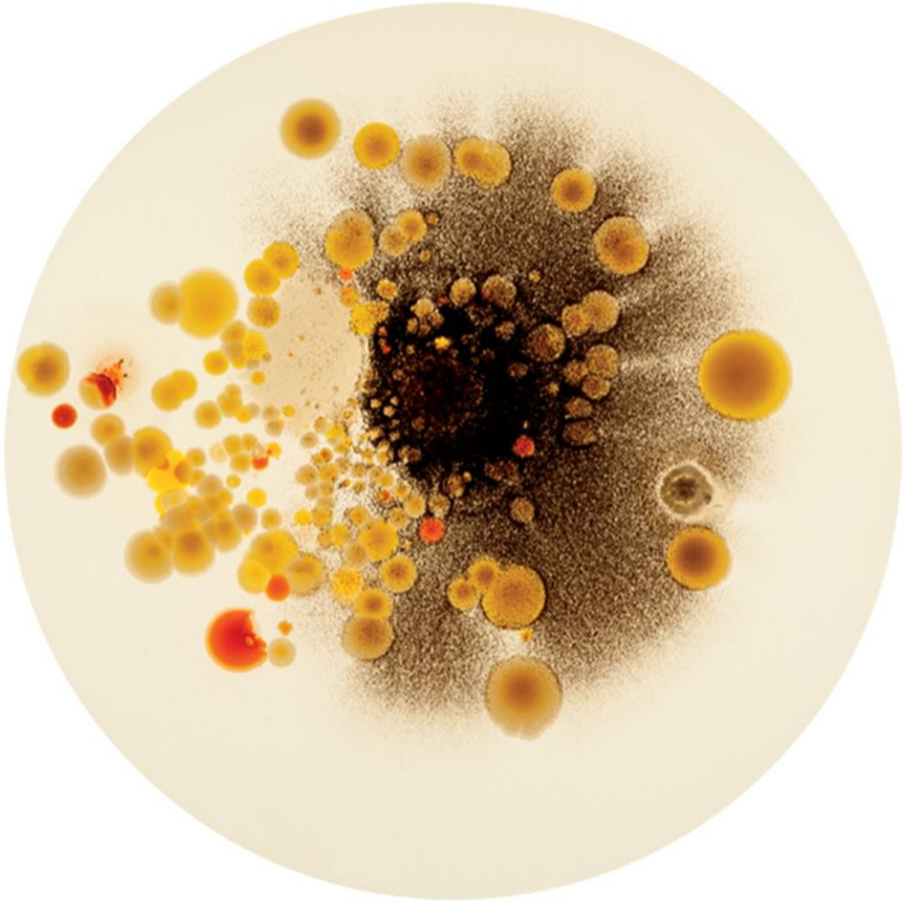
PURDUE MICROBIOME SYMPOSIUM 2022

Systems Biology and Computational Approaches to Understand Microbiome Function

May 9-11, 2022

Beck Agricultural Center
Purdue University
West Lafayette, IN



PAMS
PURDUE APPLIED MICROBIOME SCIENCES

PURDUE UNIVERSITY

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TRAINEES EVENT

Grad Student & Postdoc Mixer

The Graduate Student & Postdoc Mixer will be hosted at **Lafayette Brewing Company**, on **Tuesday, May 10 and begins at 7 pm**.

Food will be provided. Drinks are available for purchase.

Lafayette Brewing Company
622 Main St
Lafayette, IN 47901

SYMPOSIUM SCHEDULE

Monday May 9th, 2022 Fowler Hall at the Stewart Center on Purdue Campus	
19:00-20:00	**James Hamblin,** Yale University and freelance writer Keynote Address: Telling stories about the microbiome

Tuesday May 10th, 2022	
8:00-8:45	Breakfast
8:30-9:00	**Theresa S. Mayer,** Executive Vice President for Research and Partnerships Welcome & Opening remarks
9:00-10:00	**Greg Caporaso,** Northern Arizona University Keynote Address: Toward microbiome multiomics
10:00-10:30	**Tzu-Wen Cross,** Purdue University The interplay of sex steroids and the gut microbiome
10:30-11:00	Coffee and tea (Sponsored by BiomEdit)
11:00-11:15	**Jiarong Guo,** The Ohio State University VirSorter2: a multi-classifier and expert guided approach to detect diverse DNA and RNA viruses
11:15-11:30	**Javier Munoz,** Purdue University Latent Variable Interaction Effects Modeling of Microbiome Multi-omics in Crohn's Disease
11:30-11:45	**Marshall Porterfield,** Purdue University Emerging Opportunities in Integrated Omics: Lessons from NASA Gene Lab
11:45-12:00	**Yanbin Yin,** University of Nebraska-Lincoln Genome mining of carbohydrate active enzymes in microbiomes
12:00-13:30	Lunch
13:30-14:30	**Jennifer Pett-Ridge**, Lawrence Livermore National Laboratory Keynote Address: Life and Death in the Soil Microbiome: Linking Cross Kingdom Interactions with Carbon Cycling
14:30-15:00	**Douglas Brubaker**, Purdue University Multi-omics Modeling to Quantify Microbiome Modulation of Host Cell Signaling Pathways
15:00-15:30	Coffee and tea (Sponsored by QIAGEN)
15:30-15:45	**Ruth Eunice Centeno Martinez,** Purdue University Bovine Respiratory Disease: Insight into nasal fungal community
15:45-16:00	**Samuel Barnett,** Michigan State University Resistance may be futile, but resilience is not: Soil bacterial communities under press disturbance from a subterranean coal mine fire
16:00-16:15	**Matthew P. Ostrowski,** University of Michigan Cooperation and Competition for Xanthan Gum in Gut Microbiomes
16:15-16:30	**Kimberley K. Buhman,** Interim Associate Dean for Research, College of Health and Human Sciences Closing remarks
16:30-18:30	Poster Session (Sponsored by CP Kelco)
19:00	Trainee Networking Event (Lafayette Brewing Company, 622 Main Street, Lafayette)

Wednesday May 11th, 2022	
8:00-8:45	Breakfast
8:45-9:00	**Bernard A. Engel,** Senior Associate Dean of Research and Graduate Education, College of Agriculture Welcome & Opening remarks
9:00-10:00	**Amy Willis**, University of Washington Keynote Address: Modeling complex measurement error in microbiome experiments
10:00-10:30	**Leopold Green**, Purdue University A Synthetic Approach to Microbial-based Therapeutics
10:30-11:00	Coffee and tea (Sponsored by GenBiome Consulting)
11:00-11:15	**Suzanne Alvernaz,** University of Illinois Associations of Maternal Dietary Inflammatory Potential, Gut Microbiome, and Plasma Metabolome
11:15-11:30	**Robert Glowacki,** Purdue University Strain-level differences in Bacteroides thetaiotaomicron mediate survival in an in vivo model of Inflammatory Bowel Disease.
11:30-11:45	**Yiying Zhao,** Purdue University Delta tocotrienol 13' carboxychromanol (δTE-13'), a vitamin E metabolite, interacts with gut microbes as indicated by its synergy with a lactic acid bacterium against colitis
11:45-12:00	**Anna Clapp Organski,** Purdue University Gut Microbial and Metabolic Perturbations Associated with Oral Contraceptives
12:00-13:30	Lunch with the **career panel**
13:30-14:30	**Andy Benson**, University of Nebraska-Lincoln Keynote Address: Complex Trait Analysis of Bioactive Components in Food Crops that Affect the Human Gut Microbiome.
14:30-15:00	**Roland Wilhelm,** Purdue University Ecological inference from 16S rRNA gene data using the genomic signatures of life-history traits and environment-wide association surveys
15:00-15:30	Coffee and tea
15:30-16:00	**Caitlin Proctor**, Purdue University Managing Microbes in Drinking Water Systems
16:00-16:15	**Qinnan Yang,** University of Nebraska-Lincoln Near isogenic lines of sorghum carrying wild type or waxy alleles of the granule-bound starch synthase gene have distinct effects on human gut microbiome phenotypes and host physiological characteristics
16:15-16:30	**Yijing Liu,** The Ohio State University Biofilms: Filtration, Chlorine and UV LED disinfection
16:30-16:45	**Karen I. Plaut,** Glenn W. Sample Dean, College of Agriculture at Purdue Closing Remarks

KEYNOTE BIOGRAPHIES

James Hamblin

Society Keynote: "Telling stories about the microbiome"

Dr. **James Hamblin** is a preventive medicine physician, former staff writer at The Atlantic, and lecturer at Yale School of Public Health. He is the author of "If Our Bodies Could Talk" (Doubleday, 2016) and "Clean: The New Science of Skin" (Riverhead, 2020). He hosted the video series "***If Our Bodies Could Talk***", for which he was a finalist in the Webby awards for Best Web Personality. A number of his topics focus on the microbiome, including **personal microbiome testing**, **probiotics**, and **stopping showering**.

His writing and videos have been featured in the New York Times, Washington Post, Los Angeles Times, Politico, NPR, The Guardian, Elle, Mother Jones, The Awl, and Marketplace, among others. Time named him among the 140 people to follow on Twitter, Greatist named him among the most influential people in health media, and BuzzFeed called him "the most delightful MD ever".

Greg Caporaso *Northern Arizona University*

Dr. Greg Caporaso is an Associate Professor at Northern Arizona University, and Director of the Center for Applied Microbiome Science. Dr. Caporaso has published over 100 papers on the human microbiome, environmental microbiomes, and microbiome bioinformatics. He is the principal investigator on the popular QIIME microbiome bioinformatics platform (**https://qiime2.org**), a widely used tool in microbiome research that has been cited nearly 30,000 times since its publication in 2009, and the author of An Introduction to Applied Bioinformatics (**http://readIAB.org**).

Jennifer Pett-Ridge *Lawrence Livermore National Laboratory*

Dr. Jennifer Pett-Ridge is a senior staff scientist and group leader at LLNL known for developing and applying isotopic tools that help us discover and quantify how changing climate shapes the roles of microorganisms and plants in environmental biogeochemical cycles. She has pioneered the use of NanoSIMS isotopic imaging in the fields of microbial biology and soil biogeochemistry, and in 2014 received a DOE Early Career award to work on responses of tropical soil microbes to climate change. As lead scientist of the LLNL DOE BER Genomic Science Biofuels Scientific Focus Area (SFA) from 2009–2018, and more recently the LLNL Soil Microbiome SFA (2018-present), she helps to coordinate multi-disciplinary teams that integrate biogeochemistry, stable isotope probing, NanoSIMS imaging, molecular microbial ecology, and computational modeling to understand biotic interactions and energy flow in microbial communities critical to soil nutrient cycling and sustainable biofuel production. She is the group lead for the Environmental Isotope Systems group in the Nuclear and Chemical Sciences division, the lead of LLNL's National Getting to Neutral analysis of carbon sequestration capacity in the USA, and manages a portfolio of over $25 million in DOE, NSF, NASA, and other funding. She helps to mentor a group of staff scientists, postdocs, and graduate students working on terrestrial and marine carbon cycling, plant-soil interactions, and development of novel isotope tracing methods (EI-FISH, Chip-SIP, STXM-SIMS) and collaborates frequently with scientists at academic institutions and other national labs. Pett-Ridge has published over 110 peer-review articles, including a patent ROI for the "ChipSIP" approach linking microbial identity and function using NanoSIMS analysis of microarrays.

Amy Willis *University of Washington*

Dr. Amy Willis is the Principal Investigator of the Statistical Diversity Lab and an Assistant Professor in the Department of Biostatistics at the University of Washington. Amy brings her expertise in statistical methods development, high dimensional data, statistical machine learning, phylogenetics and computational biology to develop tools for the analysis of microbiome and biodiversity data. She leads a team of statisticians creating methods and software to advance our understanding of microbial ecosystems, spanning a wide variety of application areas in human and environmental health. Her group works on what they believe to be the most critical methodological needs in microbial science, and the most serious shortcomings of existing analytical methods. She is also heavily involved in outreach, education, and collaboration as a core part of the SDL's mission.

Andy Benson *University of Nebraska- Lincoln*

Located at the University of Nebraska Food for Health Center, **Dr. Andy Benson's** research group studies the complex sets of host and dietary factors that collectively influence composition and function of the gut microbiome. In collaboration with statisticians, computational biologists, and animal geneticists, his research program has focused on understanding how individual genetics can influence the microbiome, and how dietary factors can modify the impact of host genetics, most likely through a direct impact of diet on the gut microbiome. Benson's group is also spearheading the discovery component of the Nebraska Food for Health Center using complex trait analysis in crop plants to define components and molecules that can impact the gut microbiome of humans. Working closely with center members in plant genetics, statistics, and glycobiology, his team uses in vitro microbiomes in high-throughput screens of milled grains from large genetic resource populations of crop plants. This approach to complex phenotyping enables rapid and quantitative measurements of thousands of genetic variants to define pathways and molecules that are capable of influencing one or more members of the gut microbiome.

ABSTRACTS

Keynote Speakers

Complex Trait Analysis of Bioactive Components in Food Crops that Affect the Human Gut Microbiome.

Andrew K. Benson
Department of Food Science and Technology and Nebraska Food for Health Center, University of Nebraska

Composition and function of the gut microbiome is controlled by several different factors, including genetic variation of the host. By far, however, diet appears to the most significant effect. Although bioactive components of the human diet can have major effects on the gut microbiome, the catalogue of bioactives in food crops is far from complete and we have little understanding of how bioactives can vary within and between species of food crops. We have developed a powerful new approach to systematically study bioactives that affect the human gut microbiome in food crops, where we consider these bioactives as complex traits of a food crop species. Our approach uses the human gut microbiome to quantify effects of bioactive components in the grain itself through a new method called **A**utomated ***i****n vitro* **M**icrobiome **S**creening (AiMS). AiMS reactions are based on *in vitro* microbiome fermentations where the reaction volume has been miniaturized and many steps in the sample preparation are automated. AiMS can therefore measure phenotypes from an individual's microbiome across grains from hundreds-thousands of genetically diverse lines of a crop species and the resulting differences in microbiome phenotypes are used as "traits" for genetic analysis. This talk will illustrate the concept using a genetic analysis of AiMS-based phenotypes from a population of recombinant inbred lines of *Sorghum bicolor*. This study identified 10 different genomic regions defined as significant Quantitative Trait Loci (QTL) that collectively affect abundances of 16 different microbial taxa in AiMS fermentations. The work will outline how co-segregation analysis of alleles and microbiome traits can be combined with validation studies Near-Isogenic Lines (NILs) to confirm that overlapping QTL peaks for seed color, and seed composition, and microbiome phenotypes are controlled by segregation of parental alleles at specific loci. In this illustrative example, we will show how allelic variation at the *Tan2* (chromosome 2) and *Tan1* (chromosome 4) genes, which together regulate the tannin biosynthetic pathway, also controls microbiome phenotypes. The talk will also highlight candidate genes at other QTLs to demonstrate that variation in a diverse array of plant molecules can drive major effects on the gut microbiome.

Toward microbiome multi-omics

Greg Caporaso
Northern Arizona University

In the past two decades we have experienced a rapid increase in our understanding of the microbial world. This has included an awareness of the vast diversity of microbial life, as well as the role of microbiomes in human and environmental health. These advances have been driven in large part by improvements in our DNA sequencing and bioinformatics technologies. In this talk I will present work done in my laboratory on developing and applying the next generation of microbiome bioinformatics, including the results of several microbiome studies in my lab. I will conclude with a discussion of the QIIME 2 microbiome bioinformatics platform, which is developed in my lab, and how it can help you move toward a mechanistic understanding of microbiomes and ensure the reproducibility of your work.

Life and Death in the Soil Microbiome: Linking Cross Kingdom Interactions with Carbon Cycling

Jennifer Pett-Ridge
Lawrence Livermore National Laboratory

\ While soil food webs are conceptually well established, a quantitative and mechanistic understanding of how biotic interactions control soil organic matter dynamics is only recently emerging. Many bio-interactions may be mutually beneficial, but others are the proximal cause of microbial death and turnover, producing microbial 'necromass' that is thought to play a critical role in the persistence of soil organic matter (SOM). Several factors mediate microbial population dynamics, such as top-down pressure from phage and soil microfauna, and environmental shifts in moisture or resource availability. I will present evidence from studies where we see cross-kingdom responses to different environmental drivers—shifts in resource availability in an aging rhizosphere or detritusphere, and changes in soil moisture potential during a post-drought wet-up. In all of these systems, we have used stable isotope probing (SIP) to assess the active microbial and viral community and hypothesize that during periods of increasing resource availability, the potential for

biotic interactions will accelerate. During community regrowth following a wet-up event, we observe density-dependent population growth (that is highly correlated with total soil CO_2 production) suggesting that competitive interactions are shaping community assembly. Similarly, in a growing rhizosphere, microbial communities and gene expression evolve rapidly, and in genome-resolved SIP metagenomes, we consistently find many phage sequences in the isotope heavy fraction, with multiple predicted hosts, indicating that they infected and replicated within the experimental timeline. Eukaryotic hosts and viral communities are also highly diverse and dynamic in the resource-rich rhizosphere, as are nematodes and protists that likely feed on roots, bacteria and fungi. These data suggest that cross-kingdom interactions, involving bacteria, fungi, archaea, protists, microfauna and viruses, shape carbon (C) availability and loss pathways and are differentially influenced by both soil habitat (rhizosphere, detritusphere, bulk soil) and natural fluctuations in the physicochemical environment.

Modeling complex measurement error in microbiome experiments

Amy Willis
University of Washington

The relative abundances of bacterial species in a microbiome are an important and common parameter to estimate in both amplicon and whole-genome sequencing studies. By analyzing data from artificially constructed microbiomes, we show that high-throughput sequencing distorts the true composition of microbial communities, and discuss the consequences of this fact on assessing differential relative abundance. We propose a statistical model for microbiome data that reflects this observation, a stable algorithm for estimating model parameters, and briefly discuss hypothesis testing. Notably, our model and estimation procedure permit relative abundances to lie on the boundary of the simplex. We conclude with examples of the utility of the method, and recommendations for the design and analysis of microbiome studies. Our approach can be leveraged to select experimental protocols, design experiments with appropriate control data, and remove sample-specific contamination.

Invited Speakers

The interplay of sex steroids and the gut microbiome

Tzu-Wen L. Cross
Department of Nutrition Science, Purdue University

Sex is one of the most powerful modifiers of disease development, with men and women displaying divergent phenotypes in susceptibility and response to treatments in many diseases. The gut microbiome has the metabolic capability to produce or modulate endocrine molecules, termed “sterolbiome”, which affects systemic levels, potency, and half-life of sex steroid metabolites. When raised without any exposure to microorganisms, animal models demonstrated abnormal patterns of estrous cycles, reproductive function, and altered enterohepatic recycling of sex steroids, highlighting the crucial role of the sterolbiome. However, the specific mechanisms and how these host-microbial interactions can be manipulated to alter disease susceptibility are not known. Dr. Cross will discuss their recent discovery on the interactions between the gut microbiome and host endocrine system, particularly pertaining to the reproductive axis and estrogen signaling pathways. Through understanding these fundamental mechanisms and their impact on disease development, her team aims to use a precision nutrition approach to develop gut microbiome modulators through dietary strategies to prevent disease and achieve optimal health.

Multi-omics Modeling to Quantify Microbiome Modulation of Host Cell Signaling Pathways

Douglas Brubaker
Department of Biomedical Engineering, Purdue University

A central challenge in understanding the role of microbiome dysbiosis in inflammatory disease is understanding how shifts in the composition of human microbiomes, together with changes in secreted metabolites and proteins, regulate host cell signaling pathways associated with disease pathobiology. Here, we present work in my lab developing computational methods to integrate multi-omic datasets from microbiomes to understand potentially causal links between dysbiosis and host cellular responses. Here, I will discuss our work developing and applying these methods to understand drivers of bacterial vaginosis via integrated proteomic, transcriptomic and metabolomic profiling of cervicovaginal samples from 405 women from Kenya and Uganda, along with other related research directions in my lab.

A synthetic approach to 'smart' microbial-based therapeutics

Leopold N. Green
Department of Biomedical Engineering, Purdue University

Current therapies for treating inflammatory bowel disease (IBD) and other chronic intestinal illnesses are effective in less than 30% of patients due to the lack of adherence to onerous prescription schedules and off-target effects. Synthetic biology offers a unique opportunity to engineer microbial drug delivery platforms that can significantly enhance drug safety and efficacy by overcoming current IBD treatment limitations. The engineered systems can colonize the gut and provide in situ surveillance by monitoring changes in the local environment. As an immunotherapy strategy, we aim to implement a synthetic circuit in probiotic E. coli strain Nissle 1917 capable of sensing chronic conditions and modulating host immune responses. Here, we adopt the previously characterized split activator AND logic gate to multiplex two input signals: the inflammatory biomarker tetrathionate and the IPTG inducer signal. We report 4 to 6-fold induction with a minimal leak when both stimuli are present. We also demonstrate the tunability of the logic-based genetic circuit by varying the ribosome binding site sequences. We are currently engineering the split activator to drive the expression of an anti-inflammatory effector. We will test if the engineered circuit can protect against intestinal inflammation in IBD mouse models in future work.

Managing Microbes in Drinking Water Systems

Caitlin Proctor
Agricultural and Biological, Environmental and Ecological Engineering, Purdue University

Engineers have been managing microbes within the urban water cycle since the inception of water treatment. Introducing processes and chemicals that alter growth conditions, often with the goal of excluding pathogens, has quantifiable effects on the entire microbiome. While much of the growth occurring in drinking water systems is benign, niches have emerged for pathogens that can cause disease, especially amongst immunocompromised populations. These pathogens are difficult to control with the current infrastructure, which is often reliant upon disinfectants. However, there is tremendous potential to use principles of ecology to influence biofilms and thus drinking water. Several factors, including pipe material and operation conditions have been explored as potential ways to control growth without reliance upon disinfectants. Such a probiotic approach has the potential to modernize drinking water treatment and plumbing to provide safe water sustainably.

Ecological inference from 16S rRNA gene data using the genomic signatures of life-history traits and environment-wide association surveys

Roland Wilhelm
Department of Agronomy, Purdue University

The ecological insights gained from 16S rRNA gene data tend to be limited by gaps in our knowledge of abundant, yet uncultured, bacterial taxa. Increasingly, the ecological characteristics of any given representative sequence can be inferred from the ever-expanding amplicon databases and repositories of genomic data obtained using cultivation-independent means (SAGs, MAGs etc.). This research explores the ecophysiological characteristics of soil bacterial populations responding to tillage disturbance using an environment-wide association survey of 16S rRNA gen libraries from studies of agricultural land management and inferred community-weighted genomic traits that correspond with life-history traits, including genome size, rrn copy number, and coding density. Disturbed communities had a higher proportion of taxa with life-history traits selected for by environmental instability and disturbance (larger genome and multiple *rrn*), while populations from less disturbed soils were associated with metabolic dependency (smaller genome and lower coding density). The environment-wide associations of several of the most abundant confirmed the importance of disturbance-adaptation of bacteria favored by tillage. These findings provide insights into the ecological relationships between bacterial and soil disturbance and illustrate new approaches for interpreting patterns in microbiome data.

Select Oral Presentations

VirSorter2: a multi-classifier and expert guided approach to detect diverse DNA and RNA viruses.

Jiarong Guo*, Ben Bolduc, Ahmed A. Zayed, Arvind Varsani, Guillermo Dominguez-Huerta, Tom O. Delmont, Akbar Adjie Pratama, M. Consuelo Gazitúa, Dean Vik, Matthew B. Sullivan & Simon Roux

Microbiology, Ohio State University

Background Viruses are a significant player in many biosphere and human ecosystems, but most signals remain “hidden” in metagenomic/metatranscriptomic sequence datasets due to the lack of universal gene markers, database representatives, and insufficiently advanced identification tools. Results Here, we introduce VirSorter2, a DNA and RNA virus identification tool that leverages genome-informed database advances across a collection of customized automatic classifiers to improve the accuracy and range of virus sequence detection. When benchmarked against genomes from both isolated and uncultivated viruses, VirSorter2 uniquely performed consistently with high accuracy (F1-score > 0.8) across viral diversity, while all other tools under-detected viruses outside of the group most represented in reference databases (i.e., those in the order Caudovirales). Among the tools evaluated, VirSorter2 was also uniquely able to minimize errors associated with atypical cellular sequences including eukaryotic genomes and plasmids. Finally, as the virosphere exploration unravels novel viral sequences, VirSorter2’s modular design makes it inherently able to expand to new types of viruses via the design of new classifiers to maintain maximal sensitivity and specificity. Conclusion With multi-classifier and modular design, VirSorter2 demonstrates higher overall accuracy across major viral groups and will advance our knowledge of virus evolution, diversity, and virus-microbe interaction in various ecosystems. Source code of VirSorter2 is freely available (https://bitbucket.org/MAVERICLab/virsorter2), and VirSorter2 is also available both on bioconda and as an iVirus app on CyVerse (https://de.cyverse.org/de). To best serve the research community, we maintain a "live protocol" (dx.doi.org/10.17504/protocols.io.bwm5pc86) for using VirSorter2 for virus sequence identification, including curating less well-studied viruses and mobile genetic elements, and establishing bona fide virus-encoded auxiliary metabolic genes.

Latent Variable Interaction Effects Modeling of Microbiome Multi-omics in Crohn’s Disease.

Javier. E. Munoz*, Douglas. K. Brubaker
PULSe, Biomedical Engineering, Agricultural and Biological Engineering, Purdue University

Microbiota alterations in Crohn’s Disease (CD) trigger inflammatory responses that damage the intestines. The mechanisms by which dysbiosis, host responses, and gut metabolites drive inflammation in CD remain poorly understood. New methods to integrate multi-omics data and model host-microbiome interactions are needed to characterize these mechanisms. Much of the current state of the art is focused on pairwise correlation approaches, techniques that do not account for correlation structure within microbiomes or metabolomes and are challenged by high multiple testing burdens when comparing all combinations of microbes and metabolites. Here, we develop a novel approach that leverages data-driven modeling, dimensionality reduction, and multi-omic interaction-effects regression to identify microbe-metabolite interactions synergistically predictive of CD status. We develop a modeling framework for microbiota and metabolic data, combining single-omic latent variables (LV) from sparse Partial Least Square Discriminant Analysis (sPLS-DA) via an interaction effects linear model. We applied this framework to 102 samples from the IBD PRISM cohort diagnosed with CD and non-CD status (control). We trained sPLS-DA models on metagenomics or metabolomics data to predict CD or non-CD status using the Mixomics R package. Sample projections on the LV’s inferred from these models were used to train a generalized linear model with interaction effects terms. Significant interaction effects indicated that the predictive power of the bacterial loadings on a particular microbiome LV was conditioned upon the loadings of metabolites on a particular metabolome LV. We then identified microbes and metabolites on interacting LV’s with significant variable importance of projection (VIP) scores for prioritized microbe-metabolite correlation analysis. The individual microbiome (mbiome) and metabolome (metab) sPLS-DA models were significantly predictive of disease status in an independent test set (mbiome AUC = 0.972, metab AUC = 0.959). The sparse PLS-DA method employs a regularization penalty when constructing LV’s, incorporating different features into different LV’s based on ability to discriminate between conditions. In our models, 2 mbiome-LV’s encoded 100 and 1 features respectively and 3 metab-LV’s, encoded 70, 40, and 90 features respectively. A main-effects only regression model of microbiome and metabolite LV’s was predictive of disease status (p = 4.714e-06), but no individual main effects were significant, suggesting multi-omic interactions. Incorporating microbiome and metabolome LV interaction effects into the model identified significant interactions between microbiome LV1 and metabolome LV3 which together separate CD patients from controls. Significant bacteria and metabolites (VIP > 1) were prioritized from these LV’s for correlation analysis to identify microbe-metabolite pairs synergistically predictive of CD status. Analysis of these 2,915 microbe-

metabolite pairs, reduced from >750,000 in the full datasets, identified 644 significant correlations (FDR q < 0.01) between microbes and metabolites significantly predictive of CD status on interacting multi-omic LV's. This workflow prioritizes significant microbe-metabolite interactions that coordinate the development of Crohn's Disease. This framework preserves global microbiome and metabolome information in latent variables while substantially reducing the multiple testing burden in pairwise analysis. Our ultimate goals are to leverage synergistic microbe-metabolite interactions as therapeutic targets to treat CD and understand microbiome regulation of host signaling pathways

Emerging Opportunities in Integrated Omics: Lessons from NASA Gene Lab

D. Marshall Porterfield*
Agriculture and Biological Engineering, Purdue University

Microbiome sciences have emerged in the wake of the omics technology revolution, based on advances in DNA/RNA sequencing technologies. Genomics and transcriptomics are linked in terms of biochemical phenotyping through proteomic and metabolomic techniques. Collectively this enables true systems level biology and represents a major technical capability needed for the future of genomic decoding and the broader advancement of biological engineering. The broad adoption of omics technologies is now pushing the development of standards and data systems to act as repositories and data analysis centers. NASA released the GeneLab strategic plan in 2014 for an integrated omics data pipeline and data repository for NASA space life science research data. Now Los Alamos is adopting that model lead the establishment of the National Microbiome Data Collaborative (NMDC). Early in the establishment of GeneLab NASA focused on developing and applying the FAIR principles to ensure the Findability, Accessibility, Interoperability, and Reuse of these critical experimental data sets. The principles emphasize machine-actionability (i.e., the capacity of computational systems to find, access, interoperate, and reuse data with none or minimal human intervention) because humans increasingly rely on computational support to deal with data as a result of the increase in volume, complexity, and creation speed of data. The NMDC is like genelab in that it is being developed based on the emerging practice of integrated omics experiments and the emphasis on true systems level computational biology. GeneLab has now enabled a large number of broad survey studies to look for trends in the data for further investigation. These have provided high impact results of large trends that are evident across many experiments and experimental systems. For example, the data sets available from mouse and rodent experiments differ from the human data in that the experiments are remarkably diverse and provide unique data that are not available from human subjects. Since most of the mouse genes have human analogs, we were able to create a Species filter to directly compare humans to mice at the system level. The opportunities to advance basic research through integrated omics is going to advance biology to a systems science. These large and diverse data repositories are emerging as a major capability to conduct large survey studies, of specific genetic trends, across a large and diverse data set enabled and underpinned by the FAIR standards. This will open the door for meta-analytic studies of massive data sets. The compiled wealth of data in these data repositories is directly proportional to your capabilities in computational biology, and real investments are required now to move us beyond introductory level bioinformatics.

Genome mining of carbohydrate active enzymes in microbiomes

Yanbin Yin*, Catie Ausland, Jinfang Zheng, Yuchen Yan
Food Science, University of Nebraska Lincoln

PULs (polysaccharide utilization loci) are discrete gene clusters of CAZymes (Carbohydrate Active EnZymes) and other genes that work together to digest and utilize carbohydrate substrates. While PULs have been extensively characterized in Bacteroidetes, there exist PULs from other bacterial phyla, as well as archaea and metagenomes, that remain to be catalogued in a database for efficient retrieval. We have developed an online database dbCAN-PUL (http://bcb.unl.edu/dbCAN_PUL/) to display experimentally verified CAZyme-containing PULs from literature with pertinent metadata, sequences, and annotation. Compared to other online CAZyme and PUL resources, dbCAN-PUL has the following new features: (i) Batch download of PUL data by target substrate, species/genome, genus, or experimental characterization method; (ii) Annotation for each PUL that displays associated metadata such as substrate(s), experimental characterization method(s) and protein sequence information, (iii) Links to external annotation pages for CAZymes (CAZy), transporters (UniProt) and other genes, (iv) Display of homologous gene clusters in GenBank sequences via integrated MultiGeneBlast tool and (v) An integrated BLASTX service available for users to query their sequences against PUL proteins in dbCAN-PUL. With these features, dbCAN-PUL will be an important repository for CAZyme and PUL research, complementing our other web servers and databases (dbCAN2, dbCAN-seq).

Paper published at https://doi.org/10.1093/nar/gkaa742

Bovine Respiratory Disease: Insight into nasal fungal community

Ruth Eunice Centeno Martinez*, Mohit Verma, Timothy. A. Johnson
Animal Science, Purdue University

Bovine Respiratory Disease (BRD) is an ongoing health and economic issue in the dairy and beef cattle industry that is difficult for producers to identify and treat effectively. Presence of Pasteurella multocida, Mycoplasma bovis, Mannheimia haemolytica, and Histophilus somni have been identified in BRD-affected cattle; however, no studies have investigated the fungal community and how it relates to BRD. Therefore, the study objective is to compare the fungal community (diversity and composition, targeting the internal transcribed space (ITS)) present in the nasal cavity of Holstein steers that are apparently healthy (n = 73) or with BRD clinical signs (n = 56). The phyla Ascomycota and Basidiomycota and the genus Trichosporon and Issatchenkia were the most abundant among both groups. Healthy pen-mates presented lower fungal evenness than the BRD group. Fungal community structure was affected by season (Fall-Winter). Use of random forest modeling to classify BRD or healthy status using the ITS sequence results agreed more with the visual diagnosis (agreement of 44 and 55%, respectively) than in combination with qPCR quantification of BRD-pathobionts (agreement of 25 and 48%, respectively) indicating that for some animals the fungal community provides additional useful information indicative of disease. The genus Neodevriesia, Aspergillus, and Fusarium sporothrochioides influenced the accuracy of the model. Results from this novel study provide a first description and comparison of the nasal fungal diversity and composition in BRD and healthy animals. Our future studies will investigate the interaction between the fungal and bacterial communities to further understand and accurately diagnose BRD.

Resistance may be futile, but resilience is not: Soil bacterial communities under press disturbance from a subterranean coal mine fire

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Anthropogenic disturbances can drastically alter the structure and function of soil microbial communities. Such disturbance-induced soil microbiome effects have widespread implications to biogeochemical cycling, soil health, and ecosystem processing. Of particular interest is the ecological resilience of soil microbial communities to press disturbances. How well do these communities recover to their original state, either compositionally or functionally, following long-term intense disturbances? To answer these questions in disturbance ecology, we use a unique model system: the soils overlying subterranean coal seam fire in Centralia, Pennsylvania. The Centralia coal seams have been burning since 1962, with fire fronts moving at an estimated rate of 2-7 m/yr, often heating the overlying soil significantly above ambient temperatures. This heat press disturbance drives massive changes to bacterial community composition, but the moving fire fronts provide opportunities to compare actively disturbed communities to both recovered and unaffected communities. Over eight years, we annually sampled fire affected soils (i.e., those above an active fire front), recovered soils (i.e., those over historic but no-longer active fire fronts), and reference soils (i.e., those never directly heated by an underlying fire). Using 16S rRNA gene amplicon surveys, we assessed the bacterial community structures and dynamics in these soils across both disturbance intensities and time. Using the first year of collected samples, we previously found that soils overlying active fires were compositionally distinct, and selected for thermophilic or thermotolerant bacterial taxa, and that the soil microbiomes were largely resilient, with bacterial community compositions from recovered soils generally similar to those of undisturbed communities. Building from this first year of data, we now add seven more years of field collection at the same site to find these initial observations largely reinforced by subsequent years' data. We observed highly diverse and dynamic bacterial communities in fire affected sites but less so in both recovered and reference sites. The fire affected sites have been cooling over time and, concurrently, we observed the bacterial communities inhabiting these sites converging towards the unaffected or recovered communities, which showed little change over time. Across the soils we also identified edaphic factors, specifically soil pH, that significantly influenced the assembly and succession of these bacterial communities. Overall, this long-term study advances our understanding of the resilience of soil microbial communities to intense anthropogenic disturbances and potential pathways to recovery for these important habitats.

Associations of Maternal Dietary Inflammatory Potential, Gut Microbiome, and Plasma Metabolome

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Introduction: Pregnancy is known to involve changes in a wide variety of physiological systems, including the maternal gut microbiota and circulating metabolite profiles. Dietary intake is known to influence the gut microbiota composition, structure and function and host metabolism. Western diets are particularly characterized by their high levels of inflammatory food, such saturated fatty acids, animal protein and added sugar. Multiple perinatal disorders have been associated with inflammation, maternal metabolic alterations and gut microbiota dysbiosis, including gestational diabetes and mood disorders. However, the effects of high inflammatory diets in the gut microbiota and host metabolism during pregnancy has yet to be thoroughly explored. Methods: 58 pregnant women were recruited prior to 16 weeks of gestation and were followed through 24-28 gestational weeks. Participants completed a food frequency questionnaire (FFQ) and provided rectal swabs and a venous blood sample. Dietary inflammatory potential was assessed using the Dietary Inflammatory Index (DII) from FFQ data. Rectal samples were analyzed using amplicon 16S rRNA sequencing and processed using DADA2. Differential taxa abundance was identified using cumulative sum scaled (CSS) normalization and we employed a zero-inflated Gaussian model to identify taxa associated with the DII score (expressed continuously and categorically). Plasma metabolites were identified with LC-MS/MS and mapped to microbial networks using sparse canonical correlation analysis. Results: The inflammatory diet associated with the maternal gut microbiome composition showed positive associations with Blautia and Ruminococcaceae, and negative associations with taxa such as Ezakiella and Prevotella. Additionally, gut taxa were mapped to circulating plasma metabolites and enriched enzymatic pathway. Conclusions: Changes in the gut microbial composition are associated with dietary inflammatory potential as well as plasma metabolite markers. Ongoing work is being conducted to map these findings to enzyme networks and brain functionality.

Strain-level differences in Bacteroides thetaiotaomicron mediate survival in an in vivo model of Inflammatory Bowel Disease.

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Background: Inflammatory bowel disease (IBD) is an inflammatory disorder of the gastrointestinal tract that affects ~1% of the U.S. population. In IBD, immune responses targeting the gut microbiome lead to chronic intestinal inflammation, characterized by infiltration of neutrophils, destruction of normal mucosal architecture, and disruption of the gut microbiota. Although effective treatments exist, many patients are non-responsive or become refractory to t therapies over time. One emerging novel avenue for IBD therapy involves introduction of engineered bacteria “synthetic probiotics” to the human gut that can limit the damaging inflammatory immune responses. However, a major impediment to such therapies is their stable engraftment within the microbiome in the face of myriad anti-microbial compounds produced by host inflammatory responses. Objective: Define the physiological pathways and genes/genetic factors that permit survival of bacterial strains in the face of inflammatory pressures within the intestine. Methodology: Using 24 strains of the common human gut bacterial species Bacteroides thetaiotaomicron (Bt) (found in >18% of the U.S. population), we examined resistance to factors associated with inflamed environments, specifically, reactive oxygen/nitrogen species (ROS/RNS) and antimicrobial peptides (AMPs), as well as bacterial responses known to promote survival in harsh environments including biofilm formation and growth on host-derived carbohydrates. Based on phenotypic variation within our in vitro screens we chose 4 strains to test for engraftment in a murine gnotobiotic IBD model where colonization of IL-10-/- mice harboring a model community of commensal microorganisms with the pathobiont Helicobacter hepaticus (Hh) leads to development of IBD. Additionally, we performed whole genome sequencing on these strains to examine loci responsible for metabolism, capsule formation, and in vivo fitness genes. Results: Bt strains showed variable tolerance towards ROS/RNS and AMPs that correlated with their ability to form robust biofilms. Strains 0940-1 and 5951 were significantly more resistant to ROS, and were more efficient biofilm formers than strains 5482 or 3164. Strain 0940-1 was a relatively poor grower on host-derived carbohydates compared to the other strains, and all strains exhibited differences in capsule and metabolism gene presence. However, in vivo, in our gnotobiotic IBD model in IL-10-/- mice, we observed that all strains survived during inflammation and even increased in relative abundance. These data suggest that resiliency in the inflamed intestine is a shared property among different strains of Bt, but that the molecular pathways that mediate this survival differ. Conclusions: Bt exhibits strain-specific strategies for mediating survival within inflamed environments, providing the opportunity to uncover distinct pathways to promote bacterial resilience in engineered probiotic strains. Future work will focus on identifying genes responsible for tolerance to ROS and biofilm formation and those necessary for survival and expansion within 0940-1 and 5951 in vivo.

Broadly, this approach can be used to identify genes and strains of Bt to be used as a platform to engineer probiotics that can be used to stably colonize the inflamed GI tract of IBD patients to effectively deliver IBD therapies and reduce the inflammatory networks and damaging effects of IBD.

Delta tocotrienol 13' carboxychromanol (δTE-13'), a vitamin E metabolite, interacts with gut microbes as indicated by its synergy with a lactic acid bacterium against colitis

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Objectives: Ulcerative colitis (UC) is an inflammatory colonic disease and microbial dysbiosis is one of its risk factors. We recently showed that δTE-13', a metabolite of the natural vitamin E form δTE, inhibited colitis-associated tumorigenesis in mice, modulated their gut microbes and increased the relative abundance of a lactic acid bacterium. Interestingly, a subspecies of this bacterium named Lactococcus lactis subsp. cremoris (L. cremoris), has been reported to attenuate UC in mice. Therefore, we reasoned that combining δTE-13' with L. cremoris may offer synergistic protection against UC in mice. Methods: We fed male balb/c mice with either 0.04% (w/w) δTE-13' in diet, or 5×10^8 CFU L. cremoris through gavage or a combination of both for 7 days. Then we induced UC in these mice by adding 2% dextran sulfate sodium to their drinking water. All treatments continued along with UC for an additional 9 days till animal sacrifice. To assess the anti-UC effects of the combination, we evaluated mice's colitis symptoms, colonic tissue damage, and cytokine levels. To further understand the role of gut microbes underlying the anti-UC effects of the combination, we processed fecal samples to analyze changes in microbial composition, and level of δTE-13' metabolites, and performed in vitro anaerobic incubation using mouse's cecum microbes. Results: Compared to the controls, δTE-13' + L. cremoris offered superior protection against UC as indicated by milder clinical symptoms, less tissue damage and decreased colonic inflammation, while neither δTE-13' nor L. cremoris alone showed any benefits. Mechanistically, combination rendered gut microbes resistant to UC-induced microbial dysbiosis and increased the fecal level of a δTE-13' metabolite. Preliminary observations from the anaerobic study suggested that gut microbes pre-selected by δTE-13' in the animal study were capable of reducing the compound, and L. cremoris appeared to promote the microbiota-mediated metabolism. Conclusions: Our study demonstrated the benefits of δTE-13' + L. cremoris against colitis. It offered a new perspective of developing anti-UC therapies by combining dietary compounds with bacteria, and presented the first evidence that gut microbes can metabolize δTE-13', a metabolite of vitamin E. Funding Source: Purdue Center for Cancer Research Key Words: ulcerative colitis, vitamin E

Gut Microbial and Metabolic Perturbations Associated with Oral Contraceptives

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Menstruating women of reproductive age have a lower risk of developing cardiometabolic diseases compared to men, a protection largely attributed to the metabolic regulation of estrogen. However, the use of oral contraceptives (OC) that contain estrogen and progestin increases the risks of cardiometabolic disease in women. Interestingly, our research team discovered that OC supplementation in mice led to greater hepatic oxidative stress, a phenotype that is modifiable by the gut microbiome. The gut microbiome is also critically involved in sex steroid homeostasis. However, the role of the gut microbiome in the OC-induced oxidative stress phenotype is unclear. OBJECTIVE: This study aims to examine the impact of chronic OC treatment on the gut microbiota and its taxonomic association with metabolic phenotypes and hepatic oxidative stress markers. Methods: Sixteen 5-6 weeks old female C57BL/6J mice (n=8) received a 45% high-fat diet with or without supplementation of OC (2mg ethinylestradiol [estrogen] and 200 mg levonorgestrel [progestin]) per kg of diet for 20 weeks. Body composition was assessed using EchoMRI before euthanasia. Cecal microbiota was assessed through 16S rRNA gene-based amplicon sequencing targeting the V4 region using the Illumina MiSeq platform and analyzed via the QIIME2 pipeline. Hepatic oxidative stress was assessed using mRNA expression of genes related to reactive oxygen species generation. RESULTS: OC treatment protected mice against high-fat diet-induced obesity yet resulted in lower (p<0.05) expression of hepatic GPX1, SOD2, and CAT genes, suggesting a state of greater oxidative stress. Markedly, dissimilarity clustering showed that the cecal microbiota of OC-treated mice significantly differ (p<0.05) from control mice. OC treated mice had lower (p=0.01) species richness of the cecal microbiota and tended to have a lower (p=0.06) Firmicutes/Bacteroidetes ratio compared to controls. Several taxa in the cecal microbiota were significantly lower in the OC treated group, including phylum Bacteroidetes, genus Lactococcus, and unknown genera from families Ruminococcaceae and Christensenellaceae. Spearman correlation analysis showed a positive correlation (ρ=0.50, p=0.04) between the relative abundance of Lactococcus and hepatic GPX1 expression.

Whereas negative correlations between the relative abundance of Clostridium and hepatic expression of SOD2 (ρ=-0.7, p=0.002), GPX1 (ρ=-0.6, p=0.004), and CAT (ρ=-0.6, p=0.007) were identified. CONCLUSION: Oral contraceptive treatment significantly impacted the gut microbial community structure with several bacterial taxa correlated with hepatic oxidative stress markers. Our data suggest that the gut microbiota may play a role in OC-induced oxidative stress and cardiometabolic disease risk.

Near isogenic lines (NIL) of sorghum carrying wild type or waxy alleles of the granule-bound starch synthase (GBSS) gene have distinct effects on human gut microbiome phenotypes and host physiological characteristics

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Background: Waxy starches contain 90% amylopectin and are derived from grain crops carrying naturally-occurring mutations that block amylose biosynthesis. The absence of amylose in waxy starches produces unique physiochemical properties that are desirable for food processing, but the effects of increased amylopectin/amylose ratios in waxy starches on the gut microbiome and physiological characteristics of the host are not well characterized. Here, we used a whole-grain model with isogenic pairs of wild type sorghum lines and their waxy derivatives to test the hypothesis that major differences in amylose/amylopectin ratio produce significant effects on the human gut microbiome. Results: Fermentation of grain from waxy versus wild type derivatives produced substantial differences in overall microbiome composition, abundances of multiple taxa, and production of microbial metabolites (butyrate). Several of the taxonomic and metabolic signatures of fermentations from parental versus waxy lines were shared across fermentations with microbiomes from different human donors, including reduced levels of butyrate production and lower abundances of Roseburia and other amylolytic, butyrate-producing members of Lachnospiraceae in fermentations of waxy lines. Using a human microbiome-associated mouse model, we also detected significant differences in microbiome composition in animals fed low-fiber diets supplemented with 20% grain from isogenic pairs of parental versus waxy derivatives of sorghum. Remarkably, these microbiome changes were accompanied by significant differences in weight gain, with animals consuming waxy sorghum gaining significantly more weight. Conclusions: We conclude that the benefits of waxy starches on food functionality can have trade-off effects on the gut microbiome and host physiology that could be particularly relevant in human populations consuming large amounts of waxy grains.

Interactive impacts of water treatments on microbial communities in water and biofilms: Filtration, Chlorine and UV LED disinfection

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Bacteria and pathogens in water environments negatively impact water quality control processes and pose threats to human health. Biofilms in water distribution systems provide microbiome good habitats to growth. Susceptible people may become infected when exposed to contaminated water. It is important to understand the impact of different disinfection strategies on water bacterial communities to facilitate the water quality control. This work compares the yearlong impacts of disinfection of sand filter effluent by chlorine versus UV light-emitting diodes (LEDs) on bacterial communities in water and biofilm samples collected from each step of water treatment at a rural, mountain, surface water treatment plant. Significant differences were observed between microbial communities among combinations of sample types (water and biofilm) and sample sources (filter influent, filter effluent, chlorine effluent, and UV LED effluent). Relative to sand filter effluent phyla, disinfection by chlorine increased relative abundance of Firmicutes, Cyanobacteria and Planctomycetes and UV LED increased relative abundance of Proteobacteria and Firmicutes. Differential abundance analysis (Deseq2) comparing disinfected samples indicated that after chlorine, Cloacibacterium spp. were more abundant in biofilms and Desulfosporosinus spp. and Paenibacillus spp. were more abundant in water, and after UV LED, Polaromonas spp. were more abundant in both water and biofilms. Relative abundance of genera containing potential opportunistic pathogens also differed after disinfection; Mycobacterium spp. were more abundant in UV LED treated biofilms and Pseudomonas spp. were more abundant in chlorine treated water. PERMANOVA analysis demonstrated significant differences of microbial composition among sample types and sample sources. Pairwise comparison (adonis) considering both water and biofilm sample communities identified a significant difference between chlorine effluent and filter effluent ($p = 0.001$), but no significant difference between UV LED effluent and filter effluent ($p = 0.532$), indicating that chlorine applied more selective pressure than UV LED. Statistical association of environmental conditions and water quality parameters (envfit) indicated that dissolved organic carbon concentration and precipitation were linked with changes in water microbial communities. Canonical analysis of principal coordinates indicated little seasonal and water quality parameters impacts on the distribution of the community. Furthermore, systematic long-term investigations on the

impacts of treatment strategies on water microbiomes are particularly critical towards determining the impact of changes in source water quality, environmental conditions, and process operations on the changes in microbial community composition in the drinking water distribution system.

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------- poster no. 1 -------

Raman spectroscopy - an analytical tool for biologics

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Raman Spectroscopy is a non-invasive technique that can analyze biomolecules qualitatively and quantitatively. Raman spectroscopy measures the inelastic scattering of light due to molecular vibrations. It can be applied to any physical form (liquid, solid, semi-solid) of the biological sample reducing the sample preparation measures. The minimal sample preparation and non-invasive nature of the Raman Spectroscopy can be applied in developing a Process analytical technology (PAT) tool and a diagnostic tool. We have demonstrated the use of Raman spectroscopy in qualitative and quantitative measurements of biologics through our previous studies. Our results indicate that Raman Spectroscopy can distinguish between several different microbes (spanning Gram-positive bacteria, Gram-negative bacteria, and fungi) and between microbes and CHO cells in a mixture. Raman Spectroscopy can also determine the concentration of the viral samples. We aim to optimize and refine the sensitivity of Raman spectroscopy through the development of in-line probes and acoustic devices. Our future works also involve coupling Raman data with machine learning tools for accurate in-line measurements.

------- poster no. 2 -------

Using natural language processing to infer ecological guild information for fungi: A machine learning test case for FUNGuild

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FUNGuild is a bioinformatic tool for parsing fungal operational taxonomic units (OTUs) by ecological guild independent of sequencing platform or analysis pipeline. The associated FUNGuild database contains information on over 11,000 fungal taxa and the platform has been widely used in studies of fungal ecology, with ca. 1500 publications citing FUNGuild to date. Although FUNGuild covers a broad range of important fungal groups, such as human and plant pathogens, with the roughly 150,000 fungal names in current use today, the FUNGuild database presently lacks ecological information on a significant number of fungal taxa. As FUNGuild is 'hand' curated resource, filling in these information gaps would likely require numerous human hours, perhaps years given the current rates of data acquisition for FUNGuild. Computer-based 'artificial intelligence' (AI) approaches, such as machine learning (ML), have greatly accelerated tasks requiring human effort; therefore, we are exploring natural language processing (NLP) techniques of AI in order to gather ecological information on fungal taxa from public online resources (NCBI's PubMed Central and Wikipedia) for determining guild types to rapidly augment the ecological data within FUNGuild. Toward this effort, we have data-mined fungi-related content from roughly 30,000 wiki-pages and 1.5 million scientific publication and processed them in an AI NLP Python-based pipeline using the word2vec family of algorithms. With these data processed into vector space word embeddings, we will now apply NLP transformer models to 'read' and ‘interpret’ ecological from our mined text data. With ecological guild types determined by the transformer models, we will then use the resultant information to further develop the FUNGuild database, thus improving this resource for the fungal ecology community.

------- poster no. 3 -------

The gut microbiota and sex impact motor function and brain proteome in mice: Implication for Parkinson’s disease

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Parkinson’s disease (PD) is a neurodegenerative disease affecting motor function due to the depletion of dopaminergic neurons. A growing body of evidence suggests that the initial pathological amyloid-like aggregation of PD originates in the gut and eventually invades the brain. Interestingly, motor deficits of PD mice improve when the gut microbiota is abolished by antibiotics, highlighting the key role of the gut microbiota in gut-brain communication in PD pathology. Sex differences exist in PD disease prevalence, clinical features, and progression, with men being 1.5 times more likely to have PD than women. However, the interplays between sex and the gut microbiota in motor deficits related to PD remain unclear. OBJECTIVE: We sought to assess the modulatory effects of the gut microbiota and sex on motor function and brain-region-specific proteome in healthy mice to understand these fundamental interactions without the presence of PD pathology. METHODS: The motor function was assessed in both male and female conventionally-raised

(CONV) and germ-free (GF) mice using four behavioral tests: vertical pole (balance and agility), adhesive removal (first contact: sensorimotor; total removal time: fine motor function), grip strength (force), and open-field (distance and velocity). The brain proteomic profile in substantia nigra was assessed using LC-MS/MS at the Purdue Proteomics Facility. RESULTS: We observed significant interactions (p<0.05) between sex and microbiota status in the vertical pole, the first contact time in the adhesive removal test, and the total distance traveled and velocity in the open field test. In male mice, GF and CONV mice performed similarly in the vertical pole test and first contact time of the adhesive removal test. However, female GF mice performed significantly more rapidly than female CONV mice for these two assessments. In the open-field test, male and female mice traveled similarly in distance and velocity with the presence of the gut microbiota. However, males traveled with reduced distances and velocities compared to females when lacking the gut microbiota. Sex-based differences (p<0.05) were observed where female mice were faster in terms of total time of adhesive removal but exerted lower grip strength compared to males. Distinct proteomic profiles of the substantia nigra were observed between GF and CONV mice. GF mice had higher levels of brain proteins closely related to PD, including GAD2, SRRM2, and SYNJ1, compared to the CONV group (p<0.05). Other groups have reported that GAD gene therapy can improve motor symptoms, whereas SRRM2 is upregulated in PD patients. Moreover, mutation of SYNJ1 is associated with autosomal recessive early-onset PD. Collectively, these observations suggest that the gut microbiota may modulate brain proteins involved in PD pathophysiology. CONCLUSION: In summary, we observed interaction effects of sex and microbiota status in balance, agility, sensorimotor function, travel distance, and velocity. Fine motor function and force were only modulated by sex. The presence of the gut microbiota altered the abundance of PD-related proteins in the substantia nigra. Our data suggest that motor function is modulated by both sex and the status of the microbiota, while brain proteins are mainly impacted by the microbiota status.

------- poster no. 4 -------

Identifying Microbes that Improve Growth of Biofuel Crop Switchgrass

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The soil microbiome is extremely diverse and plays a significant role in plant health. The microbial community closely associated with plant's roots is known as the rhizosphere. Plants send signals to the microbial community within its rhizosphere via metabolic root exudates, which influences the composition and abundance of microbes in the rhizosphere to support the plant's needs. To gain insight as to how rhizosphere microbes affect the growth of the biofuel candidate switchgrass, a collection of bacteria was isolated from the rhizosphere of switchgrass plants grown at the Kellogg Biological Station in Hickory Corners, MI. Growth curve assays were performed to determine bacterial growth kinetics in the presence and absence of switchgrass root exudates. Preliminary results show several rhizosphere isolates with improved growth when supplemented with switchgrass root exudates as compared to growth in control condition, and in at least one of three key growth parameters: biomass (measured as maximum optical density at 600 nm), decreased lag time to exponential growth, or increased rate of exponential growth. 32 isolates had increased total biomass (measured as optical density). Of these, we targeted 15 isolates (14 proteobacteria, 1 actinomycetota) for follow-up studies of pairwise interactions with other isolates to construct non-inhibitive communities that could next be tested for positive plant outcomes in greenhouse studies. Ultimately, identification of beneficial switchgrass microbes can be used to treat switchgrass in the field, ideally resulting in increased biomass of switchgrass crops.

------- poster no. 5 -------

Bile Acid Regulation of Gut Mycobiome

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Candida albicans (CA), a commensal gut fungus (mycobiome) and opportunistic eukaryotic organism, frequently inhabits the gastrointestinal (GI) tract. Analysis of the gut microbiota so far has predominantly focused attention on the microbiome, while failing to underscore the critical role of the mycobiome in health and disease. Importantly, the factors regulating the colonization of mycobiome components in the GI tract and the role of gut fungi in the health and disease remain poorly understood. To address this gap in knowledge, our lab utilizes a combination of targeted metabolomics, 16S ribosomal RNA amplicon gene sequencing, and in-vivo mouse models to divulge the complex interaction between mycobiome, the metabolome and the microbiome. Our findings indicate that taurocholic acid (TCA), a major bile acid present in both humans and mice control the balance between commensalism and invasive CA infection originating from the gut. Oral administration of TCA through drinking water is sufficient to induce colonization and dissemination of CA in mouse models. Recent findings indicate that TCA regulates CA by controlling immune cells in the intestine. Among

immune cells examined, the percentage of CD11b+ CX3CR1+ mononuclear phagocytes that play a critical role in mycobiome regulation in the intestine was significantly decreased in TCA-treated mice that had increased CA colonization and dissemination. Future studies to elucidate the mechanism(s) by which TCA regulates host defense will provide in-depth understanding of bile-mediated regulation of CA colonization in the intestine. Taken together, the gut mycobiome is gaining recognition as a fundamental part of our gut microbiota. Mycobiome have been increasingly found to contribute and play an active role in inflammatory bowel disease, microbiota-gut-brain axis, colonization and pathogenesis of enteric pathogens. Our findings will have broad implications for our understanding of the gut mycobiome in health and disease which is an underappreciated aspect of microbiome research.

------- poster no. 6 -------

Heat Exchanger System Water Quality: Progress Towards Understanding the Cooling Tower Microbiome

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Cooling towers are evaporative heat exchangers that reduce water temperatures for facility applications. Maintaining water quality is an important consideration in the tower environment in order to reduce public health risks. For example, the public can be exposed to pathogens, such as Legionella or Mycobacterium, through the inhalation of mist droplets containing bacterial cells that are emitted from the towers. Thus, biocidal chemicals are often used for control. While maintaining healthy microbiota in these towers may help sustain water quality, very little is known about cooling tower microbial communities or even the typical members that persist within them. In order to address these knowledge gaps, we set out to characterize the cooling tower microbiome in evaporative systems across the state of Indiana, asking the question: is there a distinct cooling tower community or do communities vary from tower to tower across a region? Working towards this aim, we collected numerous samples from individual cooling towers in the Fall semester of 2021 on four university campuses state-wide in Fort Wayne, Indianapolis, Hammond, and Westville. DNA extraction of ca. 250 samples are currently underway with the objective of submitting these samples for high-throughput sequencing (HTS) this summer. These HTS data will help us to understand how tower microbial community composition varies both within towers or between them and if core healthy community members are recognizable overall.

------- poster no. 7 -------

Nucleic Acid-based Detection of Viruses Associated with Respiratory Disease in Humans and Animals on Paper

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Detection and differential diagnosis of pathogens associated with respiratory disease or acute respiratory infections in both animals and humans is typically not conducted due to the cost-prohibitive nature of current laboratory-based diagnostics. Additionally, differential diagnosis at the point-of-need for these respiratory conditions could alter patient treatment potentially resulting in more rapid recovery and a lower risk of transmission. The objective of this research is to create a spatially multiplexed paper-based device utilizing loop-mediated isothermal amplification (LAMP) to sensitively and specifically detect viral pathogens associated with respiratory disease in both patients and animals. Phenol red, a pH indicator, is used to visually indicate the presence of a given viral pathogen by changing color from red to yellow. We demonstrate the utility of this device by detecting SARS-CoV-2 in diluted human saliva on paper. A series of LAMP primer sets targeting SARS-CoV-2 were screened fluorometrically in water and saliva using heat-inactivated SARS-CoV-2 and scored according to their performance. The top performing primer set, targeting a region in the orf1ab polyprotein gene, was dried on paper along with all reagents necessary to perform LAMP. 5% diluted human saliva was then used to rehydrate the reagents and primers. After heating at 65 °C for 60 minutes, the device had an accuracy of 98% with a sensitivity and specificity of 97% and 100%, respectively when using digital image analysis. When accounting for ambiguity in users' color perceptions, the accuracy lowered to 91% with a sensitivity and specificity of 76% and 100%, respectively. The device lays the foundation for the multiplexed detection of pathogens at the point-of-need without having to utilize costly assays and ship patient samples to reference laboratories. Methods are being devised to utilize similar devices for detection of viral pathogens associated with respiratory disease in humans, cattle, and pigs using both nasal and saliva samples.

------- poster no. 8 -------

Meta-analysis of the longitudinal change of swine fecal microbiome

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Well-characterized microbial succession patterns with high resolution in humans have provided useful information clinically and nutritionally. However, the dynamics of swine gut microbial development and how it develops from nursery and weaning toward an adult pattern are poorly characterized. Such missing knowledge may help us in probiotics selection, core microbiome identification, and pork-borne pathogen control. The previous studies aimed at piecewise models were sporadic with large time intervals and failed to provide a clear view of the developmental trajectory of the swine gut microbiome. Thus, the previous “snapshot” comparison is incomplete with the influence of natural variations and transient dynamics. We conducted a meta-analysis aimed at tracking the microbial colonization and succession in swine gut from birth to marketing with a high resolution. A total of 16 studies published from 2019 to 2021 were included. There were 3671 fecal samples covering 62 time points (from birth to marketing) of over 409 pigs. Sequences with raw format prior to data analysis have been deposited at the European Nucleotide Archive or Sequence Read Archive. Control groups (n=2478) from each study were used to determine the microbial succession and maturation. Effects of antibiotics, fecal microbiota transplantation (FMT), and diet restriction were determined based on the treatment groups (n=1193). Antibiotics reduced the Faith PD, Shannon entropy, and the number of observed features while FMT increased the Faith PD, and the number of observed features. The 16S rRNA gene hypervariable regions explained the variation of beta diversity at weaning when the Bray-Curtis distance matrix was used. We assume the microbial community is similar among individuals at weaning because they take the same liquid diet from the mothers with a very similar component. Weighted UniFrac is proposed when comparing the data from different hypervariable regions considering the above assumption and the more variance explained by the principle coordination axis 1 of the weighted UniFrac. FMT have little effects on the microbiota age of young pigs when regressed using the random forest algorithm. Dirichlet Multinomial Mixtures cluster results showed a tendency of the separation of the microbial community for different growth stages, although the trend is not very clear. Overall, our study confirmed the different microbial communities between different stages and demonstrated the gradual change of the swine gut microbiome. We found the heterogeneity of different sequence regions and the strength of weighted UniFrac over Bray-Curtis when comparing multiple sequence region data sets. Our future study will focus on the 1) heterogeneity of different studies and 2) comparing the effect sizes between different studies when the same treatment was put on pigs.

------- poster no. 9 -------

Water Quality and Reproducibility Among Newly Constructed Building Plumbing Systems

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In the midst of the COVID-19 pandemic, public health concern over stagnated tap water within building plumbing was brought to light as commercial and campus buildings were shut down across the nation. In response to this concern, major research efforts were conducted to test the microbial and chemical environments within the plumbing of unoccupied buildings as well as the efficacy of preventative and remedial methods. Water quality degradation was noticed in buildings that experienced extended stagnation. However, it was also noticed that results varied across studies due to the diversity of building infrastructure (i.e. pipe material and water disinfectant type). Due to these studies having mostly been conducted on variable, existing infrastructure, it is difficult to make generalizations from these results and thus further research is needed to be conducted using more controlled environments. A team of Purdue engineering graduate and undergraduate students along with faculty and staff collaboratively built 4 large plumbing wall systems here at Purdue that will represent 4 standard building plumbing infrastructure. Each plumbing wall has been built the same, made of copper pipes in triplicate and are equipped with a water heater storage tank and a water softener. These walls create a much more controlled environment as opposed to existing infrastructure and the multiple replicates allow for better comparison of results. In order for the data of multiple replicates in a large-scale experiment to be comparable, reproducibility of these replicates must first be proven. My first experiment with these walls has been on determining if replicate plumbing systems undergoing the same water quality conditions will result in reproducibility (i.e have equivalent or similar results). In order to do this, I have collected samples to measure certain parameters such as pH, temperature, chlorine, turbidity, total and dissolved organic carbon, total and dissolved metals, cell counts, and so on over a 5 week period.

------- poster no. 10 -------

Breeding for novel microbiome-associated phenotypes (MAPs) to improve nitrogen retention

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Nutrient retention in agricultural systems requires a thorough understanding of the microbiome residing within them. N-cycling members of the microbiome play a prominent role in the loss of nitrogen from agricultural systems. Unsustainable microbial N-cycling processes such as denitrification and nitrification have been estimated to contribute up to 70% of the nitrogen loss from arable lands. To prevent the loss of nitrogen, plants have been shown to have phenotypic mechanisms to inhibit the activities of nitrifying and denitrifying microbes (known as biological nitrification inhibition: BNI and biological denitrification inhibition: BDI). These BNI and BDI traits are microbial-associated phenotypes (MAPs), which have the potential to be incorporated into agricultural systems to improve nutrient retention. Microbial-associated phenotypes (MAPs) are plant phenotypes that are dependent on the composition and variation of the microbiota. Here we present our discovery of BNI and BDI MAPs in maize to manage nitrification and denitrification. We discovered these traits by using newly developed teosinte-maize near-isogenic lines (NILs). Using NIL populations allows for the fine mapping of traits to specific genetic loci in the plant genome. NILs were grown in the field setting in a randomized complete block design, and microbiomes were characterized through DNA sequencing of microbial N-cycling genes and potential nitrogen cycling assays. From this maize-teosinte NIL experimental population, we identified candidate NILs that altered the composition of the microbiome in the root zone and the nitrification and denitrification activity of the soil. Functionally, we identified five NILs that altered the microbial communities' N-cycling activity. Two of these NILs were shown to have a 40% reduction in potential nitrification rates. Three of these NILs were shown to have a 35% reduction in potential denitrification rates. Furthermore, we set out to identify the mechanisms and genes by which these maize genotypes are suppressing the activity of nitrification and denitrification. Moving forward, we are breeding these BNI and BDI MAPs into maize hybrids to quantify how these traits can potentially be used to improve agricultural sustainability and how to shape plant yield.

------- poster no. 11 -------

Plant variety and soil phosphorus level modulate the soybean rhizosphere microbiome

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Phosphorus (P) is one of the most limiting nutrients in the soil for plant growth. Although plants have developed mechanisms to thrive under low P limitations, they rely on the soil microorganism present in the rhizosphere to increase the solubility and availability of the P from the soil. We hypothesize that genetically diverse soybean varieties have different microbiome compositions in the rhizosphere that is also dependent on the soil P concentrations. The objective of this work was to compare the rhizosphere microbiome from seven genetically diverse soybean varieties in soil with low and high P levels. In 2021, these seven varieties were grown in a randomized block design with three replicates and two P soil conditions (low and high) in chronically low P level soils located at Pinney Purdue Agricultural Center (PPAC). Rhizosphere soils were collected for 16S rRNA gene analysis (V3/V4 region), sequenced using the Miseq Illumina platform, and analyzed using the QIIME 2 pipeline. Rhizosphere microbiome alpha diversity values of two soybean varieties in low P soil, PI378684A (Glycine soja, Japan) and PI592523 (Modern, US) had significantly different (p > 0.05) Shannon diversity values from the other varieties. Whereas, under high P conditions only variety PI592523 had a significantly lower Shannon value. Comparison of diversity between rhizosphere microbiomes (Beta diversity, unweighted Unifrac) indicated significant differences among varieties (perMANOVA p = 0.001). There greatest differences were between PI59323 (Modern, US) and PI378684A (Glycine soja, Japan) (pairwise perMANOVA, p < 0.05). Whereas pairwise comparisons rhizosphere microbiomes of each variety under high and low P condition were not significantly. However, there were taxa in three soybean varieties, PI378684A (Glycine soja, Japan), PI597480 (Glycine max, Japan), and PI592523 (Modern, US) that significantly differed based on LEfSe analysis in low versus high P soils. Glycine soja had the greatest number of differentially abundant taxa in low compared to high P soils. In conclusion, the comparisons showed that different soybean varieties influence the rhizosphere microbiome structure. However, communities of each soybean variety do not significantly differ between high and low P soils. This indicates despite the differences in rhizosphere microbiomes among soybean varieties, microbial communities have adapted to low P soils that is not differentiated when additional P is added. More studies are necessary to understand the effects that these microbiomes can have on plant P nutrition under stress.

------- poster no. 12 -------

Identify Gut Microbe Traits that Influence Competitiveness for Complex Carbohydrates

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Gut microbes compete in the gut for complex carbohydrates based upon their genome-encoded hydrolytic and transport potential. However, the extent to which differences in organismal traits (here, the presence of specific carbohydrate-active enzymes, or CAZymes) influence competition for oligosaccharides varying in structure and, specifically, which traits are most influential, is poorly understood. The purpose of my research is to determine what traits influence competition for a simple oligosaccharide, inulin, with respect to varying branch lengths. This project has two components; the first is experimental evolution to allow increases in the sizes of inulins consumed and the second is deliberate alteration of the localization of hydrolytic genes (e.g. intracellular vs. secreted) to determine how these traits influence competition and population sizes of fermenting organisms. I have begun with experimental evolution K. pneumoniae and E. coli in consumption of increasingly larger inulin sizes and determining what other nutrients whose concentrations might govern competition for inulins. Here, I present data on the consumption patterns of inulins and the experimental evolution of members. Interestingly, our experiments suggest that E. coli may not directly consume inulins, contrary to what was previously observed. Further research will be done to determine whether this strain can be evolved to hydrolyze inulins.

------- poster no. 13 -------

Gut microbiota, obesity, and diet in colorectal cancer risk

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Epidemiological evidence has demonstrated a rise in young onset colorectal cancer (CRC), underlying a need to understand mechanisms that contribute to CRC development. One factor consistently associated with increased CRC risk is obesity. Since the gut microbiota is a key feature of the intestine that has been shown to significantly influence CRC development, obesity mediated changes to the gut microbiota may also play an integral role in obesity associated CRC risk. Additionally, westernized diets characterized as high in saturated fat and refined sugar are shown to promote the development of obesity and affect gut microbiota composition. Thus, it is essential to consider diet composition when assessing the role of the gut microbiota in obesity associated CRC. OBJECTIVE: We aimed to isolate the role of obesity associated gut microbiota in CRC using fecal microbial transplantation (FMT). We hypothesized that microbiota from obese mice on obesogenic diets would promote CRC independent from the development of obesity. METHODS: After fifteen weeks of dietary interventions, donor mice developed obesity or remained lean through pair-feeding on a low-fat diet (LFD), high fat diet (HFD), or western diet (WD). Donor cecal and fecal material were anaerobically collected for FMTs and stored in a -80°C freezer. Thawed FMT samples were transplanted into recipient mice. One week after colonization, recipients were treated with the chemical carcinogen, azoxymethane, to induce CRC. For the duration of the study, recipients were maintained on the LFD. At euthanasia, tumors were enumerated, and colons were processed for histological analysis. Fecal samples were collected before and after tumor development for microbiota analysis using Illumina Miseq platform targeting the V4 region of the 16S rRNA gene. RESULTS: Our results showed that gut microbiota-induced CRC outcomes were driven by diet of the donors and not dependent on obesity status of the donors. HFD-recipients had increased tumor incidence and number relative to LFD-recipients, and higher incidence compared to WD-recipients. While WD-recipients did not have macroscopic signs of increased CRC development, they displayed higher levels of inflammation compared to LFD-recipients. Interestingly, colon-tumor tissue of WD-recipients also had increased expression of signal regulatory protein alpha, an immune cell receptor that inhibits phagocytosis, relative to LFD-recipients. This may be indicative of a less active immune response against cancer cells. Collectively, these results suggest that WD-recipients have a higher likelihood of developing CRC compared to LFD-recipients, but also that there may be aspects of WD-associated gut microbiota attenuating progression from cellular to macroscopic measures of tumorigenesis. Overall, gut microbiota composition prior to tumor development was different relative to post-tumor development. At both timepoints, we identified significantly enriched taxa in WD-recipients such as Enterococcus, Bacteroides, and Anaeroplasma, and in HFD-recipients such as Dorea, Sutterella, and Erysipelotrichaceae. These taxa have been associated with obesity, CRC, and inflammatory processes that may contribute to the observed outcomes in this study. CONCLUSION: Obesogenic diets have differential effects in promoting CRC development, highlighting the importance of dietary contributions in the gut microbiota-CRC relationship. Understanding these interactions will aid in designing dietary strategies that can reduce CRC risk.

------- poster no. 14 -------

Processing Method and Particle Size of Wheat Bran Modulate Composition of Fecal Community and its Metabolic Functions

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Human gut microbiome plays an important role in our daily energy and nutrient requirement, modulation of the immune system, and the production of short chain fatty acids (SCFAs) which influence whole-body metabolism. Abnormalities in the composition and function of this microbial ecosystem result in chronic diseases. Diet plays a particularly important role in determining the diversity of their colonic microbial community. For example, dietary fibers cause a shift of the bacterial community and alter its metabolic outputs. Wheat bran (WB), obtained from refined flour production, contain up to 18% of dietary fibers dominated by non-starch polysaccharides such as arabinoxylans, cellulose, and β-D-glucans. Physical modification of WB, e.g. milling, can increase exposure of nutrients and recruit specialized bacteria to ferment fiber. Therefore, the objective of this study was to investigate the effect of milling methods and particle size of WB on the composition of fecal community and its metabolic outputs. For this purpose, five fractions (ranging from <180 to 850 µm) were sieved after milling WB with lab-scale hammer, roller, and stone mills. WB fractions were then digested using α-amyloglucosidase and protease to mimic passage through the upper gastrointestinal. Sequential in vitro fermentation was performed by inoculating fermented WB fractions with gut microbiota of two healthy individuals in phosphate-buffered gut mineral medium, fortified with 10 µM of each proteinogenic amino acid and 1X ATCC (Wolfe's) vitamin mix. After 24 hours of incubation at 35 ºC, cultures were transferred to new tubes, and this repeated for 7 sequential cultures, over which SCFA and microbial community of particles and supernatant (16S rRNA gene sequencing) were measured. Our results showed that SCFA production was particle size- and milling method-dependent. Interestingly, however, the relationship of butyrate production with particle size was method dependent. These differences in SCFA production were accompanied by changes in microbial community structure. After seven sequential passages, Lachnospiraceae and Bacteroides dominated the microbial community in particle and supernatant, respectively.

------- poster no. 15 -------

Host Plant Species Influences the Composition of Milkweed and Monarch Microbiomes

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Plants produce defensive chemicals for protection against insect herbivores that may also alter plant and insect associated microbial communities. However, it is unclear how expression of plant defenses impacts the assembly of insect and plant microbiomes, for example by enhancing communities for microbes that can metabolize defensive chemicals. Monarch butterflies (Danaus plexippus) feed on milkweed species (Asclepias spp.) that vary in production of toxic cardiac glycosides, which could alter associated microbiomes. We therefore sought to understand how different milkweed species, with varying defensive chemical profiles, influence the diversity and composition of monarch and milkweed (root and leaf) bacterial communities. Using a metabarcoding approach, we compared rhizosphere, phyllosphere and monarch microbiomes across two milkweed species (Asclepias curassavica, Asclepias syriaca) and investigated top-down effects of monarch feeding on milkweed microbiomes. Overall, monarch feeding had little effect on host plant microbial communities, but each milkweed species harbored distinct rhizosphere and phyllosphere microbiomes, as did the monarchs feeding on them. There was no difference in diversity between plants species for any of the microbial communities. Taxonomic composition significantly varied between plant species for rhizospheres, phyllospheres, and monarch microbiomes and no dispersion were detected between samples. Interestingly, phyllosphere and monarch microbiomes shared a high proportion of bacterial taxa with the rhizosphere (88.78 and 95.63%, respectively), while phyllosphere and monarch microbiomes had fewer taxa in common. Overall, our results suggest milkweed species select for unique sets of microbial taxa, but to what extent differences in expression of defensive chemicals directly influences microbiome assembly remains to be tested. Host plant species also appears to drive differences in monarch caterpillar microbiomes. Further work is needed to understand how monarchs acquire microbes, for example through horizontal transfer during feeding on leaves or encountering soil when moving on or between host plants.

------- poster no. 16 -------

Mice prone to develop colorectal cancer driven by inactivating tumor-suppressor *Apc* and activating oncogenic *Kras* mutations have different gut microbial profiles compared to control mice without mutant genes

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Colorectal cancer (CRC) is the third most common cancer in the United States and worldwide. Most CRCs occur sporadically and are caused by somatic gene mutations in the large intestine. Mutations in *adenomatous polyposis coli (APC)* and *Kirsten rat sarcoma viral oncogene homologue* (*KRAS)* are considered key driver mutations, fostering uncontrolled cell proliferation. Although accumulation of genetic aberrations causes colorectal neoplasm, mounting evidence suggests that interplays between host genetic, diet and gut microbiome have significant impacts on the disease onset and progression, and therapeutic responses. There have been developed multiple genetically modified mouse models (GEMM) of CRC with mutations in *Apc* and *Kras*, which are valuable tools to study pathobiology, treatment, and prevention of CRC. However, a major limitation of most of these *Apc* mutant models is predominant tumorigenesis in the small intestine rather than the large intestine. To overcome this limitation, a mouse model called AKC was developed to generate colon-specific tumors driven by inactivation of *Apc* and activation of mutant *Kras*, which were introduced by carbonic anhydrase 1 promoter/enhancer-Cre recombinase transgene (CAC). The AKC mice are unique as they only develop tumors in the large intestine and not in the small intestine, which resembles human CRC. Despite their similarity to the human disease, only limited features of AKC mice have been characterized, which potentially hinders its utility for cancer research. The goal of this study is to characterize key aspects of disease related phenotypes including involvement of inflammation and potential changes of gut microbial profile in the AKC mice. We compared the gut microbiota profile of the AKC mice and healthy wildtype (WT) control, using 16s rRNA gene sequencing (V3-V4 regions) on fecal DNA. Taxonomic richness and/or evenness (alpha diversity) were not different between the AKC and WT mice. Interestingly, the structures and composition of microbial community (beta diversity) were distinct between AKC and WT mice, as indicated by microbial community (dis)similarity indices including Jaccard, Bray-Curtis, and unweighted/weighted-UniFrac. Using Analysis of Compositions of Microbiomes (ANCOM) and Linear discriminant analysis Effect Size (LEfSe), we identified differentially abundant taxa between the two groups. At phylum level, AKC mice had increased *Deferribacterota* and decreased *Actinobacteriota* abundances. At family level, *Bacteroidaceae, Enterobacteriaceae, Deferribacteraceae,* and *Peptostreptococcaceae* were significantly abundant, while *Bifidobacteriaceae* was diminished in AKC compared with WT. At species and genus levels, *Bacteroides vulgatus, Eubacterium_fissicatena_group,* and *Escherichia_Shigella* were significantly abundant in AKC mice feces than in WT. These findings highlight gut microbial changes promoted by tumor-promoting environment in the AKC model, which can be a useful toolkit to study the CRC biology and potential therapeutic and preventive interventions.

------- poster no. 17 -------

Host genetic variation drives the differentiation in ecological role of the native Miscanthus root-associated microbiome

Niuniu Ji*, Angela Kent

Background: Plant root interacts with microbiome which have important functions to influence plant health and development, but how host genetic variation impacts the assembly, functions and interactions of perennial plant root microbiomes are poorly understood. Here we examined both prokaryotic and fungal communities between rhizosphere soils and root endophytic compartment in two native Miscanthus species (Miscanthus sinensis and Miscanthus floridulus) of Taiwan, and further explored the potential function of root-associated microbiomes based on FARPROTAX. Results: Our results suggested that most of root endophytic microbial communities were influenced by horizontal transmission;

plant genetic variation and soil had effects on the root endophytic and rhizosphere soil microbial compositions and co-occurrence networks in both Miscanthus sinensis and Miscanthus floridulus, with the increased effect of plant genetic variation on the root endophytic communities. Further, we found that root endophytic microbial communities in both Miscanthus sinensis and Miscanthus floridulus were more strongly driven by deterministic processes rather than stochastic processes. It was found that root endophytic-enriched prokaryotic OTUs belong to Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Sphingobacteriia and [Saprospirae] both in Miscanthus sinensis and Miscanthus floridulus; while rhizosphere soil enriched prokaryotic taxa are widely distributed among different phyla. FARPROTAX analyses further indicated that root endophytic microbiomes possessed higher functional relative abundance of chemoheterotrophy, aerobic chemoheterotrophy, nitrogen fixation and plant pathogen, with functional genes related to nutrient provision enriched in the rhizosphere soil and nitrogen cycling, hydrocarbon degradation, and aromatic compound degradation in in both Miscanthus sinensis and Miscanthus floridulus. A core microbial community composed of forty rhizosphere soil prokaryotic OTUs, seven root endophytic prokaryotic OTUs in Miscanthus sinensis; and 116 rhizosphere soil prokaryotic OTUs, seventeen root endophytic prokaryotic OTUs in Miscanthus floridulus. These core prokaryotic OTUs contribute small fraction of prokaryotic community assembly in two Miscanthus species. In Miscanthus sinensis, these core prokaryotic OTUs within the rhizosphere soil have functions in nitrogen cycling, carbon degradation and heterotrophy functions; core prokaryotic OTUs within the root endophyte play roles in carbon cycling, carbon degradation and heterotrophy. In Miscanthus floridulus, core prokaryotic OTUs within the rhizosphere soil have functions in nitrogen cycling, carbon cycling, carbon degradation and heterotrophy functions; core prokaryotic OTUs within the root endophyte play roles in nitrogen cycling and plant pathogen functions. Conclusions: In this study, we provide comprehensive and empirical evidence on the relative contribution of host and environmental factors to the microbiome assembly in Miscanthus sinensis and Miscanthus floridulus. Our results demonstrate that microbiome assembly is shaped predominantly by host genetic variation and environmental factors. Further, we revealed that host selection increased and had a strong effect on reducing microbial diversity and network complexity from in root endophytic communities compared to the rhizosphere microbiome. These findings significantly advance our current understanding of the microbial community assembly in bioenergy crops such as Miscanthus under different environmental selection pressures, and highlight the importance of the host selection effect.

------- poster no. 18 -------

Nucleic acid detection of live pathogens on contaminated foods

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The obstacle for DNA-based techniques as Biosensors is that DNA in the environment can be very stable and can persist for extended periods of time (days to weeks) after cell death. The biosensors based on the amplification of target DNA, can detect the DNA from dead cells and lead to overestimation of contamination or false positives. Here, our goal is to present a technology of Loop-mediated isothermal amplification (LAMP)-based colorimetric microfluidic device for the detection of E. coli O157:H7 DNA from live cells on contaminated food products and therefore, prevent false positives from dead pathogens. Propidium monoazide (PMA) is a photoactive dye that can preferentially penetrate dead cells, or cells with damaged or permeabilized cell membranes, but not viable cells with intact cell membranes. Once inside the cell, PMA molecules then intercalate into the DNA and become covalently bound to DNA upon exposure to blue light. This photoactivation process results in the formation of a stable DNA-PMA complex that renders the DNA inaccessible for amplification. Therefore, samples after treatment with PMA will give true amplification.

------ poster no. 19 -------

Impact of Epoxy Manufacturing and Installation Conditions on Drinking Water Quality

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Epoxy linings are increasingly used to address the aging potable water infrastructure systems, including replacing lead services lines. Epoxy linings are installed by chemically manufacturing new materials inside an existing pipe or tank. The in-situ manufacture of this plastic poses unique challenges to utilities and consultants because post-installation performance verification practices are not available. Furthermore, field installations of epoxy lining are completed outside (and thus at various temperatures), and contractors may have different methods. We reviewed literature to identify knowledge gaps and best practices associated with epoxy installation. Very little information currently exists regarding chemical leaching from epoxy linings in potable water applications. However, researchers have revealed leaching of organic carbon and bisphenol compounds, as well as potential interactions with chlorine disinfectant for potable water in contact with epoxy linings. The NSF International Standard 61 is often referenced as a benchmark for safety of these materials, but data from these studies are not disclosed. In order to address these gaps, we pursued

bench-scale testing to evaluate the effects of epoxy lining conditions (specifically, curing time and resin to hardener ratio) on chemical and microbiological water quality impacts. Experiments are being conducted currently. Water quality is being analyzed for semi-volatile organic compounds using liquid chromatography mass spectrometry, specifically targeting bisphenol compounds. Thermal and physical properties will be analyzed with thermogravimetric analysis. Total organic carbon and assimilable carbon, the biologically available fraction of carbon that contributes to growth, are also being investigated. Preliminary evidence shows high organic carbon leaching, as well as increased leaching when installation conditions varied from manufacturer instructions. It is expected that deviation from manufacturer conditions will result in greater chemical leaching and biological activity. The data collected in this study will emphasize the importance of manufacture instructions, which could lead to stricter guidelines during installation.

------- poster no. 20 -------

Development of a Gnotobiotic Mouse Model with Distinct Bacterial Equol Producing Capabilities

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Equol, a microbial metabolite of the phytoestrogen daidzein, is known for having the highest binding affinity to mammalian estrogen receptors among all soy isoflavones. Not all humans harbor equol-producing microbes in their gut, and less than half of the western population can be classified as equol producers. Post-menopausal women have estrogen deficiency, and are hypothesized to gain greater benefits from soy consumption if they are equol producers. To date, soy feeding research published suffers from confounding factors that make assessing causal impact of equol production in health difficult due to: (i) large interpersonal variation of the human microbiome and human genomes and that (ii) all rodent models harboring natural microbiome being highly efficient equol producers. Therefore, we aim to establish a gnotobiotic mouse model by colonizing germ-free mice with synthetic bacterial communities with divergent equol-producing capacities. A core community of nine non-equol producing bacterial strains was designed to include Bacteroides uniformis, Bacteroides caccae, Bacteriodies fragilis, Faecalibacterium prausnitzii, Agathobacter rectalis, Coprococcus comes, Akkermansia muciniphila, Providencia stuartii, and Collinsella aerofaciens. To model the gut microbial community of an equol-producer, an equol producing strain Adlercreutzia equolifaciens was added to the core community. Female germ-free C57BL/6 mice (n=3) were colonized with either the core community or the equol-producing community for 3 weeks. Daidzein was administered by dietary supplementation (1.5% wt/wt daidzein) for 4.5 weeks or oral gavage (two doses of 6mg) 24-48h before euthanasia. As expected, equol was detected in the serum in mice colonized with equol-producing community but not in those colonized with the core community. Route of daidzein administration did not affect equol producing capacity. Our results demonstrated the ability of gnotobiotic mice to display distinct equol-producing phenotypes by colonizing with synthetic bacterial communities. A longer colonization with a dietary formulation including fermentable fiber is currently being tested using similar synthetic bacterial communities. Once validated, a causal relationship between equol production and health outcomes can then be elucidated.

------- poster no. 21 -------

Longitudinal Analyses of Urine Cultures from Healthy Dogs

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Urine was long considered sterile, but recent studies have revealed that a wide variety of microbes are present in the urine of healthy individuals. These microbes are part of the urine microbiome and play an important role in maintaining host health, however, this field remains understudied, and little is known about the urine microbiota and its stability in healthy dogs over time. Studies using 16S rRNA sequencing have been valuable to analyze bacterial composition and diversity but may overrepresent bacteria that are not viable by culture. In this study, our goals were to identify culturable bacterial taxa present in the urine of healthy dogs, assess the variability of these taxa over time, and determine if taxonomic profiles varied by dog or by sex. Mid-stream free catch urine was collected from 7 male and 7 female dogs over 12 timepoints, ranging from hours, to days, to weeks, to months apart. Each urine sample was plated onto Blood agar and MacConkey agar, incubated aerobically at 37°C and checked at 24 and 48 hours for growth. Colonies with were collected and subjected to matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) for bacterial identification. Over 12 time points in 14 dogs, viable bacteria was cultured in 50% (85/168) of the samples. Presence or absence of culturable bacteria did not differ by sex, and 4/12 dogs cultured > 105 Colony-forming units (CFU)/mL which is typically considered the “culture positive” threshold for clinically assessing urinary tract infections in voided samples. Two of these dogs, both male, cultured > 105 CFU/mL at over 5 timepoints but never exhibited lower urinary tract clinical signs or pyuria. The most commonly cultured bacteria were Streptococcus canis and Staphylococcus pseudintermedius, and the number of total bacterial taxon types (richness) cultured in each dog did not

differ significantly by sex or timepoint but did differ significantly by dog. Cultured bacterial profiles varied significantly between dogs; but was less variable within dogs over time, indicating some stability in the urinary microbiota. No differences in presence or number of taxa (richness) by sex were observed. Previous 16S sequencing suggests high bacterial diversity in female canine urine, but no significant differences were found in the presence or number of bacterial taxa in our cultures. The most commonly cultured organisms, Streptococcus canis and Staphylococcus pseudintermedius were skin microbes frequently found in voided urine. Cystocentesis or catheterized urine collection is recommended when feasible to avoid these contaminants. Multiple healthy, asymptomatic dogs cultured high abundances of bacteria (>105 CFU/mL); although, these bacteria were likely skin contaminants. Uropathogens associated with UTIs like E.coli and P. auruginosa were also cultured at low levels in healthy dogs, however the presence of these taxa in cultures from asymptomatic dogs does not necessarily warrant treatment. Future research should utilize a broader array of aerobic and anaerobic culture conditions and media for assessing the presence and diversity of bacteria in urine and analyze 16S rRNA data from the same urine samples to determine what taxa overlap between culture and sequencing data.

------- poster no. 22 -------

EXAMINING URINE PH, SPECIFIC GRAVITY, AND URINE PROTEIN OVER TIME IN HEALTHY DOGS

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Urine properties such as pH, urine specific gravity (USG), and protein profiles are routinely measured during a urinalysis to assess canine health. Clinicians have well-established reference ranges to assess urine in patients with urinary tract diseases, such as urinary tract infections. Despite this, less is known about the natural fluctuations of urine properties in healthy dogs over time. In this study, mid-stream free catch urine was collected from 14 healthy dogs (7 male, 7 female) over 12 time points that were hours, days, and months apart. We hypothesized that urine pH and USG would vary significantly over time, while protein profiles would be stable due to consistent protein filtration by the kidneys. Urine pH was measured via pH meter; USG was measured via refractometer, and proteins were profiled via 4-12% Bis-Tris gel electrophoresis. Preliminary data showed that USG varied significantly ($p < 0.0001$) between dogs but was consistent over time within dogs ($p = 0.2$) while urine pH did not vary significantly between dogs ($p = 0.588$) because pH was so highly variable within dogs over time. Urine proteins ranged from none to trace amounts. The most commonly detected proteins were at molecular weights consistent with Tamm Horsfall and albumin. Statistics on urine proteins are forthcoming. Our hypothesis for USG was unsupported as it did not vary significantly over time, indicating that a USG measurement at any time should be representative for that dog, while pH was so variable that it should be measured at multiple time points before altering clinical decisions. These findings help define normal variations in urine properties, which can inform clinical decision-making around urine sampling and urinalyses.

------- poster no. 23 -------

Longitudinal analysis of spinal cord injury-induced changes in gut viral and microbial communities

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Gut bacteria and viruses are key members of the gut-brain-immune axis. Recent data indicate that spinal cord injury (SCI) changes gut microbe and virus composition and that these changes may be associated with the onset or progression of various post-injury comorbidities and impaired recovery of function. However, to date, those studies are limited in scope, both with respect to time-post injury and information gleaned from the bioinformatics. Here, we assess SCI-induced gut dysbiosis via gene- and genome-resolved microbial metagenomics from 333 longitudinal samples from 59 mice spanning a 6-month recovery period after SCI. These analyses were paired with a subset of matched viral particle-derived metagenomes (viromes). These analyses revealed 245 Metagenome-Assembled Genomes (MAGs) and ~29k viral Operational Taxonomic Units (vOTUs), an increase of 2- and 29-fold, respectively, over existing databases. Ongoing studies will characterize the viral and microbial communities and their functional potential to impact injury-dependent dysbiosis and the loss of sympathetic tone as a potential mechanism underlying it.

------- poster no. 24 -------

A survey exploring the virus complement carried by the Lyme disease tick collected in Indiana.

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Tick-borne diseases (TBDs) are a burden to the healthcare system. Hard ticks of the family Ixodidae transmit several human and animal pathogens. The CDC has reported an increase of TBDs and identified new tick-borne pathogens (TBPs), including two viruses in recent years. There is limited information on the TPBs circulating at the regional and local level, complicating the prevention, diagnosis, and treatment of TBDs. Furthermore, the limited availability of comprehensive diagnostic tools, reliance on clinical signs, and known exposure to ticks/tick habitats for TBDs diagnosis can lead to misdiagnosis. Analysis of the array of pathogenic and non-pathogenic microorganisms (microbiome) carried by the ticks at the regional scale is needed to design detailed TBDs risk maps, improving the prevention, diagnosis, and treatment of TBDs. Microbiome studies have focused mainly on the bacteria carried by the ticks, and only a few studies analyzed the virus complement of ticks in the US. This gap needs to be filled to detect potential human pathogens circulating in the tick population proactively. In this study, we obtained the first analysis of the virome carried by the Lyme disease tick, Ixodes scapularis, circulating in Indiana. Ticks were collected at Tippecanoe River State Parks (TRSP) in 2017-2018. Four tick pools were prepared; pool 1 (2017) was used to optimize the protocol and bioinformatics pipeline, then applied to pools 2 (2017), 3 and 4 (2018). Pools 2-4, composed of 14 ticks each (male and females in equal proportion), were homogenated, filtered, and treated with nucleases to remove bacteria, tick genomic DNA, and rRNA. RNA was then extracted using Trizol LS reagent and submitted to Indiana University Medical Genomics Core for library preparation and sequencing using Illumina NovaSeq6000 (2x100b pair-end reads) at 50 million reads/pool. The RNA preparation of Pool 2 was insufficient for library preparation and was not further processed. The sequencing reads were processed by the Purdue Bioinformatics Core. Reads were quality filtered, sequentially mapped against Tick and bacterial genomes, and unmapped reads assembled using SPADE. Contigs and singletons were subjected to BLASTn search against the NCBI nt database. Unmapped contigs and singletons were subsequently subjected to BLASTx against NCBI RefSeq viral and nr protein databases. We identified sequences with homology to a total of eight viruses, belonging to the genera nairovirus-like (1 virus) and phlebovirus-like (3 viruses); family Rhabdoviridae (1 virus), and unclassified (3 viruses). The viruses identified in our pools belong to species prevalent in previous tick studies in the Northeast. Interestingly, pools 3 and 4, composed of ticks collected in 2018 in consecutive months from the same site, had a different composition of predominant and low abundance viruses. Although the significance of these results needs further investigation, this study expands on the knowledge of the microbiome of Indiana ticks to include viruses. Additionally, it contributes to creating a broad picture of the array of microbes carried by Indiana ticks. It also establishes the foundation for further in-depth studies aiming at identifying viruses of public health significance to be added to the tick-borne virus risk map.

------- poster no. 25 -------

The Influence of Resistant Dextrins on the Microbial Diversity of the Gut Microbiome

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Dietary fibers play an important role in preventing diseases, for example, irritable bowel syndrome, type II diabetes, and cardiovascular disease, in humans, which is thought to be mediated by increasing microbial diversity in the colon. While research supports the consumption of dietary fibers as a category, research also suggests that the chemical structure of dietary fibers may play a significant role in increasing microbial diversity. We hypothesized that increased structural complexity of a glucan would relate to higher sustainable microbial diversity as microbes could specialize in consuming certain structures. Resistant dextrins were used to study these effects; they are type IV resistant starches and are composed entirely of glucose. An in vitro sequential batch fermentation was performed over a week using 1.5% of resistant dextrins with three different fecal donors. Growth curves, pH, gas, SCFAs, metabolites, and 16S rRNA amplicon data were collected. 16S amplicon data revealed that more complex resistant dextrins such as polydextrose maintained more microbial diversity than less complex resistant dextrins such as tapioca mixed linkage α-glucans. Growth curves also suggest that microbial consortia selected on more complex resistant dextrins retain better fitness and adaptability to substrate switching than those selected on less complex resistant dextrins. Overall, our data so far supports that the fine differences in chemical structures of these resistant dextrins may have an influence on the maintenance of gut microbial diversity.

------- poster no. 26 -------

Development of Fecal Microbiota transplant Therapy against Post-weaning Diarrhea in Piglets

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For piglets, weaning is one of the most stressful events during swine production. The change in diet at weaning causes an extreme turnover in the gut microbial composition. Piglets are commonly administered therapeutic, prophylactic or metaphylactic antibiotics to treat or prevent post-weaning diarrhea (PWD). Use of fecal microbiota transplants (FMT) has been shown to resolve C. difficile-induced diarrhea in 80-90% of human cases. One key barrier to adoption of FMT in swine production is that FMTs are generally given via oral gavage, which is too difficult and labor intensive for large swine operations. For FMT to be a viable option in swine management, a simple method is needed to complete the transplant. The objective of the study was to determine the efficacy of three methods of administration (oral, rectal and lyophilized in-feed) of FMT and their efficiency in preventing PWD. We hypothesize that FMT could also resolve PWD in piglets, but the different route of transplant will increase colonization efficiency and subsequently increase efficiency of preventing PWD. Forty newly weaned male piglets were allotted to 4 treatments at 10 piglets per treatment (Control, Oral gavage - FMT1, rectal gavage - FMT2 and lyophilized In-feed – FMT3). Piglets were weaned on day 21 and fed conventional weaning diet. The control and FMT3 groups were given oral gavage of saline to mimic the gavage stress imposed on the groups that received FMT by oral and rectal gavage. All animals were not given antibiotics. Feces from four 12-week-old pigs screen for pathogens and parasites were used as donor material. The study was for nine days and FMT was done for the first five days. Diarrhea incidences were observed for the first week post weaning, body weight was measured on day 0, 3, and 7. Fecal samples were collected on day 0, 2, 5 and 7. Five piglets were sacrificed on day 4 and the remaining 5 on day 8. Cecal and colon content were collected from the sacrificed piglets. DNA was extracted from fecal, colon and cecal content samples and 16S rRNA gene libraries (V4 region) was prepared for bacterial community analysis. All the FMT groups had significantly higher body weight on day 3 (P < 0.05) and average daily gain between day 0 – 3 (P < 0.05) compared to the control but there was no difference between the FMT groups. There was no effect on average daily feed intake and diarrhea incidence. FMT3 (Lyophilized In-feed) had higher alpha diversity (Observed features and Faith’s phylogenetic diversity) on day 5 (P < 0.05) but there was no difference in beta diversity between the FMT groups. There was increase abundance of 27 ASVs (amplicon sequence variant) in the control while the abundance of 30 ASVs were in increased in FMT3 (Lyophilized In-feed) on day 5. In conclusion, the three FMT groups has similar colonization pattern but there was no difference in diarrhea incidence. FMT3 (Lyophilized In-feed) may not change the overall community structure of the piglets but may modulate individual taxa.

------- poster no. 27 -------

Soil microbes from cover crop fields reduce growth of Bt-resistant western corn rootworm with no impact on larval microbiome

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Planting cover crops is an increasingly prevalent sustainable management technique that can increase soil health by increasing microbial abundance and diversity. These soil microbes can improve plant health and alter plant defenses against insects but less is known how the soil microbiome affects insect pest fitness and microbiome composition. The western corn rootworm is a major belowground pest of corn throughout the US Corn Belt. Management relies heavily on the planting of transgenic crops expressing Bacillus thuringiensis (Bt). In this study, we ask how does the soil microbiome impact western corn rootworm (WCR) fitness, WCR microbiome composition, and transgenic crop effectiveness. To do this, we applied soil microbes from continuously managed cover crop fields and those from traditionally managed fields to Bt and non-Bt corn seedlings. We then reared Bt-resistant and -susceptible WCR for 5 days on treated seedlings. Using 16S sequencing, we found microbial inoculum differed between soil types. Filtering of bacteria by WCR resulted in distinct communities from the inoculum. However, WCR microbiomes were not affected by the soil inoculum or seed treatment. In growth assays, Bt-resistant larvae were smaller when reared in the presence of cover crop soil microbes. Yet, this was not the case for susceptible larvae. Still, Bt effectiveness was not impacted by soil treatment, suggesting there is value for growers in utilizing cover crops in their agricultural systems.

------- poster no. 28 -------

A field-deployable paper-based colorimetric LAMP biosensor for the detection of antimicrobial-resistant genes of Bovine respiratory disease.

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Antimicrobial resistance (AMR) is the immediate global threat to the modern medicinal world. AMR will occur due to the change over time and no longer respond to medicines making infections harder to treat and increasing the risk of diseases spreading in viruses, fungi, bacteria, and parasites. Antimicrobial resistance is one of the top 10 diseases that lead to deaths, and in the next few decades, this will become the top leading cause for the deaths. Antimicrobial-resistant organisms can grow and spread from food-producing animals to humans by direct exposure to food and the environment. Problem: Bovine respiratory disease is a complex and high-priority disease that leads to mortality and has an economic burden on the cattle field. The common bacterial pathogens related to the BRD disease are including Pasteurella Multocida, Histophulus Somni, and Mycoplasma Bovis. However, the research results predict the resistance of these bacterial cells to the drugs administered for the BRD disease and the presence of several genes responsible for the AMR in the BRD. Hence the rapid, simple, selective, and sensitive detection of these genes at the farm level is a major research concern. Approach: To address the issue, paper-based colorimetric Loop-mediated isothermal amplification (LAMP) is a simple, fast, selective, and sensitive technique with many advantages. LAMP technique will amplify the specific target with specially designed primers at 65, and because of the amplification, there will be a change in the pH of the reaction mixture, and using a pH-sensitive dye, it can visually identify the presence and absence of the AMR genes. Conclusions: The LAMP technique can be used for visual detection of the presence and absence of AMR genes related to BRD disease in a short span of 30 to 60 min. This technique can be used at the farm level by an individual with very low expertise.

------- poster no. 29 -------

Primer Design

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The microbiome of different species is specific to its diet and although beneficial for the host, can be detrimental for other species. Cases of this nature are common in farms with different animal conglomerates which lead to contaminated crops from a specific source. This aspect of food security is of crucial importance to the consumer's health as well as to the farmer's market. An early and rapid detection of the contamination source can prevent entire crops from going to waste with simple on-site assays. DNA sequence based assays are the basis to differentiate between contamination of the microbiome of a specific host. For these assays, specific primers have to be designed to be compatible with low cost technologies and specific enough for farmers to draw confident decisions with our Assay. Although this research is still ongoing, this will help in future host specific applications of different microbiomes.

------- poster no. 30 -------

Metagenomic profiling of antimicrobial resistance in onsite greywater treatment and reuse

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Reuse of treated wastewater in general and greywater in particular can relieve water scarcity and promote sustainability in arid environments. However, greywater can contain opportunistic pathogens carrying antimicrobial resistance genes (ARGs). Exposure of the greywater microbial communities to micropollutants that are ubiquitous in greywater potentially facilitates the enrichment and spread of multidrug resistance. Therefore, reuse of greywater poses a potential environmental and public health risk to the local environment and the communities that adopt onsite greywater treatment and reuse systems. Recirculating vertical flow constructed wetlands (RVFCWs) have been successfully used for greywater treatment; however, the ability of these systems to remove ARGs is unknown. Previous studies on ARGs in RVFCWs were mostly culture- and qPCR-based. In this study, we leverage shotgun metagenomic sequencing to

characterize ARG and potential human pathogen diversity and abundance in greywater microbial communities before and after treatment by RVFCWs in Israel. Treated greywater or tap water irrigated soil were also analyzed for ARGs and taxonomy. Results revealed that total ARGs decreased in abundance and diversity in treated greywater, and that ARGs were detected in both tap water and greywater irrigated soil. Antibiotic efflux was the dominant resistance mechanism of the detected ARGs, and genes confer resistance to tetracycline antibiotic were the most abundant in both raw and treated greywater. In parallel, the microbial communities became less similar following treatment. Utilizing assembly-based analyses, we observed associations between ARGs, mobile genetic elements and potentially pathogenic bacteria in both raw and treated water, with a decreasing trend after the treatment. Raw versus treated greywater results indicate that RVFCW systems have the potential to mitigate antimicrobial resistance-related health risk when reusing treated greywater, but further measures need to be taken regarding persistent mobile ARGs and potential pathogens and their impact on local soil microbial communities.

------- poster no. 31 -------

Gut Microbiota Profiling Reveals a Signature of Microbiome Dysbiosis Associated with Colitis Development in the Heterogenous Nuclear Ribonucleoprotein I (hnRNP I) Knock Out Mice

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Introduction: hnRNP I is a polypyrimidine tract-binding protein that interacts with heteronuclear RNA (hnRNA). The function of this protein is related to mRNA maturity that could affect gene expression and protein translation. Recent studies suggest that ablation of the hnRNP I gene in intestinal epithelial cells in the knockout (KO) mice have caused spontaneous colitis. Therefore, the objective of this study is to examine gut microbiota quantity and diversity after the hnRNP I gene is knocked out in mice. Method: Fecal samples of wild-type (WT) and KO mice were analyzed at 4-week and 6-week of age. At 6-week of age, bodyweight along with colon weight and length were collected after euthanasia. Fecal samples were saved at -80°C until DNA isolation. Fecal pellets (2 per animal/sampling) were extracted to obtain DNA. Gut microbiota was analyzed by qPCR using genus-specific primers. Results were analyzed using the Mann-Whitney test. Results: The abundance of gut microbiota was examined in the WT (n=21) and KO (n=26) mice. Genus-specific primers, including Bifidobacterium spp., Enterococcus spp., Lactobacillus spp., Bacteroides spp., Desulfovibrio spp., and Clostridia cluster XIVa were used in analysis. In the KO mice, Lactobacillus spp. and Enterococcus spp. were more abundant at 6 weeks of age, comparing to the WT control mice. On the contrary, Bacteroides spp., Desulfovibrio spp., and Bifidobacterium spp. showed no difference between KO and WT mice. Interestingly, the Clostridia cluster XIVa was lower in abundance in the KO mice than WT at both 4 weeks and 6 weeks of age. Conclusion: Results indicated that the KO mice had lower Clostridia cluster XIVa than WT at both time points. Clostridia cluster XIVa belongs to butyrate-producing bacteria. These bacteria relate to mucin-adhered microbiota. This set of data aligns with the previously published results indicating that this cluster of bacteria may be essential to maintain gut homeostasis since they produce butyrate. Butyrate, one of the short-chain fatty acids, is essential for colonocytes as energy, which helps proliferate and maintain the colon from colorectal cancer. The reduction of Clostridia cluster XIVa in the hnRNP I knockout mice may be related to the susceptibility of the mice to the development of colitis. Further investigation will focus on the analyses of this bacteria cluster's role in maintaining gut homeostasis and the interactions in gut bacterial receptor-mediated host-microbe communications and colon resident immune systems in the KO mice model.

------- poster no. 32 -------

Identify microbial transport and hydrolysis traits important for polysaccharide response

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Recently, many studies have shown that a Western style diet, which is characterized by high fat and low fiber consumption, is linked to low gut microbial diversity. Furthermore, in mice, it was seen that fiber-free diets resulted in irreversible loss of gut microbial diversity over few generations. Low gut microbial diversity is associated with many chronic diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), type II diabetes and colorectal cancer. In contrast, higher consumption of fermentable dietary fibers appears to exert a positive impact on increasing gut microbial diversity. This project aims to identify the microbial traits responsible for transport and hydrolysis of polysaccharides with the aid of a chemical probe. Previous work in the lab has shown that complex polysaccharides have the ability to shape the gut microbial community diversity and structure in ways that simple polysaccharides do not. It has also been observed that relatively small differences in carbohydrate structures exert strong influence on the gut microbiota of humans. The structural differences also exert an effect on the metabolic output of the colonic microbiota. Complex carbohydrates are able to stably maintain diverse fermenting communities. Here, we propose to test the

hypothesis that the same hydrolytic and oligosaccharide transport properties govern ecological outcomes in complex, undefined communities of gut microbiota as in simple, synthetic pairings of microbes. We are currently in the process of developing chemical probes that mimic the structural characteristics of a polysaccharide, beginning with a suite of inulin-mimicking probes that vary in size. These probes can be crosslinked to bound organisms and proteins and specifically extracted using biotin-streptavidin chemistry. This approach allows us to identiy the microbes that are expressing genes for transport, hydrolysis and metabolism of polysaccharides and, specifically, what proteins are bound. The outcomes of this study will shed light on the mechanisms by which gut microbes are able to utilize complex polysaccharides. This will in turn help in designing better diets and prebiotics.

------- poster no. 33 -------

Effects of Wheat Genotype on Gut Microbiota Fermentation

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Gut microbiomes play important roles in the digestive and overall health of humans and animals. Although factors such as age, sex, and disease states contribute to microbiome structure and function, diets are a constant source of support and selective pressure on gut bacteria as they consume the nutrients that are not absorbed during digestion. Wheat bran constitutes a major source of dietary fiber in western diets and is an interesting model for insoluble fiber utilization by gut microbiota. However, effects of wheat genotype on bacterial fermentation have not been addressed in previous research, with many extant studies involving wheat lacking source or class descriptions. This gap exists despite reported genotype differences in wheat fiber structure and cell wall architecture. Additionally, most past research has focused on extracted and isolated fibers compared to intact bran fiber structures which are more common in the diet. The objective of my exploratory research to determine the extent of microbiota fermentation response variability due to wheat genotype using samples across the genus Triticum. Wheat kernels from twenty-one genotypes representing seven higher taxonomic groups were obtained and stone milled to obtain whole wheat flour which underwent in vitro upper gastrointestinal enzymatic digestion to remove water, protein, starch, and lipids. The remaining whole wheat flour fiber was inoculated with fecal microbiota from three donors in in vitro fermentation experiments. After 24-hour incubation, fermentation metabolic outcomes were measured along with microbiota community analysis using 16S rRNA gene sequencing. Results from this work show a broad spectrum in fermentation responses to wheat genotype, such as in pH, gas generation, and short-chain fatty-acid production. Microbiota community structure was also affected with alpha-diversity measures differing significantly depending on genotype. Beta-diversity measurements did not identify clear treatment differentiations due to strong initial fecal donor effects. However, wheat genotype effects were observed when looking at growth and promotion of bacteria at the OTU level. Across most fermentation outcomes, wheat genotypes responded more similarly to those from their own taxonomic grouping than those from other groups. These findings suggest wide variability of wheat fiber utilization that cannot be easily represented by one or several genotypes. Wheat genotypes from finer taxonomic levels (e.g. hard wheat, durum, spelt) may follow group trends, but even then, heterogeneity in bacterial utilization within groups appears to be present. Further work is needed to understand which wheat characteristics are drivers in deferential fermentation outcomes and whether differences seen in this study equate to practical changes in human or animal systems.

------- poster no. 34 -------

Lost Phenotypes: exploring the role of maize history on rhizosphere nitrification suppression

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Maize (Zea mays subsp. mays) domestication occurred ~9000 years ago. Substantial modification to the maize genome has since occurred, including introgressions with sympatric Zea species and population bottlenecks. Simultaneously, maize reproductive and vegetative architecture was heavily selected to maximize yields. In the 20th century, selective breeding was conducted in agroecosystems artificially fertilized with nitrogenous fertilizers, removing the selective pressures of nitrogen deficiencies from maize germplasm development. In doing so, the directed evolution of maize may have inadvertently selected against microbial nutritional symbioses important for growth and fitness. Nitrification, the chemolithoautotrophic oxidation of ammonium to nitrate, is an important microbial biogeochemical process with the potential to increase rhizosphere nitrogen loss by promoting nitrate leaching and/or nitrous oxide emissions. Numerous grass species, including landrace rice (Oryza sativa), perennial wild rye (Leymus racemosus), and sorghum (Sorghum bicolor), possess the biological nitrification inhibition (BNI) phenotype, allowing plants to

enzymatically inhibit nitrification and increasing rhizosphere nitrogen availability. We hypothesize that maize breeding in replete N conditions has selected against maintenance of the BNI phenotype in modern maize germplasm. The effect of maize genotype and demography on rhizosphere nitrification potential and microbial communities was assessed using rhizosphere nitrification potential enzyme assays and 16S rRNA amplicon sequencing. . Linear mixed-effects models revealed that demographic group significantly influenced rhizosphere nitrification potential (F(7,157) = 12.424, P < 0.0001), with the lowest rhizosphere nitrification potential within the Mexican landraces, and the highest in the Ex-PVP modern inbred cultivars. The Mexican landraces did not have significantly different nitrification potential than the allythiourea-inhibited control, suggesting these genotypes may possess a nitrification inhibition phenotype. Nitrification potential significantly influenced (F(1,141) = 35.532, P < 0.0001) leaf SPAD readings (used as a proxy for leaf nitrogen content), suggesting rhizosphere nitrification rates influence maize N-assimilation. Microbiome data is being processed.

------- poster no. 35 -------

Using machine learning approaches to predict required sequencing effort from accessible sample features in shotgun metagenomics

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A practical challenge towards a robust pipeline with metagenomics is the inability to efficiently allocate sequencing resources a priori. Assessment of sequencing depth is generally practiced post hoc, if at all, for most microbiome studies, regardless of the sample type. This practice is inefficient at best, and at worst poor sequencing depth jeopardizes the interpretation of study results. To enable more informed decision-making before sequencing, we conducted a quasi-meta-analysis, using 956 shotgun microbiome samples from hospital-related environments (874 from 6 previous studies and 82 from this study). Using these data, we linked accessible features (e.g., location, building, sampling method) to the required sequencing effort given a targeted coverage, leveraging machine learning-based models and Nonpareil. Upon examination, we observed no significant difference between sink and surface samples. Though weak, we noticed a trend of diversity increase after sample pooling, raising the alarm that more caution should be taken when increasing biomass by pooling samples. When training the machine learning models, eight predictor variables (location, building, study, country, touch frequency, sample type, sampling method, sample pooling) and one response variable (nonpareil diversity, Nd) were used. Nd was first converted to a nominal variable. Implementing repeated cross-validation (5 folds, 5 times) on the training dataset (80% of the entire dataset by random sampling), 9 algorithms were examined, and random forest was selected due to its slightly better performance. Mean balanced accuracy of 87.06% and 77.17% can be achieved for 5- and 11-category classifications, respectively. Location, building, and study were the top 3 variables with the highest importance in the classification models. With Nd and metadata features connected, the required sequencing effort at a targeted coverage was then inferred by leveraging Nonpareil. Upon fitting the data, we revealed a linear relationship between the natural log of estimated sequencing effort at 95% coverage (ln(LRstar)) and Nd, with the equation being ln(LRstar)=1.14*Nd+1.21 (Adjusted R-squared = 0.6012, p < 2.2e-16). Considering the sparsity of the currently available dataset and the challenge of multiclass classification, this model demonstrated a reasonable degree of accuracy, which should improve as sample sizes and available features grow. Our study facilitates a more efficient allocation of sequencing resources in metagenomics and contributes to achieving sequencing outcomes with a desired quality.

------- poster no. 36 -------

Twelve-Month Prune Consumption Alters the Gut Microbiome in Postmenopausal Women

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Background: Prunes contain phenols, fibers, and sugars, all of which may alter the gut microbiome, and can be metabolized by the gut microbiome to beneficial compounds. Previous studies have shown that dietary interventions may cause initial perturbations in the gut microbiome that do not persist in the long-term. Thus, it is important to assess the effects of long-term prune consumption on the gut microbiome. Aim: The aim of this study was to investigate the impact of long-term prune consumption on the gut microbiome of postmenopausal women. Methods: In a parallel arm, randomized controlled trial, 143 postmenopausal women age 55-75 completed a 12-month dietary intervention of either: 50g prune/day (n=54, 4-6 prunes), 100g prune/day (n=37, 10-12 prunes), or a no prune control (n=52). Measured outcomes included targeted urinary phenolic metabolites and the gut microbiome. The fecal microbiome was characterized using 16S rRNA gene sequencing and subsequent analysis by QIIME2. Differential taxa were assessed

using LEfSe. Associations with host variables were analyzed using canonical correspondence analysis (CCA). Linear correlations between taxa and phenolic metabolites were assessed using Spearman's r. Results: Twelve months of 50g or 100g/day prune consumption altered the gut microbiome of postmenopausal women compared to control, as determined by beta diversity analysis (Bray-Curtis PERMANOVA, p<0.04). Prunes enriched for certain beneficial taxa, including Lachnospiraceae (most abundant in 50g prune group, LEfSe LDA=4.5). Analysis by CCA showed that prune treatment (p=0.03) and BMI (p=0.002) were significantly associated with microbiome composition. Some taxa correlated with urinary phenolic metabolites, including Blautia, which negatively correlated with total phenolics (Spearman's r=-0.24, p=0.03), and Oscillospiraceae UCG 005, which negatively correlated with 3-hydroxyhippuric acid (r=-0.34, p=0.02). Conclusions: Prune consumption alters the gut microbiome of postmenopausal women, and these differences are observable after 12 months of prune consumption. Some of the taxa which were differentially abundant among the prune groups were also linearly correlated with phenolic metabolites, indicating altered phenol metabolism. These differences may mediate health effects of prunes.

------- poster no. 37 -------

In-field paper-based portable device for genetic testing of potato tuber spindle viroid (PSTVd) and tomato spotted wilt virus (TSWV)

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Tomato spotted wilt virus (TSWV) and Potato tuber spindle viroid (PSTVd) are serious pathogens of economically important ornamental and vegetable crops. Typical symptoms of TSWV-infection include severe necrosis, mosaic, and ring spots on the leaves and shrunken fruits with necrotic lesions causing an estimated losses up to $1 billion annually. Similarly, PSTVd is a highly infectious to solanaceous plants and can cause serious symptoms of dwarfism, leaf chlorosis and curling, and malformation of leaves and fruit. Specifically, PSTVd can cause severe economic losses in potato, by reducing the size and number of tubers. The successful of disease management program is highly dependent on their early and efficient detection strategies. Currently, RT-PCR is a most used method for detection of PSTVd and TSWV, however there in-field use is limited due to its requirement of laboratory machine, high skilled personnel and more time-demanding. Thus, there is an urgent need to develop tools for sensitive and rapid detection for TSWV and PSTVd in the field to apply preventive measure at early stage of infection. Our study focuses on developing a new portable method to detect PSTVd and TSWV using a loop-mediated isothermal amplification (LAMP) assay. LAMP allows accurate virus detection in samples by the naked eye, accelerating more precise diagnostics on the farm. Early detection of these pathogens on-farm will help growers to take preventive measures at earlier stages so that the economic losses associated with this disease can be reduced.

------- poster no. 38 -------

Genome Analysis and Metabolic Modeling of Faecalibacterium prausnitzii Strains

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Faecalibacterium prausnitzii is one of the most prevalent species of bacteria in the human gut microbiome and is known for its butyrate production and anti-inflammatory properties. Understanding the metabolism of this species may lead to new interventions and predictive capabilities for inflammatory bowel diseases. However, as there are over 100 different strains of Faecalibacterium prausnitzii, there may be significant metabolic differences among them that cause them to distinctly influence human health. We used comparative genomics, combined with in silico metabolic modeling, to determine the metabolic uniqueness of these various strains and grouped them into clades based upon genome content. Pangenome analysis revealed that differences in gene cluster presence exist among the strains in both Core (>90% presence) and Accessory (30% - 90% presence) gene clusters. Additional genomic analysis is underway, investigating the unique Faecalibacterium prausnitzii genes within the Oscillospiraceae family. Flux Balance Analysis of Genome Scale Metabolic Models will also be used to simulate the performance of different strains in the KBase platform. In the future, these models will be used to generate hypotheses regarding metabolic distinctives of clades to be tested in vitro. Specifically, growth rate, metabolite consumption, and metabolite production will be measured in various media types. A host cell response experiment is also planned in order to investigate the anti-inflammatory properties of Faecalibacterium prausnitzii on Caco-2 and HT-29 cells as well as metabolite flux through the colonocytes.

------- poster no. 39 -------

Bacteroidales LAMP assay for fecal contamination risk assessment

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Fecal contamination of fresh produce from animal sources is a public health concern due to the risk of foodborne illnesses. The current standard laboratory procedures for microbiological analyses usually require an enrichment step that involves several hours. Molecular techniques such as polymerase chain reaction (PCR) have been used to directly detect pathogens from the samples without culturing, but due to the low quantity of pathogens present, it is likely to give a false negative result. Furthermore, these molecular techniques are restricted to the laboratory. Here we developed a rapid risk assessment assay for fecal contamination by targeting Bacteroidales using loop-mediated isothermal amplification (LAMP). The assay allows for naked-eye observation of reactions with as few as 16.7 copies of Bacteroidales per cm2 of the surface in the field. We evaluated this assay with complex field samples as well as on-site field studies. Our research demonstrates that the Bacteroidales LAMP assay enables us to easily and quickly (< 60 minutes) assess the risk of fecal contamination from animal operations without expensive instruments.

------- poster no. 40 -------

Machine learning improves antibiotic resistance genotype-phenotype concordance for bacterial pathogens associated with bovine respiratory disease

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Bovine respiratory disease (BRD) is a broad term for diseases affecting the respiratory tract of cattle. Not only is BRD one of the costliest diseases for producers, but it has a high rate of mortality for both dairy and beef cattle. Since antibiotic resistance is common in BRD pathogens, antibiotic therapy must be administered on a trial-and-error basis, as microbiological testing takes longer than can be afforded to prevent morbidity and mortality. Identification of genes in pathogenic bacteria for rapid assays can also be useful for determining resistance but relies on resistance genes that are known. Additionally, for lesser studied bacteria such as BRD pathogens, the known resistance genes do not match the genomes with as high confidence as a well-studied pathogen such as Salmonella Typhimurium. In this study, we chose to use machine learning to find determinant sequences of resistance in three BRD pathogens, Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni. Genotype-phenotype concordance for these pathogens is low for many tested antibiotics, suggesting there are unknown genes or sequences determining resistance. The error rates of concordance for tulathromycin and tilmicosin, two antibiotics used to treat BRD, were 52% and 28%, respectively. With the marker sequences produced through machine learning, the error rates were decreased to 7.4% and 15% for the two antibiotics, respectively. This improvement of error rate was seen in all tested antibiotics with the exception of one, danofloxacin, for which the error rate increased from 31% to 33%. The marker sequences found for the tested antibiotics included transposases, tetracycline resistance gene tetR, and some amino acid biosynthesis genes. Regions surrounding the resistance markers (i.e., found on the same contig) contained other known resistance genes (sul2, bla-ROB, arsR) and some had conjugation machinery. Finding markers of resistance that are not necessarily resistance genes themselves (e.g., transposases) poses a challenge. This is a limitation of the study as mobile genetic elements (MGEs) such as transposases can be associated with many different resistance genes. However, using machine learning to find markers of antibiotic resistance increased the chances of accurately determining the resistance phenotype of the bacterial isolates. With the marker sequences from this study, we are one step closer to being able to provide more accurate assays which will help improve treatment success in cattle with BRD.

------- poster no. 41 -------

ARABINOXYLAN BRANCHING STRUCTURE GOVERNS COMMUNITY COMPOSITION AND METABOLISM OF FERMENTING HUMAN GUT MICROBIOTA

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Arabinoxylans (AXs) are complex, hemicellulosic polysaccharides and common dietary fibers that are fermented by colonic bacteria. AXs have complex molecular structures, including large molecular sizes, multiple diverse sugar composition and glycosidic linkage patterns, and diverse branching chains that decorate the β-1,4-linked xylan backbone. These structural characteristics can be the basis for selective effects for specific taxa within the microbiome, and theoretically, the stringency of these responses may influence the ability of an AX to specifically target certain gut

microbes. Herein, we hypothesized that simplification of a branching fiber structure would reduce the microbial diversity sustained by the polysaccharide due to the removal of the corresponding specialist niches, which are afforded by more complex structure. To test this hypothesis, we applied a two-step enzymatic modification to remove arabinose branches from the original sorghum arabinoxylan (SAX), without much reduction in molecular size. Both native and modified SAXs were subjected to a 7-day in vitro sequential passage fecal fermentations with inocula from three separate individuals, and for which we measured community composition (by 16S amplicon sequencing) and intermediate metabolic outcomes (gas, pH, and SCFAs) daily. The detailed chemical structures and molecular size distribution of the two SAX substrates were determined by GC with partial methylation alditol acetate derivatization and high-pressure size exclusion chromatography (HPSEC). Our results suggest that the phylotypes selected from the two SAX variants were shared at the operational taxonomic unit (OTU) level among all three individual's microbiota, implying that substrate molecular structure deterministically governs microbial succession. Furthermore, we found that SCFA production was precisely predicted by branching linkages, suggesting a hypothetical approach to manipulate in situ gut metabolites predictably via highly specific, and potentially modified, fiber structures.

------- poster no. 42 -------

Purdue Imaging Facility

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Introduction of the instruments and their capabilities in the Purdue Imaging Facility.