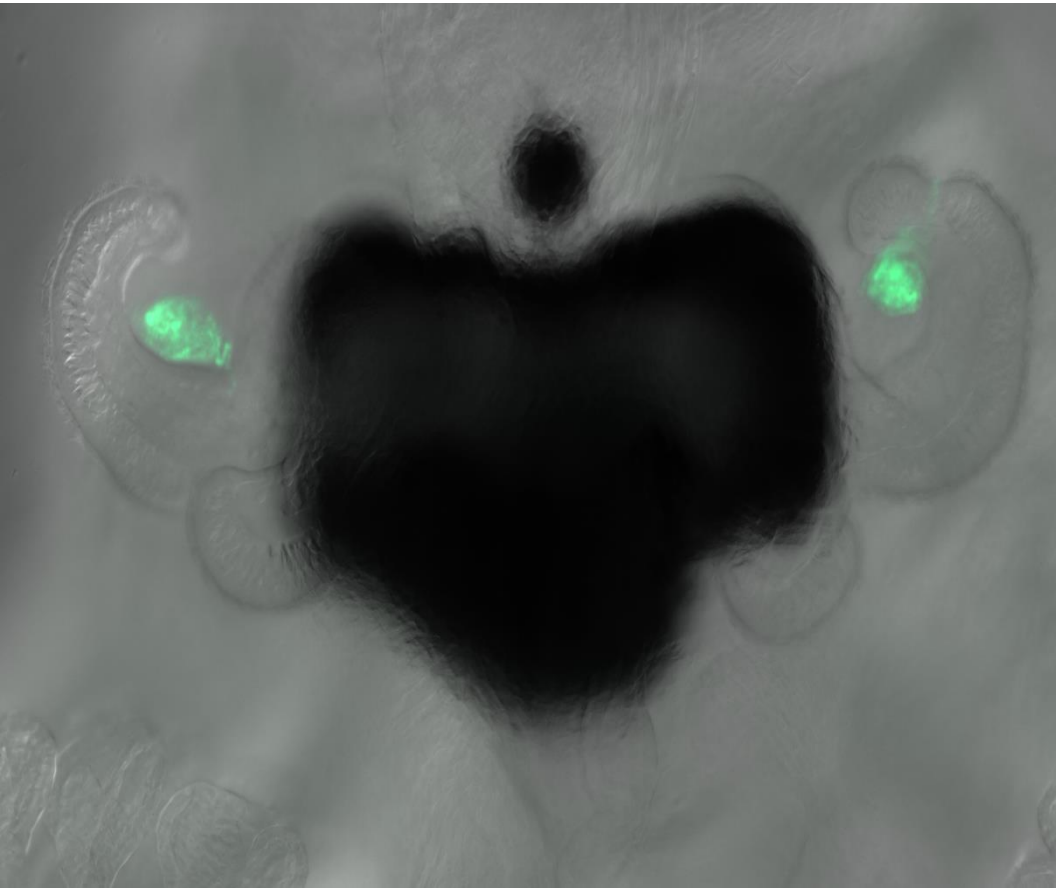

7th Conference on Beneficial Microbes

July 8th-11th, 2018
University of Wisconsin-Madison
Madison, Wisconsin

2018 CONFERENCE ON BENEFICIAL MICROBES

July 8-11, 2018
Madison, Wisconsin

Conference Co-chairs
Karen Guillemin, *University of Oregon*
Mark Mandel, *University of Wisconsin-Madison*

Meeting Organizers
Rosie Alegado, *University of Hawaii*
Robert Britton, *Baylor College of Medicine*
Nichole Broderick, *University of Connecticut*
Lora Hooper, *UT Southwestern Medical Center*
Rob Knight, *University of California San Diego*
Eric Martens, *University of Michigan*
Spencer Nyholm, *University of Connecticut*

Cover Art: GFP-expressing Vibrio fischeri aggregate in ciliated fields on the surface of the Euprymna scolopes light organ (Denise Tarnowski).

Video recording, audio recording, and still photography of scientific content is prohibited. We encourage sharing information from talks unless the presenter has indicated otherwise. For posters, ask the presenter's permission before sharing any information publicly.

Use Twitter hastag #BeneficialMicrobesMtg

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AT THE UNIVERSITY OF WISCONSIN-MADISON

The Department of Medical and Microbiology and Immunology of University of Wisconsin-Madison

The Department of Bacteriology of the University of Wisconsin-Madison

Conference Schedule

July 8	Sunday	
1:00 PM – 7:00 PM	Registration	Annex Room
5:30 PM	**Welcome** **Karen Guillemin and Mark Mandel**	**Great Hall**
5:30 PM	**Keynote: Host-Microbiome Interactions in Health and Disease**	**Eran Elinav**
6:30 PM	Opening Reception	Tripp Commons

July 9	Monday Morning	
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9:30 AM	Characterizing Gut Microbiota Associated with Childhood Diarrhea in the Developing World: The Good, The Bad, and The Ugly	Jacquelyn Meisel
9:45 AM	How Facultative Mutualism Evolves: Experimental Lactobacilli Evolution in Gnotobiotic Flies	Maria Elena Martino
10:00 AM	Butyrate-Producing Bacteria Modulate Atherosclerosis in a Diet-Dependent Manner	Kazuyuki Kasahara
10:15 AM	Break	Reception Room
10:45 AM	The Microbiota Modulate Zebrafish Behavior and Brain Development	Judith Eisen
11:00 AM	Probiotic Treatment of Post-Early Life Traumatic Brain Injury (TBI) and the Impact of TBI on the Intestinal Microbiome in Adolescent Rats	Bethany Rader
11:15 AM	How Does Carriage of Protists Affect the Microbiome and the Development of a Healthy Immune Response	Laura Knoll
11:45 AM – 1:00 PM	Lunch Buffet	Profile Room

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3:15 PM	Indigenous Gut Bacteria Use Immunoglobulin A for Mucosal Colonization	Gregory Donaldson
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ORAL PRESENTATIONS

Oral Presentation #1

Host Micro Biome Interactions in Health and Disease

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The mammalian intestine contains trillions of microbes, a community that is dominated by members of the domain Bacteria but also includes members of Archaea, Eukarya, and viruses. The vast repertoire of this microbiome functions in ways that benefit the host. The mucosal immune system co-evolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the community while maintaining the capacity to respond to invading pathogens. The gut microbiota is shaped and regulated by multiple factors including our genomic composition, the local intestinal niche and multiple environmental factors including our nutritional repertoire and bio-geographical location. Moreover, it has been recently highlighted that dysregulation of these genetic or environmental factors leads to aberrant hostmicrobiome interactions, ultimately predisposing to pathologies ranging from chronic inflammation, obesity, the metabolic syndrome and even cancer. We have identified various possible mechanisms participating in the reciprocal regulation between the host and the intestinal microbial ecosystem, and demonstrate that disruption of these factors, in mice and humans, lead to dysbiosis and susceptibility to common multi-factorial disease. Understanding the molecular basis of host-microbiome interactions may lead to development of new microbiome-targeting treatments.

Oral Presentation #2

Squid Pro Quo: Crosstalk in the Squid-Vibrio System

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Oral Presentation #3

Characterizing Gut Microbiota Associated with Childhood Diarrhea in the Developing World: The Good, The Bad, and The Ugly

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In low-income countries, moderate to severe diarrhea (MSD) significantly contributes to infant mortality. While many individual pathogens are linked to disease etiology, the importance of the overall intestinal microbial community structure is less well understood. A 2014 marker gene study characterized the fecal microbiota of 992 children from four developing countries in Africa and Southeast Asia, comparing MSD cases to healthy controls (Pop et al., Genome Biol). The microbiota of children with diarrhea were less diverse than those of their healthy counterparts and were enriched in facultatively anaerobic or microaerophilic bacteria. Escherichia/Shigella and Granulicatella species, and Streptococcus mitis/pneumoniae groups were positively associated with MSD, while Prevotella copri and Lactobacillus ruminis were potentially protective against diarrheal disease. Here, we build upon this initial work and characterize the fecal microbiota of over 3,000 children from the same MSD cohort. With increased sample size and taxonomic resolution, we delineate beneficial bacteria and potential pathogens contributing to diarrhea, as well as microbes correlated with age, physical growth, and environmental factors. We further validate these findings with qPCR on an expanded dataset. Finally, we interrogate Streptococcus species associated with MSD, finding that phylogenetically similar isolates may contribute to disease by disrupting the barrier function of the human colonic epithelial monolayer.

Oral Presentation #4

How facultative Mutualism Evolves: Experimental Lactobacilli Evolution in Gnotobiotic Flies

Maria Elena Martino[1], Pauline Joncour[2], Ryan Leenay[3], Hugo Gervais[2], Malay Shah[3], Sandrine Hughes[2], Benjamin Gillet[2], Chase Beisel[4], François Leulier[2]
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Animal-microbe facultative symbioses play a fundamental role in ecosystem and organismal health. Yet, due to the flexible nature of their association, the selection pressures acting on animals and their facultative symbionts remain elusive. Here, by applying experimental evolution to a well-established model of facultative symbiosis: Drosophila melanogaster associated with Lactobacillus plantarum, one of its growth promoting symbiont, we show that the diet, instead of the host, is a predominant driving force in the evolution of this symbiosis and identify a mechanism resulting from the bacterial adaptation to the diet, which confers host growth benefits. Our study reveals that adaptation to the diet can be the foremost step in the determination of the evolutionary course of a facultative symbiosis.

Oral Presentation #5

Butyrate-Producing Bacteria Modulate Atherosclerosis in a Diet-Dependent Manner

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Humans with metabolic and inflammatory diseases frequently harbor lower levels of butyrate-producing bacteria in their gut. However, it is not known whether variation in the levels of these organisms is causally linked with disease development and whether diet modifies these effects. Here we show that prominent gut-associated butyrate-producing bacteria (Roseburia sp.) are inversely correlated with atherosclerotic lesion development in a genetically diverse mouse population. Furthermore, we use germ-free apolipoprotein E-deficient mice colonized with synthetic microbial communities that differ in their capacity to generate butyrate to demonstrate that Roseburia intestinalis interacts with dietary components to (i) effect global changes in histone post-translational modifications and gene expression in the intestine, (ii) improve intestinal barrier function, (iii) lower systemic inflammation and (iv) ameliorate atherosclerosis. Furthermore, intestinal delivery and release of butyrate from tributyrin results in the improvement of gut barrier function and atherosclerosis development. Altogether, our results illustrate how modifiable diet-by-microbiota interactions impact cardiovascular disease, and suggest that strategies aimed at increasing the representation of butyrate-producing bacteria may provide protection against atherosclerosis.

Oral Presentation #6

The Microbiota Modulate Zebrafish Behavior and Brain Development

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There is a growing awareness of the profound influence of host-associated microbiotas on central nervous system development and function. We investigated the importance of the microbiota for brain development by assaying several different behaviors in developing zebrafish which, because of their transparency, rapid development, experimental accessibility, and large sibship size serve as an outstanding model for understanding neurodevelopmental mechanisms. We found that zebrafish reared germ-free exhibit normal spontaneous locomotor activity and visual responses compared to their conventionalized siblings. However, germ-free fish show specific deficits in other behaviors. To learn more about the mechanisms by which the microbiota modulate host behavior, we first examined transcriptional differences between the brains of germ-free and conventionalized larvae and then focused on cellular and molecular differences. We found that germ-free and conventionalized fish showed significantly different brain transcriptional profiles, as well as changes in cellular architecture in specific brain regions. Our studies reveal the importance of the microbiota for establishing the brain organization necessary for an animal to develop its normal behavioral repertoire.

Oral Presentation #7

Probiotic Treatment of Post-Early Life Traumatic Brain Injury (TBI) and the Impact of TBI on the Intestinal Microbiome in Adolescent Rats

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Traumatic brain injury (TBI) affects 2.5 million Americans each year and is a leading cause of death and disability in children and adolescents. We sought to determine if probiotic treatment post-TBI enhances cognitive function and tissue recovery by inducing a controlled cortical impact injury to the parietal lobe of just-weaned Sprague-Dawley rats and introducing Lactobacillus reuteri through daily oral gavage for 28 days post-injury. While cognitive function was minimally effected by L. reuteri treatment, L. reuteri treatment decreased contusion volume at 5 and 28 days post-injury, and decreased inflammation at 28 days post-injury. In addition, treatment with L. reuteri conditioned growth media resulted in a decrease in contusion volume and inflammation. Furthermore, disruption of the microbiome using antibiotics resulted in an increase in contusion volume, suggesting that response to injury is dependent on the context of the intestinal microbiome. Finally, 16S rRNA sequencing showed that at day 3 post-injury rat fecal bacterial community structure differed by injury status, at week 1 post-injury by probiotic treatment group; and at week 4 post-injury there was no apparent impact of injury status or probiotic treatment. In addition, fecal species richness increased over time post-injury for all rats, and an OTU classifying to L. reuteri was negatively correlated with species diversity and richness. Taken together, these data show that TBI impacts the community structure of the gut microbial community, that the structure of that community matters during TBI recovery, and that introducing a probiotic Lactobacillus strain post-injury provides enhanced neuroprotection.

Oral Presentation #8

How Does Carriage of Protists Affect the Microbiome and the Development of a Healthy Immune Response

Laura Knoll[1], Bruno Martorelli Di Genova[1], Carolina Mendoza Cavazos[1], Patrick Cervantes[1], Oscar Noya[3], Monica Contreras[3], Maria Gloria Dominguez-Bello[2]
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People in developed countries today carry fewer luminal protists than before hygienic practices were common place. Simultaneously, the incidence of autoimmune diseases, including inflammatory bowel disease, has increased in these populations. Carriage of parasitic nematodes has been shown to control autoimmune disorders, but few studies have investigated the contribution of non-pathogenic protists to gut health. Because these protists are not linked with disease there is inadequate information on their growth conditions, no mouse models and minimal genomic information. To address the role of intestinal protists in health, we have isolated the most common non-pathogenic intestinal protists from stool samples from indigenous people in the Amazon region. From healthy indigenous people, we have identified the most common protists: Endolimax nana, Balantidium coli, Iodamoeba butschlii, Blastocystis hominis, Dientamoeba fragilis, Chilomastix mesnili, Entamoeba coli and dispar. We are currently determining the purification and growth conditions for these protists. As Endolimax nana was the most common protist in the healthy indigenous people, we will sequence its genome so it can be identified from within metagenome data. To develop a mouse model, we have found mice deficient in Z-DNA binding protein-1 (ZBP-1-/-) can be colonized with these “non-pathogenic” protists, without signs of intestinal inflammation. Our preliminary data shows that these ZBP-1-/- mice have reduced Paneth and goblet cells. This project lays the ground for examining how carriage of non-pathogenic intestinal protists affect the microbiome and the development of the immune response.

Oral Presentation #9

Microbial Roles in Maintenance and Defense of the Skin Barrier

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Oral Presentation #10

C. elegans as a Model for Studying Genetic Factors Shaping the Gut Microbiota – the Role of TGFβ Signaling

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The gut microbiota contributes to host health and fitness, and imbalances in its composition are associated with pathology. However, what shapes microbiota composition is not clear, in particular the role of genetic factors. To facilitate identification of genes involved in shaping microbiota composition we took advantage of the genetic tractability of Caenorhabditis elegans. Previous work, analyzing worms raised in compost microcosms or wild isolates, identified a characteristic and geographically-conserved worm gut microbiota, demonstrated significant contributions of host genetics to its shaping, and identified numerous beneficial commensals, contributing mostly to host development and immunity. Recent RNAseq analysis indicated involvement of host immunity in interactions with gut microbes, prompting examination of the role of central immune regulators. Mutant analysis of worms raised on synthetic communities consisting of 30 worm gut isolates, combined with calibrated qPCR and CFU counts to assess abundance of core microbiota taxa, revealed a prominent role for the DBL-1/TGFβ pathway. Disruption of TGF□-dependent immune functions caused a 3-fold expansion in microbiota size, specifically due to a bloom in Enterobacter sp., common inhabitants of the worm gut. Survival analysis further demonstrated that dbl-1 disruption turned an infection-protective Enterobacter commensal to an opportunistic pathogen. These results reveal specificity in gene-commensal interactions, and the importance of microbial homeostasis, which when disrupted can directly lead to pathogenesis

Oral Presentation #11

The Microbiota Influences Life History Variation in Drosophila Melanogaster

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Local adaptation is primarily attributed to environmental selection on an animal's genotype, a model that does not but should account for the role of associated microorganisms ('microbiota'). To explore the link between the microbiota and local adaptation we use laboratory and wild populations of the fruit fly Drosophila melanogaster, to show that the fly microbiota naturally varies with latitude in a way that is consistent with the hosts' geography-specific traits. We first show that, when reared individually with different bacterial species, an isogenic fly line displays life history tradeoffs that favor either but not both of somatic maintenance (i.e. longevity) or early reproduction. We also show that the abundance of key taxa in wild D. melanogaster microbiota is correlated with the latitude at which the flies were sampled and that D. melanogaster genotype can select a latitude-specific microbiota. Finally, through laboratory experiments that eliminated or manipulated the microbiota of high- and low-latitude wild D. melanogaster, we reveal that both the microbiota and host genotype contribute to latitude-specific life history traits. For example, bacteria-free high latitude fly lines invested in somatic maintenance to a greater extent than bacteria-free low latitude fly lines; but the life history influence of the microbiota could override these genetic adaptations as shown by microbiota-swap experiments. Taken together, these findings suggest wild D. melanogaster can and must manage their microbes to adapt to varying environments and reinforce that the microbiota are an essential consideration in local adaptation.

Oral Presentation #12

Spatial Organization Illuminates Taxon-Taxon Interactions and Host Modulation of Microbiome Structure

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Spatial organization of a host-associated microbial community is a reflection of dynamic interactions within the community and between the community and its eukaryotic host. We used multiplexed fluorescence in situ hybridization and spectral imaging to investigate the spatial organization of microbial communities in the human mouth and the gnotobiotic mouse gut. Our probe sets were designed to identify simultaneously the majority of microbial cells in the samples so as to illuminate overall community structure and taxon-taxon relationships. We used probes targeting genus- and species-level taxa, permitting an assessment of the range of micro-habitats occupied by each genus or species. Our results showed dramatically different organization in the microbiotas of dental plaque, tongue dorsum, buccal mucosa, and gut. Spatial structure and taxon-taxon relationships within these communities suggested that the growth of certain microbial taxa creates environmental conditions in which other taxa can thrive. These modulations of the environment could be either physical, creating substrates for attachment, or metabolic, for example by altering oxygen concentration. Imaging of the structure of diverse communities also suggested mechanisms by which the host, in controlling the environment in which the community grows, establishes key parameters that determine community composition and dynamics. Such parameters may include surface properties and rate of shedding of the epithelium, physical and chemical properties of mucins, and rates of flow. Our imaging results thus serve to generate testable hypotheses about specific taxon-taxon interactions and about the powerful role of the host in shaping community structure in the healthy human microbiome.

Oral Presentation #13

The Hawaiian Bobtail Squid as a Model for Studying Defensive Symbioses and Development

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The Hawaiian bobtail squid, Euprymna scolopes, houses a diverse bacterial community in a female reproductive organ, the accessory nidamental gland (ANG). Bacteria from the Rhodobacteraceae and Verrucomicrobia dominate the ANG and are deposited into the egg jelly coat layer (JC) where they are hypothesized to defend eggs from potential pathogens and biofouling during embryogenesis. Eggs treated with antibiotics developed a biofilm, primarily composed of the fungus Fusarium keratoplasticum, which led to the death of the embryos. Thirty ANG/JC bacterial strains inhibited F. keratoplasticum in culture while 24 extracts from these bacteria also exhibited antifungal activity against F. keratoplasticum and/or the human pathogen Candida albicans. Molecular network analysis of inhibitory isolates and egg clutches challenged with fungi revealed compounds that may be involved with preventing fouling by microorganisms, including mycinamicins, lincomycins, and two glycerophosphocholines. To better understand the development of the ANG symbiosis, we used TEM and confocal microscopy to show that the nascent organ is poised to recruit bacteria from the environment, likely via the colonization of numerous ciliated ducts. Furthermore, squid raised with sterilized substrate and artificial seawater failed to develop an ANG or had severely stunted organs (n = 15), while animals raised with substrate from the squid's natural habitat developed normal ANGs (n = 9). Taken together, these data suggest that the ANG symbiosis is environmentally transmitted, and that bacteria from the host's environment induce development of the organ while providing for egg defense in the mature association.

Oral Presentation #14

Microbiota-Induced Serum amyloid A in the Intestine Directs Systemic Neutrophil Function

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The intestinal microbiota influences diverse aspects of host physiology, including the development and function of myeloid lineages. Numerous host factors are known to poise neutrophils and other granulocytes for response to pathogens and danger signals through a process called priming. However, mechanisms by which intestinal microbiota regulate neutrophil priming are unknown. Using gnotobiotic zebrafish, we identified the immune effector Serum amyloid A (Saa) as the most highly induced transcript in digestive tissues following microbiota colonization. Saa is a conserved secreted protein produced in the intestine and liver with described effects on neutrophils in vitro, however it's in vivo functions remain elusive. We generated saa mutant zebrafish and evaluated Saa's effects on innate immunity in vivo. saa deficient zebrafish displayed impaired neutrophil responses to wounding but augmented clearance of pathogenic bacteria. At baseline, saa mutants exhibited moderate neutrophilia and altered neutrophil tissue distribution. Molecular and functional analyses of isolated neutrophils revealed that Saa suppresses expression of pro-inflammatory mRNAs and bactericidal activity. Saa's effects on neutrophils depends on microbiota colonization, suggesting this protein contributes to the microbiota's influence on host innate immunity. To test tissue-specific roles of Saa on neutrophil function, we engineered zebrafish over-expressing saa in the intestine. Transgenic intestinal saa expression was sufficient to partially complement the observed neutrophil phenotypes in saa mutants. These results indicate Saa produced by the intestine in response to the microbiota signals to neutrophils to restrict aberrant priming, decreasing inflammatory tone and bacterial killing potential while simultaneously enhancing their ability to migrate to wounds.

Oral Presentation #15

Indigenous Gut Bacteria Use Immunoglobulin A for Mucosal Colonization

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While the immune system primarily responds to infection, it also tolerates indigenous species of the microbiome. How the immune system responds to the microbiome and how this affects the steady-state stability of the gut community remains poorly understood. Using a reductionist approach with colonization assays in gnotobiotic mice, we have discovered a mucosal sensor/regulatory system in commensal Bacteroides fragilis that modulates capsular polysaccharide production to invite binding of immunoglobulin A (IgA). This capsular attraction of IgA promotes both bacterial adherence to tissue-cultured intestinal epithelial cells and intimate association with the epithelial surface of the colon in vivo. By inhibiting the B cell response or using an isotype-specific IgA knockout mouse, we found that generation of IgA that binds the surface of B. fragilis is required for bacteria to occupy a defined mucosal niche that mediates stable colonization of the gut. Furthermore, when a mouse microbiome was transplanted into germ-free wildtype and IgA knockout animals, B. fragilis and IgA-coated Rikenellaceae benefited from the presence of IgA. Therefore, in addition to clearing pathogens, IgA responses promote colonization by the indigenous mucosal community.

Oral Presentation #16

The Role of the *Sinorhizobium meliloti* Exopolysaccharide Succinoglycan in Invasion of Symbiotic Plant Hosts

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The alphaproteobacterium *Sinorhizobium meliloti* fixes nitrogen in partnership with legume plant hosts including *Medicago truncatula* (barrel medic). On host roots, *S. meliloti* induces formation of nodules, invades these nodules, and is ultimately endocytosed by root cortical cells. Here, the bacteria differentiate into the 'bacteroid' form and begin to fix nitrogen gas to ammonia, supplying the nitrogen needs of the plant. For successful invasion of plant roots by rhizobia, compatible symbiont/host pairs must exchange multiple signals that promote bacterial entry. The first *S. meliloti* signal is the well-known Nod factor that induces a signaling cascade within the plant. Also critical for the symbiosis is the *S. meliloti* exopolysaccharide succinoglycan. On *M. truncatula*, succinoglycan is the only *S. meliloti* exopolysaccharide that can induce formation of 'infection thread' structures through which the bacteria penetrate successive layers of root tissue. We have found that in *M. truncatula*, the native level of succinoglycan production is limiting, and invasion is enhanced by increased production. Succinoglycan structure is also important for its role in infection thread formation. Production of high molecular weight succinoglycan is sufficient for function, but the low molecular weight form enhances infection rate. Succinylation of succinoglycan is absolutely required for invasion on *M. truncatula*. Strains that produce unsuccinylated succinoglycan are not impaired in free-living survival, motility or biofilm-formation. This suggests that the importance of succinoglycan succinylation is specific to the interaction with the host. The effects of succinoglycan structure on the interaction with predicted *M. truncatula* LysM receptor-like kinases and other factors are currently being investigated.

Oral Presentation #17

Bacterial Communities Within the Intestinal Mucus Layer Exhibit Distinct Spatial Variation in Composition and Diversity

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Colonic intestinal mucus consists of inner dense and outer loose mucus layers that play important roles in engineering the bacterial ecosystem. The inner mucus is sterile and limits bacterial contact with underlying epithelium, while outer mucus allows bacteria to penetrate and thrive. The community at the interface of the inner and outer mucus layers has the biggest impact on host physiology and at the same time is constantly acted upon by the host immune response. Not much is known about structure and composition of the bacterial community inhabiting the interface of the inner and outer mucus layers due to a lack of methodologies for studying the gut microbiome in a spatial context. We developed a novel methodology that isolates defined regions of the mucus layer as it exists in-situ by coupling laser capture microdissection and 16S rRNA sequencing, providing an unbiased approach to understanding microbial ecology on a fine spatial scale. Using this methodology, we discovered the microbial community closest to the host is compositionally distinct and bacteria belonging to class Gammaproteobacteria are specifically expelled from this community. We also uncovered bacterial communities closest to the intestinal epithelium are significantly more diverse than those further away. The greater diversity closer to the host suggests a role for the inner margin of the mucus layer in promoting stability of the bacterial ecosystem by enhancing spatial dissimilarity in the gut. Future studies will determine how this compositionally distinct and diverse bacterial community closest to the host responds to perturbations.

Oral Presentation #18

Evolutionary Origins of Bacterial Commensalism and Pathogenesis in the Plant Rhizosphere

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Strains from the Pseudomonas fluorescens (Pfl) species complex colonize plant roots and promote plant growth. These processes have been investigated for decades; however, our understanding is fragmented by the use of multiple model organisms and methodologies. Here we employ a standardized gnotobiotic assay using Arabidopsis thaliana to screen Pfl strains for effects on plant growth. Surprisingly, one strain killed Arabidopsis despite being a close relative of beneficial strains. To identify the genetic basis of pathogenicity, we developed a novel computational pipeline to mine the pangenome of thousands of publicly available Pseudomonas genomes, leading to the identification of a genomic island containing lipopeptide biosynthesis gene clusters. Reverse genetics confirmed that the clusters are necessary for pathogenesis.
While inactivating the cluster is sufficient to restore plant health, we suspected that related beneficial strains may have gained additional genes that promote plant health. Within a clade of 85 Pfl isolates from a single OTU, we identified two genomic islands anticorrelated with the lipopeptide cluster which encode a type III secretion system and a cluster that synthesizes an antifungal compound. Phylogenomic evidence suggests that all islands were present in the ancestral pangenome and that lifestyle switches are driven by homologous recombination leading to genomic island acquisition and loss. This approach demonstrates the power of comparative genomics to identify the basis of novel phenotypes. Furthermore, we show that beneficial Pfl strains emerge from pathogens and vice versa, yielding new insights into mechanisms that determine plant-associated lifestyles in this important clade of rhizosphere bacteria.

Oral Presentation #19

Quorum Sensing: Tipping the Scales in Symbiosis

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Key quorum sensing (QS) components are N-acyl homoserine lactone (AHL) synthases and response regulators. The free-living Sodalis praecaptivus, sister to Sodalis-allied symbionts, is exemplary of an ancestral progenitor establishing multiple times, and independently within many insects. Consequently, S. praecaptivus provides a model to characterize early molecular adaptations that enable symbiosis. S. praecaptivus requires QS for establishment within weevils (Coleoptera: Curculionidae) and, when disrupted, results in lethality [1]. However, until now, the utility of QS for long-term colonization within other insects remained unknown. Here we show that S. praecaptivus is also able to infect the tsetse fly (Diptera: Glossinidae) with QS necessary for the maintenance of this symbiosis. Microinjection with a S. praecaptivus double mutant in response regulators (ΔyenR ΔypeR) resulted in high tsetse virulence and death (i.e. >50% death in the first week). In contrast, an AHL synthase mutant (ΔypeI) exhibited milder virulence, suggesting AHL complementation from endogenous Sodalis within tsetse. A ΔypeI ΔSant_1962 (δ-endotoxin) mutant exhibited high virulence, suggesting a novel role for this endotoxin. The mutant ΔypeI ΔpirAB ΔregC, corresponding to mutations in AHL synthase, putative toxins and prophage P2, respectively, restored survival and establishment. Lastly, mutations affecting resistance to host immunity (ΔphoP or ΔmarR), while not impacting tsetse mortality, lowered colonization density. These results support the general utility of QS towards nascent stages of symbiosis, serving to regulate virulence, and proving fundamental towards maintenance especially prior to symbiont genome adaptations.

Oral Presentation #20

Bacteria-By-Ethanol Interactions Impact Drosophila Melanogaster Fitness and Physiology

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The microbiome affects how animals respond to ingested toxins. We investigated how bacteria shape the interactions between the fruit fly Drosophila melanogaster and dietary ethanol, a common compound in the natural fly diet. We found that the reproductive output of bacterially-colonized flies remains high with low amounts of dietary ethanol, while that of bacteria-free flies decreases precipitously after ethanol ingestion. We also observed that bacterial colonization and ethanol both negatively affect fly lifespan, but the effects of ethanol are more pronounced in bacteria-free flies. Together these results show that bacteria mask the negative effects of ethanol on fly fitness and suggest that ethanol-dependent effects can be investigated in bacteria-free flies. We next looked for bacteria-by-ethanol effects on fly physiology. We found that ethanol decreases intestinal stem cell turnover in bacterially-colonized, but not bacteria-free flies, suggesting bacteria-by-ethanol effects are tied to intestinal homeostasis. Next, we found that regardless of bacterial colonization, ethanol decreases intestinal barrier failure and increases fly body fat content, suggesting these mechanisms are not directly responsible for bacteria-dependent fitness differences. Measurements of dietary and fly ethanol content find that bacterial metabolism only partially explains the observed fitness effects. Bacteria-by-ethanol effects on host gene expression suggest that immunity is triggered by ethanol, providing a possible mechanism for ethanol-related decline in host health. Because ethanol is common in the wild D. melanogaster diet, these results have important implications for fruit fly ecology and evolution. More generally, they underscore the importance of the microbiome in shaping animals' interactions with their diet.

Oral Presentation #21

Microbial Nitrogen Limitation in the Mammalian Large Intestine

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Resource limitation is a fundamental factor governing ecosystems. However, the role of resource supply in structuring the intestinal microbiome has not been established and represents a challenge for mammals that rely on microbial symbionts for digestion: too little supply might starve the microbiome while too much supply might starve the host. We used stoichiometric and stable-isotope tracing approaches in vitro and in vivo to ask whether nitrogen is limiting in the mammalian gut and how nitrogen availability is managed by the host.

We found that gut microbiota confront a stoichiometric environment consistent with nitrogen limitation in the large intestines of 30 mammal species. Manipulating dietary protein levels in mice confirmed that nitrogen was limiting and constrained the total number of bacterial cells. Limitation resulted from hosts' absorption of dietary nutrients, which produced a stoichiometric gradient along the gut. Crucially, however, we found evidence that animals mitigate nitrogen limitation of gut microbes via internal secretions, which respond to microbial feedback. Single-cell spectrometry suggested that members of the phylum Bacteroidetes are the primary consumers of nitrogen in the large intestine, and these taxa were particularly responsive to changes in nitrogen availability.

Collectively, our findings support a model where nitrogen limitation arises from preferential host utilization of dietary nutrients, and we speculate that this resource limitation could enable hosts to regulate microbial communities in the large intestine. Furthermore, the commensal microbiota may have adapted to nitrogen-limited settings, suggesting why excess dietary protein has been associated with degraded gut microbial ecosystems.

Oral Presentation #22

Bacterial Competition Mediated by Siderophore Production in the Human Nasal Cavity

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Resources available in the human nasal cavity are limited. Therefore, to successfully colonize the nasal cavity, bacteria must compete for scant nutrients. Competition occurs directly through interference (e.g., antibiotic production) or indirectly by nutrient sequestration. We were interested in characterizing interactions between members of the human nasal microbiota. Culture-independent studies have revealed that the majority of bacteria colonizing the nasal cavity are from the phyla Actinobacteria and Firmicutes. We obtained ~900 bacterial isolates from frozen nasal lavage samples taken from healthy donors and found ~25% of the identified strains were Actinobacteria and the remaining ~75% were Staphylococcus spp. (a Firmicute). To investigate bacterial competition, we performed inhibition screens between nasal isolates of Actinobacteria and Staphylococcus spp. We found that Staphylococcus aureus isolates were resistant to nasal Actinobacteria but Staphylococcus epidermidis isolates were sensitive. Among Actinobacteria, we noted that Corynebacterium spp. isolates were variable in their ability to inhibit S. epidermidis. We sequenced the genomes of three Corynebacterium spp. Isolates that strongly inhibited S. epidermidis and two isolates that weakly inhibited S. epidermidis. Using antiSMASH, we predicted biosynthetic gene clusters (BGCs) and found that the genomes of the strong inhibitors encoded a BGC that was homologous to the corynebactin siderophore BGC from Corynebacterium diphtheriae. Using a chromeazurol S assay, we confirmed siderophore production. We showed that iron supplementation rescued S. epidermidis from siderophore-mediated inhibition. Finally, we confirmed that the siderophore BGC is expressed in vivo. Together, our results suggest that bacteria compete for limited available iron in the nasal cavity.

Oral Presentation #23

Transmission of Human-Associated Microbes Along Family and Social Networks

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Oral Presentation #24

Cargo Transport Shapes the Spatial Organization of a Microbial Community

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The human microbiome is an assemblage of diverse bacteria that interact with one another to design a community. Bacteria that form a community are arranged in a three-dimensional matrix with many degrees of freedom. Snapshots of microbial communities display well-defined structures. How a non-ordered community reaches an ordered state is not clear. Bacteria that possess the ability to actively move over surfaces are abundant in human microbial communities. Some of these bacteria have genes for the bacterial Type IX Secretion System and for gliding motility. Abundant non-motile bacteria found in human oral microbial communities attach to single gliding bacterial cells via a mobile cell surface adhesin, SprB. The attached bacteria are propelled as 'cargo' along the length of a gliding cell. Multi-color fluorescent spectral imaging of live bacterial cells within a polymicrobial community showed long range transport of non-motile cargo bacteria by a moving swarm. Tracking of fluorescently labeled single cells and of fluid flow patterns via gas bubbles showed hierarchy within a swarm. The synchronized transport of bacteria provides a specific spatial structure to a microbial community. Some non-motile bacteria use this mode of public transport more efficiently than others.

Oral Presentation #25

Achieving a Multi-Strain Symbiosis: Strain Dominance or Sharing in the Host

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The luminous marine bacterium Vibrio fischeri establishes its niche in the crypts of a nascent light-emitting organ within the newly hatched Hawaiian bobtail squid, Euprymna scolopes, which then provides nutrients to its symbionts and uses their bioluminescence for counterillumination. By performing co-colonization competition assays in juvenile squid, we identified two strain behaviors: the niche-sharing 'S strains' generally co-inhabited the light organ with other S strains, while the niche-dominant 'D strains' were typically alone in the light organ after a co-colonization assay with either an S or another, less dominant, D strain.
To understand what underlies the colonization advantage of D strains, we determined the minimum time that different strains needed to initiate colonization, and used confocal microscopy to localize the symbionts in the light organ at 1.5, 3 and 6 h after initiating infection. Further, we asked whether symbiont-induced host morphogenic events occurred earlier during a D-strain colonization. We concluded that D strains reached the squid crypts earlier and achieved colonization more quickly than S strains. Despite the dominant behavior characteristic of D strains, light-organ populations in field-caught squid often contain both D and S strains. To understand how this population heterogeneity is achieved, we determined that the timing of strain encounter affected how strains can establish a co-colonization.
In summary, D strains dominate a symbiont population by navigating the early steps in colonization more rapidly than S strains; however, the strain diversity found within natural symbioses can be explained by the differential timing of exposure to multiple strains.

Oral Presentation #26

Experimental Evolution of a Bacterial Symbiont to its Vertebrate Host Reveals a Primary Role for Immigration in Host Adaptation

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Animals are colonized by microorganisms that profoundly impact their health and development; however, the traits that promote a microbe's ability to be host-associated (found in or on a host) are not fully understood. Most attempts to identify these traits have focused specifically within the host, yet a growing body of literature suggests that the entire ecological system (both intra- and extra-host niches) should be considered. To address this, we developed a highly tractable vertebrate model system, which includes both intra- and extra-host factors. We evolutionarily adapted a zebrafish bacterial isolate, Aeromonas veronii, to the germ-free larval zebrafish gut. In order to facilitate adaptation, we used an A. veronii strain engineered to have a high mutation rate. Strikingly, the initial adaptations did not increase within-host fitness, but rather, enhanced immigration from the environment. Within-host adaptations only arose later in evolution, demonstrated by host genotype-specific fitness outcomes. Subsequent evolution of a non-mutator A. veronii resulted in identification of a gene mutated in all replicate evolved populations. This gene codes for a diguanylate cyclase protein coupled to a small molecule sensing PAS/Cache domain. Clean deletion of this protein in the ancestral strain recapitulated the increased competitive fitness observed in the evolved isolates, as well as a hyper-motile phenotype. Current experiments are focused on biochemical characterization of this protein as well as characterization of its impact on the physiology and colonization dynamics of this symbiotic bacterium. This work contributes important insights into the selective forces in host-microbe systems and mechanisms of increased host association.

Oral Presentation #27

Shedding Light on Symbioses: Lessons from a Bioluminescent Vertebrate-Microbe Association

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Virtually all organisms are dependent on microbial symbionts for their success, yet the processes by which these essential associations are established and persist through time remain largely undescribed. I examined a highly specific, pairwise symbiosis involving the coral reef cardinalfish, Siphamia tubifer, and the luminous bacterium, Photobacterium mandapamensis, to characterize the ecological mechanisms involved in maintaining symbiont specificity over host generations. Integrating field studies designed to define key attributes of the host's life history and behavior in Okinawa, Japan with recently developed genomic methods (restriction site-associated sequencing, "RAD-Seq"), I tested the hypothesis that the ecology of the host fish helps to structure populations of its facultative symbiont over time and space, consequently promoting the specificity of the association. Results indicate that due to their site fidelity and homing behavior, resident populations of adult fish at a reef enrich the surrounding seawater daily with excess luminous symbionts and that larval fish disperse significant distances as a cohort to non-natal reefs and a symbiont from the locally enriched water near their settlement site. Consequently, these behaviors promote the genetic divergence of the luminous symbiont between reefs. This study highlights the critical role that the ecology of a host animal can play in structuring natural populations of its bacterial symbiont, thereby promoting the specificity of its symbiosis over host generations.

Oral Presentation #28

Convergent Evolution of Complex Structures for Ant-Bacterial Defensive Symbiosis in Fungus-Farming Ants

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Major evolutionary adaptations for maintaining beneficial microbes occur in many animal groups, representing hallmarks of interspecies cooperation. Fungus-farming "attine" ants cultivate fungi for food. During the ~60 million-year evolution of ant agriculture, many have acquired complex cuticular modifications and specialized glands that house and nourish antibiotic-producing Actinobacteria symbionts, which protect their fungus-gardens from pathogens. Here we reconstruct the ant-Actinobacteria mutualistic evolutionary history by applying dated phylogenomic analyses and electron microscopy to the full range of variation within Attina subtribe. Ancestral-state analyses indicate ant-Actinobacteria symbiosis arose twice ~49 million years ago, a conclusion consistent with our direct observations of Actinobacteria on fossil ants. qPCR and microbial isolation evidences indicates that the dominant Actinobacteria belong to the genus Pseudonocardia. Tracing the evolutionary trajectories of Pseudonocardia-maintaining mechanisms across attine reveals a continuum of adaptation. In Myrmicocrypta species, which retain many ancestral morphological and behavioral traits, Pseudonocardia occur in specific locations on the legs and antennae, unassociated with any specialized structures. Whereas specialized cuticular structures, including crypts and tubercles, evolved at least three times in derived attine ant lineages. Conspicuous castes differences in Actinobacteria-maintaining structures, with specialized structures present in worker ants and queens but reduced or lost in males, reflect vertical Pseudonocardia transmission. Although the majority of attine ants are associated with Pseudonocardia, there have been multiple subsequent losses of bacteria-symbionts and bacteria-maintaining structures during evolutionary time. The early origin of the ant-Pseudonocardia mutualism and evolutionary convergences on strikingly similar anatomical adaptations for symbiont-maintenance reflect the critical role of Pseudonocardia in ant agriculture.

Oral Presentation #29

2-Year Follow-Up Study Reveals Consistent Benefits of Microbiota Transfer Therapy on Autism and Gut Symptoms

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Recent studies in human cohorts and mouse models have demonstrated a link between gut microbiota and autism. We hypothesized that if we could alter the gut microbiota in children with autism spectrum disorders (ASD) in a beneficial direction, their gastrointestinal and behavioral symptoms would improve. Fecal microbiota transplant is a promising therapy to modify the gut microbiome and repair dysbiotic gut microbiota by transferring thousands of bacterial species to a recipient's gut. We previously pioneered an open-label trial of Microbiota Transfer Therapy (MTT) that combined two-week vancomycin and followed by eight-week high-initial/low-maintenance dose of standardized human gut microbiota. In this previous trial, we provided therapy to 18 autism-diagnosed children who had gastrointestinal problems, and observed significant improvements in gastrointestinal- and autism-related symptoms, and gut microbiota. Here, we report on a follow-up with all 18 participants two years after treatment stopped. Notably, gastrointestinal symptoms were significantly reduced compared with the beginning of the original trial, and autism-related symptoms improved significantly after the end of treatment. DNA-sequencing analyses revealed that changes in gut microbiota at the end of treatment still remained at follow-up, including significant increases in bacterial diversity and relative abundances of Bifidobacteria, Prevotella, and Desulfovibrio. Our observations demonstrate the long-term efficacy of MTT for treating children with ASD who have GI problems. The microbiota transfer therapy performed in this trial thus is a promising approach for sustainably altering the gut bacterial communities, and improving gastrointestinal and behavioral symptoms associated with ASD.

Oral Presentation #30

Deciphering the Human Microbiota with Chemistry

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Oral Presentation #31

Modeling Host-Microbial Interactions at the Gut Interface

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Host-associated microbes, particularly bacteria, are inextricably involved in the life history and evolution of their hosts. Current molecular and taxonomic tools facilitate identifying both the membership and potential functions of host-associated microbial communities. The gut epithelium is among the few sites in which host, their microbes and the environment converge with significant evolutionary and ecological consequences. Given the potentially high species diversity and richness in natural gut microbial communities and the overall complexity of host-microbe and microbe-microbe interactions, experimental platforms, like germ-free animals, that facilitate deterministic species assembly can be extremely useful for testing hypotheses about host gut outcomes linked to specific microbial species and/or microbe-microbe interactions. We have developed a germ-free invertebrate system that, combined with newly isolated endemic gut symbionts, allows us to identify key bacteria that are necessary for normal development of animal digestive tracts and dietary plant carbohydrate accessibility. Our model organism, Periplaneta americana, is omnivorous and acquires its gut microbiota horizontally and vertically via filial and conspecific coprophagy. Germ-free P. americana exhibit prolonged development and gut dysmorphia, both of which were significantly resolved following exposure to conspecific feces. Genome analysis of abundant endemic Bacteroidetes gut isolates that are detectable in feces reveal broad high molecular weight carbohydrate (HMWC) degradation capabilities. Subsequent HMWC biochemical assays indicated that these isolates could degrade several substrates commonly comprising the P. americana diet.

Oral Presentation #32

Toxin Production by Clostridioides Difficile Alters Microbial Community Metabolism

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The human gut microbiota typically forms a robust community that confers protection against invasion; however, some enteric pathogens, such as Clostridioides difficile can invade and cause disease after community disturbance. C. difficile is responsible for a toxin-mediated colitis that causes 15,000 deaths in the U.S. yearly. Other enteric pathogens, such as Salmonella Typhimurium, are known to benefit from electron acceptors and nutrients generated by the oxidized environment during infection. Much less is known about potential advantages conferred to C. difficile by the inflammation generated by its toxins. Conversely, it is estimated that between 6 and 21% of adult humans are asymptomatically colonized with C. difficile, indicating that, under certain conditions, C. difficile is able to persist in the absence of toxin-induced inflammation. To understand these distinct lifestyles, we use RNA-seq combined with untargeted metabolomics in a gnotobiotic mouse model with defined bacterial communities to investigate how wild-type C. difficile and isogenic mutants lacking toxins impact community-wide metabolism. Toxigenic C. difficile elicits extensive differential gene expression in commensals, while expression profiles are similar to uninfected communities during colonization with avirulent C. difficile. Comparison of hypervirulent and lab-adapted strains of C. difficile, which differ in the magnitude of inflammation generated in a mouse model, allowed identification of specific substrates supportive of C. difficile metabolism in the presence and absence of toxin-induced inflammation. The C. difficile and commensal pathways identified will lead to a better understand of how enteric pathogens reshape gut microbiota metabolism during infection.

Oral Presentation #33

Novel Tools for in Vivo Functional Analysis of Host-Microbiome Interactions

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Host diet influences the structure of gut microbiotas in species that regularly consume food. To measure functional contribution of microbiotas to host physiology, we developed a metabolome-microbiome platform (MMP) using stable isotope-assisted labeling (SIAL) to measure microbiota metabolism of select substrates in vivo and trace bacterial contributions to the host metabolome. We combined cavity ringdown spectroscopy to measure real-time breath (13CO2/12CO2 (δ13C)) biomarker with NMR-based metabolomics to analyze metabolome profiles in cecal contents and host tissues. We applied MMP to hibernating ground squirrels to assess the contribution of gut bacteria to seasonal changes in dietary intake. Previously, we showed that the long-term fast of hibernation reduces relative abundance of bacterial taxa that are capable of degrading complex plant glycans and increases abundance of taxa that metabolize host substrates, such as mucins. Here, we determined the functional significance of seasonally changing microbiotas by gavaging Spring and Summer squirrels and aroused hibernators in Winter with 13C-labeled glycans. Measurement of δ13C in breath was used as an index of bacterial degradation of 13C-substrates, and δ13C changes were linked to changes in 1H-[13C]-metabolome profiles. Compared with robust responses in Summer, changes in δ13C after 13C-inulin gavage were nearly abolished in Winter hibernators, whereas Spring squirrels showed variable responses consistent with their transitional microbiotas. Metabolome profiles mirrored seasonal changes in 13C-labeled short-chain fatty acids. The results suggest MMP is a powerful tool in analysis of host-microbe relationships.
Supported by NSF (IOS1558044) and UW-Madison OVCGRE.

Oral Presentation #34

Ribose Metabolism in Bacteroides Thetaiotaomicron Plays an Important Role in Vivo in a Diet-Dependent Manner, and May Represent a Nutrient Niche

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Gut bacteria of the prominent Bacteroidetes phylum devote large portions of their genome towards host and dietary carbohydrate degradation through partially homologous gene clusters, Polysaccharide Utilization Loci (PULs). Here, I describe a PUL targeting ribose-containing substrates, the ribose utilization system, (rus). Previous data shows the rus of Bacteroides thetaiotaomicron (Bt) to be upregulated in vivo in several dietary conditions. We used Bt to examine the mechanism of ribose utilization via rus. Growth on ribose induced expression of Bt-rus and homologous loci in other Bacteroides species. Our results support the hypothesis that Bt metabolizes ribose-containing compounds through rus-encoded functions as deletion of rus, completely eliminates growth. In vivo, the Δrus strain is outcompeted by wild-type Bt, in mice fed a fiber-rich diet. A similar defect was observed in mice fed a fiber-free diet supplemented with water containing 1% ribose, but not RNA or nucleosides. This effect is also seen in vivo for the double kinase knockout strain competed against wild-type. Comparative genomics, growth and gene expression studies revealed rus homologues of variable enzymatic potential to be broadly represented in Bacteroidetes. This suggests the presence of a rus locus may represent a competitive advantage in vivo. We conclude that the ability to catabolize ribose within Bacteroidetes is conferred via the rus PUL and that rus activation allows access to other nutrients containing ribose, although the critical molecules foraged in vivo are unknown. Taken together, these results suggest this PUL operates as a Swiss-army knife for foraging other ribose-containing compounds.

Oral Presentation #35

Nitrogen Fixation in a Landrace of Maize is Supported by a Mucilage-Associated Diazotrophic Microbiota.

Vania Pankievicz[1], Allen Van Deynze[2], Pablo Zamora[2], Pierre-Marc Delaux[1], Cristobal Heitmann[2], Donald Gibson[2], Kevin D. Schwartz[2], Alison M. Berry[2], Danielle Graham[1], Dhileepkumar Jayaraman[1], Shanmugam Rajasekar[1], Junko Maeda[1], Srijak Bhatnagar[2], Guillaume Jospin[2], Aaron Darling[2], Richard Jeannotte[2], Javier Lopez[3], Bart C. Weimer[2], Jonathan A. Eisen[2], Howard-Yana Shapiro[2,3], Alan B. Bennett[2], Jean-Michel Ané[1]
[1]University of Wisconsin, [2]University of California, [3]Mars, incorporated

Plants are associated with a complex microbiota that contributes to nutrient acquisition, plant growth, and plant defense. Nitrogen-fixing microbial associations are well characterized in legumes but are largely absent from cereals, including maize. We studied an indigenous landrace of maize grown in nitrogen depleted soils in the Sierra Juarez region of Oaxaca, Mexico. This landrace is characterized by extensive development of aerial roots that secrete a carbohydrate-rich mucilage. Analysis of the mucilage microbiota indicated that it was enriched in taxa for which many known species are diazotrophic; was enriched for homologs of genes encoding nitrogenase subunits; and harbored active nitrogenase activity as assessed by acetylene reduction and $^{15}N_2$ incorporation assays. Field experiments in Sierra Juarez using ^{15}N natural abundance or ^{15}N-enrichment assessments over five years indicated that atmospheric nitrogen fixation contributed 30-82% of the nitrogen nutrition of Sierra Juarez maize.

Oral Presentation #36

The Production of Glycine Lipids is an Important Fitness Determinant in Bacteroides Thetaiotaomicron

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The acyltransferase activity responsible for the production of the novel monoacylated glycine species, N-acyl-3-hydroxy-palmitoyl glycine or commendamide, has been shown to be encoded by the choA gene. Genomic analysis has shown that homologues of choA are found throughout the Order Bacteroidales, including important members of the mammalian gut microbiota such as Bacteroides, Prevotella and Alistipes. In this study we used liquid chromatography-mass spectrometry (LC-MS) to show that the choA gene is required for the production of novel diacylated glycine lipid (GL) species in Bacteroides thetaiotaomicron. In all of the Bacteroidales genomes so far sequenced the choA gene is located immediately downstream from a gene, choB, also predicted to encode an acyltransferase. Using a heterologous expression system we show that co-expression of the choA and choB genes from Bacteroides is required for the production of GLs in Escherichia coli. We constructed a deletion mutant of the choA gene in B. thetaiotaomicron and we show that GLs are required for normal membrane lipid and protein composition. Moreover, we show that GLs in B. thetaiotaomicron are important for bacterial growth, the ability to adapt to stress and colonization of the mammalian gut. The ability to produce acylated amino acids, such as ornithine lipids, is widespread throughout the Domain Bacteria. However, this is the first report describing the production of glycine lipids and further studies into the role of this novel lipid species are currently underway.

Oral Presentation #37

Engineered Microbe for the Intestinal Delivery of Interleukin-22

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With improved genetic engineering technology in commensal gut organisms the ability to engineer the delivery of potent therapeutic proteins locally in the intestinal tract using native microbes is now reality. One such protein, IL-22, plays key roles in wound healing, intestinal barrier function, and prevention of infectious disease through the regulation of antimicrobial proteins. We are engineering the gut commensal and probiotic organism *Lactobacillus reuteri* LJO1 to deliver functional human IL-22 to the intestine. IL-22 was codon optimized for expression and secretion in *L. reuteri* LJO1 and we have successfully shown this secreted IL-22 is functional in both cell culture and intestinal enteroids. In this talk I will discuss some of the challenges we had to overcome in developing a suitable biotherapeutic delivery system for IL-22 and what obstacles still need to be addressed. As more academic and industrial research is moving in the direction of engineered therapeutic microbes it will be important to develop standards that not only insure proper delivery of payloads but also safety considerations including biocontainment of these therapeutics.

Oral Presentation #38

Symbiont-Mediated RNAi in Apis Mellifera with Engineered Gut Microbiota

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Western honey bees pollinate crops that feed billions of people across the world, but bees suffer from poorly understand health threats like Deformed Wing Virus and Varroa mites. Bees also possess a conserved gut community of microbes that supports host weight gain, insulin signaling, and pathogen resistance. In this work, we try to understand how we can engineer this community to further improve host health. We developed broad-host-range genetic tools for recently cultured bee gut microbes from diverse species of Proteobacteria. This toolkit allows rapid identification of selectable markers, promoters, and reporter genes that function in these poorly characterized organisms. Engineered bee gut microbes effectively recolonize and persist in the bee gut under laboratory conditions, and bacteria expressing fluorescent proteins can be directly visualized in dissected tissues. Snodgrassella alvi is an abundant species that directly colonizes the wall of the hindgut, forming a continuous biofilm. When S. alvi is engineered to express double-stranded RNA, it induces a potent RNA-interference (RNAi) response in the bee host, enabling us to modulate mRNA levels of target bee genes. Additionally, we explore the use of this symbiont-mediated RNAi as a tool to study bee gene function, alter bee behavior, and suppress bee pathogens.

Oral Presentation #39

Introducing THOR, A Model Microbiome for Genetic Dissection of Community Phenotypes

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The quest for strategies to manipulate microbiomes for human benefit has intensified. Deliberate design of such strategies will be advanced by mechanistic understanding of community phenotypes and in particular community assembly process and robustness. Model communities that can be dissected genetically will advance this goal. Here we report development of a model community containing Bacillus cereus, a common member of rhizosphere communities, and Pseudomonas koreensis and Flavobacterium johnsoniae, which are “hitchhikers”—bacteria that frequently co-isolate with B. cereus from field-grown soybean roots. In addition to their co-isolation, B. cereus, P. koreensis, and F. johnsoniae interact in several ways. We previously reported that growth of F. johnsoniae in root exudate is enabled by B. cereus. We also found that in culture P. koreensis inhibits growth of F. johnsoniae, but not in the presence of B. cereus. The inhibition is mediated by a novel family of alkaloids produced by P. koreensis, and the B. cereus protection is mediated by selective reduction of certain P. koreensis alkaloids. On solid media, both F. johnsoniae and P. koreensis induced dramatic changes in B. cereus colony morphology, causing B. cereus colony expansion following a dendritic pattern. On polystyrene surfaces, B. cereus and F. johnsoniae increased the maximum production of a P. koreensis biofilm. We designate this community THOR, because the members are the hitchhikers of the rhizosphere. The genetic, genomic, and biochemical tools available for dissection of THOR provide the means to gain a new level of understanding of microbial community behavior.

Oral Presentation #40

Bacterial Motility and Chemotaxis Promote Stable Intestinal Colonization and Host Inflammation

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Many beneficial and pathogenic bacteria use motility and chemotaxis to colonize their host. Within the vertebrate intestine, an open question is whether motility and chemotaxis act exclusively to facilitate growth and survival or if there are additional functions that these behaviors provide. To address this question, we used live imaging and gnotobiotic zebrafish colonized with genetically engineered derivatives of a model Vibrio cholerae symbiont to directly monitor and manipulate the intestinal ecosystem. Surprisingly, the in vivo growth rates of mutants lacking either flagellar motility or chemotaxis are unchanged compared to wild type. Instead, we found that motility and chemotaxis mutants assemble spatially altered population structures and are more susceptible to expulsion due to the peristaltic activity of the intestine. Intriguingly, we also discovered that motility and chemotaxis—rather than the expression and biogenesis of flagella per se—induce inflammation, suggesting that the host can sense these behaviors and/or the spatial organization of resident bacterial populations. Together, our observations highlight motility and chemotaxis as potential targets for the in situ manipulation of bacterial population stability and host inflammation. To test this idea, we used bacteria carrying inducible genetic switches to track and control motility within established populations. We demonstrate that sudden loss of motility leads to spatial reorganization and collapse events, whereas switching on motility in mutant populations restores wild-type colonization patterns along with increasing host inflammation.

Oral Presentation #41

Engineered Substrate Usage Allows Prebiotic Control of Microbial Community Population and Gene Expression

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The synbiotic relationship between human milk oligosaccharides (HMOs) and probiotic Bifidobacteria exemplifies prebiotic control of microbial community dynamics. Inspired by this example, we have engineered the well-known probiotic, E. coli Nissle, to metabolize HMOs and used this metabolism to control population dynamics and protein expression in mixed cultures of E. coli. We accomplish this using a unique whole-cell biosensor which provides linkage-specific, quantitative detection of various HMOs (Enam and Mansell, Cell Chemical Biology, in press). Addition of these complex substrates to synthetic microbial consortia orthogonally controls growth rate or protein expression of particular strains. In addition, we performed further metabolic engineering on our probiotic, enabling production of the short-chain fatty acid butyrate from HMOs as sole carbon sources, recapitulating an important function of the infant gut-associated Bifidobacteria. This work lays the groundwork for the application of directed evolution to biosynthesis of complex carbohydrates as well as the prebiotic manipulation of population dynamics in natural and engineered microbial communities.

Oral Presentation #42

Disentangling Host and Microbiome Contributions to Drug Pharmacokinetics and Toxicity

Michael Zimmermann[1], Maria Zimmermann-Kogadeeva[1], Rebekka Wegmann[1], Andrew L. Goodman[1]
[1]Department of Microbial Pathogenesis and Microbial Sciences Institute, Yale University School of Medicine

The gut microbiota is implicated in the metabolism of many medical drugs, which can have important consequences for efficacy, toxicity, and interpersonal variation in drug response. However, dissecting host and microbial contributions to drug metabolism is challenging, particularly in cases where host and microbiome carry out the same metabolic transformation. Using antiviral nucleoside analogues as an example, we combined genetic manipulation of human gut commensal bacteria with gnotobiotics to quantify the contribution of the microbiome to drug and metabolite kinetics in circulation, liver and intestine. Informed by these measurements, we built a physiology based pharmacokinetic model that accurately predicts host and microbiome contributions to the levels of drugs and their toxic metabolite in circulation, define the underlying mechanism in the microbiome, and identify measurable parameters that dictate host and microbiome contributions to the metabolism of xenobiotics. This approach is applicable to other drugs, nutrients, and endogenous compounds and could improve our understanding of the environmental and genetic factors that influence drug response variability.

Oral Presentation #43

Development of Lactobacillus Reuteri as a Biotherapeutic Delivery Vehicle

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[2]Department of Biochemistry, University of Wisconsin Madison

Lactic acid bacteria constitute a genetically diverse group of organisms that have been widely applied in the food industry. The long history of safe use, combined with health-promoting properties, make select strains attractive candidates to be developed as therapeutic delivery vehicles. The goal of this work is to engineer the probiotic strain Lactobacillus reuteri VPL1014 as a biotherapeutic delivery vehicle. We selected L. reuteri VPL1014 because the strain is safe for human consumption, encodes a variety of probiotic features, and we can efficiently modify the genome. Additionally, VPL1014 exhibits an extremely low mutation rate compared to other Gram-positive bacteria, which we expect will contribute to the stability of our genetically modified stains. We hypothesized that intracellular accumulated therapeutic protein can be delivered in situ following bacteriophage-mediated lysis. L. reuteri VPL1014 encodes two biologically active prophages, which are induced during gastrointestinal transit. In vitro prophage induction of recombinant VPL1014 released our model-protein, leptin, into the extracellular milieu, as determined by ELISA and Western blot analyses. Increased bacteriophage production was correlated with increased release of therapeutic protein. These data provide new avenues to exploit native prophages to deliver therapeutic molecules, which we will optimize by manipulating the regulatory mechanisms of prophage excision. An added advantage is that our approach of therapeutic delivery contributes to biological containment. We envision that L. reuteri can serve as an efficient and cost-effective biotherapeutic delivery platform.

Oral Presentation #44

Intersection of Microbial Diversity and the Rest of Biology

Jo Handelsman[1]
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POSTER PRESENTATIONS

Poster Session I:

Odd Numbered, 3:30-5:30 PM, Monday, July 9

Poster Session II:

Even Numbered, 3:30-5:30, Tuesday, July 10

Main Lounge: Poster Numbers 45-92

Tripp Commons: Poster Numbers 93-178

Poster #45

Host Specificity Influences Chemical response in In Vivo Symbiotic Interactions

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[1]UW-Madison, Department of Integrated Biology, [2]UW-Madison, Department of Bacteriology, [3]UW-Madison, Department of Chemistry, [4]Dow Agrosciences, [5]UW-Madison, Department of Genetics, [6]UW-Madison, School of Pharmacy

Microbial species interactions, largely mediated by small molecules, are important in influencing the evolutionary trajectories of populations, although these complex and diverse interactions are currently poorly characterized. While most ecologically relevant microbial interactions are often difficult to ascertain, well-defined symbioses provide a useful framework to explore the diversity and specificity of chemical communication between microbes. Leaf-cutter ants engage in such a symbiosis and have coevolved with a mutualistic bacterium, Pseudonocardia that resides on the ant exoskeleton, and a pathogenic fungus, Escovopsis. Here we explore the diversity of small molecules produced by Pseudonocardia on the ant exoskeleton during various Escovopsis infections by employing mass spectrometry imaging, using a high-resolution, accurate mass matrix-assisted laser desorption/ionization Orbitrap, to examine metabolic profiles expressed under each condition. Interestingly, chemical profiles of genomically similar strains of Pseudonocardia (99.98% average nucleotide identity) differ strikingly from each other when they reside on their separate ant host. Furthermore, we examined the specificity of the ant-bacterial association by employing host-symbiont switching experiments to determine the level of association important in maintaining a chemical response to pathogens. The small molecule response is largely conserved when Pseudonocardia resides on hosts of the same ant species, however the chemical profile is significantly altered when Pseudonocardia is switched to a different host species than its native association. Together these results suggest an important role of the ant host species in influencing bacterial chemical responses in a defensive symbiosis.

Poster #46

Neonatal Exposure to the Symbiotic Yeast Pichia Kudriavzevii Exacerbates Asthmatic Immunopathology in a Murine Model

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Bacteria and bacterial-derived metabolites within the infant gut microbiota have been shown to play an important role in training the development of a healthy immune system and preventing the onset of inflammatory diseases such as asthma. Fungi, however, are also present in the gut microbiota, and imbalances in fungal communities within the infant gut have been associated with both altered gut bacterial abundance and an increased risk of developing asthma. Recently, increased presence of the yeast Pichia kudriavzevii and reduced levels of the bacterial-derived short chain fatty acid acetate were detected in the guts of three-month-old Ecuadorian infants at high risk of asthma relative to healthy infants. To investigate a causal relationship between P. kudriavzevii exposure in infancy and asthma exacerbation, we induced asthma in 6-week-old mice exposed during the neonatal period to either P. kudriavzevii or vehicle and used flow cytometry to assess for differences in lung inflammatory cell infiltrates between these groups. Neonatal exposure to P. kudriavzevii results in an increase in the proportion of lung-infiltrating CD4+ T helper cells expressing the transcription factor retinoic acid receptor-related orphan receptor gamma (RORγT) following asthma induction, an immune cell type often implicated in especially severe forms of asthma. Moreover, we demonstrate using in vitro growth curve experiments that acetate inhibits the growth of P. kudriavzevii. Together these data suggest that fungi exert immunomodulatory effects on the host and that “beneficial” bacteria may further promote health by preventing the overgrowth of certain fungi within the infant gut.

Poster #47

Spatiotemporal Expression of Host Genes in Response to Colonization in the Euprymna scolopes-Vibrio Fischeri Symbiosis

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Most symbiotic interactions occur along epithelial tissues that form a barrier between host and environment. During symbiosis onset, microbial partners experience diverse microenvironments along pathways of host-epithelium colonization. Using the symbiosis between the squid, Euprymna scolopes, and the bacterial partner, Vibrio fischeri, we explored spatiotemporal aspects of host responses to symbionts migrating through microenvironments. In this 6-8 h journey, V. fischeri cells traverse a complex landscape of ~100 µm, from superficial pores to epithelium-lined crypts, where they grow and permanently reside. We visualized host gene expression using hybridization chain reaction fluorescent in situ hybridization and protein abundance with immunocytochemistry and confocal microscopy. We focused on a gene encoding a protocadherin, which is essential for epithelial integrity and organization, mapping expression through host development. The pcdh1 expression began 9 h following inoculation and localized to regions through which the bacteria had already passed, but not in the crypts where they were residing. Additionally, expression was first observed when bacteria were in the crypts, but not yet luminescing. Expression originated adjacent to crypts and expanded over time back across the ~100 µm to the pores, the bacterial entry point. The PCDH1 protein was abundant in both aposymbiotic and symbiotic animals and was prevalent at the pores, opposite of the message pattern, suggesting that bacterial exposure drives rapid PCDH1 turnover. Using molecular markers of barrier function, we can probe factors that shape symbiont-induced modifications to host epithelial integrity and structural consequences of animal-microbe associations.

Poster #48

Probing Nutritional Mutualism Between Host and Facultative Commensals Using Chemically Defined Diets in Drosophila

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Animals have evolved and are living in constant association with beneficial microbes. One of the most consequential features of such symbiosis is the improvement of host nutrition, but how symbionts achieve this remains partly elusive.

In this study, we mono-associated Drosophila melanogaster with one of its natural commensals, Lactobacillus plantarum, as a model of facultative nutritional mutualism.
Indeed unlike obligate symbiosis, Drosophila larvae can survive and develop in the absence of their symbiont. However, when they face a nutritional challenge, Germ-Free (GF) larvae show important developmental delay, which can be buffered by the presence of certain strains of their commensal partner L. plantarum.

To better comprehend the nutritional basis of this beneficial association, we replaced the commonly used laboratory diet based on inactivated yeast and cornmeal flour with a chemically defined diet. This approach enabled us to specifically deplete the amino acids one-by-one. We observed that each depletion causes developmental delay in the GF larvae, which can all be rescued by L. plantarum. Surprisingly, L. plantarum can compensate for the lack of amino acids that it is unable to synthetize, such as L-Leucine.

Therefore, we hypothesize that the nutritional mutualism between L. plantarum and Drosophila may rely on the symbiont providing the specific amino acids to its host, but also on the symbiont producing additional factors mimicking the effect of amino acids on its host physiology. Our system therefore provides a powerful model to dissect the nutritional basis of how facultative symbionts shape their animal host's development and physiology.

Poster #49

Early Life Effects of Juvenile Western Diet and Exercise on Adult Microbiome Composition in Mice

Monica P. Louis[1], James Borneman[1], Paul M. Ruegger[1], Theodore Jr. Garland[1]
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Increases in availability of energy-dense foods and simultaneous reductions in physical activity of Western industrialized societies has created an environment that promotes obesity, but alterations in the gut microbiome may also play a role. As with other organismal characteristics, the microbiome may be influenced by factors experienced early in life. We previously demonstrated that early-life wheel access for 3 weeks, starting at weaning, increases adult wheel running in both selectively bred High Runner (HR) and non-selected Control (C) lines of mice. We extended these studies by examining effects of early-life treatments on the gut microbiome, as well as potential correlations between microbial composition and individual variation in activity levels, behavior, body weight regulation, and physiology. Mice were subjected to early-life wheel access and/or Western diet from weaning until sexual maturation, then housed individually without wheels until 14 weeks of age, when fecal samples were taken. Total DNA was extracted from fecal samples using MoBio Laboratories PowerSoil DNA Isolation kit, 16S rRNA was PCR amplified, libraries were sequenced using an Illumina MiSeq, and OTUs were chosen using QIIME. We expect to find significant baseline differences in gut microbiome composition between HR and C mice. We also expect significant effects of early-life exercise and diet manipulation. Some of these effects may be interactive, e.g., because mice from HR lines run ~3-fold more per day than those from C lines and are partially protected from the obesogenic effects of Western diet. Supported in part by NIH grant R21HD084856.

Poster #50

Degradation of Dietary Pectic Glycans by Coordinated Enzyme Pathways in Bacteroides

Ana S. Luis[1], Jonathon Briggs[2], Xiaoyang Zhang[2], Benjamin Farnell[3], Didier Ndeh1[2], Aurore Labourel[2], Arnaud Baslé[2], Alan Cartmell[2], Nicolas Terrapon[4], Katherine Stott[5], Elisabeth C. Lowe[2], Richard McLean[3], Kaitlyn Shearer[3], Julia Schückel[6], Immacolata Venditto[2], Marie-Christine Ralet[7], Bernard Henrissat[4], Eric C. Martens[1], Steven C. Mosimann[8], D. Wade Abbott[3], Harry J. Gilbert[2]

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The human gastrointestinal tract is colonized by a microbial community (microbiota), which has a significant impact on human health and nutrition. The Gram-negative bacterium Bacteroides thetaiotaomicron is highly adapted to the gut microenvironment reflecting its ability to utilize an extensive repertoire of complex carbohydrates, such as pectins, found in plant cell walls. In B. thetaiotaomicron the genes encoding specific glycan degrading systems are grouped into genetic loci (Polysaccharide Utilization Loci or PULs).

Pectins are found in fruit and vegetables and the two major components are homogalacturonan (HG) and rhamnogalacturonan-I (RGI). RGI is characterized by an alternating α-1,2-L-rhamose (L-Rha) and α-1,4-D-galacturonic acid backbone, where the L-Rha residues can be extensive decorated with galactans and/or arabinans. B. thetaiotaomicron was previously shown to utilise all known pectin structures. Here we report the mechanism by which the bacterium depolymerises the various pectin structures. The enzymes encoded by HG, RGI, galactan and arabinan PULs were characterized. The RG-I degradation apparatus was shown to be particularly complex with nine enzymes targeting the glycan backbone. This system was also shown to be adapted to trim the remnants of HG and galactan side chains from the RG-I backbone. The identification of the key surface enzyme that initiates the degradative process revealed that the canonical model, in which an endo-enzyme has an essential role, is not universal within the pectin systems. Additionally, cross-feeding experiments revealed that oligosaccharides released by B. thetaiotaomicron were utilized by other Bacteroides spp., highlighting a dynamic ecological relationship in the human gut microbiota.

Poster #51

Investigating the Mechanisms by Which BefA Induces Beta Cell Expansion

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As our knowledge of the intricate interactions between hosts and their commensal microbial communities (i.e. microbiota) grows, so has our understanding for the necessity of microbiota for normal host development. Using larval zebrafish, we have shown that pancreatic insulin-producing beta cells fail to expand in the absence of their microbiota (germ free). Further, we have discovered that a novel bacterial-secreted protein, beta cell expansion factor A (BefA), rescues beta cell development within germ free zebrafish. However, a major question behind the mechanism of BefA is how a protein produced by gut bacteria signals to cells within the pancreas and subsequently induces proliferation specifically within beta cells. Utilizing tissue culture techniques, nano-manipulations on larval zebrafish, and single cell RNA transcriptomics (scRNAseq), we aim to reveal how BefA causes beta cell expansion. We found that administering BefA directly into the proximal intestine or circulatory system induces beta cell expansion in germ free zebrafish. Our tissue culture studies indicate that BefA can become internalized within beta cells. Together, this suggests that BefA acts directly on beta cells and can access them through multiple routes of action. Repeating the experiment with sox9b-/- zebrafish that lack the main connection of the pancreas to the intestine (i.e. pancreatic duct) shows that BefA rescues beta cell expansion only when administered to the circulatory system. We are currently analyzing the transcriptomic profile of beta cells treated with and without BefA.

Poster #52

Host Genetic Background Contributes to Resistance to Microbiota Disruption and Host Development in an Evolution Model Organism

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The host genetic background can influence both microbiota composition and the immune response to microbes. Disruption of the microbiota can lead to painful inflammation in the host, which can become chronic, as in the case of inflammatory bowel disease. To determine the extent that the host genetic background contributes to resistance to microbiota disruption and the ability of the microbiota to contribute to host development, we adapted the evolution and biomedical model organism, threespine stickleback fish (Gasterosteus aculeatus), for gnotobiotic experiments. Stickleback are ideal model organisms for these studies due to their large clutch sizes, genetic variation within and between populations that is similar to human genetic variation within and between populations, and the tools available to study these interactions. We compared the development and behavior in fish raised germ free, with conventional microbiota, with mock communities of up to 8 microbiota members, or with microbiota disrupted by antibiotic or environmental contaminants. We found that the populations varied in their response to these manipulations, indicating that the genetic variation between the populations contributed greater to the relationship between microbes and the host than the variation within the populations. We will use these results as a basis for future studies to identify the critical windows in development in which disruptions to gut microbiota result in short- and long-term consequences to host health, and determine the extent to which the host genetic background contributes to the ability of healthy gut microbial communities influence to fitness.

Poster #53

Neonatal Priming Shapes Preferential Capacity for Immune Tolerance to Skin Commensal vs. Pathogenic Bacteria

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Life in a microbial world requires both tolerance to commensal microbes and protective responses against pathogens. However, mechanisms enabling the host to establish a privileged relationship with commensals remain largely unknown. Using tetramers for a model bacterial antigen, 2w, we can track the antigen-specific CD4+ T cell response to a skin commensal, S. epidermidis (SE-2w), and a prototypical pathogen, S. aureus USA300 (SA-2W). As previously shown, SE-2w colonization of neonatal skin establishes immune tolerance to this commensal and enriches for 2w-specific (2w+) regulatory T cells (Tregs). In contrast, we find that neonatal SA-2w colonization does not protect against skin inflammation nor enrich 2w+ Tregs upon subsequent SA-2w challenge. Consistent with a model in which differential T cell priming upon initial microbial antigen exposure sets the course for tolerance or immunity, neonatal SE-2w but not SA-2w colonization was sufficient to expand 2w+ Tregs. Moreover, distinct responses to SE and SA were maintained following concurrent colonization, suggesting that the host can simultaneously distinguish friend from foe and that this occurs at the level of naïve T cell priming by dendritic cells (DCs). Notably, the ability to mount preferential tolerance to SE over SA was mitigated in animals lacking MyD88 signaling, and neonatal colonization with an Δagr SA-2w mutant enabled enrichment of 2w+ Tregs. Using fluorescently-marked bacteria to track DC uptake, transgenic animal models, and additional SA mutants, we are now further dissecting the relevant immune cell populations, host pathways and bacterial molecules involved in educating the early immune response to skin microbes.

Poster #54

Genetic Analysis of Colonization Fitness in a Zebrafish Gut Commensal

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The microbial communities that colonize mucosal surfaces in animal hosts are known to impact many aspects of host physiology including metabolism and immune function. However, the majority of these microbial species lack molecular genetic tools and thus the mechanisms that mediate their colonization and influence on host remain undefined. Our previous studies identified a Firmicutes member of the zebrafish gut microbiota, Exiguobacterium acetylicum, that promotes intestinal lipid absorption. Lacking genetic tools, we constructed a draft genome sequence and generated a library of isogenic mutants using chemical mutagenesis. By integrating in vitro motility assays and high-throughput sequencing, we identified novel genes implicated in bacterial motility. Currently we are defining the essential gene set for E. acetylicum by performing parallel in vitro and in vivo competitions of E. acetylicum mutants, identifying mutant strains that are enriched specifically in vivo. We are elucidating the ability of mutant strains to colonize the intestine in subsequent studies with gnotobiotic zebrafish utilizing a combination of pairwise in vivo competitions and high-resolution microscopy with labeled strains. We have isolated a non-motile mutant that hyper-colonizes in vivo, and preliminary evidence suggests that a motile isogenic suppressor strain is depleted. Our data indicate that phenotypes traditionally thought to be positively associated with host colonization, such as motility, may not be the primary drivers of successful colonization. Collectively, our data demonstrate in vivo screening of bacterial mutant libraries can identify novel genes that promote colonization. More broadly this platform could be applied to other “genetically intractable” gut bacterial strains.

Poster #55

Evolution and Engineering of Symbioses Between Plants and Beneficial Microbes

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Associations between plants and beneficial microbes play an essential role in the productivity and sustainability of agriculture. Arbuscular mycorrhizal fungi colonize the vast majority of crops and land plants. They improve the uptake of nutrients and water by the host plant and protect it against a wide range of stresses. This symbiosis is very ancient and probably appeared with the first land plants about 450 million years ago. By contrast, nitrogen-fixing bacteria such as rhizobia and actinobacteria of the genus Frankia lead to the development of nodules on the roots of a limited number of plants clades from the Fabales (legumes), Fagales, Cucurbitales, and Rosales. These root-nodule symbioses allow efficient biological nitrogen fixation and appeared more recently, about 100 million years ago. Interestingly, rhizobia, Frankia, and arbuscular mycorrhizal fungi all produce cocktails of modified chitooligosaccharides (COs) as signaling molecules to be recognized by their host plants and initiate symbiotic associations. Host plants of these microbes also use a unique and highly conserved signaling pathway to perceive and transduce modified COs suggesting that the root nodule symbioses hijacked the pre-existing pathway of mycorrhizal associations. We will present data and hypotheses on the role and possible origin of modified COs in the fungal and the bacterial kingdoms. We will show our latest findings on the evolution of the signaling pathway allowing plants to recognize these microbial COs. We will finally discuss how all this molecular and evolutionary information provides a blueprint for engineering new nitrogen-fixing associations.

Poster #56

A High-Throughput in Vitro System to Test Compound Effects on the Human Microbiome

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Kaleido Biosciences is a clinical-stage biotechnology company developing novel chemistries that drive the function of the human microbiome to treat disease and manage health. One of the main functions of the mammalian gut microbiome is to ferment complex carbohydrates that are undigested by host enzymes, which can change the bacterial composition and metabolic production of the microbiome. To assess these effects Kaleido has developed a high-throughput in vitro model to test our expansive library of chemistries. Current in vitro models of the gut microbiome are either low throughput or are lacking complexity to efficiently model the gut microbiome in a drug discovery setting. Here we describe a 96 well in vitro model, termed “ex vivo” which is a reproducible system that has screened >500 compounds for modulation of community growth, composition, and bacterially derived metabolites with the capacity to easily manipulate relevant variables. Kaleido’s proprietary chemistries have modulated the growth, composition of human fecal communities, and metabolite responses such as short chain fatty acids (SCFAs) and ammonia. This ex vivo system allows for reproducible and reliable testing of large chemical libraries and a diversity of human samples mimicking different gut environments.

Poster #57

Exploring Acetobacter-Lactobacillus Interactions in the Drosophila Gut

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The gut microbiome is an important contributor to animal health and homeostasis. Drosophila is an excellent model system for studying the gut microbiota and its interactions with the host. In particular, Drosophila has a simplified gut microbiome compared to other animals, containing between five and twenty members instead of tens or hundreds. Acetobacter pasteurianus, Lactobacillus brevis, and Lactobacillus plantarum are bacterial species commonly found in the fly gut. The interactions between these species confer greater benefit to the host than mono-association with any one species. Our data indicates that gut colonization by A. pasterianus is enhanced by the presence of Lactobacillus spp. This growth enhancement is also observed in liquid and solid media, and on fly food. Our results suggest that Lactobacillus spp. improve the growth environment for A. pasteurianus across many different conditions. Additionally, the different Lactobacillus species have slightly different effects on the growth enhancement of Acetobacter and are themselves enhanced when in co-culture. Further work will determine the specificity of this interaction and identify the mechanisms underlying the growth promotion. Specifically, we are exploring the metabolites and genes that are differently regulated when Acetobacter and Lactobacillus are grown in mono- and co-culture, and whether other bacteria are able to also enhance Acetobacter growth. Our long-term goal is to understand the different microbial and host factors that leads to successful establishment of the microbiome and to explore their long-term impacts on the function and evolution of the gut bacterial community.

Poster #58

Prevotella β-Mannan Utilization Loci: From the Rumen to the Human

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β-mannans are a diverse and abundant family of plant structural and storage glycans that comprise a significant part of human and livestock diets. However, aspects of β-mannan utilization in animals remain unclear, including the diversity of molecular systems devised by gut microbiota to address these complex carbohydrates. We present here the identification and detailed functional characterization of a large β-mannan utilization locus (β-MUL) from the historically important ruminant bacterium Prevotella bryantii B_14, using combined bioinformatic, biochemical, and structural biological approaches. We demonstrated that this locus encodes the complete repertoire of glycan-binding and catalytic activities necessary for the saccharification of (galacto)mannans and (galacto)glucomannans, which host animal genomes otherwise lack. Holistic understanding of Prevotella β-MUL function and organization enabled the identification of diverse putative β-MULs across the Bacteroidetes phylum based on a defining mannobiose-specific symporter-epimerase-phosphorylase triad as a molecular marker. Moreover, we were able to use the ruminal P. bryantii B_14 β-MUL as a query to identify homologous sequence signatures among cattle (rumen) and human (monogastric) gut metagenomes, thereby indicating the concordant distribution of Prevotella across these diverse niches.

Poster #59

Investigating the Impact of Mutualistic Symbiosis on the Transcriptomic Profiles of the Entomopathogenic Nematode Steinernema Carpocapsae

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The entomopathogenic (insect-parasitic) nematode Steinernema carpocapsae associates with Xenorhabdus nematophila bacteria in a mutualistic symbiosis. The non-feeding transmission stage infective juvenile (IJ) nematode carries symbiotic bacteria in an intestinal pocket called the receptacle. To actively seek and invade insects, the IJs exhibit specific behaviors such as nictation (standing on its tail and waving its head for sensing environmental cues), jumping, and chemotaxis in response to insect-derived CO2. Within an infected insect, the nematode releases bacterial symbionts and together they kill the insect and reproduce in the cadaver. In response to nutrient depletion and crowding, S. carpocapsae re-associate with bacteria, develop into IJs, and leave the insect cadaver via IJ-specific dispersal behavior. We hypothesize that the presence of the bacterial symbiont will influence the physiology of the nematode IJs, which will be reflected by differences in gene expression profiles among IJs developed under different cultivation regimes: axenic (without bacteria), with wild type bacteria that colonize the IJ receptacle, and with a non-colonizing bacterial mutant. We will report our current progress in RNA-seq analysis focusing on the impact of the cultivation regime on host metabolism and neuronal regulation. We also will explore if X. nematophila colonization affects IJ host-seeking behaviors using phenotypic assays. Our research will reveal the role of mutualistic bacteria in shaping the physiology and behaviors of their nematode host

Poster #60

Effect of Thermal Stress on Host Physiology and Gut Microbiota of the Eastern Subterranean Termites Reticulitermes Flavipes

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Understanding the effects of environmental disturbances on the health and physiology of insects is crucial in predicting the possible impact of climate change on the distribution, abundance and ecology of these organisms. In addition to physiological adaptations to environmental changes, insects have developed complex associations with microorganisms which may help to mediate some of these responses. In this work, we evaluate the effect of three temperature treatments on feeding, survival, cold tolerance, and gut microbiota in the subterranean termite, Reticulitermes flavipes. Subcolonies with 100 R. flavipes workers were exposed for 4 weeks to one of three temperatures, characterized as low (15°C), medium (27°C), or high (35°C) with 5 replicates per temperature treatment. We observed significantly higher mortality in the 35°C treatment (29%) compared to the 27°C (8%) and 15°C (7%) treatments. The high temperature treatment group termites had 60% less gut total bacteria counts than the control or low temperature treatments. However, termites in the high temperature harbored 2.3 times more chitin-degrading bacteria and 6 times more uric acid-degrading bacteria than the other treatments. We are currently sequencing the 16S rRNA gene of the microbial community of termite gut to assess the impact of the temperature treatment in the bacterial diversity. Overall, our results show that exposure to high temperatures have a negative impact both on the termites and their gut microbiota. Remarkably, high temperature stress decreased the total bacterial abundance in the termite gut, but increased the abundance of bacteria involved in nutrient recycling.

Poster #61

Insect-Associated Streptomyces Are a Rich Source of New Antimicrobials with Activity Against Resistant Human Pathogens

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Streptomyces bacteria produce antimicrobial secondary metabolites with vast functional and structural diversity. Most clinically-approved antimicrobials are derived from Streptomyces; however, no new classes of antibiotics have been discovered in many years. Together with the rise of antibiotic resistance among human pathogens, this underscores the need to discover new antimicrobial drugs. In nature, the chemical communication mediated by microbial secondary metabolites enables complex interactions between species. Small-scale studies demonstrate that bacteria involved in symbioses with insects represent an attractive discovery resource for antimicrobial compounds. Herein, I describe a systematic assessment of the antimicrobial potential of geographically and ecologically diverse insect-associated Streptomyces. The biosynthetic potential of a subset of these strains was determined through genomic mining for biosynthetic gene clusters (BGCs). Their evolutionary patterns provide new insight into how secondary metabolism relates to phylogeny and host-association. Genomics, metabolomics, inhibition assays, and in vivo models of infection have revealed insect-associated Streptomyces as a prolific source of compounds with bioactivity against Gram-positive, Gram-negative, and fungal pathogens of high clinical interest, offering a rational strategy to guide the discovery of new antimicrobials.

Poster #62

The Hibernating Squirrel Microbiome Responds to Seasonal Dietary Shifts by Altering Its Functional Potential

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Hibernating animals undergo dramatic, seasonal, dietary shifts that change substrate availability for their associated gut microbiota. The circannual hibernation cycle involves periods of summer host hyperphagia when the microbiota has access to dietary substrates for energy. In contrast, during winter the host fasts and hibernates, forcing the microbiota to rely on host-derived substrates. To examine seasonal shifts in the functional potential of hibernator microbiomes, we generated metagenomes from cecum contents of 13-lined ground squirrels (Ictidomys tridecemlineatus). We hypothesize that seasonal metagenomes have distinct features that reflect microbial adaptation to changes in substrate availability. Samples were sequenced from three squirrels each season: summer, winter, and spring. Winter metagenomes trended towards higher proportions of genes involved in glycan, cofactor, and vitamin metabolism, as compared to spring or summer. This suggests that these microbiomes respond to decreased dietary substrate availability during hibernation by shifting towards the metabolism of host-derived glycans, their primary winter energy source. Additionally, increased cofactor and vitamin metabolic potential may compensate for the absence of diet-based nutrients. We found that spring metagenomes had significantly higher proportions of genes involved in membrane transport, relative to summer or winter. In spring, the microbiome may respond to reintroduction of dietary substrates by increasing its capability to secrete catabolic enzymes and uptake molecules for metabolism. Our results demonstrate that the hibernator microbiome adapts to host dietary shifts by altering functional potential, highlights the importance of microbe-host symbioses in animals with complex phenotypes, and improves understanding of hibernation for applications such as deep space travel.

Poster #63

Age, Early Life Adversity, Fitness and the Host-Associated Microbiome

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The vertebrate gastrointestinal tract is home to a complex community of microbes that provide a number of ecosystem services to its host, but a lack of prospective, longitudinal data on gut microbial dynamics means little is understood about how the gut microbiome changes across an host's life, or whether these changes serve as bellwethers of host health, development, and aging. For my PhD thesis, I propose to address this gap using a longitudinal data set spanning 19,885 unique freeze-dried fecal samples from 617 known individual baboons over the course of 15 years. These samples have corresponding data about demographics, health measures, and social interactions. Because of the unprecedented nature of this baboon gut microbiome dataset, we will be able to understand, for the first time, how the gut microbiome changes over entire lifespans, and whether the gut microbiome exhibits predictable signs of aging. Next, we will test which forces explain inter-individual differences in gut microbial development and aging, focusing on the roles of nutrient limitation and social isolation. Lastly, we will test the roles of gut microbial diversity, composition, and stability on longer term measures of host fitness, such as overall lifespan and reproductive success. By determining factors that influence a favorable gut microbiome composition, my results will reveal what constitutes a healthy microbiome across life and thus direct microbiome interventions that efficiently improve human health. This research will also contribute to the field of evolutionary biology by testing the correlation between microbiome composition and markers of Darwinian fitness.

Poster #64

Phages Drive Microbial Structure During Xenobiotic Use to Perturb Metabolic Health

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Xenobiotics are substances foreign to the body, such as antibiotics, dietary supplements, and prescription medications, that can mediate microbiome shifts beneficial or detrimental to host health. Current models of xenobiotic-induced dysbiosis emphasize the xenobiotic-bacteria-host triad of interactions. However, bacteriophages comprise a significant portion of the microbiome and are capable of killing and transforming bacterial populations. In this study, we investigated the changes in the viral and bacterial components of the microbiome in response to the xenobiotic risperidone, a common antipsychotic associated with weight gain and related sequelae. Using a mouse model (C57bl6), treatment with risperidone resulted in 10-fold increase of virus-like particles (VLPs). Shotgun metagenomics sequencing revealed that the enteric phages (phageome) are altered in risperidone-treated animals in both the types of phages present and in the genes that they carry. A phage material transfer (PMT) of phages from risperidone treated animals to naïve recipients was sufficient to shift the bacterial microbiome to a community resembling risperidone treatment. These shifts include a decrease in butyrate producers and increases in LPS producers, suggesting an inflammatory microbiome. Last, shotgun metagenomics sequencing on the stool of the PMT treated animals revealed an altered functional potential of the resulting microbial community. These results implicate phage as major drivers of the risperidone-mediated microbiome shifts and should be regarded as controllers of the gut microbial structure, which has an impact on the health of the host.

Poster #65

Functional Profiling of the Skin Microbiota

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Skin is an essential component of mammalian health as it provides protection from the environment. The skin is host to a community of microbial species, the skin microbiome, which is hypothesized to contribute to the functionality of the skin and provide protection against pathogens. However, the individual contributions of skin microbiota that provide protection against pathogens or contribute to different disease states are widely unknown. In fact, basic functional analysis of the majority of microbial species colonizing healthy skin has not been performed.

To address this knowledge gap, we used skin 16S rRNA amplicon and metagenomic sequencing datasets to inform a targeted culture approach to generate a diverse culture collection of over 100 mammalian skin bacterial isolates. We compared isolates from the most abundant skin genera including, Corynebacterium, Staphylococcus, and Streptococcus to nine Staphylococcus aureus clinical isolates cultured from atopic dermatitis lesions and the common soft tissue infection strain, Methicillin Resistant Staphylococcus aureus (MRSA). Growth dynamic analysis showed that variable patterns exist within genera and between commensals and pathogens. Next, we co-cultured isolates, both commensal and pathogenic, to determine which could coexist in a community. Mapping these interactions through network analysis revealed that pathogenic isolates commonly prevent the growth of commensal isolates. Using a number of computational tools including metagenomic profiling, genome annotation, and gene prediction, we are developing detailed functional profiles for each isolate. Ultimately, this work will provide insight into the individual and group functionality of bacterial skin isolates and how skin commensals form and stabilize microbial communities.

Poster #66

Environmental Temperature Alters the Gut Microbiota and Digestive Performance of the Eastern Red-Backed Salamander (Plethodon Cinereus)

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Environmental temperature can impact diverse aspects of ectotherm physiology, including digestive performance. Additionally, changes in temperature can alter ectotherm gut microbial community composition and diversity. Because gut microbiota typically enhance host digestion, such temperature induced changes in gut microbiota may underlie some of the relationship between temperature and host digestive performance. Here, we investigated the effect of environmental temperature on the gut microbial community and digestive performance of the eastern red-backed salamander. Additionally, we investigated potential connections between specific gut microbial taxa and salamander digestive performance. We found that temperature significantly impacted salamander digestive performance, which was optimal at intermediate temperatures. Temperature also significantly impacted diversity and community composition of salamander gut microbiota. Lastly, the abundance of specific gut bacterial taxa were correlated with energy assimilation of the host. Our results suggest that whole animal performance can be negatively impacted by changing temperatures, which may be mediated by alterations to the gut microbiome. This suggests a new way in which ectothermic vertebrates may be negatively impacted by increasing global temperatures.

Poster #67

Potential Roles of Burkholderia in the Fungus-Farming Ant System

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Fungus-growing ants farm a mutualistic fungal symbiont that serves as their sole food source. The fungus garden harbors an established bacterial community composed of several genera of Proteobacteria, including Burkholderia, although the role of these bacteria is still unknown. Due to its known role as a fungal symbiont in other systems, we hypothesize that Burkholderia may have a beneficial role in the fungus garden of fungus-farming ants. Here, we investigated whether Burkholderia can inhibit Escovopsis, a fungal parasite of the fungus garden, and if Burkholderia can degrade plant defense compounds (PDC), which are toxic to the fungus garden. In this study, we isolated 33 strains of Burkholderia from fungus gardens of four different genera of attine ants. Seven genomes were sequenced, representing at least one strain from each ant lineage. Escovopsis inhibition assays and ANTISMASH analysis indicates that Burkholderia produce antifungals, with strains from one lineage of ants demonstrating increased inhibition in the Escovopsis assays. Finally, Burkholderia has the potential to detoxify PDC that leaf-cutter ants may encounter. Burkholderia strains contained 14-18 of 20 genes found in the diterpene degradation cluster, in addition to 10-18 cytochrome p450s known to be involved in the transformation of terpenes. Additionally, Burkholderia grew in the presence of eight PDC and one strain grew on all eight PDC as the sole carbon source. These results suggest that Burkholderia may fill a beneficial role in the fungus-farming ant system. Future work will involve in vivo characterization of Burkholderia in response to PDC and Escovopsis.

Poster #68

Host Fitness Costs of a Bacterial Symbiont Defensive Protein

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Microbial beneficial symbionts can impact host fitness by defending their hosts against opportunistic organisms such as parasites and pathogens. One mechanism of defense is symbiont-encoded ribosome-inactivating proteins (RIPs) that target and inhibit parasites of their hosts. RIPs are broadly distributed, occurring in both eukaryotes and prokaryotes, and are classified by their ability to target and inactivate ribosomes. Most work on symbiont-encoded RIPs has focused on their activity against non-native hosts (parasites). However, since RIPs target a conserved molecule present in all organisms, symbiont-encoded RIPs may also have a direct effect on the mutualistic hosts of the symbionts that produce the RIP. In this work, we determined the activity and impact of bacterial symbiont-encoded RIPs against mutualistic hosts. We used a well-studied model system for mutualism: the gram-negative bacterial symbiont Xenorhabdus bovienii and its nematode hosts Steinernema spp. We found that Steinernema nematodes associated with a X. bovienii RIP deletion mutant had increased progeny production and long-term survival relative to those paired with wild type, RIP-encoding X. bovienii. These results indicate that RIP encoding by the X. bovienii negatively impacts Steinernema nematode host reproduction and survival. Ongoing work focuses on determining the mechanism(s) by which X. bovienii RIP causes these host phenotypes and assessing if RIP has a dual function in host defense. Overall, our work indicates host fitness costs of associating with a symbiont that encodes a protein known for its role in defensive symbiosis. Our findings support the idea that host-benefit trade-offs occur in host-defensive symbiont associations.

Poster #69

Spiroplasma is an Unrecognized Symbiont of Trachymyrmex Septentrionalis

Emily A. Green[1], *Jonathan Klassen*[1]
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Intracellular parasites are commonly found in many insects and are known to infect ants. Although the cultivar and Pseudonocardia symbionts of the fungus growing ant Trachymyrmex septentrionalis have been studied extensively, the presence and function of bacterial symbionts remains unknown. We detected the common insect parasite Spiroplasma in T. septentrionalis ants using community amplicon sequencing of the 16S rRNA gene. Spiroplasma occurred in only some sampled ants, even within the same colony. Spiroplasma prevalence differed between the geographic regions from which T. septentrionalis ants were collected, and between colonies maintained in the lab versus those freshly-collected from their natural environment. A previously-published PCR screening method confirmed these trends, although some non-specific amplification was observed. This study suggests that Spiroplasma may be a hitherto unrecognized symbiont of T. septentrionalis ants.

Poster #70

Evaluating the Role of Diet, Taxonomy and Sex on the Oral and Rectal Microbiome of Puerto Rican Bats

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Bats play a major role in ecosystems by pollinating plants and regulating insect populations through their feeding habits. Moreover, bats harbor a complex community of microbiota that have coevolved with their hosts due to selective pressures imposed by their host's life history characteristics. Because bats are potential reservoirs for human pathogens, a characterization of their digestive tract community as well as the microbe-microbe and microbe-host relationships is key. Puerto Rico harbors 13 bat species belonging to the 5 families (Noctilionidae, Moormoopidae, Phyllostomidae, Vestertilionidae, and Molossidae) that differ greatly in diet, some feeding primarily on nectar, fruit, arthropods, or fish, while others are euryphagic. In this initial analysis, oral and rectal swab samples were collected from two phyllostomid species, Artibeus jamaicensus (frugivore) and Brachyphylla cavernarum (omnivore consuming fruits, insects, nectar and pollen), that were captured in three caves in Puerto Rico. The V4 region of the 16S rRNA gene was amplified and sequenced using an Illumina MiSeq. The data was analyzed using Qiime 1.9 and the reads were binned into OTUs (operational taxonomic units) of 97% sequence similarity. The oral microbiome was dominated by the genera Streptococcus, Eikenella, Actinobacillus and Moraxella whereas the rectal microbiome more diverse, and dominated by the taxa Lactococcus, Chlamydiaceae, Streptococcus, Pasteurellaceae, Mycoplasmataceae, Enterbacteriaceae and Helicobacteriaceae. Analyses of β diversity revealed a distinct clustering of microbiomes based on host species and site collection (oral versus rectal). Future research will consider a greater number of bat species that differ in life history characteristics, including foraging ecology.

Poster #71

Learning from Dysbiosis: Conserved Host Genetic Regulators of the Microbiome Converge on Gut Motility

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The gut microbiota exerts considerable influence on the physiology of hosts across the animal kingdom. A perturbed gut microbial community (dysbiosis) can become detrimental to the host, leading to development of pathologies like inflammatory bowel disease (IBD) where microbiome overgrowth, over-activation of the immune system and impaired gut motility are common manifestations. Variation in host genetics and microbiome composition (Enterobacteriaceae blooms) have been linked to IBD, but the molecular interplay of the host and microbiome in IBD remains largely unknown.

To address this question, we employ the high-throughput amenable nematode Caenorhabditis elegans. Its robust genetics, conserved innate immune, intestinal functions and simple Enterobacteriaceae-rich microbiome make it an ideal organismal model for these studies. First, we screened orthologs of 136 IBD risk factors for microbiome colonization defects by RNAi. Individual knockdowns (>70%) of increased E. coli gut colonization of C. elegans (N2), indicating their role microbiome homeostasis. Second, we established genetic dysbiosis model with defects in gut motility (75% reduced), bacterial control (50% higher) and immune over-activation (10-100X higher) versus controls. Fewer RNAi knockdowns (~50%) enhance colonization of dysbiotic animals. Colonization defects were suppressed by several genes (9) and also varied based on the E. coli genotype [12 HMP strains, control and IBD associated]. Finally, we developed a high-throughput assay bead-based assay for gut motility and found that both host genetic and microbial variation converge on regulation of gut motility. Together, this system allows for molecular dissection the interactions of host and microbial factors that maintain microbiome health.

Poster #72

Bacteroides Thetaiotaomicron as a Chassis for Understanding Gut-Resident Bacteriophages

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Our emerging view of the gut microbiome largely ignores the roles and identities of bacteriophages (phages) in this ecosystem. Though phages are abundant in the gut, methods of data generation and analysis routinely used in microbiome science neglect phage biology, leaving important basic questions unaddressed. Which bacterium does a predicted phage infect? How are ecosystem dynamics impacted by phages? How do phages impact the health of the eukaryotic host? To help fill this gap in understanding, we isolated a large collection of phages from the United States and Bangladesh that are specific to the prominent human gut symbiont, Bacteroides thetaiotaomicron. Using a panel of isogenic B. thetaiotaomicron mutants, we show that these phages vary in host range with respect to expression of 8 differentially regulated capsular polysaccharide loci (cps). Because the expression of cps by Bacteroides in the gut is affected by inputs such as inflammation and host diet, we are using these phages to test foundational hypotheses on the roles of phages in the response of the gut microbiome to ecological disturbances. Additionally, genomic analysis of these phages and comparison to existing metagenomic datasets revealed unexplored sequence space. Taken together, this work provides a foundation and direction for uncovering the identities and roles for phages in the gut microbiome, with relevance for how we treat the microbiome-host interactions central to our health.

Poster #73

Four Novel Polysaccharide-Degrading Bacteroides from the Gut of the Cockroach Periplaneta Americana.

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Four Bacteroides isolates from the gut of the Periplaneta americana cockroach have been successfully cultivated, and they exhibit cladogenesis amongst Bacteroides previously cultivated from animals and the environment and form a unique clade with uncultivated Bacteroides clones from cockroaches. Compared to other Bacteroides spp., these four isolates had an average amino acid identity (AAI) below 75%, which suggests they each represent new species. Near-complete genome sequencing reveals broad capabilities for plant polysaccharide degradation, vitamin biosynthesis, and energy generation, as well as host colonization genetic determinants. The presence of electron transport chain components and terminal oxidases suggests potential for energy generation via anaerobic and nanoaerophilic respiration, though one isolate presents evidence of a primarily fermentative metabolism. Numerous Carbohydrate Active Enzymes (CAZymes) were organized within canonical Bacteroidetes Polysaccharide Utilization Loci (PULs), with several non-PUL-associated, extracellular glycoside hydrolases present. CAZyme content varied across isolates, ranging from 120 to 378 glycoside hydrolases, with hydrolases predicted to target numerous polysaccharides, including cellulose, xylan, starch, pectin, host glycans and chitin. In-vitro polysaccharide degradation reflects genomic predictions and is not correlated with phylogenetic position. All four isolates are detected in >80% of lab-reared adult and nymph cockroaches, which suggests they are stable members of the cockroach gut microbiota and contribute to host dietary metabolism.

Poster #74

Back to Nature: Assembly of the Gut Microbiome in Natural Populations of Drosophila

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Successful implementation of therapies to modify the gut microbiome for improved health are contingent upon a thorough understanding of processes underlying microbial community assembly. These approaches are complicated by the variability of microbial gut community composition between individuals as well within the same individual over time. This is further exacerbated by the addition of microbes from external inputs such as food, as well as the loss of microbes through fecal shedding.

Our previous research on the Drosophila gut microbiome has demonstrated the importance of mutualistic and competitive interactions among microbes, as well as host genotype, as important determinants of the microbial composition in laboratory cultures. However, growing evidence suggests that these processes provide incomplete explanations for the microbial community composition in natural populations of Drosophila. Here we show that natural microbial populations are taxonomically different and more diverse than in laboratory flies, and also present more functional variability with respect to motility and nutrition. Furthermore, co-occurring taxa in the guts of wild flies tend to be closely-related, suggestive of niche overlap rather than mutualistic interactions. For most bacterial taxa, the relationship between abundance in individual flies and their prevalence across the population conforms to predictions of a neutral model, implicating passive dispersal and ecological drift as key drivers of natural community composition. This suggests that microbial transmission dynamics and population processes in the external environment may play important roles in shaping community assembly – and, if our findings on flies are relevant to humans - the efficacy of microbial therapies.

Poster #75

Does Glyphosate Application Affect the Plant Rhizosphere Bacteria: A CURE, Year 1

Cynthia Keler[1]
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This long term one semester microbiology laboratory course is designed to engage students in a relevant scientific question; Does long term use of glyphosate affect the population of plant growth promoting bacteria in the rhizosphere? Five, 10' x 10' plots separated by 10' were set up in an area on Delaware Valley University's campus that had not been used for at least 10 years. Three plots were treated with Buccaneer Plus (41% glyphosate) as pre-planting burndown and two were left untreated as controls. The plots were planted as follows: 1. Traditional corn Cruiser Maxx250 no burndown, 2. GMO corn DroughtGard Double Pro Aecclerar 280 no burndown, 3. Traditional corn with pre-planting burndown, 4. GMO corn pre-planting burndown, no further application, 5. GMO corn with pre-planting burndown and treatment with glyphosate at V4 and V7. Samples we collected of the soil for before burndown and after burndown but before planting. Samples of the bacteria surrounding the roots of the plants were collected before the V4 treatment and then by the students after the V7 application. Total DNA was isolated and sent for next-gen 16S ribosomal gene sequencing. Students also isolated endospore forming bacteria PGPB from the plant roots after the V7 application by heating for 80oC for 10 minutes and plating on M9 with ACC as the sole nitrogen source. Students then tested their pure cultures for root elongation, nitrogen fixation, glyphosate utilization, glyphosate inhibition, and fungal inhibition. The results of the first year of class data will be presented.

Poster #76

Microbial Mediation of Herbivory in Leaf-cutter Ant Fungus Gardens

Lily Khadempour[1], *Cameron Currie*[1]
[1]University of Wisconsin-Madison

Most metazoans lack the physiological capacity to use plants as their sole source of energy and nutrients. To compensate for this, metazoans associate with microbial symbionts, which aid their hosts with the breakdown of recalcitrant biomass, remediation of plant defense compounds, and nutrient supplementation. Leaf-cutter ants, dominant herbivores in the Neotropics, are a paradigmatic example of this microbial mediation of herbivory. The ants cut foliar biomass from their surroundings and provide it as a growth substrate to their fungal cultivar Leucoagaricus gongylophorus. The cultivar breaks down the plant biomass and provides specialized hyphal swellings called gongylidia, which the ants consume as their primary source of energy. We explored the relationships between the different types of substrates ants incorporate into their fungus gardens and how both the fungal cultivar and the bacterial community facilitate the ants' breadth in substrate use. Using metaproteomics we compared the proteins that the cultivar secretes when provided with different plant substrates showing that the fungus responds in a flexible, substrate-specific manner to the material that the ants incorporate into their gardens. Next, we used metagenomics on the bacterial community in the fungus gardens of ants to show how they may facilitate the ants' transition from using dicots to grasses through changes in community composition and functional capacity. And finally, we use fungal genome sequencing to explore the genomic transition of the ants' fungal cultivars as the ants transition from one substrate to another.

Poster #77

Exploring the Biosynthetic Potential of Complex Microbial Communities

Jason Kwan[1], Ian Miller[1], Evan Rees[1], Jennifer Ross[1], Izaak Miller[1], Jared Baxa[1], Juan Lopera[1], Robert Kerby[1], Federico Rey[1], Kerry McPhail[2]
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Microbes universally live in communities, where the antagonistic and symbiotic interactions between different species shape the evolution of bioactive small molecules. The exact nature of such interactions may shed light on the functions, and hence potential therapeutic uses, of small molecules in the environment. However, most environmental microbes have never been cultured, making the study of their natural behavior and interactions challenging. One of the methods that can help illuminate uncultured microbes is culture-independent sequencing, of both DNA and RNA (metagenomics and metatranscriptomics, respectively). In this talk, I will outline how before we can use meta'omics to ask "who is doing what?" in microbial communities, we must first determine who each sequence belongs to, in a process called "binning". I will discuss a binning pipeline that my lab has developed, termed "Autometa", that is capable of deconvoluting highly complex metagenomes with over 1,000 microbial species present and associated with non-model hosts. I will also present examples of the applications of this pipeline in the study of small molecule biosynthesis in uncultured microbial symbionts. For instance, we recently discovered a novel verrucomicrobial symbiont of a marine tunicate, that dedicates over 25% of its genome to biosynthesis of a defensive polyketide compound. Despite ongoing genome reduction, the secondary metabolite pathway is repeated seven times in the genome, suggesting intense selection pressure for increased gene dosage and compound production.

Poster #78

Season and Geography Drive High Microbial Variability in Fungus Gardens Grown by Trachymyrmex septentrionalis Ants

Kevin M. Lee[1], *Jonathan L. Klassen*[1]
[1]University of Connecticut

The fungus-growing ant Trachymyrmex septentrionalis maintains a symbiotic relationship with a specific cultivar fungus as its primary food source. The fungus is propagated vertically and raised in fungus gardens primarily consisting of the cultivar fungus and materials provided by the ants as a food source for the fungus. T. septentrionalis are indigenous to the Eastern United States from East Texas to Long Island, New York and are seasonally active from May through October. Other tropical fungus-growing ant species host low-diversity fungus garden microbial communities, but how this compares to T. septentrionalis fungus gardens remains unknown. We hypothesized that T. septentrionalis fungus garden microbial communities vary both geographically and seasonally, based on their wide geographic range and the seasonal availability of forage materials. Our survey of T. septentrionalis fungus gardens and their associated nest soils across the Eastern United States using targeted amplicon sequencing of the 16S rRNA gene showed that T. septentrionalis fungus garden microbial communities varied greatly between colonies. This variation correlated with both geography and season. In contrast, nest soil microbial communities were less variable and did not strongly overlap with the fungus garden microbial communities. Microbial community diversity in T. septentrionalis fungus gardens is therefore likely not driven by immigration from soil but instead by the heterologous input of food substrates by the ants. These results highlight how immigration can drive variance in microbial communities that is poorly captured by single time-point sampling.

Poster #79

Biogeography and Microscale Diversity Shapes the Biosynthetic Potential of Fungus-growing Ant-associated Pseudonocardia

Bradon R. McDonald[1,2,10], Marc G. Chevrette[3], Jonathan Klassen[4], Heidi A. Horn[2], Eric J. Caldera[2], Evelyn Wendt-Pienkowski[2], Matias J. Cafaro[5], Antonio C. Ruzzini[6], Ethan B. Van Arnam[6], George M. Weinstock[7], Nicole M. Gerardo[8], Michael G. Poulsen[9], Garret Suen[2,10], Jon Clardy[6,11], Cameron R. Currie[2,10]

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The geographic and phylogenetic scale of ecologically relevant microbial diversity is still poorly understood. Here we use a model mutualism, fungus-growing ants and their defensive bacterial associate Pseudonocardia, to investigate population-level diversity of bacteria across kilometer-scale geographic space. We analyzed genetic diversity and biosynthetic potential in 46 strains isolated from ant colonies in a 20km transect near Barro Colorado Island in Panama. Despite an average pairwise core genome similarity of greater than 99%, population genomic analysis revealed several distinct bacterial populations matching host geographic distribution. We identified both genetic diversity signatures and divergent genes distinct to each lineage. We also identify natural product biosynthesis clusters specific to geographic locations. These patterns were observable despite the populations living in close proximity and evidence of ongoing genetic exchange. Our results add to the growing body of literature suggesting that significant variation in traits of interest can be found at extremely fine phylogenetic scales.

Poster #80

The Impact of Resource Identity and Diversity on Coexistence of Bacteroides Species

Firas Midani[1], *Max Villa*[1], *Lawrence David*[1]
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Members of the Bacteroides genus dominate the gut microbiota of humans on a western diet. Within a single host, these members show remarkable co-localization in the intestinal mucosa. Yet, human-associated strains also exhibit interspecies antagonism through type VI secretion systems. Patterns of coexistence of Bacteroides therefore remain incompletely understood. Each individual member however can encode utilization loci for tens of different carbon substrates. Therefore, we hypothesize that resource partitioning and metabolic cooperation allow these Bacteroides to coexist in nutrient-limited environments. To this end, we developed a high-throughput in vitro system for probing the interaction of Bacteroides using multi-color flow cytometery. We can accordingly measure community structure under single or multiple carbon substrates and infer metabolic cross-feeding rates. We are now confirming whether Bacteroides coexist on select resources and whether the complexity of a resource (mono-, oligo-, or poly-saccharide) is correlated with metabolic cross-feeding. Finally, we will test whether two- and three-species interactions between Bacteroides can predict their higher-order assemblages. Using this simplified community of closely related bacteria, our study addresses outstanding questions about drivers of microbial biodiversity and evolution of metabolic cooperation.

Poster #81

Microbes and Postharvest Processing of Industrial Hemp

Luke Moe[1], *Audrey Law*[1], *Ruth McNees*[1], *Adrienne Arnold*[1]
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Incorporation of industrial hemp (Cannabis sativa L.) into conventional crop rotations has increased dramatically in Kentucky since passage of the 2014 US "Farm Bill" legalized its cultivation, with fiber production being a key area of agricultural interest. Hemp fiber is comprised of ~75% weight/weight cellulose, with lower amounts of hemicellulose, pectin, and lignin. Hemp is a "bast fiber" plant, meaning that the harvestable fiber (phloem) is between the epidermal layer of the plant and the woody core (hurd, xylem) located in the middle of the stem. Bast fibers are separated from the hurd post-harvest in a microbial degradation process referred to as "retting", during which pectin degradation of field-cut stalks facilitates fiber separation, which is necessary for industrial decortication. Our research seeks to understand the function and ecology of microbes in the retting process. We conducted a greenhouse retting trial of three hemp varieties (Futura, Felina, HTC). Each variety underwent three treatments, in which moisture was added daily using three mechanisms meant to simulate dew (misting with sterile water), rain (heavier water treatment), and heavy rain (treated with a soil slurry). At designated time points, stalks were sampled to assess retting completion and microbial community composition using 16S gene amplicon sequencing. Additionally, culture-based analysis was used to identify and catalog bacteria and fungi with pectinase-positive phenotypes. Significant shifts were observed between varieties in microbial community structure with time and treatment, with fungi predominating in "over-retted" samples. Culture-based analysis revealed a predominance of bacterial pectinase producers of widely varying taxonomy.

Poster #82

Changes in the Rumen Microbiome During Dairy Cow Dry Off

Audrey R. Morgan[1], *Cynthia Keler*[1]
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The rumen microbiome is influenced and altered by the ruminants diet, temperature, production stage, and age. The microbes living inside the rumen are important and necessary in the animal's digestion and energy maintenance. The research was designed to evaluate the rumen microbiome and look for changes in the diversity and density as the cow's lactation dries off. Rumen microbial samples were collected from a cannulated Holstein at the Delaware Valley University Dairy. Rumen content including liquid and particle samples were collected prior to the cow drying off, the day she dried off, the fourth day dry, and finally the eighth day dry. The genomic DNA was extracted using the PowerSoil DNA isolation kit and sent off for 16S sequencing. The DNA samples were sent for next generation 16S rRNA gene sequencing. The data was organized into the prominent phylas, classes, and families. After evaluating the returned results, the following results were found. As the drying off progressed an increase in Bacteroidetes was observed along with an increase in Firmicutes. Most notably, a decrease in Proteobacteria occurred with a prominent drop on the fourth day dry. The dominant class of Proteobacteria in the rumen contents proved to be Gammaproteobacteria. A rise in Proteobacteria has previously been seen in cows coming onto lactation. While a decrease was seen when this cow transitioned from a lactating diet to dry, more research is needed to better understand the association of Proteobacteria and dairy cow lactation.

Poster #83

Differential Adaptation of Lactobacillus Crispatus and Lactobacillus Gasseri to the Intestinal and Vaginal Environments

Meichen Pan[1], Claudio Hidalgo-Cantabrana[1], Rodolphe Barrangou[1]
[1]North Carolina State University

A healthy vaginal microbiome is associated with a Lactobacillus-dominated microbial community, which lowers the pH of the vaginal cavity to pH 3.5-4.5, due to lactic acid production. Lactobacillus crispatus and Lactobacillus gasseri are commonly found in both healthy human vaginal and intestinal microbiomes. These two environments are different in nutrients, pH, and microbiota composition. We hypothesize that, in order to survive and thrive in both environments, Lactobacillus crispatus and Lactobacillus gasseri have evolved to develop distinct genotypic and phenotypic features, leading to specific adaptation to each environment. This study investigated the acid resistance of vaginal and intestinal lactobacillus isolates in the presence of lactic acid and hydrochloric acid at log and stationary phase. We formulated a simulated vaginal fluid (SVF) that models the vaginal environment to test the growth potential of various Lactobacillus strains. Finally, we investigated their ability to adhere to Caco2 cells and to protect the intestinal barrier against damage induced by TNF-alpha. Overall, vaginal isolates tend to be more resistant to acids than intestinal strains at stationary phase. Interestingly, vaginal strains outperformed intestinal strains in SVF. Intestinal Lactobacillus strains showed higher adhesion rate than vaginal Lactobacillus strains to Caco2 cells and stronger protection against TNF-alpha. Our results indicated that vaginal and intestinal Lactobacillus strains differ in their abilities to perform in each environment. These results suggested that vaginal isolates may be better probiotic candidates for the urogenital environment.

Poster #84

Isolation of Novel Seaweed-Degrading Human Gut Bacteria Reveals Extensive Genomic Exchange

Nicholas Pudlo[1], Ahmed Ali[1], Melissa Cid[2], Austin Campbell[1], Karthik Urs[1], Yao Xiao[1], Ryan Adams[1], Duña Martin[1], Thomas Schmidt[1], Jan-Hendrik Hehemann[2], Eric Martens[1]
[1]University of Michigan, [2]Max Planck Institute for Marine Biology

Metabolism of terrestrial plant polysaccharides is common in symbiotic gut bacteria, adding significant breadth to human digestive potential. Relatively recently in human history, regional populations have adopted consumption of edible seaweeds that contain unique fibers such as porphyran, agarose and carrageenan. This raises the questions of how extensively human gut bacteria have evolved to digest these fibers and the mechanisms by which these traits penetrate the microbiome. Previous analysis of two human gut Bacteroides with the ability to degrade agarose and porphyran demonstrated that the genes involved share homology with marine bacteria and in one case are present on a mobile element, suggestive of lateral gene transfer (LGT). We employed a culture-based approach on healthy human fecal samples to isolate new human gut bacterial strains that grow on seaweed polysaccharides as sole carbon sources. Genomic and transcriptomic analysis revealed a more diverse range of seaweed-degrading genetic assemblies and gene expression than previously discovered within the human gut microbiome, indicative of an extensive network of intergenomic transfers. Analysis of one new Bacteroides xylanisolvens (Bacteroidetes) and two Faecalicatena contorta (Firmicutes) isolates, which all degrade porphyran, reveal that ocean-to-gut gene exchange has transferred the same trait multiple times and involves both major phyla of gut bacteria. Our results are important for understanding the metabolic plasticity of the gut microbiome as well as identifying new functions that can be introduced or engineered to improve human health.

Poster #85

Bacteria of Genus Vibrio Colonize the Esophagus of the Cuttlefish (Sepia Officinalis)

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Micron-scale biogeography shapes the interaction of microorganisms with their environment and host. We studied micron-scale structure of the microbiome of the cuttlefish (Sepia officinalis) using a combination of imaging and massively-parallel tag sequencing. Samples from esophagus, intestine and cecum of 9 cuttlefish were collected and divided for sequencing and imaging. Tissues for imaging were fixed in 2% paraformaldehyde, embedded in methacrylate, and sectioned to 5 um. Fluorescence in situ hybridization was performed using probes targeting Bacteria, gamma proteobacteria, the family Vibrionaceae, and the most abundant species in the sequencing data; fluorophore-labeled wheat germ agglutinin was used to localize mucus and DAPI was used to detect nucleic acids. Samples were imaged on a laser-scanning confocal microscope with a spectral detector and processed by linear unmixing. DNA extraction and sequencing of the 16S rRNA gene were performed using standard protocols.
Sequencing data revealed a highly simplified community in the esophagus, dominated by a single OTU that showed 97% identity to genus Vibrio. The second most abundant OTU was identical to numerous species from the genus Photobacterium. FISH revealed a striking organization of these bacteria in the esophagus of cuttlefish, distributed in a layer lining the interior and associated with mucus. Imaging with specific probes confirmed the identity of these bacteria as Vibrionaceae. In the stomach and cecum, these bacteria are present not in a layer but in lower density throughout the lumen. We conclude that bacteria belonging to the Vibrionaceae are the major symbiont of the cuttlefish Sepia officinalis.

Poster #86

Bacteriophage Coding Regions Contribute to Xenorhabdus bovienii Bacteria Strain-level Variation

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In host-microbe symbioses, microbes can provide a fitness benefit that supports the evolutionary maintenance of the relationship. Gram-negative Xenorhabdus spp. bacteria provide benefits to Steinernema spp entomopathogenic nematodes, as together they infect, kill, and propagate within insect prey. Previous studies found that X. bovienii strains vary in their symbiotic impacts on Steinernema spp. In this study, we extended this analysis to another Steinernema species: S. affine nematodes. When grown on various X. bovienii strains, S. affine nematodes developed differently (normal, delayed, or no development), indicating that bacterial strain variation impacts S. affine-X. bovienii interactions. To investigate what drives variation among X. bovienii strains that may impact their interactions with Steinernema nematodes, we used BRIG to compare 18 X. bovienii genomes. We found that each genome is roughly 20% different from other strains. We next tested if X. bovienii genomic differences are explained by bacteriophage content. Using PHASTER, we identified bacteriophages in all X. bovienii genomes and found a total of 228 bacteriophages (85 complete, 51 questionable, 92 incomplete) with few shared between strains. While bacteriophages represent some but not all genomic differences among X. bovienii strains, our findings support the idea that bacteriophages may play a role in the evolutionary maintenance of native X. bovienii bacteria and Steinernema nematode mutualisms. Future work will investigate the functional roles of individual bacteriophages in bacterial host symbiotic activities in X. bovienii-Steinernema interactions.

Poster #87

Bacteroides Thetaiotaomicron Starch Metabolism Promotes Quercetin Degradation by Eubacterium Ramulus

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Consumption of flavonoids is associated with reduced risk of cardiovascular and neurodegenerative diseases. The gut microbiota contributes to the beneficial effects of flavonoids by transforming them into more absorbable and active metabolites. However, we cannot currently predict how diet impacts metabolism of these compounds. In this study, we evaluated bacterial metabolism of one of the most commonly consumed flavonoids, quercetin, by the quercetin degrader, Eubacterium ramulus. We tested whether degradation of quercetin is affected by the type of substrates available. We examined whether glucose, arabinogalactan, casein, fructooligosaccharides, galactomannan, gum arabic, inulin, pectin and starch influence quercetin degradation. We observed that despite no apparent growth of E. ramulus with the different substrates (except arabinogalactan and glucose), all of them except starch stimulated the metabolism of quercetin. While E. ramulus was unable to use starch to metabolize quercetin in monoculuture, we found that the starch-metabolizing bacterium B. thetaiotaomicron, which does not metabolize quercetin, stimulated degradation of the flavonoid when included in the culture. We hypothesized that this stimulation is caused by cross-feeding of products of starch breakdown of B. thetaiotaomicron. Consistent with this idea, we detected free glucose in cultures of B. thetaiotaomicron growing on starch as early as at 4 h of incubation at higher levels (>2mM) than what we observed is needed to stimulate quercetin degradation (>0.1mM). These observations indicate that distinct dietary substrates and interactions between different species can affect the degradation of flavonoids and influence the bioavailability and bioactivity of this compound, thus affecting its health.

Poster #88

Distinct Biofilm Regulatory Strategies Across the Vibrio Fischeri Evolutionary Tree

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Bacteria produce biofilm comprised of extracellular polymeric substances (EPS) for a variety of functions, including surface attachment, virulence, and host-microbe symbiosis. Vibrio fischeri, the sole microbial symbiont of the Hawaiian bobtail squid Euprymna scolopes, produces symbiosis polysaccharide (Syp) as part of its EPS, which is required for the colonization of the juvenile squid light organ across multiple strains. Studies have shown that in the squid symbiont ES114, Syp production is dependent on the hybrid histidine kinase RscS. However, bioinformatic analysis across multiple strains suggested that many squid symbionts do not encode a functional RscS. Isolates that diverged prior to the acquisition of RscS, including the Mediterranean squid symbiont SR5, have an intact syp locus that is required for colonization, revealing ancestral regulation of Syp independent of RscS. Additionally, there is a closely-related group of strains with rscS DNA that seem to have a frameshift mutation in rscS. Our functional studies demonstrate that this RscS is non-functional, yet Syp biofilm is still required. These results argue that (1) Syp EPS is broadly required for squid host colonization, (2) regulation of Syp has undergone at least 2 substantial evolutionary transitions, and (3) acquisition of RscS “hijacked” a pre-existing regulatory circuit. Ongoing work is focused on the interplay of this dynamic phosphorelay network across a set of diverse isolates.

Poster #89

New Tools and Approaches for the Study of Lactic Acid Bacteria Starter Cultures

Elena Vinay-Lara[1], Christophe Fremaux[1], Phillipe Horvath[1], Anne Millen[1], Dennis Romero[1]
[1]Du Pont

Dairy starter cultures are microorganisms intentionally added to bring the conversion of milk to a desired end-product. Starter cultures play a key role in the characteristics of the final product. Therefore, precise characterization of cultures, to the strain level, is important. Traditionally, culture-based methods have been the gold standard for physiological and application characterization. Advancements in molecular biology, genetics, sequencing and “omics” have led to more rapid and precise approaches to study and optimize starter cultures. Our group has utilized these advancements to address common problems in the dairy industry. In this poster, we will discuss targeted methods and their impact to the industry.
Improvements in DNA sequencing, facilitated defining the genetic determinants of lactococcal C2viruses for host infection. This has allowed us to create target-specific mutations to generate bacteriophage insensitive mutants (BIMs). Additionally, sequencing has aided in the elucidation of new mechanisms by which starter cultures acquired immunity against phages, such as CRISPR-Cas. This technology has enabled the natural generation of BIMs by acquisition of unique sequences into the CRISPR array, thereby conferring phage immunity. Moreover, unique CRISPR loci have been identified and used for strain identification.
Progress in molecular biology, bioinformatic tools and in the genomics field, have facilitated the utilization of native gene transfer systems such as natural competence and conjugation. A procedure based on natural transformation for marker-free targeted genetic modification was developed in Streptococcus thermophilus. Also, conjugative plasmids encoding novel phage resistance mechanisms have been identified by our group. These technologies enable natural and directed strain improvements, providing the dairy industry with industrially robust cultures.

Poster #90

Biodiversity, Geographical Distribuition and Phylogenetic Analysis of Geminivirus Associated Alphasatellites from Cotton Crop in Pakistan

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[1]University of the Punjab

The complete nucleotide sequence of alpha satellite associated with monopartite begomoviruses complex isolated from cotton from Pakistan was determined and evaluated. The present study represent new complexes of these satellite molecules present in nature. Isolation of 8 alpha satellite molecules including 6 from cotton were identified as strains of PaLCuA (Papaya leaf curl alpha satellite) with 90 % homology, one with 98 % similarity score being an isolate of CLCuMA (Cotton leaf curl Multan alpha satellite) and a new strain of GDSA (Gossypium dawanii symptomless alpha satellite) with 93 % similarity. These sequences could be a new strains of PaLCuA first time reported from cotton plants in Pakistan. As they were only recently discovered so knowledge about these satellite molecules, their structure and function is limited. Further study could reveal their impact on the host and their role in evolution, survival and diversification of begomoviruses.

Poster #91

Safety and Antibiotic Resistance of Gut Healthp Pomoting Bacillus Strains

Alexandra Smith[1], Renae Geier[1], Joshua Rehberger[1], Evan Hutchison[1], Thomas G. Rehberger[1]
[1]Arm and Hammer

As of January 2017, using medically important antibiotics as growth promoters in livestock is prohibited in the USA. One of the numerous alternatives to antibiotics are probiotics. The presence of transmissible antibiotic resistance genes should be determined before probiotic strains are commercialized. The antibiotic resistance profile of six probiotic Bacillus strains, selected for pathogen inhibition, was determined by the National Antimicrobial Resistance Monitoring System panel for Gram-positive bacteria. Screening for transmissible resistance genes was done through ResFinder, which identifies acquired antimicrobial resistance genes in sequenced bacteria. A complete genome was obtained for each strain by assembling paired-end Illumina reads together with long reads generated by the Oxford Nanopore MinION using Unicycler. No acquired antibiotic resistance genes were detected in the genomes. The antibiotics for which complete or partial resistance was determined belong to the lincosamides, oxazolidinones, phenicols and streptogramins. These are all ribosome binding antibiotics that interfere with protein synthesis. These four classes bind to the 50S ribosomal unit inhibiting protein translation. All six of these strains contain two genes that methylate the 23S rRNA at position A2503, which is necessary for proofreading of proteins. The strains are less susceptible to these classes of antibiotics as methylation at A2503 impedes binding of the antibiotics at the active site. Strain safety was further confirmed through the absence of B. cereus toxin genes, emetic toxin; hemolysin BL, nonhemolytic enterotoxin and cytotoxin K. The genome sequence of these strains confirmed their uniqueness, safety and potential to improve the intestinal health of livestock.

Poster #92

Microbiome Hub - Enabling a Biological Revolution at UW

Sailendharan Sudakaran[1], Michael Sussman[2], Jo Handelsman[1]
[1]Wisconsin Institute for Discovery, [2]UW Biotechnology Center

The Microbiome Hub is a joint venture between the UW Biotechnology Center (UWBC) and the Wisconsin Institute for Discovery (WID), making use of current prowess at UWBC in sequencing and bioinformatics and WID's strength in data analysis, storage, and management. Microbiome research is a rapidly growing discipline encompassing a broad range of systems including the human body, animals, soil, plants, lakes, food, wastewater treatment systems, and test tubes. Continuous advancements in sequencing technologies have enabled researchers in utilizing various -omics methodology to gain a better insight into the complex relationships between organisms, genes, and the environment and in turn this has led to the generation of vast amounts of data. Therefore, there is a need to ensure that adequate expertise and infrastructure is in place to meet the challenge of storing, analyzing, and distributing the data as well as a robust and reliable framework for interpreting it. The Microbiome Hub will serve as a campus-wide resource to address these challenges at UW-Madison. It will support researchers interested in tackling a broad spectrum of microbiome studies, investigating fundamental questions, developing new techniques, and understanding the impact of microbiome across different fields. The Hub will provide a broad spectrum of services such as technical expertise, consultation, coordinating collaborative research, data management, and conducting outreach activities. Overall, by harnessing the significant research already taking place at UW, world class expertise, and facilities we aim to develop The Microbiome Hub as a means to facilitate and broaden engagement in microbiome research at UW.

Poster #93

Fructose metabolism and Short-Chain Fatty Acids Drive Intestinal Bacteriophage Production by the Gut Symbiont Lactobacillus Reuteri

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The mammalian intestinal tract is home to one of the most densely populated bacterial communities. Most bacteria in this community are lysogens, which means they contain prophages in their genomes. While most virus-like particles (VLPs) in the gut are derived from lysogens, we have limited understanding what the triggers and dynamics are of prophage activation. By combining genetics and metabolic profiling, we showed that fructose-metabolism increases L. reuteri acetic acid production via the Pta-Ack pathway, which produced bacteriophages in a RecA-dependent manner. These data were corroborated in a mouse dietary crossover study, which also revealed that intestinal acetic acid was increased by L. reuteri acetogenesis. We further demonstrated that, aside from acetogenesis, exposure to short-chain fatty acids promote L. reuteri bacteriophage production in a dose-dependent manner. A dietary crossover study with fructooligosaccharides, which cannot be metabolized by L. reuteri, increased intestinal acetic acid and subsequent bacteriophage production. We conclude that diet-microbe interactions and environmental short-chain fatty acids are important drivers of Lactobacillus bacteriophage production in the gastrointestinal tract.

Poster #94

Transient Osmotic Perturbation Causes Long-Term Alteration to the Gut Microbiota

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Osmotic diarrhea is a prevalent condition in humans caused by food intolerance, malabsorption, and widespread laxative use. We assessed the resilience of the gut ecosystem to osmotic perturbation at multiple length and time scales using mice as model hosts. Osmotic stress caused reproducible extinction of highly abundant taxa and expansion of less prevalent members in human and mouse microbiotas. Quantitative imaging revealed decimation of the mucus barrier during osmotic perturbation, followed by recovery. The immune system exhibited temporary changes in cytokine levels and a lasting IgG response against commensal bacteria. Increased osmolality prevented growth of commensal strains in vitro, revealing one mechanism contributing to extinction. Environmental availability of microbiota members mitigated extinction events, demonstrating how species reintroduction can impact community resilience. Our findings demonstrate that even mild osmotic diarrhea can cause lasting changes to the microbiota and host, and lay the foundation for interventions that increase system-wide resilience.

Poster #95

Genomic and Metabolic Capabilities of Five New Polysaccharolytic Dysgonomonas Isolated from P. Americana Guts.

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Insect digestive tracts can harbor tremendous bacterial diversity, with uncharacterized taxa awaiting discovery. While metagenomic studies of the American cockroach Periplaneta americana gut microbial community exhibits a high diversity of Bacteroidetes phylum members, relatively few, if any, have been cultured. Here we described five new Dysgonomonas species cultured and isolated from P. americana digestive tracts. Phylogenetic analysis using either 16S rRNA or a set of 31 single copy marker genes resulted in cladogenesis of P. americana Dysgonomonas isolates amongst previously described species. Additionally, average amino acid identity (AAI) values (68-86%) in comparisons with all available Dysgonomonas genomes support the conclusion that the P. americana isolates are new species within Dysgonomonas genus. Several polysaccharide utilization pathways, including those involved in starch, pectin assimilation as well as soluble carboxymethyl(CM)-cellulose and insoluble cellulose degradation, were detected in genomes of these new species using the Carbohydrate-Active enZymes Database (CAZy). Subsequent bioassays and enzymological tests revealed that, as predicted by the genomes, all of the strains were able to produce reducing sugars with starch and pectin, and a subset of these isolates were capable of reducing cellulose. Plant-based carbohydrates are a significant component of cockroach and related termite diets, and thus it is not unexpected that P. americana would harbor species capable of utilizing a wide array of complex carbohydrates.

Poster #96

Reduced Firmicutes in the Gut of the Mexican Cavefish and Autism Patients

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The gut biota is regulated by gut morphology, gut homeostasis and diet. Similarly, the gut biota is critical in regulating intestinal development, gut homeostasis, and neural function. Astyanax mexicanus is a species composed of cave-dwelling morphs and their ancestral type, surface-dwelling morphs. Recently, due to the similarity in behaviors and genetics, cavefish were presented as a new alternative for animal models of autism. The surface-dwelling ancestor of cavefish was first trapped in caves millions of years ago, and evolved under perpetual darkness with limited food sources. Their diet, therefore, was shifted from variety of small fishes and aquatic crustaceans to rotten organic matter, bat guano, and soil arthropods. Despite the diversification, cavefish and surface fish remain interfertile, allowing us to apply genetic approaches to investigate gut and gut microbiota evolution. Here, we perform genetic analyses on gut morphology, gut microbiota, and fatty-acid composition by using cave fish, surface fish and their F1 hybrids. One month after the larval stage, the three morphs were fed three diets: nutrient-poor spirulina algae, nutrient-rich brine shrimp, and bat guano. Cave fish gut morphology showed significantly less pyloric caeca and a wider gut diameter than surface fish. Analysis by 16S rRNA gene sequencing of the gut microbiota revealed surface fish and cave fish have significantly different gut microbiota, where firmicute species are largely reduced in the cavefish. The substantial reduction of firmicutes was also reported in feces of autism patients. Surprisingly, the gut microbiota is stable in each morph of A. mexicanus regardless of diet, suggesting a major involvement of host genetics. We are currently investigating the relationship between behavior, gut morphology, gut microbiota and fatty-acid composition—the major metabolite that firmicutes provide to the host.

Poster #97

Engineered Production and Sensing of Butyrate in Probiotic Bacteria

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Short-chain fatty acids (SCFAs), especially butyric acid, have many roles in the human gut, affecting immunomodulation, cell differentiation and apoptosis. In addition, butyrate is the preferred carbon source for colon cells. Butyrate is normally produced in the colon by butyrogenic anaerobic bacteria that respond to dietary fiber or other prebiotics. However, diets rich in low-fiber, highly processed foods can result in low gut butyrate levels, which can lead to inflammation and other bowel diseases. In these disease states, populations of butyrogenic bacteria are also decreased.
Using a CRISPR-Cas based genome engineering method, we have engineered a model probiotic organism, E. coli Nissle 1917, to produce butyrate in both aerobic and anaerobic culture conditions. Additionally, to optimize butyrate concentration, we have developed a synthetic circuit that responds to intracellular butyrate concentrations, allowing for feedback control of butyrate production. We also test in an animal model by feeding of butyrate-producing probiotics to C. elegans. Nematodes receiving engineered probiotics behaved similarly to controls and had slightly improved brood size. Taken in total, this work provides a method of controlled butyrate delivery to the gut and is an important step towards engineered SCFA production in situ in the gut.

Poster #98

Gene Discovery of Bile Acid 12β-Hydroxysteroid Dehydrogenase Through Induction and RNA-Seq Analysis in Clostridium Paraputrificum AGR2156

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Bile acids are detergent molecules synthesized from cholesterol in the liver, which function to solubilize dietary lipids. Bile acid 7α-dehydroxylating gut bacteria form the toxic secondary bile acids deoxycholic acid and lithocholic acid from host-derived cholic acid and chenodeoxycholic acid, respectively. Gut bacteria encode numerous enzymatic activities that biotransform both the host and secondary bile acids. Recently, we demonstrated that bile acid 7α-dehydroxylating Clostridium scindens, Clostridium hylemonae, and Clostridium hiranonis express 12α-hydroxysteroid dehydrogenase (HSDH) activity, which catalyzes the reversible oxidation/reduction of the 12α-hydroxyl group. Bile acids oxidized at the C-12 position can also be epimerized by 12β-HSDH activity into the β configuration. 12β-HSDH has been shown to be inducible in Clostridium paraputrificum AGR2156 previously, however no genes had been found encoding this activity. In order to locate this gene, Clostridium paraputrificum was induced with 12-oxolithocholic acid and RNA-Seq analysis was performed. Transcriptomes of induced versus uninduced cultures were compared and genes annotated as short chain or medium chain dehydrogenase/reductase family were screened for 2 to 3-fold induction. One gene in the expected family was found to be upregulated and likely encodes the putative 12β-HSDH activity. Further characterization of the purified recombinant 12β-HSDH will be performed to demonstrate the identity of the gene. Future engineering of a probiotic organism, such as Lactobacillus, to encode 12β-HSDH may be important for shifting metabolism away from deoxycholic acid, a bile acid implicated in cancers of the gastrointestinal tract.

Poster #99

Manipulation of the Bile Acid Pool with Lactobacillus Bile Salt Hydrolases for Rational Design of the Gut Microbiota

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The host and gut microbiota-modified bile acid pool in the gastrointestinal tract (GIT) is linked to many human diseases. Gut microbes that encode bile salt hydrolase (BSH) enzymes deconjugate or cleave the glycine and taurine from conjugated bile acids to yield unconjugated bile acids. This reaction may benefit the host and gut microbiota by detoxifying bile acids and providing nutrients, which can lead to alterations in the gut microbiota and host physiology. Many probiotic Lactobacillus strains encode multiple non-identical BSH homologs, however their function and activity in vitro and in vivo remains unclear. A phylogenetic analysis of Lactobacillus BSH occurrence across 170 sequenced species demonstrated that these enzymes are associated with vertebrate-adapted niches. To understand how BSHs have adapted Lactobacillus species to the vertebrae GIT, BSH activity was screened using growth, plate precipitation, and enzyme assays in the presence of GIT bile acids, revealing BSH substrate specificities and contributions to fitness in vitro. In vivo effects on microbiota-bile dynamics were first investigated in germ free mice monocolonized with WT Lactobacillus acidophilus and isogenic bsh mutants, L. acidophilus □bshA□bshB. While bile acid metabolomics of the GIT did not capture differences between the strains, significant disparities in colonization levels suggest that BSHs provide a competitive advantage in the gut. Biochemical and genetic characterization of bile acid-altering probiotics in vitro and in vivo will allow us to rationally alter the bile acid composition in the gut, which will be used to manipulate the structure of the gut microbiota in a targeted manner.

Poster #100

Saccharomyces Cerevisiae as a Mycotoxin Remediator

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One of the major challenges in food sustainability is the spoilage and contamination of crops from secondary metabolites, mycotoxins, produced by fungi. Mycotoxins pose a significant danger to the health and performance of farm livestock and cause a variety of different symptoms including decreased feed intake, poor reproductive performance, reduced milk production and even death. The aim of this study was to investigate whether Saccharomyces cerevisiae could degrade mycotoxins.

S. cerevisiae (R404) was inoculated in 50ml of nutrient broth with or without 1µg/ml Zearalenone, and incubated at 37°C, at 200rpm for 48 hours. Samples were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 24, and 48 hours for mycotoxin analysis. Samples were analyzed using a Waters LC/MS for the presence of Zearalenone and its metabolites.

S. cerevisiae was able to degrade Zearalenone (ZON) to its metabolites α- and β-Zearalenol (ZOL). The presence of β-ZOL was detected after 1 hour, while the more toxic α-ZOL was detected after 3 hours. The less toxic metabolite β-ZOL was detected at a higher concentration than the more toxic α- ZOL.

S. cerevisiae is known to have a probiotic effect in animals and humans and helps to maintain the integrity of the intestinal epithelial lining. In this study, we have shown that S. cerevisiae primarily degrades ZON to its less toxic daughter metabolite β-ZOL. This suggests that S. cerevisiae can be used as a probiotic and mycotoxin remediator in the treatment of animals contaminated with ZON.

Poster #101

Mining and Expressing Biosynthetic Gene Clusters from Soil Metagenomes

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Soil microorganisms encode vast reservoirs of bioactive natural products; however, the majority are recalcitrant to cultivation and the massive number and complexity of soil microbes makes metagenomic sequence assembly of their pathways very difficult. A soil metagenomic library containing 19,200 clones with 110 Kb inserts (~1M genes/2 Gbp) was constructed in a broad host range shuttle BAC vector. Pathway-containing clones from this library were identified using a 3D pooling method in which plates, rows and columns were separately combined and sequenced. Phylogenetic analysis of the 16S genes indicates a large and diverse assembly of bacteria. Contigs were assembled from each pool and bioinformatically screened for secondary metabolite gene clusters using antiSMASH4.0. 474 clones containing a PKS and/or non-ribosomal peptide synthetase pathway among 1,516 total biosynthetic pathways were identified. These pathways are very divergent from known clusters, with the %G+C content varying from 34 to 79% and the nearest BLAST hit of keto-synthase domains ranging from 19 to 95% amino acid identity. New clades of keto-synthase domains were also found. These pathway-containing BAC clones were conjugally transferred into Streptomyces coelicolor M1154 and screened for the synthesis of antibacterial compounds against methicillin-resistant Staphylococcus aureus, Acinetobacter baumannii, Candida albicans, Escherichia coli, the fungal pathogen Cryptococcus neoformans. 69 S. coelicolor clones expressed antifungal and/or antibacterial activity, an antibiosis hit rate of ~15%. These results indicate that highly novel biosynthetic clusters can be cloned intact from complex metagenomes and heterologously expressed to produce secondary metabolites, thus expanding our available resources for natural product discovery.

Poster #102

The Effect of a Bacillus Probiotic on Herd Health, Milk Production and Clostridium Populations on a Dairy Farm in Wisconsin

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Clostridium species have been linked to enteric diseases in ruminants for example, haemorrhagic bowel syndrome (HBS) is a disease often correlated with Clostridium perfringens Type A. A probiotic, comprising three Bacillus strains selected to inhibit C. perfringens strains present in Wisconsin dairy cattle, was developed and incorporated into the total mixed ration at a dose of 2.0E+09 CFU/head/day. Herd health, milk production, fecal clostridia levels and diversity in dairy cows was monitored over 144 days of probiotic treatment and again 97 days post-treatment. Herd health was again monitored for another 144 days of treatment. Digestive deaths decreased from five in the three months pre-treatment, to a single death during the first 144 days on treatment. During the 97 days post-treatment six digestive deaths were reported and zero for the second treatment period. Productivity improved while the cows were fed the probiotic as energy corrected milk (ECM) increased on average by 1.8 kg/day/cow and milk fat by 0.3%. During the first 30 days on probiotic there was a significant reduction in total clostridia. When the probiotic was discontinued the number of animals with fecal clostridial levels over 10 000 CFU/g increased to 10% from a range of 2-6% at other time points. The proportion of the C. beijerinckii group decreased during treatment and their proportion increased again when the probiotic was discontinued. In conclusion, the blend of Bacillus strains in the probiotic product improved herd health by reducing the number of digestive deaths and increasing milk production, while impacting clostridial species.

Poster #103

Developing Lactobacillus Reuteri as a Model to Study the Ecological Role of Temperate Bacteriophages in a Gut Symbiont

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Most bacteria, including those inhabiting the mammalian intestinal tract, contain prophages that are embedded in the bacterial genome. However, little is known to what extent prophages impact the ecological fitness of a gut microbe. Our work provides insight in the distribution of biologically active prophages in the gut symbiont species Lactobacillus reuteri. We used a genetic approach to develop the human-derived strain L. reuteri 6475 as a model to study its prophages. We generated single prophage deletions, a double prophage deletion, and we restored the prophages in the double prophage deletion strain. To quantify phage production, we engineered L. reuteri to yield a lytic host. We demonstrated that, in vitro, L. reuteri continuously releases bacteriophages. The wild-type and complemented strain produced similar levels of PFUs, while single prophage deletion strains produced up to 732-fold more PFUs, suggesting an interplay between the prophages that contributes to phage production. We also identified different triggers, including carbon source utilization, that contribute to L. reuteri 6475 bacteriophage production. To understand the ecological role of these prophages, two-strain competition assays were performed in a previous germ-free mouse model, which revealed that bacteriophages provide L. reuteri 6475 with a competitive advantage. Fundamental knowledge of the interplay between bacteria and their viruses is critical to lay a foundation for the development of rational strategies to modulate the microbiota.

Poster #104

Gel-Free Targeted Cloning of Large Biosynthetic Gene Clusters

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Microbes secrete numerous small molecules influencing interactions with host and other environmental microbiota. DNA sequencing often reveals dozens of large biosynthetic gene clusters (BGC) per genome that could participate in interspecies interactions. Corelating the biological activity of a small molecule with its cognate BGC can be a slow, expensive process. Isolating a physical DNA clone for expression, refactoring, and other analyses can take months to complete, and gene synthesis is expensive and can be stymied by GC-rich and/or repetitive sequence. Here we describe a rapid technique to directly clone large BGCs from genomic DNA without using gels or agarose plugs. Using CRISPR-Cas9 on intact genomic DNA, we targeted cuts to regions flanking BGCs of interest. A linearized Streptomyces BAC shuttle vector with overlaps matching the BGC cut sites were prepared using PCR, and the vector and restricted DNA were assembled and transformed. We tested three BGCs from Streptomyces coelicolor of sizes of 21, 34, and 59 kb, and achieved successful cloning rates of 100% (8/8), 100% (14/14), and 20% (4/20) respectively. We have also cloned four additional clusters of 45, 58, 71, and 73 kb from three different marine actinomycetes. Starting with genomic isolation from a cell pellet, this technique takes ~5 days to generate a BGC shuttle vector, which is directly ready for heterologous expression studies. These results indicate that any sequenced biosynthetic gene cluster can be cloned intact from complex genomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources for natural product discovery.

Poster #105

Metabolic Division of Labor in Microbial Systems

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Metabolic pathways are often engineered in single microbial populations. However, the introduction of heterologous circuits into the host can create a substantial metabolic burden that limits the overall productivity of the system. This limitation could be overcome by metabolic division of labor (DOL), whereby distinct populations perform different steps in a metabolic pathway, reducing the burden each population will experience. While conceptually appealing, the conditions when DOL is advantageous have not been rigorously established. Here, we have analyzed 24 common architectures of metabolic pathways in which DOL can be implemented. Our analysis reveals general criteria defining the conditions that favor DOL, accounting for the burden or benefit of the pathway activity on the host populations as well as the transport and turnover of enzymes and intermediate metabolites. These criteria can help guide engineering of metabolic pathways and have implications for understanding evolution of natural microbial communities.

Poster #106

D-Ala-D-Ala Ligase as a Broad-Host-Range Counterselection Marker in Lactic Acid Bacteria

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Counterselection markers have proven invaluable to generate unmarked genome edits in bacteria, eukaryotes, and archaea. In Lactobacillus, with more than 200 species the largest genus in the group of lactic acid bacteria, the application of genetic tools is restricted to select species or strains. Here, we demonstrated that heterologous expression of dipeptide ligase in vancomycin-resistant lactobacilli increases their sensitivity to vancomycin. By quantitative PCR we demonstrated that the vancomycin sensitivity is correlated to the level of Ddl dipeptide ligase transcript. We exploited the dipeptide ligase as a counterselection marker in eight Lactobacillus spp, including in species for which no counterselection marker was previously available. We developed a liquid-based approach that, combined with our counterselection system, identifies recombinant genotypes in only five days, which is less than half the time compared to conventional approaches. Based on our phylogenetic analysis of Ddl we predict that 140 out of 173 Lactobacillus species are intrinsically resistant to vancomycin, which suggests our system is broadly applicable in Lactobacillus. We also identified 5 other lactic acid bacteria genera in which our counterselection system can be applied. Key features of our system are that no synthetic medium and/or genome editing is required prior to its application, and it represents the first ‘plug and play’ counterselection system in lactic acid bacteria.

Poster #107

Cholecystectomy Alters Cecal and Fecal Microbiota in a Mouse-Model Consuming a High-Fat Diet: A Pilot Study

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Cholecystectomy (XGB), removal of the gallbladder, is the most common abdominal surgery performed in the USA. Individuals with XGB have an increased prevalence of metabolic syndrome, gastrointestinal symptoms, chronic bile reflux, and small intestinal bacterial overgrowth. These symptoms may be related to disruption of microbiota homeostasis given the antimicrobial effects of bile acids (BA) on bile-intolerant taxa. Additionally, some bacteria can alter BA diversity through a variety of metabolic reactions. The objective of this pilot study was to identify longitudinal effects of XGB on cecal and fecal microbiota and BA.

Eight-wk-old female C57BL/6J mice (N=48) were fed a high-fat (45% kcal), low-sucrose diet for 24 wk. All mice had XGB at baseline, and mice were sacrificed every 6 wk for cecal digesta collection. Fresh fecal samples were collected at wk 0, 2, 4, 6, 12, 18, and 24.

Following XGB, a shift in unweighted UniFrac PCoA was observed in both cecal and fecal microbial communities. In fecal samples, XGB resulted in an increase in relative abundance of Firmicutes and a decrease in Bacteroidetes. Relative abundance of fecal Akkermansia muciniphila and Coprobacillus decreased while fecal Enterococcus increased. In cecal digesta, relative abundance of Actinobacteria, namely Coriobacteriaceae, and an unclassified genus of Clostridiaceae, both metabolizers of cholesterol-derived compounds, increased ~8-fold at wk 6 compared to baseline. Relative abundance of Christensenellaceae was non-detectable at baseline, but was present in almost all fecal and cecal samples post-XGB.

These findings suggest a considerable impact of XGB on the gastrointestinal microbiota community, warranting larger future studies.

Poster #108

Prebiotics and the Bifidogenic Effect: Assessing Variability and Host Response

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Background: Dietary supplementation with prebiotics promotes growth of gut beneficial microorganisms (including bifidobacteria and lactobacilli), resulting in a more resilient and balanced microbiota. However, the individual response to prebiotics in regard to increase in Bifidobacterium abundance (bifidogenic response) is often variable. Since aging has been correlated with gut microbial imbalances (dysbiosis) and decreased abundance of Bifidobacterium, we evaluated impact of β-(1,4)-galactooligosaccharides (GOS) on a mouse model of aging, and in vitro in colonoids injected with stools from bifidogenic responders and non-responders.
Methods: High-throughput qPCR, 16S rRNA amplicon sequencing, and RTqPCR were used to assess GOS-induced gut microbiome changes and host response in mice and organoids grown from colon crypts.
Results: Only 33% of aged mice showed an overall increase in Bifidobacterium in response to GOS while 75% of colonoids grown from a single mouse but injected with stools from different mice showed a bifidogenic response, suggesting that host genetics impacted GOS response. Aging animals exhibited increased stem cell proliferation (33% more proliferating cells/crypt) compared to young mice. However, bifidogenic responder mice had a 20% reduction in stem cell proliferation in colon compared to non-responders. In all aging animals, regardless of responder or non-responder status, GOS feeding induced expression of mucin-2 and tumor suppressor p53 genes.
Conclusions: Our study indicates that the bifidogenic response to GOS in aging could be impacted not only by the microbial community, but also by the host genetic background. Further studies will identify mechanisms by which host genes may modulate composition of the gut microbiota.

Poster #109

Viral Diversity on Human Skin Revealed by a Metagenomic Analysis of the DOCK8-Deficient Microbiome

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From a microbial perspective, the skin surface is a habitat that can be colonized as well as a barrier to dissemination and infection. The bacteria and fungi that colonize the skin have been characterized in large cohorts of healthy volunteers (e.g., HMP) and in the context of disease (e.g., atopic dermatitis). In contrast, technical barriers, including lack of universal marker genes and low relative-abundance, have limited studies of the virome to specific taxa or enriched preparations of virus-like particles. To explore skin virome diversity, we recruited a cohort of 27 patients with a rare primary immune deficiency with underlying mutations in the Dedicator of cytokinesis 8 (DOCK8) gene. DOCK8-deficient patients have many clinical manifestations of viral involvement, including extensive warts, rashes and infections. Using shotgun metagenomics to characterize 200 skin samples, we find that DOCK8-deficient patients exhibit a profound increase in the relative abundance of the viral component of the microbiome, compared to healthy volunteers. The composition of the virome, deciphered with both read based and de novo assembly, includes molluscum contagiosum virus, human papillomaviruses, and polyomaviruses. While members of these viral families are known to cause disease, they are also part of the microbiome of healthy individuals and reflect the natural diversity of the skin virome. We consider these results in the context of the question, 'Are there commensal human viruses?' If so, what benefit might they provide to the human host?

Poster #110

Adaptive Host-Microbial Interactions Mediated by sIgA at the Sinonsasal Mucosa: Implications for Chronic Rhinosinusitis

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Chronic rhinosinusitis (CRS) is a complex disease that results in significant expenditures; CRS is responsible for 5% of total healthcare costs in the US. Recent studies characterizing the CRS sinonasal microbiome demonstrate reduced bacterial diversity, but identifying specific taxa associated with disease is complicated by high interpersonal variation in taxonomic composition. Identifying taxa that are targeted by host immunity may yield insights into disease-driving microbiota. Secretory IgA (sIgA) is a major component of the airway mucosal immune system. Studies in the gut have demonstrated that commensal bacterial shape the production of sIgA, thus, we hypothesize that sIgA will target potential drivers of inflammation. We have optimized sIgA pulldown of microbiota from the murine sinonasal and GI tract using Magnetic Activated Cell Sorting (n=5 mice/group). sIgA was quantified in the pre-sort, sIgA(-), and sIgA(+) fractions using ELISA. The V4 region of the 16S rRNA gene was amplified from each fraction, sequenced on the Illumina MiSeq, and analyzed using QIIME2. The average pre-sort sIgA concentration was 5.6 ng/mL (fecal) and 1.7 ng/mL (sinonasal). As expected, the concentration of IgA was significantly higher in the sIgA(+) fraction compared to sIgA(-) fraction (paired t-test p=0.046). The 16S rRNA gene was successfully amplified in all specimens. Taxa enriched in the sIgA(+) fraction included Akkermansia and Erysipelotrichaceae; those enriched in the sIgA(-) fraction included Clostridiales. These studies demonstrate optimization of sIgA pulldown from multiple specimen types, including low burden samples. Understanding host targeting of specific mucosal microbiota will be critical to distinguishing disease-driving taxa in CRS.

Poster #111

Comparison of the Gut Microbiota of Mule Deer Before and After the Winter Season.

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Mule deer rely on fat storage accumulated prior to the winter season as an energy source during the winter months when other food sources are sparse. Since animal-associated microbes ('microbiota') play a significant role in nutrient metabolism of their hosts, we predicted that the deer microbiome might influence fat deposition in deer populations during seasonal changes. To test this hypothesis we performed a 16S rRNA marker gene survey of fecal samples from two deer populations in the western United States before and after onset of winter. Analysis of QIIME2-curated sequence data by PERMANOVA revealed significant differences in the deer microbiota with herd type (location). We also found that measures of deer fat levels (MaxFat) were a near-significant covariate in statistical models assessing differences in beta-diversity of animals with geographic location and season. To identify specific bacterial communities that were associated with fat deposition, we calculated correlations between OTU read counts and various measures of animal nutrition or health. We identified a positive correlation between the bacterial family Ruminoccocaeceae and deer loin thickness, but only in a sub-population of deer from a geographic location that had relatively low health scores throughout the winter season. These analyses confirm that variation in the microbiota is associated with at least one measure of animal health that changes with season, and that this effect is geography dependent. The data also suggest the possibility that the microbiome plays a role in seasonal variation in mule deer health and fat storage.

Poster #112

The Host-Shield Effect: Host Interactions Protect the Microbiome from the Effects of alcohol in Drosophila Melanogaster

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Gut microbes play an important role in the balance between host health and disease. The fruit fly, Drosophila melanogaster, is an established model to study the animal microbiome, and because its natural habitat is fermenting fruit, D.melanogaster is also a model to study alcohol. Ongoing work in our laboratory has shown that the microbiome affects fly health in the presence of alcohol. Host impacts on the microbiome are thought to mainly involve immune defenses and the provision of food supply. However, here we show that the host protects the microbiome from toxic alcohol levels. We studied how alcohol diets change intestinal bacterial composition in D.melanogaster, and we found that Acetobacter sp. were eliminated at alcohol concentrations higher than 10%, while Lactobacillus sp. persisted inside flies feeding on 15% alcohol diets. In in vitro studies, we found that Acetobacter sp. has a lower alcohol resistance than Lactobacillus sp. Interestingly, these bacteria showed no growth at alcohol concentrations higher than 5% and 10%, respectively. Finally, we compared bacterial survival in the fruit fly to the diet, and we found no bacterial survival in the 15% alcohol diet. Conversely, bacterial abundance inside the fruit fly remains constant even at 15% alcohol diet. These results suggest a host-shield effect, in which alcohol acts as an antimicrobial agent in the diet, but once the bacteria enters the fruit fly's gut, the host environment promotes bacterial survival. The mechanisms behind the host-shield effect represent an opportunity to research the nature of host-microbial interactions.

Poster #113

Structured Analysis of Dietary Patterns Reveals Daily Diet-Microbiome Associations

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Diet is a strong driver of microbiome variation, but methods for diet-microbiome analysis are limited. Both daily dietary intake and microbiome variation are highly individualized, making analysis challenging. Traditional nutrient analysis and food frequency questionnaires fail to capture the complexity and variability of daily dietary intake necessary to understand diet-driven microbiome variation. Here we introduce FoodTree, a phenetic, hierarchical tree of foods, that can reduce the high dimensionality of dietary data to recover complex dietary patterns and diversity metrics from 24-hour dietary recall data.

We applied the FoodTree method to study the relationship between diet and microbiome composition in 34 individuals who provided dense diet-microbiome data for 2.5 weeks (526 fecal samples and 558 dietary records). We found that microbiome stability (centered log-ratio transformed Euclidean β-diversity) correlated with increased dietary whole-tree α-diversity ($r=0.58$, $p=0.001$). Interestingly, FoodTree-based UniFrac distances between subjects' diet profiles, but not nutrient profiles (i.e. macro and micronutrients), corresponded with microbiome composition (Procrustes analysis; $p<0.001$ for diet profiles; $p=0.3$ for nutrient profiles). Furthermore, using FoodTree we defined five tree-based dietary patterns and found that these patterns are correlated with bacterial taxa.

Our work demonstrates that tree-based analysis of dietary data can be used to study complex diet-microbiome relationships and provides a new framework that researchers can use to both control for dietary intake as a confounder and identify meaningful associations between personalized dietary intake and microbiome variation.

Poster #114

Whole Genome Transcriptional Analysis of Sensitivity and Adaptation of Different Ruminal Bacteria to the Ionophore Antibiotic Monensin

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Monensin, an ionophore antibiotic, is widely used in ruminant animal diets to improve production efficiency. The increasing feed efficiency results from manipulating the ruminal microbial population by selectively suppressing growth of G+ bacteria while G- bacteria are insensitive to monensin. However, little is known about its cellular and molecular mode of action. To analyze the sensitivity and the mechanism of adaptation to monensin, the growth inhibitory effect of monensin was first investigated in representative ruminal strains of G- (Prevotella bryantii $B_1$4 and Fibrobacter succinogenes S85), G+ (Ruminococcus albus 7 and Streptococcus bovis JB1), and G- intermediate (Selenomonas ruminantium HD4, Megasphaera elsdenii T81 and Butyrivibrio fibrisolvens D1). After each culture was subjected to growth in the presence of increasing monensin concentrations, gene expression profiles of monensin-adapted isolates were compared with those of naïve isolates. Interestingly, although the susceptibility of the naïve isolates to monensin was in accordance with their cell wall types, their adaptability to monensin varied. Of the bacterial strains tested, S. bovis JB1 displayed outstanding adaptability enabling growth at more than 10xMIC, the concentration of which was comparable to those of the resistant isolates. The transcriptional profile of monensin-adapted S. bovis JB1 overexpressed genes encoding modification of cell wall proteins and transmembrane secretion effectors as well as energy metabolism and the acid stress responses, suggesting putative protective mechanisms against monensin. These results describe, for the first time, bioenergetic targets within gut bacteria and allow understanding of population based ecological studies and long-term efficacy of adding ionophores to animal diets.

Poster #115

Hormonal Effects on the Fecal Microbiota of Prairie Voles

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Circulating hormones have intricate effects on human physiology; however, our knowledge of how they affect the gastrointestinal (GI) microbiota is limited. The gut microbiota composition can have a profound impact in prevention or promotion of GI disorders such as inflammatory bowel diseases. To investigate the role of reproductive hormones in modulating the microbiota and associated GI disease states, we used prairie voles to examine how changes in circulating estrogen affect the GI microbiota. Prairie voles are a model in which to study social behavior and the integration of the microbiome in the gut-brain-behavior axis. Since prairie vole females lack an estrous cycle, we were able to transiently raise hormone levels from a background of low but constant circulating estrogen via implanted mini-osmotic pumps. Uterine weight was measured to assess the physiological efficacy of the hormone administration. The composition of the fecal microbiota from estrogen-treated and control voles was examined using 16S rRNA gene-based sequencing. Sequence reads were classified taxonomically and alpha- and beta-diversities of the microbiota were analyzed. While uterine weight of estrogen-treated animals peaked around day 10 of the treatment, changes in the fecal microbiota were detected throughout the time course. Specific groups of bacteria were affected by the estrogen treatment. We will report on our findings of estrogen modification of the bacterial communities. This study provides insights on the interaction of estrogen with the GI microbiota and offers potential indications for therapeutic/preventative interventions when hormone levels, e.g., postmenopausal low estrogen, are correlated with intestinal disorders.

Poster #116

Gut Microbiota Over Gestation in an Oviparous Lizard: Do the Changes Mirror Findings in Humans?

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In humans, pregnancy significantly alters the structure of the gut microbiome. Specifically, gut communities exhibit decreases in alpha diversity and increases in beta diversity (higher inter-individual variability) over the course of pregnancy. Here, we investigated whether similar trends occur in an evolutionarily distant and oviparous host. We collected repeated fecal samples from gravid Eastern fence lizards (Sceloporus undulatus) and recorded the date of egg laying to determine the time of gestation for each sample. Additionally, fecal samples were collected from non-gestating females. Bacterial inventories were conducted by sequencing the 16S rRNA gene and community profiles were determined using QIIME2. We found that over the course of gestation, lizard gut microbial communities exhibited decreases in alpha diversity (Faith's phylogenetic diversity and number of observed OTUs). Additionally, inter-individual variation was higher towards the end of gestation. The relative abundance of the candidate phylum Melainabacteria was lower in lizards towards late-gestation. The presence of Melainabacteria was detected in 60% of samples from non-gestating individuals, 70% of samples from early-gestation individuals, but less than 40% of samples from late-gestation individuals. Overall, our results are similar to previous results observed in humans, suggesting similar interactions between gestation and the gut microbiome in these disparate lineages. We hypothesize that hormonal, immunological, or metabolic changes associated with gestation may underlie these community shifts. Further studies should investigate the functional effects of these altered communities over gestation in varied animal groups, including humans.

Poster #117

Gut Microbiome Alterations Upon Nicotinamide Riboside Supplementation

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Nicotinamide riboside (NR), a vitamin B3 derivative and NAD+ precursor, is a dietary supplement with the potential to promote weight loss and improve metabolic disorders such as obesity and diabetes. Preliminary data show that mice fed a high fat diet (HFD) resist weight gain when supplemented with NR. The gut microbiome aids the metabolism of dietary compounds and xenobiotics, leading to functional consequences on the host. Thus, we hypothesized that NR-treated mice will exhibit alterations in the gut microbiota, which contribute to reduced weight gain and improved host metabolism. To study this, mice were fed a HFD supplemented with NR or vehicle. Gut microbiome changes and total energy flux were assessed over time to investigate the mechanisms of weight gain. NR-treated mice exhibited reduced weight gain, exhibited increased energy expenditure, and improved glucose tolerance. The gut microbiome of NR-treated mice exhibited an enrichment of butyrate producers, suggesting that the NR-consuming gut bacteria alters the host via production of short chain fatty acids (SCFAs). To establish cause-effect, a fecal material transplant (FMT) was done. FMT from NR-treated mice into naïve mice was sufficient to resist weight gain and improve metabolism relative to naïve mice receiving feces from control mice. Surprisingly, the FMT provided the beneficial effects faster than dietary NR supplementation. These data suggest that the beneficial effects of NR supplementation are at least partially mediated by the gut microbiome.

Poster #118

Mock Community Identification by Targeted 16S Amplicon, Full-length 16S PacBio Sequencing and Comparison of Custom Reference Database to Greengenes and Silva

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Recently, regulatory agencies have attempted to use next-generation sequencing (NGS) to identify probiotics in commercial products to insure proper labeling claims of mixed microbial constituents. Although this decision is a step towards assuring accurate labeling claims, the identification made by sequencing technologies is not without limitations or inconsistencies in taxonomic assignment at the genus and species level. In this study, we used a mock community of 10 probiotic strains to compare 16S rRNA gene amplicon sequencing (MiSeq) of 3 hypervariable regions (V3-V4, V4 and V4-V5), full-length 16S rRNA PacBio sequencing, as well a custom designed 16S rRNA reference database to compare against the Greengenes and Silva databases. Mock community of 16S amplicon sequencing revealed that V3-V4 region was better than V4 and superior than V4-V5 for genus level identification of probiotic microbes. The custom database enhanced the correct taxonomic identification over Greengenes and Silva databases. Whole 16S rRNA PacBio sequencing along with the custom database greatly improved, at the species level, the correct taxonomic identification over the Greengenes and Silva databases, however, false positive identifications were made. Overall, this study revealed that in order to make correct taxonomic assignments using NGS, validation of the most appropriate hypervariable region to use for amplicon sequencing is clearly needed, which in this study was V3-V4. More importantly, this study clearly demonstrated that updated and curated reference databases with inclusion of 16S rRNA sequences of microbes of interest is imperative in order to make correct taxonomic identifications at the genus or species level.

Poster #119

Effects of Starvation and Re-Feeding on the Microbiomes of the Zebrafish Intestine and Environment

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Evidence increasingly supports that diet has a deterministic influence on the intestinal microbiome in diverse animal hosts, yet how microbiomes respond to and recover from drastic dietary perturbations remains poorly understood. We investigated dietary disruptions in adult zebrafish (Danio rerio), from which food can be withheld for prolonged periods without mortality. We monitored bacterial communities in the intestines of conventionally-reared adult zebrafish hosts and their water environments across a three-week starvation and three-week re-feeding period. We constructed a sequence variants table from 16S rRNA gene sequence data using DADA2. The most significant differences in intestinal microbiomes between fed and starved groups were observed immediately following the two perturbations, one day after starvation onset and one day after refeeding onset. Preliminary results indicate increased similarity between intestinal and environmental microbiomes in starved fish than fed fish, but this difference was no longer detectable by 7 days post-refeeding. To evaluate the impact of this starvation/re-feeding regimen on host biology, we performed RNA-seq on whole intestine in a separate cohort of zebrafish. Functional categorization of differentially expressed genes revealed dynamic host responses to starvation and re-feeding, including transient reduction of interferon and antimicrobial pathways during starvation. Our ongoing analysis aims to identify the effects of starvation and re-feeding on specific microbial taxa and on host physiology. This project adds to a growing body of work indicating that the intestinal microbiome is both sensitive and resilient to drastic dietary perturbations, and provides a foundation for exploring how such perturbations affect host-microbiome relationships.

Poster #120

Role of Caenopores in Shaping Caenorhabditis Elegans Microbial Associations

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Microbiota research in the nematode Caenorhabditis elegans is a surprisingly young discipline. Despite of a microbe-rich natural habitat and a bacteriovorus lifestyle the identification of the native C. elegans microbiota and interacting host factors has only recently begun. Several protein families have been implicated in digestion of food bacteria and immune defenses against pathogenic bacteria, yet, their interaction with the microbiota bacteria has not been evaluated.
Hence, we aim at characterizing the role of one family of antimicrobial peptides, the caenopores (SPPs), in shaping microbial associations with C. elegans. Caenopores are primarily located in the intestine of C. elegans and have been demonstrated to permeabilize bacterial membranes. A comparative proteome analysis revealed that SPP-3 and SPP-5 were more abundant in worms exposed to microbiota bacteria. Both SPP encoding genes (spp genes) are located in a genetic cluster together with three other spp genes, namely spp-2, spp-4, and spp-6. We will complement genetic analyses of this cluster with phenotypic analyses of C. elegans overexpression and knock-out strains to understand the role of the spp cluster on microbiota bacteria in vivo. Further, recombinant expression and subsequent functional analyses of these SPPs should uncover the specific interaction between host factors and microbiota bacteria in vitro.
Our results may provide a first insight into the function of caenopores shaping microbial associations of bacteriovorus C. elegans.

Poster #121

A Game of T Cells: Interactions Between the Microbiome and Immune Recovery

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HIV is one of many conditions associated with an altered microbiome. This microbial perturbation widens with time and persists into treatment, but it is unknown if treatment reverses the perturbation. Treatment of People living with HIV (PLHIV), allows recovery of a depleted CD4 response, but only variably reverses CD8 stimulation and doesn't fully normalize (ratio ≥ 1.0) the immune system. Recent data suggests excess morbidity is largely concentrated in patients with CD4:CD8 ratios below 0.4, while immune defects coexist when the ratio is below 1.0. How the microbiome relates to normalization of CD4:CD8 ratio as well as immune recovery is unclear. We examined three distinct cohorts of PLHIV on treatment. One cohort is from Ethiopia, one is from Veteran Hospital, and one consists of patients intermittently incarcerated in Wisconsin. Normalization is variable in all three cohorts and does not occur universally despite a decade of therapy or more. Normalization of females was greater in all cohorts. In the Ethiopian cohort, 6% of females reach a CD4:CD8 ratio of 1.5 and 27% reach a ratio greater than 1.0, while only 2% of males reach a CD4:CD8 ratio of 1.5 and 11% reach a ratio greater than 1.0. Factors associated with lack of ratio recovery will be discussed. Age and sex alter CD4:CD8 across mammalian systems. As microbiome studies move from descriptive cataloging to mechanistic inquiries they will need host phenotypes, such as CD4:CD8 ratio, associated with normal or abnormal functions.

Poster #122

Cultivated Relationships: *C. Elegans* Genetic Landscapes that Shape Microbiome Form and Function

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Together with diet, host genetic landscapes shape microbiota acquisition in the animal gut. Genomic variation among individuals contributes to establishment of distinct physiological environments within the gut ('enterotypes') that support distinct microbiomes. The genetic determinants of stability and variation of enterotypes remain largely undefined.

To address this problem, we employ the ge¬netically-tractable nematode Caenorhabditis elegans. Its microbiome is deterministically acquired from its natural habitats of rotting fruits and vegetation. We can experimentally model this process using a functionally redundant, 68-member model core microbiome (BIG68) in the lab. A panel of 38 fully genome sequenced C. elegans wild strains were first made 'germ-free' then colonized to examine differences in microbiome composition (16S) and levels (CFU) longitudinally using a high-throughput pipeline. The strains clustered tightly into three distinct enterotypes: (1) a highly-selective enterotype that differed greatest from the surrounding environment [74% of strains]; (2) a 'dysbiotic' enterotype with up to 30-fold higher colonization levels [Bacteroidetes-dominant]; and (3) a non-selective enterotype. All strains tested retain deterministic selection and/or control, suggesting two complementary programs for microbiome regulation.

To genetic basis of these enterotypes, we employed a combination of conventional GWAS, allelic clustering and machine-learning based methodologies to identify candidate genetic regulators/effectors (~1000). Many are intestinally expressed, responsive to microbes and fall into highly conserved pathways common in other systems for microbiome regulation (>60%)—e.g., insulin signaling, GPCRs, mucus, gut motility and immunity. Our study provides a robust platform to comprehensively identify the host genetics of gut microbiome acquisition and assembly.

Poster #123

Diet Driven Hypertension and Gut Dysbiosis in the Dahl Rat

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The consumption of a high salt diet is known to influence the development of salt-sensitive hypertension (SS-HTN), but the mechanism behind this is not well understood. To study HTN, we have used the Dahl SS rat model. Results from past experiments suggest that the salt content and the protein source in the diet are factors influencing the severity of the SS-HTN phenotype. In addition, rats sourced from distinct facilities are observed to have different SS-HTN phenotypes. These observations suggest a role for the gut microbiome in SS-HTN, as diet composition and facility housing are known to influence the gut microbiome. Therefore, we hypothesize that changes in the gut microbiome contribute to the development of SS-HTN in the Dahl rat model. To test this, we extracted bacterial DNA from stool collected from male SS-Dahl rats that were fed a low salt diet and then challenged with a high salt diet. Preliminary data obtained using 16S Illumina sequencing, indicate that 1) the protein source in the parental diet influences gut microbial composition in the offspring, 2) SS-HTN Dahl rats supplied by different facilities have different microbiomes and 3) salt may be influencing gut microbial composition. These results demonstrate that the gut microbiome is distinct in sets of SS-HTN Dahl rats that display different SS-HTN phenotypes, which warrants further exploration of each microbial community and its contribution to the development of SS-HTN.

Poster #124

Using Genes to Predict Bacterial Colonization in Mammals

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The microbiome is becoming an area of intense research as we understand more about its potential influence on a macroscopic scale of the host. As the research continues, we will need more tools to aid the studies. Here we report on the ability of our previously published pipeline MAGNAMWAR to extend beyond the D. Melanogaster that it was tested on. We applied it to results from a mono-associated mouse study in which they used 53 strains of bacteria and found the genes that were altered by the presence of the bacteria. We ran the data through MAGNAMWAR and analyzed the KEGG enrichment results to identify potential pathways necessary for these bacteria to colonize either the intestinal tract, mLN, or SLO. We show that MAGNAMWAR was able to predict genes associated for bacterial colonization that were previously found in other studies as well as novel genes and metabolic pathways. We were able to find 4 pathways and genes previously correlated with those bacterial strains and colonizations as well as predict 9 novel pathways necessary. These results imply that the MAGNAMWAR package can be beneficial for other gene association studies involving bacteria.

Poster #125

Identifying Host Genes that Control the Gut Microbiome and Metabolism

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The population of microbes that inhabit the ecosystem of the mammalian gut has large effects on host physiology. Alterations in the gut microbiota contribute to metabolic diseases, including obesity and diabetes. The major factors that influence the gut microbiota include host genetics and diet. However, we lack a comprehensive understanding of the interrelationships among host genetics, the gut microbiome, and metabolism. To address this gap, we leveraged a powerful genetic model called the Diversity Outbred (DO) mice to identify host genetic associations with the microbiome and related traits. The DO mice were derived from eight founder strains containing most of the genetic diversity of all inbred mouse strains. Each mouse was genotyped at high density. We used “shotgun” metagenomics and metabolomics approaches to assess the functional potential of the distal-gut microbiome from 300 DO mice maintained on a Western-type diet. Quantitative trait locus (QTL) analysis revealed mouse genetic “hot spots” that associate with the presence /absence or abundance of many microbial functions. The candidate mouse genes driving these “hot spots” include regulators of cascades known to respond to the microbiome, including TGF-β and TLRs, and novel mouse candidate genes including a lesser-known cytokine and G-protein receptor. Additionally, we identified mouse genomic loci where microbial functions and metabolites co-associated. Mediation analysis suggests causal roles for these co-associating QTL. Together, our analyses will yield novel insights into the forces that shape the functional capacity of the gut microbiota and how it modulates metabolism and disease.

Poster #126

Host-Microbe Interactions Between the Tsetse Fly Innate Immune System and the Secondary Symbiont Sodalis Glossinidius

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Tsetse flies are the sole vectors of Trypanosoma parasites which cause human and animal African trypanosomiasis. Additionally, tsetse harbors a low diversity microbiome making them an ideal model to study host-symbiont interactions. In this study, we used the tsetse association with its secondary symbiont Sodalis glossinidius to understand the molecular mechanisms that govern endosymbiont-host immune interactions.

First we established a Sodalis-free (GmmSod-) tsetse fly colony, allowing us to compare host immunological parameters between Sodalis-infected and uninfected flies with identical genetic backgrounds. We then examined their immune response to infection with cultured Sodalis and E. coli using RNA-seq and qPCR. In-depth analysis of immunity genes demonstrated moderate immune responses elicited by the Sodalis symbiont, whereas the pathogenic E. coli resulted in full immune activation. These results clearly indicate the existence of a mechanism allowing host immune tolerance of this bacterial gut symbiont.

Furthermore, we demonstrated that the activated immune response has no impact on the Sodalis density, indicating the symbiont's insensitivity to its host immunity. Also, suppression of the immune deficiency signalling pathway by RNAi revealed that the symbiotic population is not regulated by the fly's immune system, suggesting that other regulatory mechanism, like nutrient availability, are involved in the control of this symbiont.

This study provides first insights in the interactions between Sodalis and the tsetse fly innate immune system. Currently, we are investigating the contribution of Sodalis to its host functioning, i.e. viability, fecundity, and metabolism, as well as its ability to modulate the fly's susceptibility towards Trypanosoma infection.

Poster #127

The Global Regulator Lrp Controls Xenorhabdus Nematophila Virulence Modulation in Response to Nutrient Availability

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Bacterial populations vary phenotypically to adapt to changing environments. Environmental parameters like nutrient availability can affect phenotypic switching and cause population heterogeneity. The bacterium Xenorhabdus nematophila is an insect pathogen and a mutualist of Steinernema carpocapsae nematodes. The bacteria colonize the nutrient-limiting intestine of the infective juvenile (IJ) stage nematode. When an IJ infects an insect, it releases its bacteria, which help kill the insect. The bacteria and nematode consume the nutrient-rich insect cadaver and reproduce until crowding and resource limitation trigger IJ development. We examined the mechanisms by which the bacterium undergoes virulence modulation (VMO), phenotypic variation between mutualistic and pathogenic lifestyles that depends on the expression level of global regulator leucine-responsive regulatory protein (Lrp). Bacteria with high levels of Lrp better colonize nematodes and support their reproduction, while low-Lrp bacteria are more virulent toward insects. We hypothesized that the mutualism-to-pathogenesis phenotypic switch occurs in the IJ prior to insect infection, possibly in response to nutrient limitation. To test if nutrient limitation induces a high-to-low Lrp switch we monitored expression of an Lrp-dependent gene fliC, using a PfliC-gfp/constitutive Plac-rfp fluorescent reporter. Flow cytometry revealed that growth in low-nutrient medium results in a subpopulation shift from high- to low-Lrp. Furthermore, cumulative GFP intensity (an indirect measure of Lrp activity) negatively correlates with virulence towards insects. Current efforts focus on measuring switching frequency in different X. nematophila lineages. Our results suggest that nutrient-responsive Lrp-dependent phenotypic switching serves as a pre-adaptive mechanism for bacteria to transition from mutualism to pathogenesis.

Poster #128

Modeling Probiotic Colonization and Infection in Drosophila

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Drosophila melanogaster is an excellent model organism for studying the gut microbiome and its effects on infection and immunity. The Drosophila immune response includes the production of reactive oxygen species (ROS) and reactive chlorine species (RCS), which kill microbes by inducing redox damage. However, these reactive compounds also harm the gut epithelia. We are studying the effects of the probiotic bacterium Escherichia coli strain Nissle 1917 on gut colonization and epithelial renewal following oxidative stress. Our data show that Nissle colonizes the fly gut at higher levels than E. coli K12, but that populations drop following a single dose over the course of a few days. The probiotic nature of this organism is beneficial in that colonization of the gut shields the epithelia from redox stress. Screening of Nissle mutants defective in the response to ROS and RCS showed that many of these mutants are diminished in their ability to colonize the Drosophila gut. To complement this data, preliminary experiments looking at colonization levels in a Drosophila line in which ROS/RCS levels have been genetically reduced through knock- down for Duox will be discussed. Future experiments will include infecting Drosophila with Nissle and Erwinia carotovora carotovora 15 (Ecc15), a non-lethal pathogen that causes oxidative damage to the gut in order to examine the effects of the probiotic on gut damage and renewal. Overall, this work will broaden our understanding of how the fly response to pathogens causes gut damage induced by reactive compounds and how probiotics can ameliorate this damage.

Poster #129

Regulation of Keratinocyte Gene Expression by the Skin Microbiome

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The skin microbiome represents a milieu of microorganismal communities adapted to their host in both composition and physiological potential, demonstrating a coevolutionary relationship that inhabits one of the largest human organs. However, cutaneous host-microbiome relationships remain poorly characterized, including the full range of host processes and functions that are modulated by microbial colonization. We analyzed the epidermal-specific host response to microbial colonization using gnotobiotic mice and RNA-seq. Keratinocytes, the outermost layer of structural cells that comprise the epidermis, were isolated from germ free (GF), conventionally raised (CR), and conventionalized (CV) mice, a model for acute colonization where GF mice were colonized for 2 weeks with CR microbiota. Expression profiles of GF and CR mice were most distinct, with over 6,000 differentially expressed (DE) genes. Many of the DE genes revealed an intermediary expression profile in CV mice, demonstrating a microbial-induced shift in CV keratinocyte transcription. Moreover, CR mice were enriched in DE genes necessary for the construction, formation, and subsequent degradation of corneodesomes, the main adhesive junction between keratinocytes. CR mice were also enriched in DE genes involved in sphingolipid metabolism, which produces key lipids responsible for epidermal structure, barrier function, and signaling. These findings, along with ongoing functional validation studies, suggest a physiological response by the murine epidermis to the microbiome whereby keratinocytes differentially regulate processes necessary for the maintenance of effective barrier function and epidermal homeostasis. A further understanding of this host-microbiome relationship may reveal novel therapeutic targets to promote skin barrier function.

Poster #130

The Role of the Genetic Regulator TcpP in V. Fischeri Colonization of the E. Scolopes Light Organ

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An animal's physiology is significantly affected by its colonizing microbial communities, and interactions between host and symbiont(s) are particularly important to host development. The symbiotic relationship between the squid Euprymna scolopes and the bioluminescent Gram-negative bacterium Vibrio fischeri, which colonizes the squid light organ, is an excellent model for the intricate interactions that take place between host and symbiont. In this work, we explore genetic regulation that occurs in V. fischeri during the switch between planktonic life and light-organ colonization. The genes encoding the regulator TcpPH (toxin-coregulated pilus biosynthesis proteins P and H), which are important for virulence in V. cholerae, have previously been shown to be more highly expressed in V. fischeri cells expelled from E. scolopes light organs than in planktonic cells. Here we present RNA-seq results showing the regulatory targets of TcpPH and the resulting effects on V. fischeri physiology, including changes in light production and metabolite uptake. We also discuss similarities and differences between the tcpPH regulatory pathways in V. fischeri and V. cholerae. Understanding the regulatory system controlling the switch between free-living and symbiotic lifestyles, and the resulting changes in transcriptional output by V. fischeri, may shed light on the important interactions between humans and their colonizing bacteria.

Poster #131

Among-Strain Variation in Functional Traits of Symbiotic Bacteria

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Routine genome sequencing of symbiotic bacteria is revealing considerable among-strain variation in predicted functional traits, but it is difficult to elucidate the impact of this variation on host-bacterial interactions in the many associations with complex microbial communities. The naturally low diversity symbiosis in aphids offers a unique opportunity to investigate how genetic variation in the bacterial partners influences host-bacterial interactions, including the known symbiotic function of essential amino acid provisioning to the host. Our specific goal was to identify how genetic variation in two bacterial symbionts, the intracellular symbiont Buchnera aphidicola and the hemolymph (blood)-borne bacterium Hamiltonella defensa, influence metabolic interactions with the aphid host. Using our panel of 200 field-collected pea aphid genotypes and multiple genomic and metabolomics methods, we established that Buchnera genotype, specifically SNPs in four key metabolism genes, is strongly correlated with host demand for dietary histidine, but no other essential amino acids tested; and that Hamiltonella has far-reaching effects on metabolic function, including nitrogen metabolism, B-vitamin metabolism and redox status of the symbiosis. Metabolic modeling, especially flux variability analysis, of the multi-partner symbiosis reveals the metabolic basis of Buchnera genotype effects on histidine nutrition and, more generally, how host metabolism is more responsive to variation in a low abundance, facultative symbiont (Hamiltonella) than to the required abundant Buchnera symbiont. We also address how the metabolic effects of Hamiltonella may underpin the known effects of this bacterium on host immunological function, conferring resistance to key natural enemies.

Poster #132

Human Gut Microbiota Metabolic and Community Response to Salmonella Enterica Typhimurium Infection

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The gut microbiome contributes to host defense by conferring resistance to pathogen colonization. Although colonization resistance has been well studied, the role of the numerous members of the microbiota and their metabolites is unclear. We used a combination of metagenomics and untargeted metabolomics to identify changes in the community and metabolome of gnotobiotic mice infected with the virulent pathogen Salmonella enterica Typhimurium or the opportunistic pathogen Candida albicans. To isolate the role of the microbiota in response to pathogens, we compared mice monocolonized with pathogen, uninfected mice humanized with a synthetic human microbiome, or infected humanized mice. We found a shift in the microbial communities in mice infected with Salmonella by day three of infection, in contrast to a lack of symptoms and changes in Candida infection. The changes corresponded to a rise in Enterobacteriaceae strains and a reduction in biosynthetic gene cluster potential. The metabolomic fingerprint of the cecum differed between mice monocolonized with either pathogen and humanized infected mice. Specifically, we identified an increase in glutathione disulfide, glutathione cysteine disulfide, and inosine monophosphate in mice infected with Salmonella in contrast to uninfected mice and mice monocolonized with Salmonella. These results provide insight into how the microbiota interacts with pathogens on a metabolic level.

Poster #133

Investigating the Role of Copper during Initiation of the Squid-Vibrio Symbiosis

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The symbiosis between Vibrio fischeri and the Hawaiian bobtail squid, Euprymna scolopes, serves as a model system to characterize the molecular dialogue that ensures highly specific colonization of host-epithelial tissues by bacteria. Using a global approach based on transposon insertion sequencing (INSeq), our lab identified 380 putative squid colonization factors in V. fischeri. Three factors identified in this screen as colonization deficient—copA, cusA, and cusC—function in copper export, which is highly toxic due to its ability to produce reactive oxygen species and outcompete other metals for binding essential metalloproteins. V. fischeri encodes several homologs of proteins characterized to play a role in copper tolerance, including copper-responsive transcription factors and a two-component signaling system These findings suggest that appropriately managing intracellular levels of copper is vital for V. fischeri to colonize its host. Here we show that CopA is required for resistance to copper during growth and for squid colonization. We describe future experiments to elucidate the role that copper plays during initiation of the squid-vibrio symbiosis. We also present an updated mutagenesis approach developed to generate precise deletions of V. fischeri chromosomal loci in a single step and insert a unique barcode sequence within each deletion scar, enabling quantification of individual mutants in a population by deep sequencing. We will apply this approach to further characterize the 380 putative colonization factors by generating a barcoded mutant library and assaying population dynamics during in vivo competition experiments.

Poster #134

Members of the Gut Microbiota Modulate Virulence of Enterohemorrhagic *E. Coli*

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The remarkably low infectious dose of Enterohemorrhagic *E. coli* (EHEC) underscores that this important human intestinal pathogen has evolved strategies to outcompete the resident microbiota. We hypothesize that EHEC takes advantage of the physiology of the microbiota to efficiently colonize and cause disease. In response to Bacteroides-produced succinate, EHEC upregulates its virulence genes, leading to more severe disease in vivo. Here we further investigate the interaction between EHEC and Bacteroides thetaiotaomicron (B. theta). We show that B. theta produced proteases cleave structural components of the EHEC type III secretion system (T3SS), and that T3SS function is altered in the presence of B. theta. We also examine the effect of phylogenetically diverse members of the microbiota on EHEC virulence. Both B. theta, from the Bacteroidetes, and Enterococcus faecalis, from the Firmicutes, increase EHEC virulence gene expression. However, the commensal E. coli strain HS has no effect on EHEC virulence gene expression. Combinations of these three bacteria yielded distinct effects from any strain alone, highlighting the complexity of these interactions. We further probed the interaction between EHEC and E. faecalis, demonstrating that an Enterococcus-induced increase in T3SS transcription translated to an increase in effector protein delivery into host cells. Interestingly, when different strains of E. faecalis were tested for this phenotype one strain, MMH594, had a weaker effect than the other strains tested. This work demonstrates that different members of the microbiota can affect the virulence of intestinal pathogens in unique ways and underscores the complexity of interactions between pathogens and commensals.

Poster #135

Microbially-Liberated Urea-Nitrogen is Utilized by Hibernating Arctic Ground Squirrels

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Arctic ground squirrels (Urocitellus parryii), obligate seasonal hibernators, experience dramatic physiological and dietary changes across their annual cycle. During hibernation they conserve energy reserves by entering a state of torpor with reduced metabolic rate, body temperature and activity. Unlike the summer active season when squirrels forage, reproduce, and fatten, the long fast of hibernation is characterized by a lack of dietary nitrogen to meet nitrogen needs. The gut microbial community may play a role in nitrogen metabolism and homeostasis via urea-nitrogen salvage (UNS), a process by which urea is metabolized by ureolytic gut microbes, liberating nitrogen for host use. The goal of my project is to discover the degree to which arctic ground squirrels rely upon UNS to meet their nitrogen needs and to isolate and characterize ureolytic gut microbes. At pre-selected time points across the annual cycle (during hibernation when host dietary supply is low; during euthermia when dietary supply is high), 15N/13C labeled urea was injected into squirrels and breath and tissue samples collected for stable isotope analysis. Cecal contents were collected for microbial characterization. Analysis of 13CO2 in breath indicates that ureolysis occurs in the gut. Analysis of tissues for 15N indicates that squirrels incorporate more microbially-liberated urea-nitrogen during hibernation than the active season. Five facultative ureolytic bacterial species have been isolated for characterization to date, and isolation and characterization of obligate anaerobes is ongoing. The results indicate ureolytic bacteria and urea nitrogen salvage may make important contributions to nitrogen homeostasis during hibernation in arctic ground squirrels.

Poster #136

Mapping the Amylosome of R. Bromii: A Keystone Species of the Gut Microbiota

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Due to altered bacterial communities observed in people with a wide range of diseases such as Crohn's Disease, obesity, and C. difficile infection, modulating the human gut microbiota has been proposed as a therapy for such ailments. One way to manipulate the gut community towards improved health is via diet as carbohydrates drive community assembly. Ruminococcus bromii, a keystone species in the human gut, degrades dietary fibers and cross-feeds other gut bacteria. It has an amylosome, a complex of secreted amylases and pullulanases, that is predicted to be responsible for its unique ability to degrade resistant starch (RS.) However, the molecular determinants within this complex that confer RS degradation and community cross-feeding are poorly understood. An immunoprecipitation of a secreted pullulanase from R. bromii, Amy12, revealed an association with an uncharacterized adaptor protein, Doc20. Doc20 is comprised of two uncharacterized domains followed by a dockerin domain. While others have proposed a theoretical framework by which R. bromii assemble via dockerins, no thorough molecular studies have confirmed their interactions. We have purified Doc20 and its domains and have confirmed via affinity PAGE that Doc20 domain 2 binds to both corn and potato amylopectin. Preliminary ELISA experiments suggest that Amy12 and Doc20 directly interact with low affinity. In conclusion, while the gut microbiota has been a hot topic with regards to human health, more studies should be conducted at the molecular level to mechanistically explain key interactions that can lead to microbiome therapeutic intervention.

Poster #137

Mining the Microbiota for Bioactive Molecules

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Bacteria use small molecule chemicals to interact with other community members and with their eukaryotic hosts. The biochemical pathways involved in the production of these molecules are typically encoded in distinct physical locations of the bacterial chromosome, in biosynthetic gene clusters (BGCs). Using in silico mining techniques, we previously identified several widespread families of BGCs in prominent members of the human gut and skin microbiome.

We are now experimentally characterizing the function of two BGC families involved in modulation of community composition and interaction with the host immune system:

- Aryl polyenes (APEs) are cell surface-attached molecules that occur in Gram-negative gut pathogens as well as symbionts. We discovered that APEs protect their producers from oxidative stress, providing them with a competitive advantage in an inflammatory environment.

- Corynomycolic acids make up a large portion of a specialized 'outer membrane' in most skin Corynebacteria. They are involved in the recruitment and activation of a γδ T cell subset that conditionally promote skin inflammation on a high fat diet. We are now identifying the contributing gut microbial metabolites as well as characterizing the effect of variations in the corynomycolic acid biosynthesis pathway.

A better mechanistic understanding of our microbial symbionts will lead to the discovery of druggable small molecules, new targets for antibacterial therapy and beneficial bacterial strains that can be employed for intervention therapies.

Poster #138

Dynamics of Chromobacterium Violaceum in Drosophila melanogaster Host Immune Responses

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The innate immune response is the first line of defense against infections in both Drosophila melanogaster and humans. The Toll and immune deficiency (Imd) pathways recognize pathogen-associated molecules, such as peptidoglycan, to activate host immune responses during infection. However, the diversity of pathogens used to study mechanisms of innate immunity in Drosophila is relatively small. To better understand the breadth of host responses to various pathogens this study characterizes the host response in Drosophila to a novel pathogen Chromobacterium violaceum, a Gram-negative bacterium commonly found in soil and freshwater. C. violaceum is unique due to its quorum sensing regulated production of the antibiotic violacein, which produces a purple pigment when induced. Drosophila survival response to three stains of C. violaceum (CV017, CV026, or CV31532), varies based on route of infection and strain type. The observation that the infectiveness of different C. violaceum strains is dependent on the mode of infection (oral versus systemic injection) suggests different mechanisms lead to host death and pathogen success. By examining the response of different innate immunity fly mutants, our results suggest that systemic infection of C. violaceum overstimulates the Drosophila Imd pathway leading to internal damage and eventually fly death. Given that the fly microbiome establishes basal immune status, preliminary data on the contribution of the host microbiome to survival will also be discussed. Overall, the unique production of the natural antibiotic violacein allows for novel research in quorum-controlled virulence, and how the pathogen interacts and alters the host microbiome and host immune responses.

Poster #139

Toward Understanding the Influence of Environmental Conditions on the Catalytic Promiscuity of 1-deoxy-D-xylulose 5-phosphate synthase

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The human microbiome is constantly exposed to environmental changes due to factors such as diet, disease, and pharmacological intervention. One mechanism that microbes use to sense and adapt to this onslaught of stimuli is restructuring metabolic pathways. Rapid responses to environmental perturbations can be executed via catalytically-promiscuous enzymes, which can contribute to microbial survival by providing an alternative metabolic activity that increases bacterial fitness. Hallmarks of this metabolic plasticity are found in 1-deoxy-D-xyluose 5-phosphate synthase (DXPS)- an enzyme that forms the central bacterial metabolite, 1-deoxy-D-xyluose 5-phosphate, from pyruvate and D-glyceraldehyde 3-phosphate. In addition to having relaxed substrate specificity and being catalytically promiscuous, DXPS catalyzes a unique mechanism and displays conformational flexibility. Together, these characteristics of DXPS suggest it is possible that cellular conditions could influence the chemistry of this enzyme. Toward understanding the potential alterative roles that DXPS could play in bacteria, we conducted mechanistic studies which revealed three intriguing activities of DXPS including 1) pH-dependent acetolactate formation, 2) O2-dependent acetate formation and 3) consumption of hydroxypyruvate. Here we discuss the unique features and intriguing implications of each activity. For example, the increase in catalytic efficiency of DXPS-dependent acetolactate formation and pyruvate consumption at low pH could imply a role for DXPS in response to acid stress and pyruvate buildup. Overall, the ability of DXPS to utilize distinct donor/acceptor pairs coupled with its unique mechanism suggests that DXPS may facilitate metabolic remodeling to enhance bacterial fitness in response to environmental changes.

Poster #140

Discovering Probiotic Bacteria to Mitigate Thermal Stress in Corals

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Corals are colonized by billions of symbiotic microorganisms that exert a profound influence on health and disease. One noted symbiont is the photosynthetic dinoflagellate Symbiodinium, which provides up to 95% of the coral's carbon. During thermal stress, reactive oxygen species (ROS) accumulate from damage to the photosystems of Symbiodinium causing cellular damage. As a protective mechanism, corals expel their Symbiodinium, a condition known as bleaching. We use the model organism, the anemone Exaiptasia pallida, to investigate the production of extracellular antioxidants by symbiotic bacteria and the capacity of these antioxidants to protect their host against bleaching. The goal of this research is to identify non-enzymatic antioxidants that bacteria secrete, which, in neutralizing excess oxygen radicals, could help maintain the host-Symbiodinium relationship and prevent bleaching. We applied a qualitative test to assess the free radical scavenging ability in over 600 E. pallida-sourced bacterial pure cultures. From this phenotypic test, over 20 strains were identified as potential beneficial bacteria, and these have been quantitatively assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH; stable free radical) assay with cell free extracts. The whole genome data of these 20 strains are being analysed to determine the genetic basis of the antioxidant production and facilitate their therapeutic application. Exposure of corals to exogenous antioxidants that scavenge ROS during temperature-induced stress has been shown to prevent coral bleaching and our research aims to extend this approach by developing a natural probiotic with high antioxidant capacity to increase climate resilience in corals.

Poster #141

Friend or Foe? Lactobacillus BrevisPpromotes Tumour Growth in the Drosophila Intestine.

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Microbial factors promote a hyperplastic expansion of mutant intestinal stem cells in experimental models. For example, I demonstrated that symbiotic bacteria are required for the growth of Notch-deficient stem cells in the intestines of adult Drosophila. Despite the importance of bacterial cues for stem cell growth, we know very little about the host and bacterial factors required for tumourigenesis. To address this question, I examined the impact of common fly symbionts on stem cell growth in Drosophila. In these studies, I established that the cell wall fraction of Lactobacillus brevis is sufficient to drive the growth of Notch-deficient tumours, indicating a role for bacterial sensing pathways in intestinal tumourigenesis. To identify the host responses that promote L. brevis-dependent tumourigenesis, I performed RNAseq on purified intestinal progenitors from wild-type and Notch-deficient flies that I raised under germ-free conditions, or in association with L. brevis. As expected, I found that L. brevis induces mitogenic responses, and inhibits tumour suppressor pathways in the host. However, I also found that L. brevis blocks antibacterial immune activity in the intestines of Notch-deficient flies. Consistent with a diminished immune response, I observed a significant increase in the intestinal loads of L. brevis in Notch-deficient flies. These results suggest a physiological feedforward loop where L. brevis stimulates the growth of Notch-deficient tumours, suppressing host immune responses, and allowing for the expansion of tumourigenic L. brevis.

Poster #142

C. Elegans as a Bioindicator to Screen Probiotic Bioremediation of Heavy Metal Toxicity

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Continued industrial use of heavy metals, such as lead, results in human exposure and contributes to several health complications, including neurological defects. One proposed solution for prevention of heavy metal exposure and toxicity is the consumption of probiotics specialized in reducing the bioavailability of heavy metal ions and reactive oxygen species. However, the time and resources required to test potential probiotics with higher order animals such as mice, rats, and swine remains a challenge, which is further exacerbated with the numerous amounts of bacterial strains available to test. To overcome these limitations, utilizing screening platforms such as the nematode, Caenorhabditis elegans, allows for rapid observation of numerous probiotic strains while still delivering complex, whole-organism level endpoints. In this study, swimming performance of young adult C. elegans worms were assessed following a 24 hour exposure to various toxicants, including lead and cadmium acetate. Nematodes challenged with heavy metal ions display significant reductions of body bend frequency compared to unchallenged controls (p-value<0.0001), especially when challenged with lead. This neurotoxicity could be substantially mitigated if the worms were coincubated with specific LHS probiotics (p-value n.s). Measurement of oxidation in challenged worms revealed that the probiotic mediation is not limited to the antioxidant activity of the probiotics. The results obtained in this study support the rational to use the C. elegans animal model to rapidly screen LHS probiotics for various health applications and future clinical trials, in this case inhibition of neuroactive heavy metals.

Poster #143

Investigating the Antagonistic Behavior Between Skin Commensal Species Reveals Novel Antimicrobial Metabolites

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The skin is a physical barrier and host to commensal microbial communities with postulated functions in colonization resistance to pathogenic species. We previously observed in murine models that commensal skin isolates were protective against colonization by the pathogen, Staphylococcus aureus. We hypothesized that a network of commensal microbial interactions underlies functional colonization resistance, and that hubs of this network represent top candidates for the identification of novel molecular mediators of microbe-microbe interactions, including antimicrobials. We screened a selection of 46 bacterial strains isolated from healthy mammalian skin for inhibitory behavior against one another in a pairwise manner. After testing all possible permutations, we assembled a network of antagonistic behavior between skin commensals. Over 60 antagonistic interactions were identified between bacteria, including self-inhibition. To further elucidate the mechanisms of antagonism, genomic profiling was carried out to identify candidate biosynthetic gene clusters encoding secondary metabolites with putative antimicrobial activity. In total, 321 potential biosynthetic gene clusters were identified, many of which were novel. Of these, approximately 160 were predicted to produce secondary metabolites possessing antimicrobial properties. Prioritizing network hubs, we are now identifying which metabolites are responsible for antagonistic interactions. Furthermore, we will test the ability of these metabolites to inhibit the growth and colonization of pathogens including S. aureus. Our long term goal is to identify molecular mediators of antagonistic behavior and characterize their utility as novel antimicrobials against cutaneous colonization and infection by pathogens.

Poster #144

Lactobacillus Gasseri Ameliorates Diet Induced Diabetes in Mice via Changing Lipid Metabolism Gene Expression in the Gut

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Gut and gut microbiota play an important role in metabolic diseases such as type 2 diabetes (T2D) and have potential to be used to manage the disease. For this, identification of specific microbes is necessary to explore their role in prevention or treatment of T2D. We used High Fat High Sucrose (HFHS) diet to induce glucose intolerance in mice and determined the phenotypes altered by HFHS diet. Using 16S rRNA sequencing of ileum, we evaluated microbial perturbations in the gut. To identify microbial candidates that can affect glucose homeostasis, we used casual inference analysis and transkingdom networks for the changed metabolic parameters and perturbed microbes. We predicted several candidate microbes with potential beneficial effects on glucose homeostasis. Lactobacillus gasseri (Lg) was identified as a top candidate and was tested in vivo by gavaging it into HFHS-fed mice. Supplementation with Lg ameliorated glucose intolerance development and reduced adiposity in HFHS fed mice. To evaluate the mechanisms of action of Lg in the gut, we sequenced ileum gene transcripts. We found that Lg regulated expression of about 100 genes in the ileum and about 90% of them were restored to normal levels. Genes involved in fatty acid and steroid biosynthesis that had been downregulated by HFHS diet were upregulated by administration of Lg. Thus these findings suggest that Lg promotes beneficial effects in diet-induced glucose intolerance by modulating gene expression in the gut. Further analyses are in progress to identify key genes modulated by Lg to restore metabolic homeostasis in T2D.

Poster #145

The Role of the Vibrio Fischeri Cytoplasmic Chaperone DnaJ in Colonization of the Hawaiian Bobtail Squid

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Host-microbe interactions are abundant in nature and can be beneficial to both partners. The Gram-negative bacterium Vibrio fischeri colonizes the light organ of the Hawaiian bobtail squid (Euprymna scolopes), where the squid gains protection from predators via V. fischeri's bioluminescence, and V. fischeri receives nutrients and shelter. To successfully colonize the host, V. fischeri produces biofilms containing symbiosis polysaccharide (Syp). A previous INSeq experiment identified 380 putative colonization factors for V. fischeri, including dnaJ. DnaJ is a co-chaperone for DnaK, bringing unfolded polypeptides to DnaK and stimulating DnaK's ATPase activity to fold the protein. Deletion of dnaJ in V. fischeri squid symbiotic strain ES114 results in reduced Syp production, biofilm formation, and squid colonization. However, the DnaJ substrates that promote biofilm formation are unknown. We aim to identify DnaJ substrates in V. fischeri and elucidate DnaJ's role in biofilm formation and squid colonization. To accomplish this, we conducted genetic suppression analysis and identified transposon insertions that promoted increased biofilm formation in a ΔdnaJ strain. Interruption of the gene encoding the cytoplasmic protease HslU led to increased biofilm formation, while overexpression of HslUV led to reduced biofilm formation. Our current model is that DnaJ folds critical client(s) in the biofilm pathway and HslUV degrades misfolded client(s) when DnaJ is absent. We are applying multiple approaches to identify the client(s), including examining candidate substrates, multicopy suppression, and investigation of HslUV degradation specificity. With this work, we plan to reveal how regulated protein folding contributes to V. fischeri biofilm formation and squid colonization.

Poster #146

Predicting Bioactive Metabolites from Shotgun Metagenome Data

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The human microbiome can profoundly influence the balance between health and disease. However, the mechanisms mediating disease outcomes correlated to microbiome disruption are largely unknown. Microbial-host interactions include communication mediated by small molecules that are expressed from genomic islands referred to as biosynthetic gene clusters (BGC). The capacity of the human microbiome to produce bioactive molecules has just recently been explored and only a small fraction of predicted compounds have been characterized, largely from cultured isolates. While predicted molecules have unknown functions and targets, they are hypothesized to have pharmacological activity that could be exploited for human use. Here we present an un-biased computational method to detect BGC from complex metagenomes. Examining data from skin, gut, and environmental sources, we identified BGC conserved across individuals and populations. We focused our analysis on the skin to reveal enrichment of cluster sub-types partitioned by body site, suggesting an ecologically important relationship between the encoded metabolite and body niche. We also determined that the number and types of BGC can differ between healthy and disease cohorts of individuals, suggesting an important role in health maintenance. Importantly, our approach allows us to identify the genome harboring the BGC, facilitating targeted isolation and subsequent characterization of the metabolite. Here we use a non-ribosomal peptide synthetase cluster in Corynebacterium genomes found on the skin as an example. Our approach will result in a deeper understanding of co-evolving molecules produced by the microbiome and their importance in maintaining a healthy symbiosis between host and microbe.

Poster #147

Functional Characterization of LBA0695, a Lactobacillus Acidophilus Surface-Layer Associated Protein

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The bacterial surface (S-) layer is a two-dimensional self-assembling crystalline array composed of proteinaceous subunits that constitute the outermost layer of select prokaryotic cell envelopes. S-layer proteins have been shown to act as a scaffold for external display of additional proteins or glycoproteins. Supplemental functionality depends on which proteins the S-layer is presenting. These S-layer-associated proteins have been recognized in a number of industrially relevant probiotic bacteria and garnered attention due to their direct physical contact with host intestinal mucosa. We recently employed multiplexing quantitative proteomics to investigate growth phase-dependent fluctuations of the S-layer associated proteome of Lactobacillus acidophilus NCFM. Results revealed several surface-localized proteins that were highly abundant throughout the cell life cycle. In particular, LBA0695, a constitutively expressed uncharacterized protein, was selected for targeted chromosomal deletion to gain insight into the functional role of the L. acidophilus noncovalent exoproteome. In comparison to the parent, the lba0695-deficient strain demonstrated abnormally elongated cells, most notably during stationary growth phase, and significantly decreased adherence to human Caco-2 intestinal cells. Furthermore, under high salt conditions, the mutant exhibited impaired growth and a visibly disrupted S-layer. Together, these outcomes suggest that LBA0695 may play a role in S-layer stability and adhesive capacity, especially under osmotic stress.

Poster #148

Microbiome Influence on Drosophila Melanogaster Life-History Evolution

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Drosophila melanogaster is a model for understanding how organisms adapt to changing environments. Recent work has established that D. melanogaster evolves rapidly and predictably in response to seasonal selection. We focus on the microbiota as a variable influence on phenotypes that evolve in response to seasonal selection in a Pennsylvania orchard. Lactic acid bacteria have been shown to favor life history traits associated with somatic maintenance, and acetic acid bacteria with life history traits associated with reproduction. We hypothesized that manipulating the microbial communities of fly populations might influence those populations' phenotypes after seasonal selection. We reared Drosophila melanogaster in diet-controlled outdoor mesocosms for five generations with different bacterial treatments. For three treatments, each prepared in triplicate, the diets were undisturbed or inoculated with either an AAB or LAB strain. Preliminary culture-based assessments of bacterial communities in the flies and their diets confirmed that diet inoculation was sufficient to lead to different microbial communities in LAB and AAB inoculated flies. Following outdoor selection, the sixth generation from the selected populations were reared in the laboratory with each of a single species of LAB, AAB, or left bacteria free, and their development rate and starvation resistance were measured. Our findings suggest that the presence of LAB (L. brevis), which normally confers slow development rates on the flies, preferentially led to accumulation off rapid development-promoting mutations in the evolved fly populations. Taken together, these findings show that differences in microbiome composition are sufficient to induce differential adaptation within a heterogenous fly population.

Poster #149

Environmental pH Drives Developmental Phenotypes Through Modulation of Outer Membrane Vesicle Proteins

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As they move between habitats, bacteria must adjust their physiology to survive changing ambient conditions. For instance, facultative symbionts alter their biology as they transit between free-living and host-associated states. Strains of the bioluminescent marine bacterium Vibrio fischeri live either planktonically or in association with a variety of marine organisms, including the Hawaiian bobtail squid, Euprymna scolopes. One environmental change that V. fischeri experiences as it moves from the ocean to the symbiotic organ of the squid is a sharp, transient drop in pH. Outer membrane vesicles (OMVs) are potent inter-organismal communication mediators, and have previously been shown to initiate phenotypes characteristic of symbiosis in the Euprymna-Vibrio association. We report that, in response to acidic pH, V. fischeri alters the protein composition of its OMVs. One major alteration is increasing the relative amount of the dominant outer membrane protein, OmpU. This increase in OmpU loading is sufficient to affect host phenotypes, indicating that environment-driven changes in OMV composition can impact symbiosis. Interestingly, these pH-driven effects are conserved across both symbiotic and free-living V. fischeri strains, and are apparently not controlled by some well-described regulators of OmpU homologs in other Vibrionaceae, such as OmpR and ToxR. We are currently exploring novel regulators of this effect, as well as examining the importance of OmpU's porin activity in symbiotic development. Our findings suggest a general model in which environmental cues cause a bacterium to shift its signaling capacity towards symbiotic stimulation through differential loading of an outer membrane protein into its OMVs.

Poster #150

Dietary Arabinoxylan Assimilation in the Gut Symbiont Eubacterium Rectale

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The Firmicutes are an important phylum that colonizes the human gut. They are considered carbohydrate-degrading specialists for their ability to use a narrow spectrum of carbon sources from our daily diet. Eubacterium rectale is a butyrate-producing Firmicute that can degrade several indigestible fibers such as arabinoxylan into oligo- or monosaccharides which may cross-feed other beneficial gut microbes. Arabinoxylan is a major component of soluble dietary fiber in the plant cell wall of cereal grains. It is composed of β-1,4 linked xylose and substituted by several 2,3-linked arabinose units. E. rectale degrades arabinoxylan via the action of a glycoside hydrolase family 10 (GH10) enzyme comprised of a catalytic domain and three family 9 carbohydrate-binding modules (CBM9A,B,C). The full-length enzyme displays robust activity towards wheat arabinoxylan and xylan (from birch wood) with little activity on corn, sorghum or rice arabinoxylan. Affinity electrophoresis revealed that CBM9A binds arabinoxylan and is required for activity on the fiber, while CBM9B/C are not essential. Once arabinoxylan is hydrolyzed at the cell surface, two ABC transporters are predicted to scavenge the released oligosaccharides. Using isothermal titration calorimetry (ITC) we have demonstrated that one of the ABC solute-binding proteins, Eur_20790, binds xylohexose suggesting this transporter may favor longer oligosaccharides. Our on-going work is focused on obtaining a molecular picture of arabinoxylan recognition via protein crystallography, as well as identifying other fiber degrading pathways in E. rectale using RNAseq. Overall these data elucidate how a prominent butyrate-producing gut species access dietary fiber in the competitive gut environment.

Poster #151

Examining Nematode Surface Structures That Could Aid Specific Bacterial Symbiont Colonization

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The Goodrich-Blair lab is interested in discovering the mechanisms by which hosts and symbionts recognize each other and begin cell-cell interactions. To study host-symbiont interactions, we use the model system of soil dwelling Steinernema nematodes, and their obligate symbionts, Xenorhabdus, which together parasitize insects. Xenorhabdus bacteria localize to a region of the nematode intestine called the anterior intestinal cecum, or AIC. My hypothesis is that the nematode excretes in this area (relative to other regions of the intestine) a mucus containing distinctive sugar moieties with which Xenorhabdus physically and specifically interacts. First, fluorescently labeled lectin dye WGA which interacts with glycans NAG and NAM was applied to three nematode species, S. carpocapsae, S. scapterisci, and S. feltiae. Results show that there is species level variation in WGA reactive glycan expression in the AIC among Steinernema species used. With S. carpocapsae expressing the highest levels of glycan expression. Secondly application of unconjugated WGA dye as well as GFP expressing bacterial symbionts concurrently shows bacterial symbiont X. nematophila, the symbiont of S. carpocapsae has reduced colonization rates as compared to S. carpocapsae that has not been exposed to unconjugated WGA. This lends itself to the hypothesis that there is species specific glycan expression in Steinernema nematode species at the AIC, and that X. nematophila binds NAG/NAM within its nematode host.

Poster #152

Probing Functions of the Host-range Specificity Determinant NilB, a DUF560 Protein

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Symbioses are ubiquitous and can involve specificity between partners. Insights into the molecular mechanisms governing specific partner recognition during acquisition of symbionts are being gained through the investigation of diverse symbiotic systems. We study the mutualistic and species-specific interaction between entomopathogenic Steinernema nematodes and the Xenorhabdus spp. bacteria that colonize their intestines. The bacterial integral outer-membrane surface-exposed protein NilB is required for X. nematophila to colonize its host, S. carpocapsae. NilB belongs to the largely uncharacterized DUF560 family of proteins, which are encoded by diverse symbionts of animals. Previous mutational analyses revealed NilB topology and functional sequences, including surface exposed loops and a periplasmic domain that are essential for colonization. Our analysis of DUF560 homologs though sequence similarity networks and genome neighborhood networks suggest several models of NilB function: (i) the surface-exposed loops of NilB may mediate adherence to nematode intestines; (ii) the beta-barrel domain of NilB may aid cross-membrane transport of molecules/proteins that are necessary for colonization. This second model is supported by the function of the only other characterized DUF560 homologs, Neisseria meningitidis SLAM proteins. SLAM proteins flip outer-membrane localized lipoproteins from periplasmically-oriented to surface-exposed in a substrate-specific manner. Our current work (funded by NSF IOS-1353674) is geared toward testing if NilB similarly acts upon the outer-membrane localized lipoprotein NilC, a colonization factor. Since experimental evidence indicates NilC is periplasmically oriented during X. nematophila growth in lab media, we hypothesize that NilB activity is regulated such that NilC is exposed to the cell surface only under certain conditions.

Poster #153

Investigating the Relationship Between Intestinal and Skin Disorders in the Context of Immunet Tolerance to Commensals

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The intestinal tract and skin are major barrier sites that house complex microbial communities capable of influencing host immunity. Under homeostatic conditions, tissue-resident microbes are thought to have a dominant impact on local immune cell function. However, the prevalence of neutrophilic skin disorders among patients with Inflammatory Bowel Disease (IBD) and following certain forms of bowel-bypass surgery suggests that this compartmentalized control may not hold under conditions of host-microbe dysbiosis. We hypothesize that in instances of intestinal inflammation, skin disease may result from inappropriate immune responses directed at skin commensal microbes. We have shown previously using an in vivo model to track S. epidermidis-specific CD4+ T cells that immune tolerance to skin commensals is established early in life. Here, we employ new tools to simultaneously track the antigen-specific responses to both skin and gut commensals in the context of chemical and genetic experimental colitis models. In the former, administration of 3.5% or 2% dextran sodium sulfate (DSS) induces acute or chronic colitis. In the latter, inducible deletion of A20 and ABIN, members of the NF-B inhibitory pathway, in the intestinal epithelium prompts acute gut inflammation. Colonizing mice with commensal bacteria engineered to express antigens of interest, we are able to measure the impact of intestinal inflammation on microbe-specific CD4+ T cells in both barrier tissues. We find that DSS-induced chronic colitis leads to loss of immune tolerance to S. epidermidis, as demonstrated by reduced frequencies of antigen-specific regulatory T cells. This and other preliminary results will be discussed.

Poster #154

Metabolic Model-Based Evaluation of Microbiome-Metabolome Association Studies

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Metabolic variation between microbiomes can have important impacts on host health, and identifying the microbial drivers of such variation is a major research goal. Paired microbiome-metabolome studies are an increasingly widespread approach to address this challenge. A common method for integrating the resulting data is univariate correlation analysis of species and metabolite abundances. To date, however, the limitations and interpretation of this paradigm have not been evaluated. To address this challenge, we used a multi-species genome-scale metabolic modeling framework to simulate taxonomic and metabolomic data from simplified gut communities with varying composition. We developed a mathematical definition of the true contributed impact of each microbial taxon on metabolite variation based on uptake and secretion fluxes, and then assessed the capacity of microbe-metabolite correlation analysis to recover the key microbial contributors in our simulated dataset. We found that microbe-metabolite correlation analysis poorly predicts key microbial contributors even in these simplified settings, with a ~74% false discovery rate and an AUC of 0.72. We investigated what factors determine whether a microbe-metabolite correlation coincides with a true contribution, identifying properties of both species and metabolites. We further demonstrated that exogenous environmental metabolite variation can strongly influence the sensitivity and specificity of detected correlations, but positive predictive value remains low across all conditions. We have used our findings to inform a set of best practices for the analysis and interpretation of microbiome-metabolome studies.

Poster #155

NOD2 Suppresses Pathogenic Inflammatory Responses Toward Saccharomyces Cerevisiae.

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Commensal fungi residing in the intestinal microbiome play an important and often overlooked role in shaping the intestinal immune environment. Emerging research has demonstrated that intestinal fungi can exacerbate pathogenic inflammation and that this fungal community often blooms in people suffering from inflammatory bowel disease. Host and fungal features that drive pathogenic inflammation are still largely unknown. Aberrant antifungal immune responses are associated with Crohn's disease (CD) and systemic antifungal antibodies (anti-saccharomyces antibodies or ASCAs) are present in 41-76% of patients. Two of the most prevalent and abundant commensal fungi, Saccharomyces cerevisiae and Candida albicans, are able to induce ASCAs in mouse models and are both recognized by human ASCAs. In this study, we investigate immune interactions between S. cerevisiae and C. albicans in mice lacking the NOD2 innate immune receptor, which is the most commonly mutated gene in CD. We find that NOD2 is particularly important for protecting mice against S. cerevisiae-induced inflammation. Using in vitro co-culture experiments, we find dendritic cells lacking NOD2 have significantly different cytokine responses toward both S. cerevisiae and C. albicans and promote more inflammatory CD4+ T cell responses. These data suggest an important role of NOD2 in suppressing inflammatory responses toward fungi and also highlight important differences between the immune responses elicited by S. cerevisiae and C. albicans.

Poster #156

Combined Low Dietary Fiber and Mucus-Degrading Symbiotic Gut Bacteria Cause Lethal Colitis in Mice

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The precise etiology of the disorders collectively known as inflammatory bowel diseases (IBDs) remains unknown. Despite >100 genetic polymorphisms associated with IBDs, host genetics explains only a fraction of disease risk suggesting that environmental factors such as diet and gut microbes play a critical, causal role. Using a gnotobiotic mouse model, in which animals were colonized with a synthetic human gut microbiota composed of fully sequenced and metabolically characterized commensal bacteria, we have begun to elucidate the mechanistic interactions between dietary fiber, the gut microbiota and the colonic mucus barrier, which serves as a primary defense against encroachment by intestinal bacteria. During dietary fiber deficiency, the gut microbiota resorts to host-secreted mucus glycoproteins as a nutrient, leading to erosion of the mucus layer. Dietary fiber deprivation, together with a fiber-deprived, mucus-eroding microbiota, promotes lethal colitis in mice deficient in interleukin 10, a cytokine that normally dampens inflammation and for which loss of function defects have been associated with human IBD. In contrast, isogenic wild-type mice do not experience disease, revealing that inflammation develops in a diet- and microbiota-specific fashion, but only in the context of an underlying host defect. Removing mucus-degrading species from the synthetic microbiota abrogates disease in genetically-susceptible, fiber-deprived mice, providing an additional link to microbial causation. Recombinant expression of enzymes hypothesized to be involved in causal mucus erosion and eventual inflammation have begun to reveal the molecular pathways involved in this disease model and provide targets for therapeutics to block disease progression.

Poster #157

A Polyketide Synthase Cluster in Lactobacillus Reuteri R2lc Activates the Aryl-Hydrocarbon Receptor

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Mechanistic understanding of host-microbe interactions is critical to developing therapeutic strategies for targeted modulation of the host immune system. Different members of the gut symbiont species Lactobacillus reuteri modulate host health by, for example, reduction of intestinal inflammation and pathogen inhibition. Previously, L. reuteri has been shown to activate the aryl-hydrocarbon receptor (AhR)—a ligand-activated transcription factor that plays an important role in the mucosal immune system—by producing tryptophan metabolites. Here, we identified a novel pathway by which L. reuteri activates AhR. We determined that L. reuteri R2lc and L. reuteri 2010, strains with a pigmented phenotype, are potent AhR activators. By whole genome sequencing and comparative genomics, we identified genes unique to R2lc and 2010. Our analyses showed that R2lc harbors two multi-copy plasmids, each encoding a distinct polyketide synthase (pks) cluster, pksR2lc01 and pksR2lc02. Inactivation pksR2lc02, but not pksR2lc01, abolished the AhR activation ability of R2lc. Similarly, L. reuteri 2010 has a homologous gene cluster to pksR2lc02 with an identical gene organization. Together, we propose that L. reuteri R2lc and 2010 produce a secondary metabolite that modulates the host immune system via activation of the AhR pathway. Understanding the mechanisms by which probiotics promote health provides opportunities for targeted strain improvement to develop next-generation probiotics.

Poster #158

The Mechanism of Resistant Starch Degradation by Bifidobacterium Adolescentis

Amanda Photenhauer[1], *Nicole Koropatkin*[1]
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The consumption of dietary resistant starch (RS) has been linked to a lower incidence of colorectal cancer and intestinal inflammation, as well as an improvement in blood glucose levels. Many of these benefits may be mediated by the gut microbial community via the production of butyrate, a short chain fatty acid that serves as an energy source for colonocytes with potent anti-inflammatory and anti-tumorigenic properties. Few bacterial species have been identified that can utilize RS as a sole carbon source. Three key primary degraders of RS include Bifidobacterium adolescentis, Bifidobacterium faecale, and Ruminococcus bromii. These bacteria increase in relative abundance upon administration of RS but the mechanism whereby these species degrade RS has not yet been elucidated.

Our previous work has demonstrated in part that the ability to degrade starch relies on either the interaction and cooperativity between enzymatic domains and carbohydrate-binding modules (CBMs) within carbohydrate active enzymes (CAZymes) or the assembly of these functions from separate polypeptides. The genome of B. adolescentis encodes several proteins that potentially function to degrade starches. Here we bioinformatically identify proteins involved in binding or hydrolyzing different starches indicated by the presence of CBMs or glycoside hydrolase family 13 (GH13) enzymes respectively. After recombinantly expressing and purifying these candidate proteins, we measured binding affinity for and enzymatic activity on different starches including resistant potato starch. With these approaches we ultimately seek to determine the strategy of RS breakdown in B. adolescentis in order to better understand the health benefits of resistant starch consumption.

Poster #159

The Role of TonB in Polysaccharide Utilization by the Bacteroidetes

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The human gut microbiota is required for the degradation of otherwise undigestible dietary and host-derived polysaccharides. These polysaccharides are a key energy source for the gut microbiota and fermentation products such as short chain fatty acids are beneficial to the human host. The Bacteroidetes are the prominent contributors to polysaccharide degradation in the gut and the model system for this degradation is the starch utilization system (Sus) in Bacteroides thetaiotaomicron (Bt). All Sus-like systems contain three key components: 1. a SusC-like protein, a hypothesized TonB-dependent transporter; 2. a SusD-like protein, an accessory polysaccharide-binding protein; and 3. a transcriptional regulator, SusR in the Sus system. Despite the importance of the SusC-like protein in these systems, little work has been done to confirm the TonB-dependence of transport through these proteins or to characterize the TonB-SusC pair. To address this question, we have generated Bt strains in which each of the 10 TonB genes are deleted. Using these single mutant strains as well as strains with multiple TonB deletions, I have shown that deletion of TonB genes affects nutrient uptake but no single TonB gene is essential for Bt growth on starch. This mechanistic understanding of how Bt and related Bacteroidetes use polysaccharides to establish their niche in the microbiota will allow us to design non-invasive approaches for optimizing the gut community and improving patient outcomes caused by a lack or overgrowth of Bacteroidetes.

Poster #160

Going Places: a Bioinformatic Analyses of the Wigglesworthia Flagellum

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Obligate symbionts purge genes unnecessary within their hosts, resulting in small genomes. For example, the tsetse fly (Diptera: Glossinidae) symbiont, Wigglesworthia spp., has a small genome that complements host biology. Interestingly, Wigglesworthia genomes still retain the capacity for flagella assembly, accounting for 6% of the minute genome. Although the majority of orthologs are present (i.e. 37 of 39), a Wigglesworthia flagellum has yet to be observed or ascribed function. Here, we perform in silico analyses comparing Wigglesworthia flagella genes to those of other bacteria spanning different lifestyles to elucidate on the adaptive value of the flagellum within tsetse. Using Tajima's relative rate test, comparable rates of evolution were observed in the majority (i.e. 92%) of Wigglesworthia flagella genes, relative to Salmonella typhimurium and Escherichia coli. When classified by functions, both "flagellum regulation" and "hook and filament" demonstrated a higher proportion of genes either missing or exhibiting atypical mutation rates suggesting biologically driven modifications for accommodating the symbiosis. Further, a strong correlation (R^2= 0.8) was observed between the proportion of structural and non-structural flagella genes preserved within genomes of endosymbionts and their free-living relatives, with the Wigglesworthia genome clustering with motile Gammaproteobacteria. Surprisingly, a relationship between genome size and the proportion of retained flagellar genes was not observed (R^2= 0.2), suggesting that the selection for genome reduction does not drive the loss of flagella. The retention of flagella genes by Wigglesworthia, despite tremendous relaxed selection on the genome, supports the utility of these genes despite unknown symbiotic roles, warranting further investigation.

Poster #161

Interactions Between the Gut Microbiome and Host Gene Regulation Shed Light on the Pathogenesis of Colorectal Cancer in Cystic Fibrosis Patients

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Cystic Fibrosis (CF) is the most common autosomal recessive genetic disease in Caucasians. It is caused by mutations in the CFTR gene, leading to poor hydration of mucus and impairment of the respiratory, digestive, and reproductive organ functions. Advancements in medical care have lead to markedly increased longevity of patients with CF, but new complications have emerged, such as early onset of colorectal cancer (CRC). Although the pathogenesis of CRC in CF remains unclear, altered host-microbe interactions might play a critical role. Here, we characterize the changes in the gut microbiome and host gene expression in colonic mucosa of CF patients relative to healthy controls. We find that CF patients show decreased microbial diversity, decreased abundance of taxa such as Butyricimonas, Sutterella and Ruminococcaceae, and increased abundance of other taxa, such as Actinobacteria and Acidaminococcus. We find that 1543 host genes, including CFTR, show differential expression in CF patients relative to healthy controls. Interestingly, we find that these genes are enriched with functions related to gastrointestinal cancer, such as metastasis of CRC, tumor suppression, cell proliferation and apoptosis. Lastly, we modelled associations between gut microbiota abundances and host gene expression, and identified CRC-related genes, including LCN2 and DUOX2, that are correlated with CRC-associated bacteria, such as Ruminococcaceae and Veillonella. Our results provide new targets for potential treatment and therapeutic research for improving patient outcomes in CF.

Poster #162

A Metagenomic Meta-analysis Reveals Functional Signatures of Health and Disease in the Gut Microbiome

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Recent research indicates that human health depends, in part, upon the symbiotic relationship between gut microbes and their host. However, the specific interactions between host and microbe that define health are poorly resolved. Metagenomic clinical studies clarify this definition by revealing gut microbial taxa and functions that stratify healthy and diseased individuals. However, the typical single-disease focus of microbiome studies limits insight into which microbiome features robustly associate with health, indicate general deviations from health, or predict specific diseases. Additionally, the focus on taxonomy may limit our understanding of how the microbiome relates to health given observations that different taxonomic members can fulfill similar functional roles. To improve our understanding of the association between the gut microbiome and health, we integrated ~2,000 gut metagenomes obtained from eight clinical studies in a statistical meta-analysis. We identify characteristics of the gut microbiome that associate generally with disease, including functional alpha-diversity, beta-diversity, and beta-dispersion. Moreover, we resolve microbiome metabolic modules that stratify diseased individuals from controls in a manner independent of study-specific effects. Many of the deferentially abundant functions overlap multiple diseases suggesting a role in health, while others are specific to a single disease and may associate with disease-specific etiologies. Our results clarify potential microbiome-mediated mechanisms of disease and reveal features of the microbiome that may be useful for the development of microbiome-based diagnostics. Ultimately, our study clarifies the definition of a healthy microbiome and how perturbations to it associate with disease.

Poster #163

Contributions of Cecal Microorganisms to an Obligate Fat Storing Hibernator

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The intestinal microbiome has been shown to greatly contribute to the health of homeothermic mammals, however little is known about the role of the microbiome during heterothermy. The thirteen-lined ground squirrel (Ictidomys tridecemlineatus) is an obligate fat storing hibernator that nearly doubles its body mass in fat prior to hibernation. During this time, the ground squirrel undergoes an extended period of depressed metabolism and relies almost entirely on these fat stores to survive the winter months with no food. This adaptive fasting eliminates the degradable substrates that are available to the intestinal microbiome during active months. To determine how the cecal microbiome survives the winter and if it contributes to host health, we employed a combination of metagenomics, metabolomics, and cultivation techniques. We found that during hibernation, the cecal microbiome undergoes a decrease in phylogenetic diversity and a ten-fold decrease in abundance by colony counts, yet no changes in spore concentrations. This suggests that the microbiome is adaptable to the lowered hibernation body temperature (~5ºC). Shotgun metagenomics from five fall active and five torpid revealed increased abundance of genes involved in vitamin production and changes in nitrogen metabolism during hibernation. This increase in microbial vitamin production is supported by metabolomics which also showed changes in bile acid, fatty acid and amino acid composition, suggesting that the microbiome is important to the hibernating squirrel's health, playing a central metabolic role during hibernation. By combining metagenomic analyses to metabolomics, we can begin to unlock the metabolic potential of the hibernator microbiome.

Poster #164

Regulation of Colonization Behavior by the Histidine Kinase BinK

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The light organ of the Hawaiian bobtail squid, Euprymna scolopes, is colonized exclusively by a single species of luminescent bacterium, Vibrio fischeri. The formation of a biofilm aggregate in host mucus at the entrance of the light organ is required for V. fischeri to robustly colonize the squid. The lab recently identified BinK as a histidine kinase that inhibits biofilm formation. My work is focused on determining how BinK modulates biofilm transitions during colonization.
BinK was identified in a transposon insertion sequencing screen for colonization factors. During colonization, ΔbinK mutants outcompete wild type. In contrast, overexpressing binK prevents colonization. ΔbinK strains exhibit increased biofilm formation in culture, enhanced aggregate formation in the host, and decreased motility in soft agar. We have evidence that BinK acts to negatively regulate transcription of the symbiosis polysaccharide (syp) genes, which are activated by a phosphorelay as part of the canonical pathway for V. fischeri biofilm formation. This suggests a model where BinK is regulated upon contact with the host to repress its inhibition of biofilm. Mutation of predicted phosphorylation sites in the BinK histidine kinase (H362Q) or receiver (D794A) domains render the protein nonfunctional, suggesting that phosphotransfer is necessary for BinK biofilm inhibition. Further work will continue this structure/function analysis of BinK and examine potential downstream signaling partners of BinK, including SypG. This will integrate BinK into a more detailed model of biofilm regulation leading to a better understanding of how V. fischeri transitions from the seawater environment to the squid host.

Poster #165

Epi-Metabolites: Discovering How Bacteria Affect Host Epigenetic States

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Introduction: Short chain fatty acids (SCFAs), a major fermentation product of gut bacteria, have many beneficial effects on host health. However, the mechanism behind these effects remains relatively unknown. We recently discovered that SCFAs can act as epigenetic metabolites, dramatically changing histone modifications in host tissues. Histones are the fundamental unit of chromatin – the structure that organizes eukaryotic DNA in the nucleus. Modifying histones changes the accessibility of DNA and thus ultimately affects transcription. The fact that bacterial metabolites may change the organization of host chromatin is intriguing, but we currently do not understand the cellular mechanism underlying this process.
Methods: We use mass spectrometry to track SCFAs through cellular metabolism and onto histones. By treating cells with ^{13}C-labeled SCFAs, we can measure labeled carbon in TCA cycle intermediates and its deposition onto histones. Results from these experiments identify important metabolic pathways, which we can then disrupt by knocking-out key enzymes. Combining knockout cell lines with ^{13}C-labeling will allow us to pinpoint which metabolic pathways contribute to SCFA metabolism and histone deposition.
Results: Preliminary results suggest that SCFA carbons are directly added to histones, causing dramatic increases in histone acetylation. The SCFAs acetate, propionate, and butyrate elicit different epigenetic effects, with butyrate producing the strongest increase in acetylation.

Poster #166

Characterization of Bile Salt Metabolism in Zebrafish

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Steroid bile salts serve important functions in digestive physiology. Produced in liver and released into intestinal lumen in bile, bile salts act as emulsifiers of dietary fats, are resorbed in ileum, and then trafficked to liver for reuse. In ileum and liver, bile salts also serve as signaling molecules that coordinate physiologic responses to nutrients through transcription factor farnesoid X receptor (Fxr/Nr1h4). Though bile salts are found in all vertebrates, our information on their functions is mainly derived from mammals. It remains unclear whether non-mammalian vertebrates possess a conserved bile salt signaling axis. We have recently developed zebrafish as a non-mammalian model to study bile salt metabolism. In silico analysis suggested that genes involved in bile metabolism are conserved in zebrafish including fxr and its transcriptional targets. CRISPR/Cas9-mediated mutation of zebrafish fxr revealed similar transcriptional signatures shown in Fxr knockout mice. This includes induction of hepatic bile salt biosynthesis enzyme cyp7a1 and repression of predicted Fxr targets shp, fabp6, and slc10a2, indicating a conserved regulatory network in zebrafish. Studies from mammals have demonstrated that intestinal microbiota regulate bile signaling pathways through chemical modification of bile salts. Our HPLC analysis of zebrafish bile revealed that zebrafish only possess one form of bile salt, suggesting that microbiota may not modify bile salt in zebrafish. Together, these results establish that the genetic and physiologic mechanisms of bile salt metabolism are conserved between fishes and mammals and provide critical foundation for using zebrafish to identify contributions of host and microbiota in bile salt metabolism.

Poster #167

Defining Nematode Responses to the Bacterial Pathogen/Symbiont Photorhabdus Luminescens

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Photorhabdus bacteria enter into a mutualistic symbiosis with Heterorhabditis nematodes to infect insect larvae. However, they rapidly kill the model nematode Caenorhabditis elegans. One hypothesis for these divergent outcomes is that the nematode defense responses differ. To begin testing this hypothesis, we have systematically analyzed available data on the transcriptional response of C. elegans to P. luminescens strain Hb. From a starting pool of over 7,000 differentially expressed genes, we carefully chose 21 Heterorhabditis-conserved genes to develop as comparative markers. Using newly designed and validated qRT-PCR primers, we measured expression of these genes in C. elegans exposed to the sequenced TT01 strain of P. luminescens, on two different media types. Almost all (18/21) of the genes showed a significant response to P. luminescens strain TT01. One response is dependent on media type, and a subset of genes may respond differentially to distinct strains. Overall, we have established useful resources and generated new hypotheses regarding how C. elegans responds to P. luminescens infection.

Poster #168

A Novel Screen and Identification of Potential Bioluminescence Regulatory Mutants in Photorhabdus Luminescens TT01

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Background
Of all host-microbe relationships involving a bioluminescent bacterial symbiont, only one terrestrial symbiosis has been discovered: nematode Heterorhabditis spp. partner with bacteria of the genus Photorhabdus. In the Heterorhabditis/Photorhabdus symbiosis, the significance of bioluminescence is not well understood. Equally poorly understood is the mechanism by which light production is regulated by the bacterial symbiont. In this study, we utilized a transposon mutagenesis approach to create mutants of P. luminescens TT01 and screen them for alteration in bioluminescence production during in vitro growth.

Methods and Results
A mini-Tn10 element was delivered to a strain of P. luminescens TT10 harboring an integrated pPINT-PluxC-mCherry element. The genomes of all Photorhabdus spp. sequenced to date contain an operon comprised of genes with homology to lux genes present in many Vibrio species. The gene luxC is the first gene in the Photorhabdus lux operon; upstream of luxC is a putative “promoter” sequence (that was cloned into the pPINT element). Mini-Tn10 mutagenesis of this strain led to the creation of a ~2000 isolate mutant library which was screened for alteration in fluorescence intensity over in vitro growth. A collection of ~10 “low” and “high” mCherry fluorescence intensity mutants were identified by the screen. These mutants were analyzed for bioluminescence production and found to have altered bioluminescence per unit growth in vitro.

Conclusion
Our results support the hypothesis that some growth-dependent expression of P. luminescens bioluminescence is regulated through sequence upstream of the start codon of the luxC gene in P. luminescens TT01.

Poster #169

Precision Editing of the Gut Microbiota Ameliorates Colorectal Tumorigenesis

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Chronic intestinal inflammation and members of gut microbiota play important roles in the development of colitis associated colorectal cancer (CAC). Enterobacteriaceae such as E. coli are among the most commonly overrepresented symbionts found in conditions involved in gut inflammation, such as CAC. Members of E. coli could promote the development of CAC via distinct mechanisms: 1) by exacerbating mucosal inflammation or 2) by producing DNA-damaging genotixin colibactin. Previously, we showed that Molybdenum-cofactor-dependent pathways, which are critical to the dysbiotic expansion of Enterobacteriaceae and are only operational during gut inflammation, can be selectively inhibited by tungstate treatment. Notably, precision editing of the microbiota reduced intestinal inflammation in mouse models of acute colitis. Here, we applied this approach to inhibit the bloom genotoxic Enterobacteriaceae family members to prevent CAC development. We showed that tungstate treatment reduced inflammation-associate expansion of E. coli population in the setting of CAC development. Importantly, tungstate-mediated microbiota editing reduced the severity of chronic intestinal inflammation, limited the production of carcinogenic genotoxins, and reduced the risk of subsequent tumor development in murine models of CAC. We conclude that precision editing of the microbiota composition is suitable strategy for ameliorating the adverse effects of dysbiosis in the setting of CAC.

Poster #170

The Role of Autoinducer-2 Quorum Signaling in Mixed Bacterial Community Structures

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Interspecies quorum sensing signal, Autoinducer-2 (AI-2), has been implicated in biofilm formation of a wide variety of Gram negative and Gram positive bacterial species. While AI-2 has been shown to alter biofilm structures through gene regulatory mechanisms common to other quorum signals, AI-2 is unique in that it has also been shown to alter biofilm structures by serving as a chemotactic cue that can disperse or recruit cells. AI-2 therefore serves as a unique opportunity to investigate how interspecies communication helps structure mixed bacterial communities, however much of the research involving AI-2 has been carried out in monoculture biofilms. I hypothesize that in multispecies communities, AI-2 chemotaxis and AI-2 quorum sensing, are an important yet distinct factors in how multi species bacterial communities spatially structure themselves. I will use zebrafish microbial communities to test my hypothesis.
I have screened through zebrafish bacterial isolates and found several isolates that produce AI-2. I then assessed biofilm formation of these isolates and found that upon the addition of exogenous AI-2, Vibrio spp. (ZWU0020) and E. coli (HS) displayed increased biofilm formation. Interestingly, this biofilm phenotype was abolished in chemotaxis mutants, suggesting that AI-2 associated biofilm phenotypes were chemotaxis dependent. Using capillary assays I then found that AI-2 is a chemoattractant for both Vibrio and HS. I have since generated AI-2 synthesis and AI-2 sensor knockouts in Vibrio and HS and plan to image the structure of in vitro mixed culture biofilms, as well as image the structures of these communities in vivo.

Poster #171

Genetic Determinants of Stability and Emergent Properties Within a Model Microbial Community

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Understanding microbial community stability and function are crucial to manipulating microbiomes for the benefit of human and agricultural health. Using a model system composed of Bacillus cereus, Flavobacterium johnsoniae and Pseudomonas spp., several emergent properties based on community interactions have been identified. Importantly, the three phyla represented in this model are the three dominant phyla on plant roots and in the human gut: Firmicutes, Bacteroidetes, and Proteobacteria. Community assembly, stability, invasion, and function can all be assessed using the same simplified model community. To assess community assembly and stability, we inoculated sterilized sand in test tubes to simulate the native environment. We found that F. johnsoniae was eliminated from the community after a week. To assess community function, we took advantage of an emergent biofilm property seen in 96-well plates. Together, these species produce more biofilm than any of the three species singly and, therefore, provide the basis for the first genetic analysis of a phenotype that requires all members of a three-membered community. Using transposon mutant libraries in each species, we hope to identify candidate genes that stabilize the presence of F. johnsoniae in the community, and start to characterize the mechanisms driving the emergent biofilm property. By viewing the community as a genetic entity, novel community-specific mechanisms could potentially be revealed that are not evident in monoculture. Conducting invasion studies using fluorescently-labeled strains in both the sand and biofilm platforms will allow us to test ecological hypotheses regarding the relationship of invasion with community stability and function, respectively.

Poster #172

Cutibacterium (Propionibacterium) Acnes Antibiotic Production Shapes Niche Competition in the Healthy Human Skin Microbiome

Katherine P Lemon[2,8], Jan Claesen[1], Jennifer B Spagnolo[2], Kenji Kurita[3], Allyson L Byrd[4], Stephany Flores-Ramos[2], Weng R Wong[5], Ryan D Hernandez[6], Julie A Segre[4], Roger G Linington[3], Michael A Fischbach[7]
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Little is known about how bacteria of the nasal and skin microbiome shape the composition and function of the community in which they live. Cutibacterium (formerly Propionibacterium) and Staphylococcus are both highly prevalent and abundant on human skin, including that of the nostrils. We identified and elucidated the structure of a Cutibacterium-produced thiopeptide, dubbed cutimycin, which shapes skin microbiome composition. Nanomolar amounts of cutimycin inhibit Staphylococcus aureus and epidermidis but not Corynebacterium and Cutibacterium species, all of which are common on the skin the nostrils. Analyzing metagenomic data from multiple skin sites in 12 people, we found that cutimycin-producing Cutibacterium are present on up to 80% of people and account for up to 30% of strains in a skin site. To test whether cutimycin in human follicles can shape microbiome composition, we used Bioré pore strips to collect human follicular content and observed a positive correlation between the presence of the cutimycin biosynthetic gene cluster (BGC) in individual follicles, as detected by PCR, and a higher Cutibacterium-to-Staphylococcus ratio, as detected by colony forming units (CFUs) of each in follicular content. There were some follicles with a high Cutibacterium-to-Staphylococcus ratio in which we did not detect the cutimycin BGC by PCR suggesting there is a second agent or mechanism that favors an increased ratio of Cutibacterium to Staphylococcus. These findings support our overarching hypothesis that the human microbiome is a rich, untapped source of discoveries that will lead to new insights into microbial community structure, as well as host immune response.

Poster #173

The Importance of Community Context in Recovery from Antibiotic Perturbation

Katharine M Ng[1], Carolina Tropini, Andres Aranda-Diaz[1], Justin L. Sonnenburg[1], Kerwyn Casey Huang[1]
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The use of antibiotics has saved countless lives since their discovery nearly a century ago. Unfortunately, the overzealous prescription of antibiotics has led to a rise in resistance and has wide-ranging effects on the communities of commensal bacteria that live on and within the host, underscoring the need for antibiotics that minimize damage to commensals. Previous efforts to understand the effects of antibiotics on commensals have focused on pure cultures of individual bacteria. These in vitro results are not always mirrored in clinical and animal models, forming a body of literature that is often contradictory and inconsistent. Understanding factors that underlie a bacteria's response to antibiotics within a complex host-associated community will require integration of in vitro and in vivo data and careful dissection of quantifiable aspects of the host environment and community. We show that the microbiota has a robust response to antibiotic perturbation, even after a massive 105-fold drop in bacterial load, showing recovery during antibiotic treatment in a housing-dependent manner. The coarse-grained taxonomic kinetics of this recovery are relatively independent of microbiota context, antibiotic target, multiple antibiotic treatments, or dietary shifts, although dietary shifts result in a much larger perturbation to the bacterial load. We demonstrate that the recovery is dominated by a few species, and the final state after stabilization exhibits signs of substantial extinction that are not predictable based on in vitro growth in pure culture. These results highlight the importance of community context in the sensitivity of bacteria to antibiotics.

Poster #174

Impact of Chemical Communication within an In Vitro Microbial Community.

Jorge Peña-Díaz[1,2], Anna Creus-Cuadros[1,2], Mihai Cirstea[1,2], Antonio Serapio-Palacios[1,2], B. Brett Finlay[1,2]
[1]University of British Columbia, [2]Michael Smith Laboratories

The human intestinal microbiota comprises approximately 100 trillion bacteria from up to 800 different species. Imbalances in microbiota composition (dysbiosis) have been associated with a wide range of human disorders including obesity, inflammatory bowel disease, Alzheimer's disease, and asthma. Despite the clear link between gut heath and microbiota composition, the importance of bacterial communication to the maintenance of overall gut homeostasis remains largely unknown. To identify possible cross-species interactions between microbiota members, an artificially defined in vitro model was engineered by selecting a consortium of 10 bacterial strains representative of the gut microbiota of healthy individuals. To identify interventions capable of altering the population dynamics of the community, we probed our model system with a variety of treatments. Preliminary results show that the addition of a specific prebiotic affects the distribution of individual members within the community, leading to a higher abundance of Bacteroides and lower proportions of Proteobacteria, which is associated with gut health. Future work will focus on determining the 16s modulation profile caused by the addition of exogenous bacterial metabolites; such small molecules have previously been shown to have a significant impact on microbial community structure. Overall this work will attempt to characterize how specific molecules can shift dysbiotic communities towards a health-associated state, and how bacterial communication shapes population dynamics within the gut microbiota.

Poster #175

Vitamin B12 in Gut Bacteroides

Emily Putnam[1], Aaron Wexler[1], Andrew Goodman[1]
[1]Yale University

Our understanding of the factors that shape gut microbial community composition is largely based on the primary economy of this ecosystem: the flow of carbon from the diet to bacterial biomass and fermentation products. However, an accompanying secondary economy of essential vitamins and other cofactors, which are much less abundant, also plays a critical role in determining bacterial growth rates and resulting microbiome dynamics. Vitamin B12 is one such molecule. Bacteroides thetaiotaomicron and other commensals rely on elaborate machinery to recognize and capture vitamin B12 and related corrinoids from the gut environment. These species encode a number of genes of unknown function that are co-regulated with known B12 transport genes. We are using a combination of genetic and biochemical techniques to understand how human gut Bacteroides transport corrinoids and how corrinoids produced by other microbes impact Bacteroides physiology.

Poster #176

In Ovo Microbial Communities: A Potential Mechanism for the Initial Acquisition of Gut Microbiota Among Oviparous Vertebrates

Brian Trevelline[1], Kirsty MacLeod[2], Sarah Knutie[3], Tracy Langkilde[2], Kevin Kohl[1]
[1]University of Pittsburgh, [2]The Pennsylvania State University, [3]University of Connecticut

Vertebrate gut microbiota mediate critical physiological processes known to affect host fitness, but the mechanisms that expose wildlife to pioneer members of this important microbial community are not well understood. For example, oviparous vertebrates are thought to acquire gut microbiota through post-natal exposure to the external environment, but recent evidence from placental mammals suggests that the vertebrate reproductive tract harbors microbiota that may inoculate offspring in utero. These findings suggest that oviparous vertebrates may be capable of acquiring pioneer microbiota in ovo, but this phenomenon remains unexplored. To fill this knowledge gap, we used culture-independent inventories to determine if the eggs of wild birds and lizards harbored in ovo microbial communities. Our approach revealed robust and distinct in ovo bacterial communities, but fungal communities were indistinguishable from controls. Further, the bacterial community structure of lizard eggs from the same clutch were more similar to each other than to unrelated individuals. These results suggest that oviparous vertebrates may acquire maternal microbiota in ovo, possibly through the inoculation of egg yolk prior to shelling. Therefore, this study may provide a first glimpse of a phenomenon with substantial implications for our understanding of the ecological and evolutionary factors shaping gut microbial communities.

Poster #177

The Microbiome of the Marine Bryozozoan *Bugula neritina* is Shaped by the Presence of its cytotoxin-Producing Symbiont

Natassia Patin[1]
[1]Georgia Institute of Technology

The bryozoan *Bugula neritina* is a marine invertebrate found in shallow temperate coastal waters on the east and west coasts of North America. Some animals, but not all, are associated with a bacterial symbiont, the uncultured *Ca*. Endobugula sertula, which produces a suite of protein kinase C inhibitors called bryostatins. Compound levels are particularly high in *B. neritina* larvae and are thought to deter predation. Past microbial studies have largely focused on *Ca*. E. sertula because of its link to the bryostatins, and very little is known about other potential microbial partners. In this study, we explored the composition and diversity of *B. neritina*'s entire microbiome using using 120 samples collected from various locations and times of year on the East Coast of North America. We found a wide range in the relative abundance of *Ca*. E. sertula, which composed anywhere from <1% to 53% of the microbiome. The presence of the symbiont appears to drive the structure of the rest of the microbiome as predicted by the machine learning Random Forest algorithm. Latitude also affects the likelihood of *Ca*. E. sertula being present in the host, with lower latitudes featuring more animals containing the symbiont. Phylogenetic analysis shows microdiversity of the 16S rRNA amplicon sequences among the samples, and we are investigating whether or not this diversity is correlated with host-specific or environmental factors. Our study highlights the complexities of a host-associated microbiome that has been largely characterized by the presence of one taxon, but which in fact features a diversity of microbial life that is likely affected in as-yet unknown ways through chemical interactions with *Ca*. Endobugula sertula.

Poster #178

Dysbiosis-Associated Change in Host Metabolism Generates Lactate to Support *Salmonella* Growth

Caroline C. Gillis[1], Elizabeth R. Hughes[1], Luisella Spiga[1], Maria G. Winter[1], Wenhan Zhu[1], Tatiane Furtado de Carvalho[2], Rachael Chanin[1], Cassie L. Behrendt[3], Lora V. Hooper[3,4], Renato L. Santos[2], Sebastian E. Winter[1]
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During *Salmonella*-induced gastroenteritis, mucosal inflammation creates a niche that favors the expansion of the pathogen population over the microbiota by unknown mechanisms. We show that *S.* Typhimurium infection was accompanied by dysbiosis, decreased butyrate levels, and substantially elevated lactate levels in the gut lumen. Administration of a lactate dehydrogenase inhibitor blunted lactate production in germ-free mice, suggesting that lactate was predominantly of host origin. Depletion of butyrate-producing Clostridia, either through oral antibiotic treatment or as part of the pathogen-induced dysbiosis, correlated with increased lactate levels. Administration of tributyrin or a PPARg agonist altered host cell metabolism and diminished lactate production. Lactate utilization by *S.* Typhimurium required the terminal oxidase CydAB. We conclude that alterations of the gut microbiota, specifically a depletion of Clostridia, reprogram host metabolism to perform lactate fermentation. This work elucidates a link between the metabolism of the gut microbiota, the host, and *S.* Typhimurium.

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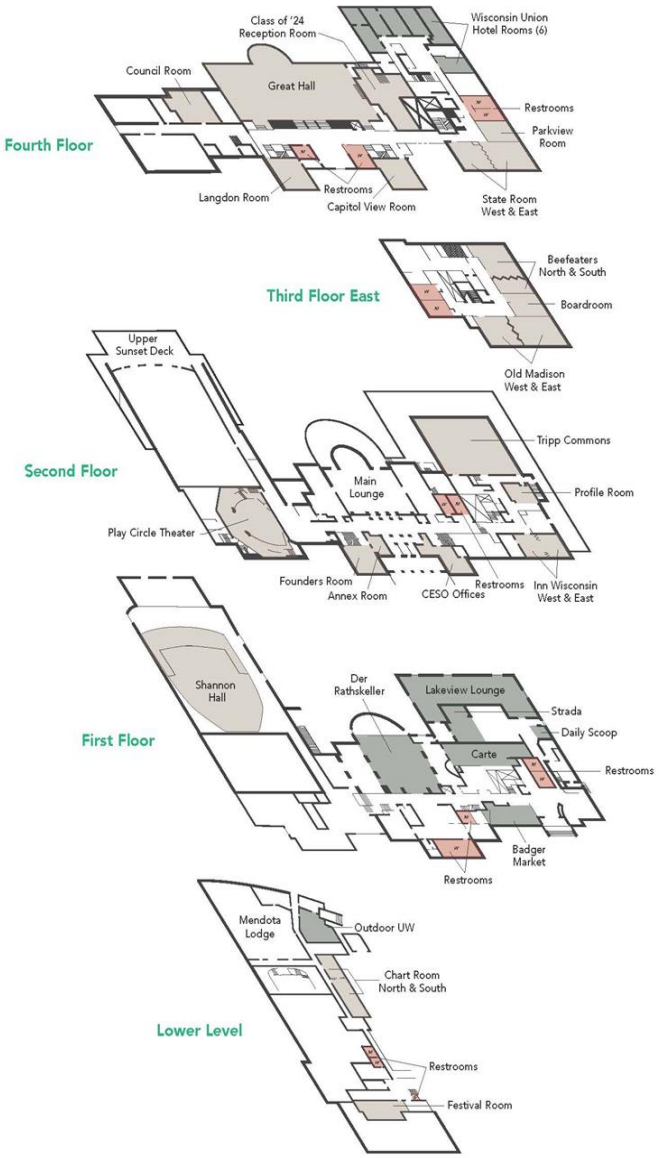
MEMORIAL UNION BUILDING MAP

MEMORIAL UNION



Conference Schedule at a Glance

	Sunday, July 8th, 2018	
1pm	Registration	*Annex Room*
5:30pm	Welcome and Keynote Address	*Great Hall*
6:30pm	Opening Reception	*Tripp Commons*
	Monday, July 9th, 2018	
8am	Registration	*Annex Room*
9am	Developmental Impact of Microbes	*Great Hall*
11:45am	Lunch Buffet	*Profile Room*
1pm	Host Factors Shaping the Microbiome	*Great Hall*
3:30pm	Poster Session	*Tripp Commons Main Lounge*
5:30pm	Dinner Buffet	*Profile Room*
7pm	Ecology and Evolution of Microbe-Host Interactions	*Great Hall*
9:15pm	Late Night Drinks	*Tripp Deck*
	Tuesday, July 10th, 2018	
8am	Registration	*Annex Room*
9am	Social Interactions and Microbial Transmission	*Great Hall*
11:45am	Lunch Buffet	*Profile Room*
1pm	Microbe-Host Interactions at the Molecular Scale	*Great Hall*
3:30pm	Poster Session	*Tripp Commons Main Lounge*
6:30pm	Banquet	*Great Hall*
8pm	Reception and Dance Party	*Tripp Commons*
	Wednesday, July 11th	
8am	Registration	*Annex Room*
8:30am	Engineering Beneficial Microbes	*Great Hall*
11am	Keynote Address	*Great Hall*
12pm	Closing Remarks	*Great Hall*
12:15pm	Boxed Lunch	*Reception Room*