbes that play critical roles in nutrient cycling and transport as well as influencing plant growth and health. Molecular methods including DNA sequencing have begun to elucidate the forces governing the assembly and maintenance of plant and soil microbial communities, offering the opportunity for these microbial communities to be nurtured and manipulated to promote plant growth and health as well as soil health and ecosystem functions.

We have investigated the factors influencing greenhouse gas emissions from natural and managed wetland systems and find that gas fluxes represent a complex interplay of biological, chemical and physical factors that vary across habitats. Our results suggest considerable heterogeneity in fluxes even in physically proximate locations that have implications for the success of wetland preservation and restoration as a carbon storage strategy, particularly in the context of sea level rise.

In agricultural systems, we find that different plant compartments (e.g. rhizosphere and root endosphere) harbor unique and dynamic microbial communities heavily influenced by the soil, surrounding environment and host genotype. Abiotic stress, such as drought and low nitrogen, can alter both the composition of these communities and their interactions with each other and the plant. Our sequence-based characterizations of plant-associated communities have identified key populations that structure the community and respond dynamically to environmental changes, representing potential targets for improvement of plant resilience.

Current projects in the group aim to identify microbial interventions that will potentially decrease greenhouse gas emissions, increase soil carbon storage and improve crop yields.

CONTRIBUTED PRESENTATIONS

(in alphabetical order by first author's last name)

Local environment shapes milk microbiomes while evolutionary history constrains milk macronutrients in captive Cercopithecine primates

Sally L. Bornbusch*, Hannah E. Shinner, Lindsey Gentry, Mia M. Keady, Virginia Glick, Carly R. Muletz-Wolz, Michael L. Power

Smithsonian Institution

Milk is a complex biochemical fluid that includes macronutrients known to facilitate infant growth and milk microbiomes that colonize infant microbial communities and mediate immune development. Milk microbiomes are increasingly recognized as a key component of the vertical transmission of microbes from mother to infant. Examining factors that shape milk microbiomes and milk-nutrient interplay across host taxa is critical to resolving the evolution of the milk environment and the degree to which milk microbes contribute to infant development. Using a comparative approach across four cercopithecine primate species housed at three facilities under similar management conditions, we test for the respective influences of host species and local environment (housing facility) on milk (a) macronutrients (fat, sugar, and protein analyses), (b) microbiomes (16S rRNA sequencing), and (c) predicted microbial functions. We found that milk macronutrients were structured according to host species, with no signal of local environment. In contrast, milk microbiomes and predicted function were strongly shaped by local environment and, to a lesser extent, host species. The milk microbiomes of rhesus macaques (*Macaca mulatta*) at two different facilities more closely resembled those of heterospecific facility-mates compared to conspecifics at a different facility. We found similar, facility-driven patterns of microbial functions linked to physiology and immune modulation, suggesting that milk microbiomes may influence population-level variation in infant development. These results provide novel insight into the complexity of milk and potential impact on infants across species and environments. Namely, these results suggest intriguing avenues for future research into how variation in milk microbiomes may shape infant development and lifelong health.

Investigating the gut microbiome of Skeleton Shrimp (*Caprella* sp.) in Delaware Bay

Malique Bowen*, Jason Rosales, Jennifer F. Biddle

University of Delaware

Marine organisms and their associated microbes often establish close ecological relationships that reflect their life history. Associated microbiomes can be influenced by morphological development, diet, and host identity in marine organisms. Our knowledge about these microbiomes excludes many anthropoid candidates in brackish coastal ecosystems. Delaware Bay hosts a number of cosmopolitan anthropoids suitable for microbiome studies, but little is known about their species diversity, specifically Caprellids of the family *Caprellidae* (order *Amphipoda*). Here, we use the skeleton shrimp (*Caprella* sp.) in the Delaware Bay to establish both its presence and species diversity and identify the core associated microbiome of individual species. Samples were collected from submerged artificial structures in Delaware Bay across three types of intertidal macroalgae. Microbiomes were characterized using 16S ribosomal RNA gene sequencing. We identified six potential Caprellid species in Delaware Bay and

observed distinct sex and species-based gut microbiome signatures from three of these species. Additionally, 16S ribosomal RNA gene sequencing revealed an abundance of sulfate reducing bacteria (SRB) and sulfur oxidizing bacteria (SOB) and microscopy showed sulfur granules inside the gut, indicating that Caprellids may interface with sulfur cycling with a role that has not yet been studied.

Optimizing Metagenomic Approaches for Enhanced Food Safety in Poultry Production Environment

Dhiraj Chundru*, Mostafa Ghanem, David Erickson, Xinyang Huang, Jianghong Meng

University of Maryland, College Park

Monitoring foodborne pathogens in agricultural environments is critical for regulatory compliance, source tracing, and outbreak management. Current methods, such as pathogen-specific culture-based or molecular assays, are time-consuming and may overlook vital foodborne pathogens not targeted by these tests. While Whole Genome Sequencing (WGS) has enhanced outbreak investigation accuracy, it still carries limitations from culture-based approaches. Shotgun Metagenomics (SGM) is proposed as a comprehensive solution for faster, holistic microbial community analysis to bolster food safety. However, implementing Metagenomics for this purpose faces challenges due to the absence of standardized workflows in laboratory procedures and data analysis pipelines. In this experiment, we aimed to validate standardized protocols for DNA extraction, and library preparation using mock microbial communities mixed with targeted samples like fecal droppings and litter samples from a broiler farm. This validation involves assessing relative and absolute taxonomic species diversity as well as alpha and beta diversity for 3 replicates of each test sample processed using two different commercially available DNA extraction, and library preparation kits. Subsequently, all libraries underwent sequencing on Illumina Hiseq. Preliminary results from analysis on TruSeq libraries on Miseq revealed better alpha diversity using the Qiagen kit for DNA extraction. Principal Coordinate Analysis (PCoA) plots (bray-Curtis) displayed dissimilarities among samples extracted using Zymo kits across various sample types. Moreover, relative taxa abundance from DNA extracted by the Qiagen kit from Zymo bacterial community standards closely matched the expected abundance. These findings highlight the impact that DNA extraction and library preparation kits could have on diversity metrics and taxa abundance on similar experiments. These experiments are initial steps to create a standardized workflow for laboratory procedures and data analysis to monitor Foodborne pathogens in poultry production environment. This could enhance food safety in agriculture by improving pathogen detection, contamination source identification, intervention assessment, and risk assessment models. It could pave the way for widespread adoption across agricultural systems, benefiting regulatory monitoring and food safety.

In vitro effects of starch with or without buffers on dairy cattle fecal microbiome

Shane Cronin*, K. Hobert, A. Biddle, T. F. Gressley

University of Delaware

During lactation, dairy cattle are fed rations with relatively high starch concentrations to increase energy levels. However, the influx of rapidly fermentable carbohydrates increases the risk of ruminal and hindgut acidosis. We sought to determine whether buffers could reduce the negative effects of hindgut acidosis on the fecal microbiome. Anaerobic bovine fecal samples (n = 4) were diluted to inoculate culture bottles with 6 dietary conditions in triplicate (FECC = no additive, STAR= 1% food grade corn

starch product, SBA = 1% starch and 0.05% buffer prototype 1 (containing 50% CaCO3, 25% MgO, and 25% crushed oyster shell), SBB = 1% starch and 0.05% buffer prototype 2 (containing 100% MgO), BA = 0.05% buffer prototype 1, BB = 0.05% buffer prototype 2). Samples were collected at 0, 3, 6, 12, 18, and 24 h to determine fermentation products, and 0, 6, and 24 h for microbial populations. Fermentation products were measured using colorimetric assays and high-performance liquid chromatography and analyzed in JMP Pro using the Fit Model procedure for standard least squares, with the main factors being treatment, time, and their interaction. DNA was extracted from pooled pellets and the V4-V5 region of the 16S rRNA gene was amplified and sequenced using Illumina MiSeq. Microbiome analysis occurred using QIIME2 and RStudio. Buffer inclusion increased pH, acetate, and propionate, while starch inclusion decreased pH and increased lactate, butyrate, and endotoxin. Microbial α-diversity was affected by treatment based on observed species ($P = 0.01$), but treatment did not affect Shannon index ($P > 0.05$). BA increased observed species compared to STAR ($P < 0.01$), while BB increased observed species as compared to FECC ($P = 0.02$). β-diversity Unweighted Unifrac was affected by treatment (PERMANOVA, $P = 0.02$; betadisper, $P < 0.01$), with BA and BB being different than STAR, SBA, and SBB at 24h ($P < 0.05$). At the 24h timepoint, differential abundance (ANCOM) and Spearman correlations identified *Desulfovibrionales* and *Clostridiales* increases in BB ($r = 0.68$ and 0.52, respectively), and *Betaproteobacteriales* and *Bacteroidales* decreases in STAR ($r = -0.53$ and -0.52, respectively). These data suggest that starch addition favored lactate production and reductions of microbial diversity, typical conditions of acidosis. Buffers promoted bacterial growth by increasing diversity while preventing lactate buildup or enhancing the utilization of lactate.

A Sensitivity Analysis of Methodological Variables for Metagenomic Sequencing

Samuel P. Forry*, Stephanie L. Servetas, Jennifer N. Dootz, Monique E. Hunter, Jason G. Kralj, James J. Filliben, Scott A. Jackson

National Institute for Standards and Technology

Background and Study Methodology:
Metagenomic sequencing (MGS) analysis results depend strongly on the methods specified for sample handling, library preparation, and data analysis. Herein, we used a full-factorial experimental design to assess the impact of several parameters on the MGS analysis of human stool samples. Specifically, MGS results were compared while systematically varying the: sample (n=5), operator (n=2), lot (n=2), extraction kit (n=2), variable region (n=2), and reference database (n=3). From these 240 datasets, a main effect was calculated for each methodological parameter, allowing direct comparison between biological variability and methodological biases.

Results and Conclusions:
Significant methodological bias was identified and attributed to the parameters of extraction kit, operator, and database. Notably, the quantified biases were similar in magnitude to the real biological differences measured between samples. Methodological bias was consistent across samples, while varying substantially between taxa within each sample, even within taxonomic clades (e.g., genera within a family). Using the quantified, taxa-specific methodological bias measurements, MGS results could be computationally corrected to improve comparability between datasets collected under differing protocols. The characterization demonstrated here can be used to optimize MGS protocols or to improve comparability between data acquired under different procedures. The results described here confirm that routine analysis of common microbiome reference materials provide a mechanism to improve comparability between MGS various methodologies.

VIRGO2: A Remastered Gene Catalog for Vaginal Microbiome Omics Analysis

Michael T. France, Issac Chaudry*, Madeline Alizadeh, Pawel Gajer, and Jacques Ravel

University of Maryland, Baltimore

A plethora of studies have implicated the vaginal microbiome in obstetric and gynecological health. However, the specific molecular mechanisms by which perturbations in the optimal microbiome-host interface contribute to disease remain poorly understood. This lack of understanding has hindered advancements in clinical standard of care. Omics approaches, especially metagenomics and metatranscriptomics, are instrumental in probing the intricate interface between host and microbiome. However, analyzing and interpreting microbiome microbial and functional composition in these large datasets is complex and challenging. A reference database that allows for rapid profiling of taxonomy, genetics, and function is essential for reproducible and accurate data analysis. Our previously published gene catalog, VIRGO, was designed to facilitate vaginal microbiome omics data analyses.

Here, we present VIRGO2, a remastered non-redundant gene catalog of vaginal microbes with expanded and improved taxonomic and functional annotations. Building upon the VIRGO, VIRGO2 offers broad geographic representation in its constituent datasets (from 5 continents) and more extensive taxonomic annotations (90% of the genes are taxonomically annotated). The catalog is built from 2,560 geographically diverse vaginal shotgun metagenomes and over 4,000 cultured isolate genomes (CIGs). Assembled contigs from metagenome samples were binned using a multiprong strategy, resulting in 85% of the contigs being incorporated into 15,142 metagenome-assembled genomes (MAGs).

Metagenomic and metatranscriptomic reads can be mapped to VIRGO2 to yield information on taxa relative abundance or gene expression, respectively. We validated the catalog using simulated metagenomic datasets, demonstrating significant improvement over VIRGO in determining microbiome taxonomic composition. Ultimately, VIRGO2 comprises nearly 2 million non-redundant genes, with over 90% of the genes having unambiguous taxonomic annotation. Non-bacterial species, such as viruses, and fungi that are common inhabitants of the vagina, are comprehensively represented in VIRGO2. In constructing VIRGO2, we identified novel *Fannyhessea*, *Beryella*, and *Prevotella* species, and explore in depth the structure and function of vaginal microbiome, and that across a broad geography.

Enrichment of apple microbiome and its survivability during simulated gastric digestion

Zhujun Gao*, Ryan A. Blaustein, Gail M. Bornhorst, Nitin Nitin, Rohan V. Tikekar

University of Maryland, College Park

Introduction:
Food and nutraceutical industries have concentrated on fermented foods or probiotic supplements using manually selected strains. This study investigated the potential of utilizing the natural microbiome on apples and understanding its survivability during simulated gastric digestion.

Method:
Fresh Golden Delicious and Empire apples were cut into wedges with peel on and transferred into Tryptic Soy Broth (TSB) with pre-adjusted pH at 7 or 5; or, the apples were blended with TSB. The samples were incubated at 30 °C for 1 to 4 day(s), and the microbiome was collected by centrifugation at 5750 rcf for 10 minutes. The microbiome was transferred into different matrices including deionized

water, apple sauce, sweet potato puree, chicken puree, or a water-in-oil emulsion. The augmented food matrix samples enriched with apple microbiome were 1:1 mixed with simulated gastric fluid (HCl, NaCl, gastric mucin, pepsin, and amano lipase A) and kept on an orbital shaker in a 37 °C incubator for up to 180 min. The pH of the digesta was adjusted to pH 4 at 0 min, pH 3 at 30 min, pH 2 at 90 min, and pH 1.5 at 150 min. Samples were taken every 30 min for bacteria enumeration.

Results:
The enriched apple microbiomes reduced from 8.44 ± 0.39 log CFU/ml to 7.55 ± 0.38 log CFU/ml in the first 90 minutes in deionized water for both apple cultivars from both enrichment condition. As pH decreased beyond the initial stage, microbiome inactivation was observed to be matrix-dependent. That is, the inclusion of fat and protein in the augmented food matrices significantly prolonged survivability of the apple microbiomes during the pH 3 digestion stage by 3 log CFU/ml compared to sweet potato puree (p<0.05), and the water-in-oil emulsion appeared to significantly protect the microbiome, as concentrations remained above 4 log CFU/ml throughout the entire digestion process. There was no significant difference in inactivation rates observed between the two apple cultivars or two enrichment pH levels within the same food matrix (p<0.05).

Significance:
The results demonstrated the feasibility and possible benefits of enriching natural microbiome from apples and the potential of reintroducing the natural microbiome back into other food products. This work will be extended to understand the ecological dynamics of the apple microbiome and implications for food quality and consumer health.

The Role of Periodontitis in Inflammatory Bowel Disease

Jeba Mercy Gnanasekaran*, Himanshi Tanwar, Jair Sinisterra, Devon Allison, Amitabh Das, Sathish Yesupatham, Mario Aimetti, Giacomo Baima, Massimo Costalonga, Yasir Dilshad, Man-Kyo Chung, Robert Ernst, Jean-Pierre Raufman, Cynthia Sears, Xuesong He, and Vivek Thumbigere-Math

University of Maryland, Baltimore

Objective: Our recent clinical study demonstrated an increased prevalence of periodontitis in individuals with inflammatory bowel disease (IBD). We sought to understand if periodontitis plays a causal role in IBD using pre-clinical models.

Methods: We employed interferon regulatory factor 8 (Irf8) knockout (KO) mice with inherent intestinal barrier defects and wild-type (WT) mice with intestinal barriers weakened by piroxicam medication. We induced periodontitis in WT and Irf8 KO mice using the ligature-induced periodontitis (LIP) model, facilitating the natural translocation of oral bacteria from the mouth to the gut. Additionally, oral commensals and periodontal pathogens were orally gavaged into piroxicam-treated WT mice and germ-free Irf8 KO mice. The impact of oral bacteria on the colonic mucosa was assessed by histology, RT-qPCR, Western Blot, RNA-seq, flow cytometry, ELISA, and other in vitro assays.

Results: LIP in Irf8 KO vs WT mice promoted increased translocation and colonization of native oral bacteria in the intestine, leading to colitis as evidenced by body weight loss, colon shrinkage, colonic inflammatory infiltrates, and loss of epithelial crypts. Similarly, inoculation of periodontal pathogens induced colitis in piroxicam-treated WT mice and germ-free Irf8 KO mice compared to untreated mice or those inoculated with oral commensals. Aggravated colitis was marked by increased colonic expression

of MHC class II, T cell receptors, and Th1/Th17 differentiation markers, suggesting that oral pathogens might promote colitis by potentially activating T cells via MHC II-TCR interactions. Furthermore, oral pathogens promoted increased IL17 secretion in the colonic lamina propria of LIP Irf8 KO vs WT mice.

Conclusion: The oral cavity may serve as a reservoir for colitogenic pathogens. In hosts with impaired intestinal barrier functions, concurrent periodontitis may enable certain oral pathogens to opportunistically colonize the intestine and trigger intestinal inflammation via Th1/Th17 pathways. Hence, oral health should be considered as an integral part of IBD evaluation and care.

13C-DNA stable isotope probing reveals active methanogen are more numerous in a restored freshwater wetland

Nora D. Hamovit*, Stephanie Yarwood,Taniya RoyChowdhury, Denise Akob, Gregory McCarty, Xuesong Zhang

University of Maryland, College Park

Wetlands are the largest natural source of methane (CH4), a potent greenhouse gas, produced by methanogens in suboxic soils. Methanogenesis rates are controlled by environmental and edaphic factors such as redox potential and alternative electron acceptor availability. Presumably rates are also dependent on the quantity and composition of the active methanogen community, but few studies have targeted these active communities directly. Following on previous research that observed greater CH4 emissions from restored compared to natural wetlands, we collected intact soil cores from both a restored and natural freshwater depressional wetlands on Maryland's Delmarva Peninsula (USA). The restored site is a prior converted agricultural field restored in 2004 and both sites are part of a broader watershed emptying into the Chesapeake Bay. Intact cores, encased in glass sleeves, were incubated under either a suboxic or oxic condition, mimicking the sites seasonal hydrology, with the addition of 13C labelled acetate. At the end of the incubation DNA was extracted and fractioned using 13C-DNA stable isotope probing. Bacteria and archaea actively metabolizing acetate were identified by performing quantitative stable isotope probing on 16S rRNA gene amplicon sequences quantified using the same 16S rRNA gene primers. Regardless of the redox potential, the restored site contained a higher abundance of acetotrophic methanogens putatively identified as *Methanosarcina*. In contrast, soils from the natural wetland that were incubated under suboxic conditions contained high abundances of iron and sulfate reducers but few active methanogens. These findings suggest that restored wetlands may support more methanogens, some of which may be oxygen insensitive, compared to their natural counterparts, where the presence of alternative electron acceptors may lead to metal reducers decreasing CH4 production. These results highlight that fundamental microbial interactions present in the natural site have not yet been re-established in the restored wetland.

Changes in growth rate and gut microbiome of an important animal waste upcycler reared on horse manure

Kasey Hobert*, Destiny Mann*, Michael Crossley, Amy Biddle

University of Delaware

As insects become a sustainable protein source for people and animals, it is important to understand the factors affecting their growth and development. The black soldier fly (*Hermetia illucens*) is a common insect used for agricultural waste upcycling. They are known to alter the microbiome of their gut and

surrounding environment which offers insight to the mechanisms assisting in the breakdown of waste streams. Various animal manure has been evaluated as rearing substrates for black soldier fly larvae (BSFL), but their performance on horse manure and subsequent effects on their core microbiome have yet to be evaluated. This study describes BSFL growth and changes to the gut microbiome associated with horse manure to better understand the potential of using these insects to upcycle animal waste. BSFL colonies were raised on 4 substrates (manure from two horses, chicken and fly feed). Every 7 days BSFL were measured for growth and culled for microbiome sampling via 16S rRNA sequencing. QIIME2 (v. 2023.2) and R packages *stats* and *phyloseq* were utilized for statistical analysis. When reared on chicken or fly feed BSFL grew to over twice the average mass of those reared on horse manure. Percent larval mortality and proportion of larvae reaching the prepupal stage showed no evidence of differences between substrates, indicating that the larvae were developing at similar rates. β diversity, measured according to weighted and unweighted UniFrac, revealed significant changes in microbial communities between substrates over time in both adonis and betadisper testing. Differential abundance testing with ALDEx2 identified 41 genus level taxa that significantly differed in at least one substrate with *Actinomyces* being higher in fly feed, *Enterococcus* higher in chicken and fly feed, and *Bacillus* higher in manure samples. Overall, though BSFL accumulated less biomass when reared on horse manure, survival and developmental rates were similar among substrates, suggesting potential for some degree of upcycling of horse manure with BSFL. It is unclear whether differences in microbiome profiles of BSFL grown on horse manure vs feed samples indicate levels of specific nutrient availability, or interactions between the microbiomes of the BSFL and each substrate. Disentangling the relative contributions of substrate quality and microbial contribution to BSFL performance is critical for understanding the opportunities and limitations of upcycling of animal wastes with insects.

Integrating compositional and functional content to describe vaginal microbiomes in health and disease

Johanna B. Holm*, Amaury Maros, Michael T. France, Pawel Gajer, Bing Ma, Rebecca M. Brotman, Michelle Shardell, Larry Forney, and Jacques Ravel

University of Maryland, Baltimore

A *Lactobacillus*-dominated vaginal microbiome provides the first line of defense against adverse genital tract health outcomes. However, there is limited understanding of the mechanisms by which the vaginal microbiome modulates protection, as prior work mostly described its composition through morphologic assessment and marker gene sequencing methods that do not capture functional information. To address this gap, we developed metagenomic community state types (mgCSTs) which uses metagenomic sequences to describe and define vaginal microbiomes based on both composition and functional potential. MgCSTs are categories of microbiomes classified using taxonomy and the functional potential encoded in their metagenomes and reflect unique combinations of metagenomic subspecies (mgSs), which are assemblages of bacterial strains of the same species, within a microbiome.

We demonstrate that specific mgCSTs are associated with demographics such as age and race, as well as vaginal pH and Gram stain assessment of vaginal smears. A subset of mgCSTs, including three of the six predominated by *Gardnerella* mgSs, as well as a mgSs of *L. iners*, were associated with a greater likelihood of bacterial vaginosis diagnosed by Amsel clinical criteria. The *L. iners* mgSs encoded enhanced genetic capabilities for epithelial cell attachment that could facilitate cytotoxin-mediated cell lysis. In other cohort studies, mgCST 22 (predominated by *Gardnerella* mgSs 2) was associated with an increased incidence of sexually transmitted infections and recurrent bacterial vaginosis.

MgSs and mgCST classifiers were developed for which source code is provided and may be adapted for use by the microbiome research community. MgCSTs are a novel and easily implemented approach to reduce the dimension of complex metagenomic datasets, while maintaining their functional uniqueness. MgCSTs enable investigation of multiple strains of the same species and the functional diversity in that species. Importantly, our findings support the hypothesis that functional differences between vaginal microbiomes, including those that may look compositionally similar, are critical considerations in vaginal health. Ultimately, mgCSTs may lead to novel hypotheses concerning the role of the vaginal microbiome in promoting health and disease, and identify targets for novel prognostic, diagnostic, and therapeutic strategies to improve women's genital health.

Exploring the Diversity of Culturable Endophytic Bacteria in leafy *Brassica* spp. intended for fresh consumption

Diksha Klair*, Marissa Lee Sang, Shirley A. Micallef

University of Maryland, College Park

Endophytic microbiota, encompassing the diverse microorganisms residing within plant tissues, play a pivotal role in influencing plant health, growth, tolerance to stresses and overall ecosystem robustness. The endophytes within fresh leafy vegetables may also influence human gut health, as these microorganisms are not destroyed during food preparation. Yet, the diversity of the endophytic component of fresh crop microbiomes remains understudied. Our research focuses on describing the diversity of endophytic bacteria within leafy Brassica species, Bok choy (*Brassica rapa* subsp. *chinensis*, Greenand Red mustard (*Brassica juncea*), Tatsoi (*Brassica rapa* var. *narinosa*), and Green and Red mizuna (*Brassica rapa* var. *nipposinica*). Crops were grown at the University of Maryland TerpFarm, Central Maryland Research and Education Centre, Upper Marlboro, Maryland, in fall 2022 and 2023 in high tunnels. Sample collection entailed uprooting plants, preserving root integrity, and transporting on ice for laboratory processing. Leaves and roots underwent thorough surface sterilization. Utilizing culture-based methods, tissue extracts were plated on nutrient agar medium, incubated at 28°C and 37°C, and colonies with distinctive morphologies were picked and streaked for isolation. DNA extraction, PCR amplification of the full length 16S rRNA gene region, and sequencing was performed on all isolates. From the Bok choy leaves, 4 out of 12 isolates were classified as *Acinetobacter*, alongside *Pseudomonas*, *Chryseobacterium*, *Paenibacillus*, *Sphingomonas*, *Rhizobium* and *Priesta* genera. Intriguingly, all eight endophytes from the root tissue exclusively belonged to *Pseudomonas*, indicating its dominance, and suggesting influence over other symbiotic microorganisms in plant roots. The difference in endophyte diversity detected between roots and leaves suggests that bacteria carry our distinct functions and contribute to different ecosystem services in the two plant niches below and above ground. The identification of specific endophytic microbiota associated with leafy *Brassica* crops has direct implications for food safety and crop resilience to biotic and abiotic stresses, offering opportunities to develop biocontrol strategies and climate resilient approaches and reduce reliance on chemical interventions. Unraveling the dynamics of endophytic microbiota within diverse *Brassica* spp. advances our comprehension of plant-microbe interactions for sustainable agriculture and food safety.

What's brewing? Impacts of cultivation management on the *Coffea arabica* soil microbiome

Steve Kutos*, Ruth Bennett, Carly Muletz Wolz

Smithsonian Institution

Coffea arabica (coffee) is cultivated on ~28-million acres and is essential to local economies in the tropics. Globally, most coffee is farmed in open monocultures despite evidence that agroforestry systems with shade trees can increase resiliency of coffee productivity and associated livelihoods. Open questions remain, however, on how aboveground coffee farm management affects local ecosystem properties, especially soil microbial communities. To test if soil microbiomes respond to different management, we collected soil samples at coffee farms in Colombia and El Salvador that differed in management system (sun grown vs shade grown) and soil samples in Peru that varied in agroforestry shade tree type (native vs. non-native tree species). From these soils, we sequenced microbial DNA on an Illumina MiSeq to identify bacterial and fungal community diversity and composition. We found that each country had distinct soil microbial communities, as expected. In El Salvador and Colombia, both bacterial and fungal community composition differed between sun and shade grown farms, while alpha diversity only differed in bacterial communities in El Salvador with lower diversity in sun grown coffee soils. Within Peru agroforestry, bacterial and fungal composition differed between non-native shade trees (*Eucalyptus grandis* and *Pinus tecunumanii*) and native shade trees (dominated by *Inga* spp.) farms, but alpha diversity was similar between shade tree farm types. Across all farms, composite microbial networks showed similar topology, but distinctions in connectors and hub taxa showcasing regional differences in potentially key microbial taxa. Our findings indicates that aboveground coffee farm management impacts belowground microbial biodiversity.

Characterization of Soil and Lettuce Resistomes from Harvest Through Storage in Modified Atmosphere Packaging

Susan R. Leonard*, Taylor K.S. Richter, Mark K. Mammel, Ivan Simko, and Maria T. Brandl

US Food and Drug Administration

It has been demonstrated that soil microbial communities contribute to the lettuce phyllosphere microbiome. Leafy vegetables carrying bacteria that harbor antimicrobial resistance genes (ARGs) may provide a pathway for those genes to enter the human microbiome and metal and biocide resistance genes (MRGs and BRGs) may contribute to co-selection of ARGs depending on the selection pressure encountered. In this work, the distribution of resistomes associated with lettuce from field through cold storage in modified atmosphere packaging (MAP) was investigated. ARGs, MRGs, and BRGs in soil and lettuce phyllosphere microbiomes were profiled and compared to determine possible transfer between soil and lettuce as well as persistence during lettuce processing and storage. Shotgun metagenomic sequencing was performed on a total of 225 samples consisting of surface soil, harvested lettuce heads, processed lettuce (cut and washed), and processed lettuce cold-stored in MAP for five different harvests in Salinas, California. Sequencing was performed on an Illumina NextSeq platform generating paired-end 150 bp reads, and classification of resistance determinants in the sequence datasets was accomplished using MEGARes 2.0. Overall, a higher number of resistance genes per million reads was found in processed lettuce after storage (9.8) compared to processed lettuce before storage (0.74) (Wilcoxon Rank Sum, P<0.001). MRGs were prevalent in both soil and lettuce, with copper resistance particularly high in lettuce. BRGs were most frequent in processed lettuce before storage. In the five separate

harvests, between three and 59 different resistance gene alleles were identified in both soil and at least one lettuce sample. Omitting genes conferring both drug and biocide resistance, and including all samples, the greatest percentage of ARGs belonged to the beta-lactam class (30%) followed by aminoglycosides (6.7%), and specific allele sequences from both classes were observed in common between soil and lettuce. These results provide insight into the transfer of antimicrobial resistance genes from soil to the lettuce phyllosphere and ready-to-eat packaged lettuce, thus their spread in the environment and potential to enter the human food chain.

City and Neighborhood Scale Wastewater Based Epidemiology Monitoring and Modeling of SARS- CoV-2, Influenza A and RSV in Maryland

Yuzhu Mao, Birthe Veno Kjellerup

University of Maryland, College Park

Wastewater-based epidemiology has become a popular and important public health tool to monitor the communities' viral spread after the outbreak of SARS-CoV-2, which is cost-effective, unbiased, and can provide warnings before the large-scale outbreaks. However, most research set sampling locations at WWTPs, which represents a big scale's wastewater situation, may not offer detailed regional health insights, and most modeling took place during the global health emergency, marked by high infection rates and accurate clinical data. Therefore, the goal of this study is to compare the PMMoV concentrations from different scale's sewer systems, find the correlation of its concentration with populations; and analysis the concentration of SARS-CoV-2, RAV, and IAV in wastewater and clinical case counts to develop a time series model to estimate case counts pre and post the global health emergency period. To address these objectives, wastewater samples were collected from 5 public WWTPs and 5 pumping stations proximate to communities in Montgomery County, Maryland, after concentration, the presence of PMMoV, SARS-CoV-2, Influenza A, RSV virus RNA in the supernatant was analyzed by RT-qPCR using the CDC N1 primer sets, CDC Influenza primer 1, revised WHO assay 1, respectively. The PMMoV concentrations from county and neighborhood were linear regression with served populations but show very weak correlation. There were significant correlations found between the 3 viruses concentration and the number of daily new cases in corresponding catchment areas, besides, the PMMoV normalization largely enhanced the correlation. VAR model has been successfully applied and predicted the exact clinical data using the wastewater data after CDC stopped the emergency for SARS-COV-2. These findings have important implications for public health to have a better understanding of real clinical situation and provides a reference for the neighborhood scale's sampling location chosen.

Characterizing the Vaginal Microenvironment of Transmasculine Individuals Accessing Testosterone Therapy

Bern Monari*, Hannah Wilcox, Pawel Gajer, Jason Hallarn, Greta Bauer, Jessica Prodger, Jacques Ravel

University of Maryland, Baltimore

Recent estimates from UCLA's School of Law Williams Institute indicate that more than 1.3 million adults in the United States identify as transgender or gender diverse (TGD), meaning that their gender identity does not align with their sex assigned at birth. There are several options for TGD individuals to align their gender presentation with internal sense of gender, including social shifts, hormone therapy, and surgical procedures. While not all TGD persons will choose medical transition, more than 75% of TGD persons report desiring or accessing hormone therapy. Transmasculine individuals experience male or

masculine gender identity, despite being assigned female at birth, and may use testosterone therapy to masculinize the body. Sustained use of testosterone (>1 year) in transmasculine persons has been shown to increase serum testosterone levels to cis-male levels while significantly lowering serum estrogen, and has been associated with a variety of adverse genital symptoms, including vaginal atrophy, dryness, irritation, non-menstrual bleeding, and pain during sex. As genital surgery remains rare (<2% of transmasculine persons), a growing proportion of transmasculine individuals possess a vagina with high levels of testosterone and low levels of estrogen. The vaginal microbiota in cisgender women is optimally characterized by high relative abundance of *Lactobacillus* species, while non-optimal states (such as bacterial vaginosis) are characterized by anaerobes including *Gardnerella*, *Fannyhessea*, and *Prevotella* species. A published report on twenty-eight transmasculine individuals revealed a paucity of Lactobacillus species and enrichment of strict and facultative anaerobes in the vaginal microbiota, but did not make any casual associations with symptoms or local immune status. We therefore launched the TransBiota study to characterize the microbiota and immune status of 91 transmasculine persons accessing sustained testosterone therapy. We will report on the longitudinal study design, participant characteristics, composition and structure of vaginal microbiota, and its association with local immune status and symptomology.

Host and environmental microbiomes in a disease system in Appalachian salamanders

Carly R. Muletz-Wolz.*, Jimenez, R.R., Osborne, O., Byrne, A.Q., Kutos, S.J., Cleland, T.P., Gratwicke, B., Ellison, A.

Smithsonian Institution

Host evolutionary history and environment can shape animal microbiomes. The degree to which these factors drive host microbiome variation is important to quantifying and predicting host-microbial and host-pathogen dynamics. Amphibians serve as a useful model to study host-environmental microbial sharing and disease dynamics as environmental microbiomes may be a rich source of anti-pathogen taxa. Losses of amphibian have occurred globally driven by fungal infection with *Batrachochytrium dendrobatidis* (Bd). We determined the eco-evolutionary processes structuring skin microbiomes and their association with Bd infection in 10 salamander species. We sampled species from six genera (*Ambystoma, Desmognathus, Eurcyea, Gyrinophilus, Notophthalmus,* and *Plethodon*) at 12 sites in Central Appalachians, USA (n = 222) and their environment (pond, stream or forest, n = 118). We found Bd infection in six species (*E. bislineata, P. cinereus, P. glutinosus, D. fuscus, G. porphyriticus, N. viridescens*), with *N. viridescens* having the highest Bd prevalence and loads. High relative abundance of putative Bd-inhibitory bacteria was correlated with decreased Bd prevalence across salamander species. Both host evolutionary history and environment impacted skin microbiomes; host evolutionary history had a stronger effect and we detected phylosymbiosis (correlated microbiome change with evolutionary divergence), even after correcting for environmental microbiomes. In three salamander species with robust sampling (*N. viridescens, E. bislineata* and *P. cinereus*), we investigated environmental selection and bacterial associations effects on skin microbiomes. Generally, salamanders hosted more unique bacteria taxa overall and more functional Bd-inhibitory bacteria than found in the environment. Some Bd-inhibitory bacteria were shared between environment and salamander species, ranging from 27 to 29 bacterial taxa, and were detected as hub taxa (highly connected) in networks. Bd-infected *N. viridescens* had a high proportion of negative associations in bacterial networks (40%), while uninfected *N. viridescens, P. cinereus* and *E. bislineata* had fewer negative associations (30%, 30% and 13%,

16

respectively). Our findings suggests that environmental bacterial reservoirs are important to maintaining microbiomes within the constraints of host evolutionary history and that microbial-Bd interactions drive competition in the microbiome.

Fructose Epimer Utilization by Gut Bacteria

Glory Minabou Ndjite, Audrey Burnim, Xiaofang Jiang, Brantley Hall

University of Maryland, College Park

Due to the rise in obesity and type II diabetes, there has been an increased interest in the search for sweeteners that do not contribute to the post-prandial glucose response nor lead to weight gain. These include rare sugars such as D-Allulose and D-Tagatose, the C-3 and C-4 epimers of fructose, respectively. These molecules are recognized by taste receptors as sweet but are unable to be metabolized and, therefore, do not contribute to postprandial glucose response. This, combined with improvements in their production by industrial-scale bacterial fermentation, has made D-Allulose and D-tagatose increasingly commercially viable sugar substitutes. Due to their relative novelty and rarity in nature, the effects of these sugars on the gut microbiome have yet to be thoroughly investigated. Therefore, we sought to study which microbes can metabolize D-Allulose and D-Tagatose as a carbon source. We show that D-Tagatose has higher rates of bacterial growth than D-Allulose as a greater variety of bacteria displayed the ability to utilize it as a carbon source. These findings will have long-term implications in food science and gut microbiome studies and allow us to better understand how our diet modules our gut microbes.

The Vaginal Microbiome: Implications for Treating Vaginal Disease

Baris Ozdinc, Holly Hack, Dana Walsh, Kelly Moffat*

CosmosID

Vaginal microbiome communities can be categorized into community state types (CST) based on dominance by specific lactobacilli species. These CSTs are globally present and may indicate risk of vaginal infection. In this work we compare the vaginal microbiota of women from three different countries: Sweden (N = 45), China (N = 35) and two groups from the USA (N = 51 and N = 30). We imported this whole genome sequencing data from publicly available studies and performed a comparative microbiome taxonomic and functional analysis using the CosmosID-HUB. We found every CST within each group and the samples clustered according to CST rather than country, implying a broad applicability of the CST classification system. However, when we predicted functionality of the microbial communities, we found that they clustered by country rather than CST. This suggests that vaginal community functionality may be influenced by location, regardless of CST. One functional group consisted of samples with enriched abundance of genes coding for mucin degradation, which has been previously associated with pathogenic potential. The women in these group were from a mix of the three countries. These results suggest that, in a healthy state, CSTs are globally present, but their functionality may be influenced by location-specific factors. However, there may be a subset of functions with greater pathogenic potential that is not location-associated. These women may be more prone to vaginal infections and their identification may aid physicians in catching and treating vaginal disease early on.

Probiotic bacterial extracellular vesicles treat mouse model of colitis after oral delivery

Nicholas Pirolli*, Zuzanna Mamczarz, Dan Levy, Talia Solomon, Emily Powsner, Raith Nowak, Chris Bridgeman, Sulayman Khan, Laura Reus, William Bentley, Steven Jay

University of Maryland, College Park

Probiotic bacterial extracellular vesicles (BEVs) have emerged as a promising alternative to live microbiome therapeutics for treatment of inflammatory diseases. Probiotic BEVs are cell-secreted membrane nanovesicles that contain abundant protein and RNA cargo and can be administered in a "drug-like" fashion with more reliable PK/PD compared to live therapies. After administration, probiotic BEVs offer cargo protection from degradation in harsh environments and delivery across biologic barriers to reach target cells. Furthermore, probiotic BEVs can potentially be loaded with designer therapeutic cargo for delivery to a variety of tissues. Thus, probiotic BEVs hold potential as both standalone therapeutics and as engineered drug delivery vehicles.

Our lab has found probiotic BEVs derived from several species of lactic acid bacteria (LAB; historically, Lactobacillus) are particularly effective treatments in the dextran sodium sulfate (DSS) mouse model of colitis. In our model, oral gavage of LAB BEVs produced superior therapeutic efficacy compared to both BEV-depleted probiotic conditioned media and BEVs secreted by a non-probiotic strain of Escherichia coli (DH5α). Additionally, qPCR and flow cytometry analysis reveal probiotic BEVs (i) increase regulatory T cells and limit inflammatory responses (e.g., IFNy, IL-1β, TNF-α), and (ii) increase genes related to epithelial barrier integrity (E-cadherin, IL-22). Finally, using an in vitro macrophage cytokine release assay and a DSS colitis mouse model, we identified certain species of LAB BEVs with more potent therapeutic efficacy.

In addition to LAB BEV's therapeutic effects, we have also elucidated their exciting potential for oral delivery of biologics by demonstrating LAB BEVs can (i) protect biologic cargo in simulated GI fluids and (ii) cross a model human GI barrier comprised of co-culture of Caco-2 epithelial cells and mucus-producing HT29-MTX cells. Our current work is focused on evaluating the role of the microbiome in therapeutic efficacy of LAB BEVs, and engineering probiotic BEVs for designer cargo loading to improve treatment of colitis.

Early-life fecal transplantation from high muscle yield rainbow trout to low muscle yield recipients accelerates somatic growth through respiratory and mitochondrial efficiency modulation

Guglielmo Raymo* Ali Ali, Ridwan Ahmed, Mohamed Salem

University of Maryland, College Park

Previous studies conducted in our lab revealed microbial assemblages to vary significantly between high (ARS-FY-H) and low-fillet-yield (ARS-FY-L) genetic lines in adult rainbow trout. We hypothesized that a high ARS-FY-H donor microbiome can accelerate somatic growth in microbiome-depleted rainbow trout larvae of the ARS-FYL line. Germ-depleted larvae of low ARS-FY-L line trout reared in sterile environments were exposed to high or low-fillet yield-derived microbiomes starting at first feeding for 27 weeks. Despite weight-normalized diets, somatic mass was significantly increased in larvae receiving

high fillet yield microbiome cocktails at 27 weeks post-hatch. RNAseq from fish tails reveals enrichment in NADH dehydrogenase activity, oxygen carrier, hemoglobin complex, gas transport, and respiratory pathways in high fillet-yield recolonized larvae. Transcriptome interrogation suggests a relationship between electron transport chain inputs and body weight assimilation, mediated by the gut microbiome. These findings suggest that microbiome payload originating from high fillet yield adult donors primarily accelerates juvenile somatic mass assimilation through respiratory and mitochondrial input modulation. Further microbiome studies are warranted to assess how increasing beneficial microbial taxa could be a basis for formulating appropriate pre-, pro-, or post-biotics in the form of feed additives and lead to fecal transplantation protocols for accelerated feed conversion and fillet yield in aquaculture.

Long-term impacts of untreated dairy manure on the microbiome and pathogenic *E. coli* persistence in agricultural soil

Taylor K.S. Richter*, Michael Kauffman, Mark K. Mammel, David W. Lacher, Gireesh Rajashekara, and Susan R. Leonard

US Food and Drug Administration

Untreated dairy manure is a source of nutrients for agricultural soils that improves soil health and aids in produce production. However, as a natural reservoir for Shiga toxin-producing *Escherichia coli* (STEC), untreated dairy manure can be a source of this pathogen to the agricultural environment and to fresh produce. Given the impacts of foodborne STEC outbreaks on growers and on public health, it is necessary to understand the longevity of the impacts of manure application on the pathogen risk in the soil as well as better understand the ecological and environmental conditions that contribute to STEC survival in the agricultural soil environment. Using shotgun metagenomic sequencing, this project explores the microbiome of soil with and without a manure amendment, focusing on the *E. coli* population over time alongside changes in the soil microbiome and abiotic properties. Two nearby farms in Ohio, one using an untreated dairy manure amendment and one that does not use a biological soil amendment of animal origin, were sampled at least monthly for a year. Soil and manure samples were used for DNA extraction and shotgun metagenomic sequencing of the microbiome, enrichment of the samples targeting the *E. coli* community and shotgun sequencing of the enriched culture, as well as physical and chemical analyses of the soil. Microbial taxonomy was determined using an in-house k-mer-based program. *E. coli* serogroup and Shiga toxin genes (stx1 and stx2) were identified using publicly available and privately curated databases. The results show that the impacts of the manure on the soil lasted for four weeks by several measures including higher water content, higher bacterial alpha diversity, higher *E. coli* diversity (the number of *E. coli* O serogroups), and more frequent STEC detection when compared to the nonamended soil. Metagenomic analysis consistently identified STEC toxin genes in manure samples as well as in the amended soil in the four weeks following amendment, which dissipated prior to tilling and planting. Outside of this initial amendment period, stx genes were identified periodically in both fields throughout the year. STEC detection was significantly correlated with higher *E. coli* diversity. This work expands upon the knowledge of conditions that support STEC persistence in the produce growing environment and its longevity following amendment, though more work is required to determine the additional sources of STEC emergence in the soil.

Antimicrobial Resistance in Urban Agriculture Environments

Autumn Salcedo*, Qingyue Zeng, Kevin Lam, Derek Konsen, Ryan Blaustein*

University of Maryland, College Park

Urban agriculture offers benefits from engaging communities to expanding local food production and nutritional security. Organic soil amendments are typically used to restore soil health in these systems, though implications for food safety are not well established. We conducted a microbiological field survey of local farms and community gardens in the DMV area (n=7 sites) to test the hypothesis that site-specific factors (e.g., land history, intrinsic soil properties, management practices) impact levels of food safety indicators and antibiotic-resistant bacteria present. As expected, concentrations of total bacteria in amended soils were significantly greater than those in bulk soil at respective farm sites. The amendment-induced 'priming effect' associated with reduced fractions of tetracycline-resistant (Tet) bacteria, as well as site-specific cases for reductions in ampicillin-resistant (Amp) bacteria and coliforms. We further observed a high frequency of multi-drug resistant phenotypes exhibited among the Amp and Tet isolates, suggesting universal responses of the native soil biota to putative chemical stressors in the environment. Future research will focus on understanding microbial interactions that occur in response to soil management with applications to promote overall healthier food systems.

A genome catalog of the infant skin microbiome indicates microbial diversity, function, and transmission in early life

Zeyang Shen*, Lukian Robert, Milan Stolpman, You Che, Katrina J. Allen, Richard Saffery, Audrey Walsh, Angela Young, Jana Eckert, Clay Deming, Qiong Chen, Sean Conlan, Karen Laky, Jenny Min Li, Lindsay Chatman, Sara Saheb Kashaf, NISC Comparative Sequencing Program, VITALITY team, Heidi H. Kong, Pamela A. Frischmeyer-Guerrerio, Kirsten P. Perrett, Julia A. Segre

National Institutes of Health

Metagenome-assembled genomes have greatly expanded the reference genomes for skin microbiome. However, the current reference genomes are largely based on samples from adults in North America and lack representation from infants and individuals from other continents. Here we use deep shotgun metagenomic sequencing to profile the skin microbiota of 215 infants at age 2–3 months and 12 months who are part of the VITALITY trial in Australia as well as 67 maternally matched samples. Based on the infant samples, we present the Early-Life Skin Genomes (ELSG) catalog, comprising 9483 prokaryotic genomes from 1056 species, 206 fungal genomes from 13 species, and 39 eukaryotic viral sequences. This genome catalog substantially expands the diversity of species previously known to comprise human skin microbiome and improves the classification rate of sequenced data by 21%. The protein catalog derived from these genomes provides insights into the functional elements such as defense mechanisms that distinguish early-life skin microbiome. We also find evidence for microbial sharing at the community, bacterial species, and strain levels between mothers and infants. Overall, the ELSG catalog uncovers the skin microbiome of a previously underrepresented age group and population and provides a comprehensive view of human skin microbiome diversity, function, and development in early life.

Vaginal bacterial extracellular vesicles as mediators of gynecologic health

Darby Steinman, Robert Kirian, Hannah Zierden*

University of Maryland, College Park

The composition of the vaginal microbiome significantly impacts gynecologic and obstetric outcomes. In contrast to the gastrointestinal microbiome where diversity is considered optimal, a healthy vaginal environment is one dominated by a single species of bacteria, typically one of four *Lactobacillus* spp, whereas a dysbiotic vaginal environment is characterized by a polymicrobial overgrowth of facultative anaerobes. Clinically referred to as bacterial vaginosis (BV), this affects ~30% of women in the United States, and is associated with an increase in risk for sexually transmitted infections, urinary tract infections, infertility, pelvic inflammatory disease, spontaneous miscarriage, and preterm birth. Despite these established associations, the exact mechanisms by which bacteria facilitate these outcomes remains unclear. Emerging work suggests that bacterial extracellular vesicles may contribute to microbe:host communication in the female reproductive tract.

Bacterial extracellular vesicles (bEVs) are cell-derived, lipid-bound, nano-sized particles produced by both Gram-positive and Gram-negative bacteria, including those present in the vaginal microbiome. bEVs carry small molecules, nucleic acids, and proteins long distances in the body, enabling functional changes to host tissues. bEVs have inherent targeting and barrier crossing abilities, further facilitating their role in microbe:host communication. Here, we report differences in the size, ζ-potential, and concentration of bEVs isolated from cultures of different strains of vaginal bacteria (*L. crispatus*, *L. iners*, *Gardnerella vaginalis*) which may contribute to differences in clinical outcomes based on the composition of the vaginal microbiome. By establishing differences in bEVs, bEV cargoes, and bEV mobility through human CVM, we may revolutionize the way that a dysbiotic vaginal environment is treated, and identify novel therapeutic mechanisms for promoting healthy gynecologic and obstetric outcomes.

Role of Gut Microbiota in Osteoclast Regulation and Skeletal Homeostasis

Himanshi Tanwar*, Jeba Mercy Gnanasekaran, Devon Allison, and Vivek Thumbigere-Math

University of Maryland, Baltimore

Objective: With the advent of human microbiome project, it is becoming clear that the gut microbiota plays a critical role in osteoclast regulation (OC) and skeletal homeostasis. However, the underlying contributory mechanisms remain unclear. Previously, we have shown that interferon regulatory factor 8 (Irf8) functions as a negative regulator of OC. Specific pathogen-free (SPF) Irf8 KO mice harbor dysbiotic gut microbiota and exhibit increased OC activity and osteoporosis phenotype. To delineate the gut microbiota's immunoregulatory effects on OC regulation and skeletal homeostasis, we generated novel germ-free (GF) Irf8 KO mice.

Methods: GF wild-type (WT) and Irf8 KO mice, and their respective SPF mice were subjected to micro-CT analysis, OC formation and resorption assays, and flow cytometric analysis of immune cells from blood. Further, GF WT and Irf8 KO mice were colonized with gut microbiota from their respective age-matched SPF mice to generate conventionalized (Conv) mice.

Results: Both GF WT and Irf8 KO mice displayed increases in in-vivo trabecular bone volume, trabecular

number, trabecular thickness, and correspondingly decreases in in-vitro OC numbers, resorption activity, and OC-related genes/proteins when compared with their respective SPF mice. These phenotypes were more prominent in GF Irf8 KO vs WT mice. Further, we noted diminished monocyte population in GF vs SPF WT mice, which may partially explain the decreased osteoclastogenesis noted in GF WT mice. However, GF vs SPF Irf8 KO mice exhibited relatively increased monocytes, which suggests that apart from regulating the volume of OC precursor production, gut microbiota may further shape osteoclastogenesis through other mechanisms, such as transcriptional and epigenetic pathways. The altered skeletal, osteoclastogenesis, and immune cell phenotypes observed in GF WT and Irf8 KO mice were normalized following gut microbiota colonization in Conv mice.

Conclusion: This preliminary study provides novel insights into the role of gut microbiota in OC regulation and skeletal homeostasis.

Characterization of Upper Respiratory Tract Microbiome of Chickens following *Avibacterium paragallinarum* Infection

Madhusudan Timilsina*, Dhiraj Chundru, Mostafa Ghanem

University of Maryland, College Park

Avibacterium paragalinarum (AP), is the causative agent of infectious coryza, an upper respiratory tract (URT) disease of chickens that leads to decreased egg production in layers and early marketing of broilers. Pathogens could influence the microbiome of the chicken's respiratory tract leading to more severe diseases in chickens. The impact of AP infection on the microbiome of URT of chicken has not been studied and whether different AP strains influence the microbiome differently is also unknown. The aim of this study was to investigate the effect AP infection on the URT microbiome and if different strains of AP influence the URT microbiome differently. In this study, four different field strains of *Avibacterium paragallinarum* were used to infect four groups of specific pathogen-free chickens at four weeks of age, a fifth group was used a control. Pooled choanal swabs were collected from infected chickens and the control group at various time points post-challenge, and DNA extraction was performed for next-generation sequencing based on the microbial 16s rRNA gene. The results showed that the microbiome of the chicken's URT was primarily composed of *Firmicutes*, followed by *Proteobacteria, Actinobacteria, Bacteroidota*, and other phyla. Following AP infection, there was a significant decrease in the alpha-diversity of the URT microbiome. Additionally, beta-diversity analysis revealed significant differences between the infected and non-infected groups, indicating a distinct microbial composition in response to AP infection. The microbial diversity reached the lowest level on 4th day-post infection (DPI) and returned to normal level on 9th DPI. There was no significant difference in the alpha-diversity in the microbiome due to infection by different strains of AP, but there was significant difference in beta-diversity. These findings suggest that AP infection has a significant impact on the composition and diversity of the URT microbiome in chickens, highlighting the need for further studies on the effect of different respiratory pathogens on URT microbiome and their impact on poultry health and disease severity.

Antimicrobial Peptides in Appalachian Salamanders

Julian Urrutia-Carter*, Randall Jiménez, Owen Osborne, Amy Ellison, Brian Gratwicke, Carly Muletz Wolz

Smithsonian Institution

Antimicrobial "host-defense" peptides (AMPs) are an innate defense mechanism found in nearly all organisms. In amphibians, AMPs are secreted from granular glands in their skin, serving as a protective layer against invading pathogens. AMPs work alongside other microbial symbionts to form the "first layer of defense" in amphibians. Amphibian AMPs are highly variable in their primary structure and function, allowing them to enact various mechanisms of action. Many amphibian AMPs have been identified, however there are very few salamander representatives in most AMP databases.

Skin tissue samples were extracted from three salamander species native to the Appalachian Mountain Range: *Eurycea bislineata* (n = 2), *Plethodon cinereus* (n = 5), and *Notophthalmus viridescens* (n = 6). Gene expression RNA-seq was utilized to assemble transcriptomes for each species and transcripts were translated to amino-acid residues. Putative AMPs were identified by mapping transcripts to published AMP databases. The putative AMPs were clustered based on amino-acid sequence identity (95%) and alignment length (90%) to identify unique (present only in one species) and shared (present in two or more species) AMPs. Bioinformatic tools predicted amino-acid cleavage sites, conserved regions, and mature antimicrobial regions.

From this transcriptomic approach, 279 putative AMPs were identified which were grouped into 128 clusters. Most of these clusters were from a single species, where a representative sequence was selected for further analysis. 119 AMPs were classified as cathelicidin-like (cath-like). The cathelicidin, histone H2A (H2A), Reactive oxygen species modulator 1 (ROMO1), and ubiquitin protein families were present in all three species. One cath-like, one H2A, and one ROMO1 AMP shared 100% sequence identity between all three species. *E. bislineata* had the most total AMPs (66), while *P. cinereus* had the most diverse set of protein families (8). Identification and classification of AMPs through gene expression RNA-seq analysis shows promise.

INDEX