Distinguished Faculty Speakers

Dr. Mandy Muller

Department of Microbiology, University of Massachusetts Amherst

Manipulation of RNA stability: a cornerstone of the viral-host battle

Dr. Muller received her Master's degree in Biology, at Ecole Normale Supérieure (ENS) in Lyon, France, her PhD in Virology from Institut Pasteur, in Paris, France, followed by postdoctoral work at UC Berkeley. The Muller lab studies Gamma-herpesviruses and in particular Kaposi Sarcoma Associated Herpesvirus, and how these viruses induce major changes in mRNA stability during infection, as well as how the host cell reacts to the viral takeover.

Dr. Sarah Hird

Department of Molecular & Cell Biology, University of Connecticut

The Avian Microbiome: Past, Present and Future

Dr. Hird received her BS and MS from the University of Idaho, her at PhD at Louisiana State University, and did her postdoctoral work at UC Davis. The Hird lab studies the evolution of host-associated microbiomes and microbial phylogeography. The lab is particularly interested in what factors shape and maintain the gut microbiomes of wild birds, as well as novel bioinformatic tools to answer these questions.

Dr. Marco Keiluweit

School of Earth & Sustainability and Stockbridge School of Agriculture, University of Massachusetts Amherst

Spatial Variations in Microbial Processes Controlling Carbon Oxidation Rates in Soils

Dr. Keiluweit received his BS in Geoecology from the University of Tübingen (Germany), a MS in Soil Chemistry, and a PhD in Soil Biogeochemistry from Oregon State University. Before moving to Massachusetts, he was a Lawrence Scholar at Lawrence Livermore National Laboratory and a Postdoctoral Scholar at Stanford University. The main goal of his research is to understand the effect of changing environmental conditions on fundamental, microbially-mediated geochemical reactions that control the fate of essential elements, most importantly the cycling and storage of carbon.

Talks

10:05 – 10:20am

Channel proteins are implicated in ATP synthase clearing from the forespore engulfing membrane during early stages of sporulation in *Bacillus subtilis*

Megan Littlehale, Elizabeth Mearls, Rachel Salemi, and Amy Hitchock Camp
Mount Holyoke College, South Hadley, MA

Bacillus subtilis is a model organism especially suited to the study of bacterial development and differentiation. During the process of sporulation, *B. subtilis* differentiates into two different cell types that lie side-by-side; a forespore, which becomes metabolically dormant, and a mother cell, which ultimately lyses and dies. While many aspects of the sporulation process have been well characterized, the mechanism by which the forespore orchestrates metabolic shut-down in preparation for dormancy is still poorly understood. We aim to characterize metabolic shut-down in the forespore by direct visualization and dynamic monitoring of metabolic proteins. In comparison with a membrane-bound GFP control, we've observed a significant decrease in the presence of ATP synthase components in the forespore engulfing membrane. However, this observation is not seen in cells depleted of a forespore channel protein, SpoIIQ. The clearing phenotype was seen in single mutants of mother cell channel proteins SpoIIIA and GerM but was again reversed in a double mutant of these two proteins. These results suggest that channel proteins may be involved in removing ATP synthase from the forespore in preparation for dormancy. They further suggest that ATP synthase may not be essential for energy generation in the forespore and loss of ATP synthase may be part of a mechanism that ultimately leads to the metabolic dormancy of the forespore. Ongoing investigations aim to uncover which compartment ATP synthase is degraded in and characterize the mechanism by which it is cleared from the engulfing septal membrane.

10:20 – 10:35 am

Life on the Leaves: Understanding Dynamic Plant Microbe Relationships on Pasture Grass

Emily Bechtold and Klaus Nusslein
Dept. of Microbiology, UMass Amherst, Amherst, MA

Grasslands are an important component of global food security acting as the foundation for ruminant milk and meat production. Despite their significant role in food production, grasslands are at risk of degradation due to human activity. As grassland conditions decrease or are converted to urban environments, new land must be cleared to maintain current agricultural outputs. Finding ways to increase productivity and lifespan of existing pastures is crucial to stopping agricultural expansion and creating sustainable farming practices that can keep up with the growing global population while slowing the rate of global warming. One solution is through the plant-leaf microbiome or phyllosphere. Plant-microbe interactions that occur on the leaf surface, or phyllosphere, are an understudied and underutilized resource. Phyllosphere bacteria can impact ecosystem function by influencing resistance to pathogens, producing plant hormones, and increasing available nitrogen. Understanding the phyllosphere community structure, function, and response to stress is important to determine how we can use the phyllosphere to increase sustainable farming practices. In this study, three forage grass species were grown in a greenhouse under current climate conditions and two different drought conditions that mimic future climate predictions. Bacterial community analysis, performed using high-throughput sequencing methods, was compared to plant traits and phylogeny. Bacterial communities were diverse with over 6000 unique OTUs identified. Additionally, bacterial abundance and community diversity decreased under severe drought stress conditions. Host traits such as chlorophyll content, water content, and dead material were correlated with the phyllosphere community structure. Further research to understand community function in relation to plant productivity will help us understand how we can promote phyllosphere communities that lead to healthier and more productive ecosystems.

10:35 – 10:50 am

Human milk oligosaccharides alter the metabolism of gut microbes in a modeled ecosystem

Ezgi Özcan[1] , Margaret Hilliard[1] , and David A. Sela[1,2,3]
[1]Dept. of Food Science, [2]Dept. of Microbiology, UMass Amherst, [3]Dept. of Microbiology & Physiological Systems and Center for Microbiome Research, UMass Medical School, Worcester

Bifidobacteria have evolved to metabolize human milk oligosaccharides (HMOs) in breastfed infants and they secrete metabolites to benefit their host. Lacto-*N*-tetraose (LNT) and lacto-*N*-neotetraose (LNnT) are tetrasaccharide isomers abundant in human milk that vary by a single glycosidic linkage. This structural variation has an unknown impact to microbial physiology. We previously demonstrated that *B. infantis*, a typical member of the infant gut microbiome, differentially metabolizes LNT and LNnT to secrete formate during inefficient metabolism. This increased formate production is predicted to increase acetogenic bacteria and attendant metabolic networks in the infant gut. We hypothesize that differential metabolism of LNT and LNnT by *B. infantis* will influence the overall structure and function of the microbial community in a modeled system. To test this hypothesis, a pH-controlled batch reactor system was employed to model the infant gut microbiome. Infant fecal samples spiked with or without *B. infantis* ATCC 15697 were inoculated into bioreactors with lactose, LNT, or LNnT. Changes in community structure and function were analyzed using qRT-PCR, 16s profiling, and HPLC. Gut microbes in bioreactors exhibited differential growth patterns while subsisting on lactose relative to HMOs. Lactate and acetate increased over time while formate concentrations drastically dropped between hours 15 and 24 of the fermentation in response to both LNT and LNnT. Interestingly, propionate increased over time during fermentation of both HMOs while there was no butyrate observed until hour 24 during LNT and lactose fermentation but not with LNnT. Bifidobacterial concentrations remained steady throughout the incubation regardless of the carbohydrate substrate. This study provides *in vitro* evidence for HMOs influence on select bifidobacteria and their secreted organic acids within the infant gut microbiome. In addition, this adds to the biological knowledge of the function of human milk and the potential impacts to infant health through its microbiome.

11:10 – 11:25 am

A Scalable Approach to Logic Gate Design using TAL Effector-Based Synthetic Repressors

Nathaniel Howitz and Lauren B. Andrews
Dept. of Chemical Engineering, UMass Amherst, Amherst, MA

Cells continuously perform complex signal processing of environmental stimuli. This capability can be used for a variety of applications, including diagnostics, treatment, environmental remediation, agriculture, and industrial purposes. To do this, cells can be re-programmed to respond to stimuli in a prescribed manner using designable genetic circuits. The genetic circuit is a synthetic gene regulatory network that allows for precisely controlling gene expression in response to the combination and temporal sequence of stimuli. TetR-family of protein repressors have been developed for use in genetic circuits. However, they are limited in number and suffer from toxicity and specificity issues. These drawbacks limit the size and complexity of designable genetic circuits. Here, we present a new algorithmic approach to design and build orthogonal synthetic repressors *de novo*. Using programmable DNA-binding proteins called transcription-activator like effectors (TALEs), we designed these synthetic proteins to act as repressors that bind to our custom synthetic repressible promoters. We developed a constraints-based approach to design a set of synthetic operator sequences that are orthogonal from one another and the host genome. In this work, we applied this approach to develop a library of synthetic repressors for *E. coli*. We designed and characterized the synthetic operator sequences in bacterial cells. We developed a DNA assembly strategy that allows for efficiently constructing TALEs that bind any 19 bp sequence. We constructed and characterized the function of the TALEs as repressor proteins, optimized their function as repressors via ribosome binding site tuning, and used them to construct genetic circuits. We expect this approach to synthetic logic gate design will enable automated genetic circuit design to achieve complex cellular behaviors that have been limited by the current genetic circuit components and ultimately allow the precise programming of genetic circuits for diverse organisms.

11:25 – 11:40 am

Development and Field Testing of Highly Sensitive qPCR Assays to Detect Ross River Virus and other Arboviruses in Mosquitoes

Jessica Grant[1*], Beth Yigzaw[1*], Rebecca Kuzma[2*], Nils Pilotte[1, 3], Steven A. Williams[1, 3]
[1]Dept. of Biological Sciences, Smith College, Northampton, MA, [2]Dept. of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Boston, MA, [3]Program in Molecular and Cellular Biology, UMass Amherst, Amherst, MA, *These authors contributed equally to the work.

Ross River virus (RRV) is a common mosquito-borne virus endemic to Australia and Papua New Guinea. Macropod marsupials have long been thought to be the sole reservoir hosts for RRV, limiting the geographic range available to the virus. However, recent studies show that RRV circulates endemically in American Samoa where there are no macropods, suggesting that RRV has the potential to become a health threat globally. In addition to RRV, other mosquito-borne viruses are of growing concern in the region. There has been an increase in confirmed cases of Dengue, Chikungunya and Zika virus in the last several years. We have developed a qPCR assay that targets the region of the genome that is most conserved among RRV strains, but divergent in other viruses. Thus, our assay detects RRV but not Getah, the most closely related virus to RRV. Optimization of our assay and validation using laboratory-infected mosquitoes are complete and have shown the test to be highly effective at detecting RRV in pools with as few as one infected mosquito in a pool of 25. We are currently using the test to screen wild mosquito populations from Samoa and American Samoa. Sensitive qPCR assays for other mosquito-borne viruses – Zika, Chikungunya, Dengue and Getah – have also been developed and are being optimized. Our results support the use of these assays for the detection of virus in mosquito vectors and validate our bioinformatic approach to assay design. However, outside of severe outbreaks, the prevalence of any one of these viruses in the mosquito population is low, and screening for multiple viruses when most are negative is costly. Thus, we are using our approach to develop a single qPCR assay that targets a broad diversity of arboviruses. This will allow one test to detect positive mosquito pools, with the specific virus to be determined afterward by testing only positive pools with species-specific assays.

11:40 – 11:55 am

The role of RNase E in mRNA metabolism in *Mycobacterium smegmatis*

Ying Zhou, M. Carla Martini, Scarlet S. Shell
Dept. of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (Mtb). A major challenge in TB treatment is the high tolerance of Mtb to various types of stress, including antibiotics and hypoxia. We hypothesize that adaptation to stress conditions involves both transcriptional and post-transcriptional regulatory mechanisms. mRNA cleavage may regulate gene expression post-transcriptionally by affecting the stability (half-life) of mRNA. Multiple ribonucleases (RNases) may perform endonucleolytic cleavages of primary mRNAs producing fragments with different stabilities and thus differentially alter transcript abundance. We sought to understand the role of mRNA cleavage in mycobacteria by investigating the role of RNase E, an enzyme involved in both mRNA degradation and selective mRNA stabilization in *E. coli*. Using the non-pathogenic model species *Mycobacterium smegmatis*, we constructed inducible repression systems to knock down RNase E. Repression of RNase E slows growth, consistent with the prediction that RNase E is an essential gene, and also results in decreased mRNA cleavage at the *esx-1* operon, which plays vital roles in conjugation in *M. smegmatis* as well as infection of macrophages by Mtb. Altered relative transcripts abundances also demonstrated a complex role of RNase E in both stabilizing and destabilizing portions of this transcript. We also examined the half-lives of several mRNAs and found that most were increased when RNase E was repressed, suggesting that RNase E plays a global role in mRNA degradation. Further, we plan to measure mRNA stability transcriptome-wide in the context of RNase E repression to further define its contribution to mRNA metabolism. In addition, RNase E is hypothesized to interact with other proteins to form a multi-protein complex (RNA degradosome) in *M. smegmatis*. Native chromosomal RNase E was tagged with FLAG and *in vitro* pull down assay together with LC-MS/MS identified protein candidates, including PNPase and RNA helicase that may play important roles in mRNA regulation.

11:55 – 12:10 pm

Exploring the role of microbiome-pathogen interactions during disease in *Drosophila melanogaster*

Danielle Andrzejewski Lesperance, Sthefany Calle, & Nichole A. Broderick
Dept. of Molecular and Cell Biology, University of Connecticut

Several insect studies have explored the role of the resident microbial community during pathogen infection. While some studies suggest a protective effect of the microbiota during infection, others find that gut microbes actually contribute to increased susceptibility to pathogens. In this study, we explore how the microbial community of *Drosophila melanogaster* affects lethality of a natural insect pathogen, *Pseudomonas entomophila (Pe)*. Cross-streaks were used to visualize *in vitro* microbe-microbe interactions between *Pe* and two common members of the *Drosophila* microbiome, *Lactobacillus plantarum* and *Acetobacter* pomorum. Additionally, flies with defined microbial communities (gnotobiotics) were generated using the same microbiota strains, then infected with *Pe* to show how the observed *in vitro* interactions were impacted by the host environment. Survival was also recorded at this time in both gnotobiotic and axenic flies (flies with no microbiome) in order to assess the effect of pathogen-microbiome interactions on disease susceptibility. *Lactobacillus plantarum* inhibited *Pe* growth both *in vitro* and *in vivo*, as determined from colony counts post infection. *Acetobacter pomorum*, on the other hand, enhanced growth of *Pe in vitro* but did not have a discernable effect on *Pe* titers *in vivo*. Axenic flies had the highest levels of *Pe* two days post infection, suggesting a role for the microbiota in clearing *Pe* from the gut. Surprisingly, however, inhibition patterns and *Pe* titers did not directly correlate with survivability. Axenic flies were the least susceptible to lethality while *Lactobacillus*-associated flies experienced high mortality especially at early time points. These results indicate that complex interactions occur between the microbiota, *Pe*, and the host that influence disease progression and impact fly survival.

2:10 – 2:25pm

Re-examining electron transfer in anaerobic microbial environments

David J.F. Walker[1,2], Dawn E. Holmes[1,3], Eric Martz[1], Ramesh Y Adhikari[4], Zimu Zhou[5], Stephen S. Nonnenmann[5], Amelia E. Rotaru[6], Michael J. McInerney[7], Derek R. Lovley[1,2]
[1]Dept. of Microbiology, UMass Amherst, [2]Institute for Applied Life Sciences, UMass Amherst, [3]Dept. of Physical and Biological Science, Western New England University, [4]Dept. of Physics, UMass Amherst, [5]Dept. of Mechanical and Industrial Engineering, UMass Amherst, [6]Dept. of Biology, University of Southern Denmark, Denmark, [7]Dept. of Microbiology and Plant Biology, University of Oklahoma

Hydrogen/formate interspecies electron transfer (HFIT), in which H_2 and/or formate function as diffusible electron carriers, has been considered to be the predominant form of electron-based syntrophy. It is becoming increasingly apparent that microorganisms can make direct electrical connections with other cells as well as abiological materials, such as minerals and electrodes. The use of electrically conductive pili (e-pili) to make these electrical connections has been studied intensively in the Fe(III) respiring genus Geobacter. Type IVa PilA subunits associated with Geobacter pili are small (60-90 aa) tightly packed proteins with aromatic and charged amino acid residues positioned in a manner that promotes metallic-like electron conduction along the length of the pilin. It appears that aromatic amino acid positioning seems to be a determinant of conductivity. In this study, we developed an empirical formula and identified 5 bacteria from diverse phylogenetic backgrounds that encode e-pili. All of these PilA were heterologously expressed in a PilA deletion mutant strain of *G. sulfurreducens* and shown to produce a high-current in H-cells, indicative of e-pili. Electrical analysis of the e-pili proved they were electrically conductive. One such organism, *Syntrophus aciditrophicus*, the most intensively studied organism for hydrogen/formate interspecies electron transport (HFIT), was further characterized and found to be able to partake in Direct Interspecies Electron Transport (DIET) with a mutant *G. sulfurreducens* strain deficient in HFIT. This proves that *S. aciditrophicus* can alternate between HFIT and DIET depending on its environment and syntrophic partner. These results refute the theory that electrically conductive e-pili are unique to the genus Geobacter and that diverse syntrophs may have the option to establish direct electrical connections for DIET, necessitating a reexamination of 50 year-old- concept that HFIT is the predominant mechanism for syntrophic electron exchange within anaerobic microbial communities.

2:25 – 2:40 pm

Examining the Role of External Nutrient Availability on the Expression of a *Vibrio cholerae* Metabolic Virulence Factor in the Model Host System *Drosophila melanogaster*

Elisa Bello[1] and Alexandra Purdy[1,2]

[1]Program in Biochemistry and Biophysics, [2]Dept. of Biology, Amherst College, Amherst, MA

The pathogenicity of the gram-negative bacterium *Vibrio cholerae* is at least in part mediated by the enzyme acetyl CoA synthetase (Acs), which incorporates external acetate into acetyl CoA for energy metabolism. Acs expressed over the course of infection thus switches the bacterium from excreting acetate to assimilating it as an energy source. In the model host *Drosophila melanogaster*, *V. cholerae* colonizes the gut of the fly, expresses *acs*, and begins to deplete acetate from the surrounding intestinal microenvironment. Depletion of acetate in the host gut is associated with dysregulation of host metabolic function and eventual host death. While the effects of this switch have been characterized in the *D. melanogaster* model, the external conditions governing the switch are still largely unknown. It has been shown that *acs* expression is controlled by the two-component system CrbS/R, and is further modulated by the global regulator CRP. While the external stimulus for CrbS activation has not yet been identified, CRP involvement suggests that the switch is sensitive to external nutrient concentrations. Our goal is to determine whether the availability of nutrients in the *Drosophila* gastrointestinal tract alters the switch to acetate consumption via *acs*, thus contributing to bacterial virulence. We are investigating this question through Hybridization Chain Reaction-Fluorescence *in situ* Hybridization (HCR-FISH), which will allow us to detect spatial and temporal variations in *acs* expression in the fly gastrointestinal tract in response to varied nutrient conditions. This will reveal changes in *acs* expression in response to external nutrient availability and allow us to visualize those changes in context at high levels of resolution.

2:40 – 2:55 pm

Using single-cell 'omics to study biodiversity and population genetics of testate amoebae

Agnes K.M. Weiner[1] and Laura A. Katz[1,2]

[1]Dept. of Biological Science, Smith College, Northampton, MA, [2]Program in Organismic and Evolutionary Biology, UMass Amherst, Amherst, MA

Testate amoebae of the order Arcellinida (Amoebozoa) are microbial eukaryotes that are highly abundant in freshwater ecosystems, such as bogs, fens and lakes. Within the microbial community inhabiting *Sphagnum* moss in bogs and fens, these shell-forming amoebae represent top predators. Because of their abundance, their sensitivity to abiotic environmental factors and the preservation of their tests in the fossil record, they serve as excellent bioindicators of past and present climate changes in these freshwater habitats. Diversity estimates in these studies thereby generally rely on morphospecies concepts, using attributes like test size, shape and composition for species designations. Yet, recent molecular work has challenged the validity of morphospecies concepts among testate amoebae, as both cryptic species and convergent morphologies have been identified. We are now using single-cell transcriptomics to assess the biodiversity of Arcellinida, to try to delineate species based on multiple genes and to thus resolve the discordance between morphological and molecular estimates of testate biodiversity. We have characterized transcriptomes from multiple (~50) individuals of Arcellinida, which we assemble and analyze using our custom-made pipeline PhyloTOL, which results in numerous gene-trees. In addition, we are using these transcriptome data to analyze effective population size of testate amoebae, as our data showed surprisingly low levels of intra-population genetic variability across time and space. Single-cell transcriptomics seems to be a powerful tool for studying Arcellinida biodiversity on the molecular level and elucidating species boundaries as well as for obtaining insights in the molecular evolution and population dynamics of this important group of protists.

4:45 – 5:00 pm

Probing the molecular mechanism of ProQ-RNA interactions using a bacterial three-hybrid assay

Smriti Pandey, Chandra Gravel, Chukwuamaka Onyewadume, Katherine E. Berry
Dept. of Chemistry and Program in Biochemistry, Mount Holyoke College, South Hadley, MA

In bacteria, small RNAs (sRNAs) play important roles in gene regulation; sRNAs can regulate the translation and stability of target mRNAs via imperfect base pairing. These sRNA-mRNA interactions are often facilitated by RNA chaperone proteins. ProQ- was recently identified as a global RNA-binding protein that binds to dozens of sRNAs and hundreds of mRNAs in multiple proteobacteria. It's been shown to facilitate base pairing between the mRNA *hupA* and sRNA RaiZ with the effect of regulating *hupA* expression. These observations have led to the proposal that ProQ may act as a widespread regulator of bacterial gene expression. Our goal is to understand the molecular mechanisms of ProQ's interaction with regulatory RNAs, mapping the amino acids on ProQ's surface and nucleotides of RNAs that contribute to binding and regulation by using a bacterial three-hybrid (B3H) assay to detect ProQ-RNA interactions genetically. In the B3H assay, ProQ is fused to RNA polymerase (RNAP) and a hybrid RNA containing an sRNA or 3'UTR of interest is tethered to a DNA sequence upstream of a test promoter. Interaction of ProQ with the RNA stabilizes the binding of RNAP to the test promoter and activates transcription of a reporter gene. We have detected preliminary B3H interactions of ProQ with several of its RNA partners. Our data support a model where the conserved N-terminal-domain (NTD) and an unstructured linker are the primary regions that mediate ProQ-RNA binding. Further, we have identified point mutations in ProQ that affect its RNA interactions, using immunodetection to verify ProQ protein levels and ensure that these mutations are not destabilizing. Current efforts are focused on screening for additional ProQ point mutations to locate the binding interface(s) for the sRNAs and mRNAs with which it interacts as well as exploring the structure and sequences in RNA that are required for ProQ interaction.

5:00 – 5:15 pm

Testing glucosylceramide transferase as a potential target for developing anti-giardial therapy

Jasmine Moss, Vanessa Enriquez, Breanna Pence and Siddhartha Das
Infectious Disease and Immunology Cluster, Border Biomedical Research Center (BBRC), Dept. of Biological Sciences, University of Texas at El Paso, El Paso, TX

Background: *Giardia lamblia*, is a flagellated protozoan and a major cause of waterborne diarrhea (giardiasis) worldwide. *Giardia* colonizes and thrives in the small intestine of humans and undergoes encystation or cyst formation when intestinal condition changes. Our laboratory has reported earlier that *Giardia* expresses glucosylceramide transferase 1 (GlcT1), the activity of which is elevated during encystation. Goal: The goal of this project is to further examine how the changes of gGlcT1 expression alter the growth and cyst formation by *Giardia*, and whether this enzyme can serve as a potential target for developing anti-giardial agents. Methods: We used various plasmid-containing clones of *Giardia* expressing different levels of GlcT1 and monitored their growth/cyst formation in culture. D-*threo*-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP, 10 μM), a common inhibitor of GlcT1 enzyme in mammalian cells was tested to examine if giardial GlcT1 is also inhibited by this compound. Drug efficacy was determined by counting live trophozoites and mature oval-shaped cysts. Results: Wild type and high level of GlcT1 expressing parasites exhibited reduced inhibition by PPMP compared to α-tubulin-GlcT1-AU1 which shows low level expression. Interestingly, PPMP was found to be more effective on parasites expressing the low level GlcT1. Conclusion: Our results suggest that the regulated expression of GlcT1 is critical for encystation and cyst production by *Giardia*. We also observed that giardial GlcT1 is sensitive to PPMP. Furthermore, PPMP-induced inhibition of growth and encystation is dependent on the level of intracellular GlcT1, implicating that GlcT1 can serve as an effective target for developing anti-giardial therapies in the future.

5:15 – 5:30 pm

***Mycobacterium tuberculosis* susceptibility and survival under antibiotic pressure**

Michelle Bellerose[1], Seung-Hun Baek[2], Caitlin Moss[3], Megan Proulx[1], Eun-Ik Koh[1], Clare Smith[1], Richard Baker[1], Christopher Sassetti[1]

[1]Dept. of Microbiology and Physiological Systems, UMass Medical School, [2]Institute for Immunology and Immunological Diseases, Yonsei University College of Medicine, [3]Dept. of Microbial Pathogenesis, Yale School of Medicine

Mycobacterium tuberculosis (Mtb) assumes a phenotypically drug-tolerant state during infection, which contributes to both the relative inefficacy of antibiotics and to the development of drug resistance. To probe mechanisms underlying drug tolerance, we performed a comprehensive genetic screen for Mtb mutants with altered susceptibility to antibiotics during an *in vivo* infection setting. Mice were infected with a saturated Mtb transposon mutant library and then treated with the four first-line TB drugs alone and in combination. Following *in vivo* selection, we observed mutants that were both over- and under-represented. Mutants more susceptible to treatment identified mechanisms of tolerance, whereas mutants less susceptible provided insights into the development of resistance. These chemical-genetic interactions identified genes involved in a range of functions, such as cell wall formation, efflux pumps, and metabolism. For example, glycerol metabolism mutants were less susceptible to treatment with pyrazinamide and combination therapy. We found that similar glycerol catabolic mutations are common in extensively drug-resistant clinical Mtb isolates, indicating that loss of glycerol utilization may enhance phenotypic drug tolerance and could play a role in development of resistance. The better understanding of Mtb tolerance and response to antimicrobials gained by this genome-wide approach may identify targets for novel treatment strategies.

Posters

1. Probing the molecular mechanism of ProQ-RNA interactions using a bacterial three-hybrid assay

Smriti Pandey, Chandra Gravel, Chukwuamaka Onyewadume, Katherine E. Berry
Dept. of Chemistry and Program in Biochemistry, Mount Holyoke College, South Hadley, MA

In bacteria, small RNAs (sRNAs) play important roles in gene regulation; sRNAs can regulate the translation and stability of target mRNAs via imperfect base pairing. These sRNA-mRNA interactions are often facilitated by RNA chaperone proteins. ProQ- was recently identified as a global RNA-binding protein that binds to dozens of sRNAs and hundreds of mRNAs in multiple proteobacteria. It's been shown to facilitate base pairing between the mRNA *hupA* and sRNA RaiZ with the effect of regulating *hupA* expression. These observations have led to the proposal that ProQ may act as a widespread regulator of bacterial gene expression. Our goal is to understand the molecular mechanisms of ProQ's interaction with regulatory RNAs, mapping the amino acids on ProQ's surface and nucleotides of RNAs that contribute to binding and regulation by using a bacterial three-hybrid (B3H) assay to detect ProQ-RNA interactions genetically. In the B3H assay, ProQ is fused to RNA polymerase (RNAP) and a hybrid RNA containing an sRNA or 3'UTR of interest is tethered to a DNA sequence upstream of a test promoter. Interaction of ProQ with the RNA stabilizes the binding of RNAP to the test promoter and activates transcription of a reporter gene. We have detected preliminary B3H interactions of ProQ with several of its RNA partners. Our data support a model where the conserved N-terminal-domain (NTD) and an unstructured linker are the primary regions that mediate ProQ-RNA binding. Further, we have identified point mutations in ProQ that affect its RNA interactions, using immunodetection to verify ProQ protein levels and ensure that these mutations are not destabilizing. Current efforts are focused on screening for additional ProQ point mutations to locate the binding interface(s) for the sRNAs and mRNAs with which it interacts as well as exploring the structure and sequences in RNA that are required for ProQ interaction.

2. Upregulating the master motility regulator, *flhDC*, in *Salmonella* increases intracellular invasion and colonization of tumor masses

Vishnu Raman[1], Nele Van Dessel[1], Owen O'Connor[1], Neil S. Forbes[1, 2]
[1]Dept. of Chemical Engineering, [2]Institute for Applied Life Sciences, UMass Amherst, Amherst, MA

Salmonella have potential as anticancer therapeutic because of their innate tumor specificity. In clinical studies, this specificity has been hampered by heterogeneous responses. Understanding the mechanisms that control tumor colonization would enable the design of more robust therapeutic strains. Two mechanisms that could affect tumor colonization are intracellular invasion and intratumoral motility. Both of these mechanisms have elements that are controlled by the master motility regulator *flhDC*. We hypothesized that 1) overexpressing *flhDC* in Salmonella increases intracellular invasion in tumor cell masses, and 2) intracellular invasion of Salmonella drives tumor colonization *in vitro*. To test these hypotheses, we transformed *Salmonella* with genetic circuits that induce *flhDC* and express green fluorescent protein after cell invasion. The genetically modified *Salmonella* was perfused into an *in vitro* tumor-on-a-chip device. Time-lapse fluorescence microscopy was used to quantify the dynamics of intracellular invasion and tumor colonization. A mathematical model was used to determine how these mechanisms are related to each other. Overexpression of *flhDC* increased intracellular invasion and tumor colonization 2.5 and 5 times more than control *Salmonella*, respectively ($P < 0.05$). Minimally invasive, *ΔsipB*, *Salmonella* colonized tumor masses 2.5 times less than controls ($P < 0.05$). Mathematical modeling of tumor colonization dynamics demonstrated that intracellular invasion increased retention of *Salmonella* in tumors by effectively causing the bacteria to bind to cancer cells and preventing leakage out of the tumors. These results demonstrated that increasing intracellular invasion increased overall tumor colonization and that *flhDC* could be used to control both. This study demonstrates a mechanistic link between motility, intracellular invasion and tumor colonization. Based on our results, we envision that therapeutic strains of *Salmonella* could use inducible *flhDC* to drive tumor colonization. More intratumoral bacteria would enable delivery of higher therapeutic payloads into tumors and would improve treatment efficacy.

3. Divergence of the human milk oligosaccharide (HMO) utilization cluster among *Bifidobacterium longum* subspecies *infantis* strains

Korin Albert[1,2] Asha Rani[2], and David A. Sela[1,2,3]
[1] Molecular and Cellular Biology Graduate Program, [2] Dept. of Food Science, UMass Amherst, Amherst, MA, [3] Dept. of Microbiology and Physiological Systems, UMass Medical School, Worcester, MA

Bifidobacterium longum subspecies *infantis* (*B. infantis*) is a constituent of the infant gut microbiome commonly associated with positive health outcomes. *B. infantis* metabolizes host-indigestible carbohydrates, termed human milk oligosaccharides (HMOs), via a ~40 kb gene cluster first identified in the type strain *B. infantis* ATCC15697. The ability of *B. infantis* to subsist on human milk represents a potential diet-mediated coevolutionary relationship between mother, infant, and gut microbiome. To investigate the evolutionary trajectory of HMO utilization in *B. infantis*, we analyzed the genomes of 28 *B. infantis* strains with publicly available genome data. Using a comparative genomics approach, we determined the extent of variation within the HMO utilization gene cluster and explored the phylogenetic relationships between strains. Our analyses revealed significant HMO gene cluster divergence with respect to the number, location within the locus, and sequence similarity of carbohydrate transporters. In addition, the *B. infantis* strains were found to be subdivided into two separate groups using several different measures, including pangenome matrices, average nucleotide identity values, single-copy concatenated gene phylogenies, and ecotype modeling. Members of both groups were further compared phenotypically via scanning electron microscopy and growth on various human milk-associated carbohydrates. Overall, our results suggest that *B. infantis* strains show significant genomic and phenotypic diversity with respect to both the HMO utilization cluster and other features of the genome.

4. Understanding the mechanism of the SOS response to help design better antibiotics

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Most antibiotics target essential processes in bacteria to inhibit their growth or kill them. Commonly used antibiotics, such as aminoglycosides and β-lactams, inadvertently also cause DNA damage. DNA damage inducible responses occur in all organisms and frequently up-regulate mutagenic DNA repair mechanisms. This in turn increases the frequency of mutation that in turn give rise to superbugs, multi-drug resistant bacteria. Thus better understanding of the regulation of these mechanism might help to design better antibiotics that do not also cause DNA damage. The archetypal DNA damage response system is the SOS response of *E. coli*. LexA represses approximately 50 genes at the level of transcription. The sensor for the response is the RecA protein polymerizing onto damaged DNA creating a protein DNA helical filament. LexA then binds in the major groove of this helical filament to auto-cleave, thus lowering the concentration of LexA in the cell and inducing the SOS response. The specific interactions between RecA and LexA in this process are yet unknown. LexA exists in two forms in solution: cleavable and uncleavable. RecA filaments stabilize the cleavable form. It is hypothesized that LexA regulates recombination by protein-protein interactions as well as transcriptionally. We have found that overproduction of LexA3 (G85D), a mutant of lexA that is stuck in the uncleavable form inhibits RecA-dependent DNA repair processes. We will discuss the mechanism of this inhibition and mutants of LexA3 that no longer inhibit this process. Through understanding this mechanism, we hope to help develop better antibiotics that no longer inadvertently induce DNA damage and create multi-drug resistant bacteria.

5. Structural Analysis of the Phyllosphere of Key Amazonian Forage Grasses Based on Leaf Senescence

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Due to its tropical climate, the Amazon rainforests have become a hub for agricultural industry. With a growing world population and expanding market, Amazon deforestation has reached a rapid rate to make room for livestock pastures and plantations. In an effort to maximize yield and to combat the extreme climate conditions, farmers have turned to planting new forage species as replacement, particularly the African grass species *Brachiaria*. Able to withstand a variety of environments, *Brachiaria* has become a pioneer species in providing nutritious forage to depleted areas. Its pivotal role in the agricultural industry makes it an essential plant to study on the microbial level. As a microbial lab studying land-use change in the Amazon, we are particularly interested in the phyllosphere of the *Brachiaria* species. The phyllosphere, the portion of the plant above the soil and all of its associated microorganisms, is a critical component of ecosystem nutrient exchange and plant health. Our research aims to identify the natural composition and function of this microbial community, in addition to how the community may change when exposed to environmental stressors such as drought or elevated temperatures. Our ultimate goal is to create a better understanding of the phyllosphere as a dynamic microbial community. By analyzing the microbial abundance of *Brachiaria*, specifically those involved in nitrogen fixation, we intend to explore methods to maximize the beneficial plant-microbe interactions. On a global scale, we hope to find a way to apply our research to address broader issues like sustainable agricultural practices in order to protect the world's tropical forests.

6. Quantitative understanding of lipid-mediated DNA probe modification on mammalian cell membranes

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Modification of functional probes on live cell membranes have found a range of applications in bioanalysis, nanobiotechnology, cell biology, and medicine. Among different approaches, lipid-mediated cell membrane insertion of DNA probes become popular for studying cell membrane signaling, intercellular interactions and tissue engineering. In this method, lipid-DNA conjugate was first chemically synthesized. After brief incubation with mammalian cells, efficient and spontaneous modifications on the cell membranes are realized based on the similar hydrophobicity of the synthetic lipids with natural membrane lipid bilayers. In this project, we have performed a systematic study to understand and further improve the membrane persistence and insertion efficiency of these lipid-DNA probes. We have quantified the insertion efficiency, kinetics, membrane durability, and distribution of several probes mimicking eight natural lipids on live MDCK cell membranes. Our results indicated the importance of the types of lipids in these membrane modifications. For example, unsaturated lipid structures dramatically decrease the probe insertion efficiency. Quite excitingly, we have developed two methods to enhance probe persistence on cell membranes for long-term bioanalysis and regulation. To the best of our knowledge, this is the first quantitative study on how various effects of lipid structure/ hydrophobicity, DNA length, probe concentration, cell membrane composition, and temperature can be used to improve the lipid-mediated DNA modification on live cell membranes. Results of this project will be used as a general guideline to identify optimal conditions to modify DNA probes in different membrane applications.

7. The Story of C19ORF66, an Uncharacterized Host Factor, Resisting KSHV-induced mRNA decay and Restricting Viral Infection

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Herpesviruses induce lifelong infections that place them under a strong evolutionary pressure to maintain a "successful" balance with their host. Maintaining a tight control over the host gene expression environment and finding ways to gain access to host resources is central to a productive infection. To do so, viruses induce "Host-Shutoff" as an effective way to dampen host gene expression and immune response. We focus on Kaposi's Sarcoma-Associated Herpesvirus (KSHV), a gamma-herpesvirus known to be involved with the development of several malignancies. Host shutoff in KSHV is triggered by a single viral protein, SOX, an RNA endonuclease that triggers widespread mRNA degradation during lytic reactivation of the virus. Despite this ability of the virus to drastically depreciate over two-thirds of the total host mRNAs, their remains a fraction of host mRNAs that escape this viral-induced decay which we term "escapees". One of the most prominently expressed escapees, identified via RNA seq analyses, is a recently characterized IFN-α stimulated gene, C19ORF66. This protein has been previously shown to be upregulated during infection of a number of viruses including adenovirus, influenza virus, Lassa virus, Ebola and Marburg viruses, as well as post-infection with human herpesvirus 8 (KSHV) and human herpesvirus 1 (HSV-1). Our recent data show that knocking out C19ORF66 significantly amplified lytic reactivation, suggesting that C19ORF66 may restrict progression of the KSHV life cycle. We are currently investigating the mechanism behind the effect of C19ORF66 on KSHV infection, addressing possible changes in its expression and subcellular localization upon KSHV reactivation as well as mapping its protein-protein network. By deciphering the role of this and other escapees, we hope to further our understanding of the complex interplay between KSHV and its host.

8. New pathway for hydroxyl radical generation associated with *Cryptococcus neoformans* pathogenesis in mice cortical tissues

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The pathogenic fungal yeast *Cryptococcus neoformans* infects about 220,000 people/year and causes approximately 181,000 deaths/year in immunocompromised patients. The exact mechanisms leading to host brain lesion(cryptococcoma) formation are still incompletely understood. We propose that oxidative reduction of iron by 3-hydroxyanthranilic acid (3-HAA), an intermediate in the tryptophan degradation pathway, plays an important role in lesion formation. 3-HAA is an iron chelator produced by *Cryptococcus neoformans* that is typically reported to function as an antioxidant. However, our research suggests that 3-HAA can participate in a type of Fenton chemistry to reduce ferric iron which would then react with oxygen, or hydrogen peroxide produced by host immune cells, leading to hydroxyl radical generation. This has not been previously reported in *C. neoformans*, and it is possible that the generation of a sustained stream hydroxyl radicals might be involved in cryptococcoma formation. Using organotypic brain slice cultures(OBS), we observed that the pH of infected mouse brain slice culture fluids post 10-day infection is lowered from a physiological pH of 7.4 to 6.0. Electron paramagnetic resonance spectroscopy data suggests that though 3-HAA is an antioxidant at physiological pH, it is a pro-oxidant at pH 5.5 and produces hydroxyl radicals(•OH). Culture supernatants of *C. neoformans* grown in limited iron media(LIM) at pH 5.5 show similar production of •OH which is absent in cultures grown at pH 7.4. HPLC and Mass Spectrometry revealed a seven-fold increase in 3-HAA levels produced by *Cryptococcus neoformans* at pH 5.5 compared to pH 7.4 *in-vitro* in LIM. Using confocal microscopy, a comparative analysis of brain slices after different days of infection showed a progressive increase in hydroxyl radical generation. Exploring the role of 3-HAA in triggering •OH generation leading to cryptococcoma formation will broaden our understanding of the fungal virulence and might provide targets for future gene therapy.

9. Transmembrane Domain in a Syntaxin Protein involved in Legume-Rhizobia Symbiotic Interaction May Determine its Differential Subcellular Localization

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Syntaxins are a conserved family of proteins involved in the fusion of vesicles with target membranes to deliver proteins essential for different physiological functions including growth, development and plant-microbe interactions. In rhizobia and arbuscular mycorrhizal (AM) symbioses, syntaxins contribute to the secretion of proteins needed for the development of the plant cells that interact with the beneficial microbe. This interaction subsequently contributes to the acquisition of growth-promoting nutrients from the soil. In particular, nitrogen fixation symbiosis (NFS) allows legume plants to thrive in nitrogen-limited soils because they form a symbiotic association with nitrogen-fixing bacteria called rhizobia, which convert atmospheric inert nitrogen gas into bio-available ammonia. This NFS is achieved in specialized root organs called nodules where hundreds of rhizobia divide and differentiate into mature nitrogen-fixing forms called bacteroids. The bacteroids are completely and individually enclosed in a plant-derived organelle-like compartment called symbiosome and are surrounded by the symbiosome membrane. Formation of symbiosomes require abundant proteins and lipid material. We are interested in the question of how legume plants influence the successful formation and development of symbiosomes. Previous studies have shown that a symbiosome protein in *Medicago truncatula* legume species called syntaxin132 (MtSYP132), is part of the nodule-specific secretory pathway, necessary for supplying the symbiosome with plant-host proteins essential for its formation and development. Our lab recently showed that the *MtSYP132* gene undergoes alternative cleavage and polyadenylation during transcription, giving rise to two isoforms; MtSYP132A and MtSYP132C (Pan, Oztas et al, 2016). In AM symbiosis, MtSYP132A was required for the penetration of fungi into the plant roots and the development of the peri-arbuscular membrane. In rhizobia symbiosis, knock-down of the symbiosome-localized MtSYP132A resulted in nodules that did not fix nitrogen and differentiation of symbiosomes inside these nodules was blocked. Additionally, these isoforms exhibited differential subcellular localizations; MtSYP132C localizes on the plant plasma membrane, while MtSYP132A localizes on the symbiosome membrane. Our goal is to understand how these two SYP132 protein isoforms localize to distinct membrane compartments: the plasma membrane and the symbiosome membrane. Sequence alignment showed that the two SYP132 isoforms are conserved in many species forming symbioses with rhizobia or AM fungi but there are striking differences in some amino acids in the transmembrane (TM) domain between SYP132A and SYP132C. We hypothesize that these amino acids are important for the difference in subcellular localization patterns. To test this hypothesis, plant transformation with constructs containing GFP-tagged-SYP132A/C ‘swaps’ in the TM domain is underway, and the localization patterns of these mutants will be examined using confocal microscopy. We expect that domain swapping between the TM domains will result in novel localization patterns for the corresponding proteins. Because SYP132 protein is a component of peri-microbial compartments in both NFS and AM symbioses, detailed knowledge of this protein can be potentially used to genetically manipulate crop plants in order to enhance their nitrogen and phosphorus acquisition, respectively, for crop productivity.

10. Microbial Succession of a Newly Developed Aquaponics System

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Aquaponics, a farming system that combines growing hydroponic vegetables with fish aquaculture, is expected to increase in importance because it allows growing food in areas not conducive to conventional farming. Aquaponics can also grow more plants in less space; this efficiency is due to nitrogen cycling from fish waste to plant fertilizer. Microbes are central to this nitrogen cycling. The Spring Valley Student Farm at the University of Connecticut installed an aquaponics system spring of 2017, introducing fish summer of 2018. We collected water samples from the system through both summers to monitor the establishment of the microbial community. We tested DNA extraction methods and found that sufficient DNA could be extracted from water samples that were simply dip collected and stored in typical household freezers. We amplified and sequenced v4 of 16S rRNA targeting both Bacteria and Archaea. During the first summer (before the fish were added to the system) the microbial community structure was stochastic suggesting that the community succession was not stable. During the second sampling season, the fish developed a serious illness and were euthanized. We will continue monitoring this aquaponic system as it is restarted to evaluate the hypothesis of system instability during the initial phases of community development.

11. Regulation of mRNA stability in mycobacteria as an adaptive response to stress conditions

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Mycobacterium tuberculosis (MTB) is the causative agent of tuberculosis (TB), a significant cause of human mortality. MTB is a successful pathogen due to its ability to survive harsh environments, such as hypoxia or nutrient deprivation, by entering non-growing states. In these states of low metabolism, MTB can tolerate antibiotics and can develop genetically encoded antibiotic resistance, making its metabolic adaptation to stress crucial for survival. Numerous bacteria have been shown to reduce their rates of mRNA turnover under stress. While the existence of this response appears to be conserved in bacteria, the mechanisms underlying mRNA stabilization remain unknown. To identify the mechanisms that govern mRNA stabilization in mycobacteria, we used the non-pathogenic model *Mycobacterium smegmatis*. We found that mRNA half-lives were longer in hypoxia and carbon starvation compared to log phase. Interestingly, hypoxia-adapted cultures that were re-exposed to oxygen for two minutes displayed increased transcription of some genes accompanied by dramatic shortening of mRNA half-lives. Furthermore, experiments using a translational inhibitor in re-aerated hypoxia cultures suggested that the increased mRNA turnover occurs in absence of protein synthesis. Hence, we hypothesize that metabolic changes during growth cessation impact the activity of degradation proteins, increasing mRNA stability. However, other mechanisms may also be involved. For example, we found that in *M. smegmatis* total RNA levels remain similar in non-growing states, while mRNA levels are significantly reduced. Thus, mRNA stability could result from ribosomes masking RNase cleavage sites. Together, our data are consistent with a model in which mRNA stability is controlled at the level of RNase activity and/or accessibility of transcripts to the RNA degradation machinery.

12. Investigating the Glycerol Degradation Pathway as a Regulator of Acetyl-CoA Synthetase Expression in *Vibrio fischeri*

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Vibrio fischeri is an aquatic microbe that engages in a monospecific symbiotic relationship with the Hawaiian bobtail squid, *Euprymna scolopes*. *V. fischeri* colonize the light organ of the squid, obtaining shelter and nutrients while providing the animal with camouflage. This symbiosis is mediated by the exchange of specific carbon sources and metabolites, including acetate, which *V. fischeri* regulates via the expression of acetyl-CoA synthetase encoded by the *acs* gene. When *V. fischeri* are grown on glycerol-containing SWT media, *acs* expression is suppressed. However, when the gene *glpK* is deleted from the bacteria's glycerol degradation pathway, *acs* expression is restored. The aim of this study is to determine whether a specific metabolic intermediate in the glycerol degradative pathway represses *acs* expression. To accomplish this, we are constructing in-frame deletions of the *glpD* gene and *glpACB* operon, which catalyze the aerobic and anaerobic catabolic pathways, respectively. Our goal is to understand the regulatory network controlling *acs*, which will not only provide insight into the symbiotic relationship between *V. fischeri* and *E. scolopes*, but may also reveal mechanisms conserved in the pathogenic interactions of related host-associated bacteria.

13. Controlling *Salmonella* on alfalfa sprouts using lactic cultures

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Over the last decade, foodborne outbreaks related to sprouts have been a persistent public safety concern. *Salmonella* and the enterohemorrhagic *Escherichia coli* are the main causative agents responsible for outbreaks involving alfalfa, mung bean, cress and clover sprouts. Contaminated seeds and irrigation water are a potential source of the pathogen. In addition, the germination process favors pathogen proliferation to high numbers (>8 log CFU/g). Considering that sprouts are usually consumed raw and that washing procedures do not achieve significant reduction or removal of the pathogens, different approaches, including chemical and biological interventions, have been evaluated. In this study, a cocktail of five *Salmonella enterica* serovars was used to inoculate seeds (6 log CFU/g) followed by a pre-germination wash containing Lactic acid bacteria (LAB) namely, *Lactobacillus acidophilus* (LA), *Lactobacillus reuteri* (LR) and *Streptococcus lactis* var Diacetylactis (SLD) as a biocontrol measure to reduce pathogen load in seeds and sprouts. Seed treatment with different LAB significantly reduced *Salmonella* populations on seeds and sprouts. Treatment with LP, SLD and LR reduced *Salmonella* populations to below detectable limits by day 3 of storage. However ~7.6 log CFU/g of *Salmonella* was recovered from the *Salmonella* control samples (no probiotic treatment) at the end of the experiment. Furthermore, application of LAB was associated with a higher germination efficiency when compared to the untreated seeds. Overall, this study demonstrates the potential application of LAB in promoting the microbiological safety of sprouts.

14. Identification and Quantification of *Saccharomyces cerevisiae* in an Equine Probiotic

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GutWerks™, manufactured by HorseTech™, is a digestive health supplement for the horse that contains probiotics. One of the probiotics in the supplement is yeast (*Saccharomyces cerevisiae*). The concentration of *Saccharomyces* cerevisiae in the supplement is 0.88 billion CFU per gram according to HorseTech™. HorseTech™ recommends administering 28.4 to 56.8 grams of the supplement per day for horses with a weight of approximately 1000 lbs. The purpose of yeast is not clearly defined by HorseTech™ or any other manufacturers, yet it is a common additive in horse feed and supplements. Some manufacturers indicate that yeast plays a role in decreasing the acidity in the hindgut and reducing lactic acid levels. In contrast, several benefits have been confirmed for inclusion of yeast in human diets: antioxidant activity; improved energy levels; maintenance of healthy skin, hair, eyes, and other organs; improved immune function; nervous system support; and improved control of sugar levels for individuals with type 2 diabetes mellitus. The purpose of this study is to validate the presence and advertised concentration of *Saccharomyces cerevisiae*. Yeast will be identified with end point PCR. The advertised concentration of yeast will be verified by culturing serial dilutions of the probiotic and determining the number of CFUs.

15. Defining the CrbS/R regulatory landscape in *Vibrio* and *Pseudomonas* strains

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Vibrio cholerae is responsible for causing the diarrheal disease cholera. When *Drosophila* are infected with *V. cholerae*, acetate is depleted in the gastrointestinal tract, which leads to death of the flies. Assimilation of acetate is controlled by the acetyl-CoA synthetase protein, encoded by *acs*. Previous work has shown that the CrbR/S two-component system activates transcription of *acs*. Aside from *acs*, the set of genes controlled by CrbR/S in *Pseudomonas* and *Vibrio*, two important environmental pathogens, may otherwise vary considerably. This project investigates the regulatory landscape of CrbR/S in order to reveal mechanisms driving major evolutionary changes in the CrbR regulon across and within the *Pseudomonas* and *Vibrio* genera. We are characterizing the CrbR/S regulon in *V. cholerae* and *V. fischeri* in addition to *Pseudomonas aeruginosa* and *Pseudomonas entomophila* with both ChIP-Seq and RNA-Seq techniques. With these results, we will reveal mechanisms through which the CrbR/S regulon is evolving across multiple timescales.

16. Protistan Ecology of Narragansett Bay Benthic Habitats

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Many anaerobic protists host methanogenic symbionts that contribute to nutrient cycling in aquatic ecosystems. Among these are the ciliate classes Plagiopylea and Armophorea. In order to understand the distribution and relative abundance of such protists, as well as to survey the general biodiversity of the protistan community in Narragansett Bay, sediment cores were collected from three locations (Providence River Estuary, Wickford Harbor, and Mid-Bay). Triplicate cores from each site were subsampled into centimeter thick horizons to a maximum depth of six cm. DNA was extracted from the sediment of the top two horizons from all cores, as well as the additional four horizons from the Wickford Harbor cores. The DNA was then amplified using two sets of 18S rRNA gene primers, a universal primer set and a Stramenopile-Alveolata-Rhizaria (SAR) specific primer set. Resulting 18S rRNA gene amplicons were sequenced with Illumina MiSeq. Results will be used to examine the diversity and relative abundance of protists in the benthic habitats of Narragansett Bay according to location and depth.

17. Anti-filarial Activity of Natural Neurolenin D and Synthetic Neurolenin Derivatives

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Lymphatic filariasis (LF) is a neglected tropical disease that threatens 52 countries and over 1 billion people. There are drugs available to treat LF; however, they are ineffective against adult worms, potentially toxic when people have other infections, and prolonged administration has led to emerging drug resistance. Thus, there is an urgent need to develop a novel, safe and affordable drug that is able to kill adult worms without causing side effects. Our work has focused on studying the bioactivity of neurolenin D, available from *Neurolaena lobata*, and synthetic analogs against the lymphatic filarial parasite *B. pahangi*. These analogs were created by modifications of the neurolenin scaffold including esterification at the reactive secondary alcohol position. Their bioactivity was measured *in vitro* against male and female adult nematodes by adding one dose (3 µg/ml) of the respective drug and then monitoring nematode mortality over a period of 100 hours. Interestingly, the activity of neurolenin analogs varies between male and female nematodes, indicating the presence of some mechanism resulting in gender selectivity. The bioactivity of neurolenin D and the most promising analogs will be further tested by means of RNAseq analysis comparing untreated and treated *B. pahangi* male and female adults. Studying the effect of the drugs on the transcriptome will provide us with information on the mechanism of action of these compounds. These results will provide further useful data on neurolenin and its analogues as promising alternatives for the treatment of LF and perhaps other neglected tropical diseases caused by nematode parasites.

18. Surveying microbial eukaryote diversity at different scales

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Microbial eukaryotes represent the majority of eukaryotic diversity, encompassing a spectrum of microorganisms such as fungi, protists, and algae. These eukaryotic microbes are important for ecosystem functioning, essential for monitoring and predicting environmental change, and also excellent models for understanding biological interactions and evolutionary history. However, many lineages of microbial eukaryotes remain understudied, as most are uncultivable. Recent advances in genomic techniques have allowed previously uncultivable, and therefore under-sampled, lineages to be explored. In this study we describe methods to survey microbial eukaryote diversity in freshwater and marine habitats. We focus on diversity within the SAR clade (Stramenopiles, Alveolates, Rhizaria) which includes many photosynthetic algae (e.g., Diatoms, Dinoflagellates, Brown algae), parasites (e.g., Oomycetes), and heterotrophic organisms (e.g., Ciliates, Cercozoa) and many other uncultivable lineages. By using

specific primers designed to amplify the 18S (SSU) rRNA gene within these groups we describe a method that allows characterization of community diversity across these lineages. These techniques capture diversity in many lineages, however, certain groups such as Foraminifera (marine shell-forming protists mostly defined by their test morphology) are highly derived and require a customized approach to characterize diversity. By developing and combining these broad scale and lineage specific techniques we demonstrate promising methods to survey microbial eukaryote diversity. We apply our methods across multiple study systems spanning several ecological scales, including the fluid of the purple pitcher plant (*Sarracenia purpurea*), intertidal habitats of the North Atlantic Ocean, and open ocean habitats worldwide.

19. A Metagenomic Approach to Evaluating Surface Water Quality in Haiti

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The cholera epidemic that occurred in Haiti post-earthquake in 2010 has resulted in over 9,000 deaths during the past eight years. Currently, morbidity and mortality rates for cholera have declined, but cholera cases still occur on a daily basis. One continuing issue is an inability to accurately predict and identify when cholera outbreaks might occur. To explore this surveillance gap, a metagenomic approach employing environmental samples was taken. In this study, surface water samples were collected at two time points from several sites near the original epicenter of the cholera outbreak in the Central Plateau of Haiti. These samples underwent whole genome sequencing and subsequent metagenomic analysis to characterize the microbial community of bacteria, fungi, protists, and viruses, and to identify antibiotic resistance and virulence associated genes. Replicates from sites were analyzed by principle components analysis, and distinct genomic profiles were obtained for each site. Cholera toxin converting phage was detected at one site, but members of the Acinetobacter family were frequently detected in samples, including members implicated in waterborne diseases. These results indicate a metagenomic approach to evaluating water samples can be useful for source tracking and the surveillance of pathogens such as Vibrio cholerae over time, as well as for monitoring virulence factors such as cholera toxin.

20. Enzyme production by the ectomycorrhizal fungus *C. geophilum* across varying substrate availability and soil moisture conditions

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Spatial variation in substrate availability and abiotic conditions are likely drivers of the distribution and activity of ectomycorrhizal (ECM) fungi. To understand how these environmental conditions may affect ECM function, I am testing the effects of neighboring tree species and soil hydrology on ECM community composition and enzyme activity. Preliminary surveys of ECM community composition at the Hubbard Brook Experimental Forest in Woodstock, NH show that the ECM community is largely dominated by a single species, *Cenococcum geophilum*, which comprises 87% of ECM-colonized root tips in the forest, with an average of 38% of total root tips colonized by ECM. I seek to determine whether tree community composition—a proxy for nutrient and labile carbon availability—or soil moisture regime best predicts *C. geophilum* enzyme activity. To test for variation in carbon (C), nitrogen (N), and phosphorus (P) acquisition, I am measuring potential production of a suite of enzymes that target cellulose, hemicellulose, chitin, phosphate, lignin, and other phenolic carbon compounds. To represent natural variation in the availability of these compounds, I sampled soil beneath clusters of two tree species with varying litter C, N, and P content: American beech (*Fagus grandifolia*) and white ash (*Fraxinus americana*). I also sampled beneath these species in areas with two different soil hydrological classifications, representing sites with generally higher water tables and soil moisture in addition to well-drained sites with more variable soil moisture. A better

understanding of how ECM enzyme production varies across these environmental and substrate conditions will be instrumental for generating predictive models of soil nutrient and carbon cycling in northern forests.

21. Material Stiffness and Hydration Influence Bacterial Attachment

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Bacterial adhesion and subsequent biofilm development are ubiquitous problems that cause infections and death. In hospitals, antibiotics, which kill bacteria and cause antibiotic-resistant bacteria, are used to combat infections caused by bacteria that adhere to implants, catheters, and medical equipment. Because of the few treatment options associated with antibiotic-resistant bacteria, polymer coatings which reduce bacterial adhesion are of increasing interest. Notably, understanding the adherence of model bacteria to materials as a function of their intrinsic properties would allow for future design of bacteria-resistant materials. The non-motile organism *Staphylococcus aureus* (*S. aureus*), a growing concern due to antibiotic-resistance, serves as a model microorganism in our studies. In this study, we systematically test two soft materials common for medical devices and coatings, hydrophilic poly(ethylene glycol) (PEG) and hydrophobic polydimethylsiloxane (PDMS). Properties such as stiffness, homogeneity, and hydration were assessed using Fourier-transform infrared spectroscopy, atomic force microscopy, and contact angle measurements. By optimizing their polymer concentration, paired PEG and PDMS gels were prepared with three distinct stiffnesses (32, 470, and 1100 kPa). We found the stiffness of fully-hydrated PEG hydrogels affected the adhesion of motile bacteria: the fewest bacterial cells adhere to thick, soft, and hydrophilic hydrogels. Whereas on PDMS, fewer *S. aureus* adhere to thick, stiff, and hydrophobic elastomers. This work provides insight into the future design of bacteria-resistant surfaces with broad implications for applications including medical implants and equipment.

22. Testate Amoebae Community Diversity Across Seasonal Change in New England Bogs and Fens

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Testate amoebae (TA) are eukaryotic microorganisms that build shells, known as tests, out of materials they gather from their environment. TA can be used as bioindicators in changing environments due to their sensitivity to changes in abiotic environmental factors such as a temperature and pH. My research focuses on understanding the diversity of TA communities found in New England Bogs and Fens using morphological and genetic analysis. Although TA are incredibly abundant, very little is known about genetic makeup of the communities. Solely delineating species based on morphology becomes difficult as there is a lot of cryptic diversity. This is why we are employing molecular techniques such as high throughput sequencing in order to more accurately characterize these communities. A large part of our project focuses on how TA communities grow and change in regards to species diversity across the span of a year. We have sampled once a month in three different locations, two in Western Massachusetts and one in Acadia National Park in Maine. At each site we gather samples of sphagnum peat moss from which we isolate our samples. We are currently working on analyzing our morphological data and isolating the DNA and RNA from the community samples. Hopefully, this data will allow us to gain more insight into the way these communities react to dynamic changes in their environments, and become a valuable resource for understanding the effects of environmental change on the ecology of sphagnum bogs.

23. Temperature adaptation and genome dynamics in Thermotogae

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Life is found in a wide range of temperature conditions, yet a specific organism can grow only in a limited temperature interval. While some cellular and genomic features that contribute to growth and survival at high and low temperatures are known, detailed mechanisms behind both microbial response to changing temperatures and

adaptation to new growth temperatures remain to be discovered. The bacterial phylum Thermotogae is an excellent system for studying the evolution of temperature preference. The phylum comprises ubiquitous deep-biosphere microorganisms isolated from terrestrial and marine settings. Collectively, Thermotogae are shown to grow from 20°C to 90°C. The comparison of genomes of such closely related mesophilic, thermophilic, and hyperthermophilic isolates provides an opportunity to identify new candidate genes and explore evolutionary processes that lead to adaptation to temperature. By examining a pan-genome of 114 Thermotogae, we explore how gene content vary across taxa that grow at different temperatures, and correlate gene family loss and expansion with optimal growth temperature.

24. Analysis of the CYP51 paralogs and their potential role in differential sensitivity to fungicides in *Calonectria pseudonaviculata* and *C. henricotiae*

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Fungal pathogens commonly threaten the growth of commercial and consumable crops. *Calonectria pseudonaviculata* and *C. henricotiae* are two closely related fungal species responsible for the development of boxwood blight disease in ornamental shrubs (*Buxus spp.*) in the U.S. and Europe. Previous studies have shown the latter species, which is restricted to Europe, to be less sensitive to select demethylation inhibitor (DMI) fungicides than the former. An unpublished study has shown that isolates of *C. pseudonaviculata* displayed an early stop codon in one of three *CYP51* paralogs known to confer resistance to fungicides, *CYP51A*. In this study we plan to analyze the *CYP51* paralogs for polymorphism in geographically disparate populations of *C. pseudonaviculata* and *C. henricotiae*, since the prevalence of an early stop codon in isolates of *C. pseudonaviculata* could emphasize the importance of the pseudogene in dictating sensitivity to DMI fungicides. We also plan to analyze RNA-Seq data from both species to determine if expression levels of *CYP51* paralogs and/or putative fungicide detoxification enzymes could explain the observed differential sensitivity.

25. Elucidating the Impact of Allosteric Regulation on the AAA+ Protease Lon

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Lon is an ATP-dependent protease charged with recognizing, unfolding, and degrading aberrant or cell cycle-dependent proteins in *Caulobacter crescentus*. Within the unique dimorphic life cycle of *C. crescentus*, target substrates of Lon have been shown to include master regulators of cell cycle checkpoints. In previous studies, the knockout of Lon invoked detrimental phenotypes, such as DNA damage sensitivity and hyper-filamentation. Although degradation is often thought to be Lon's primary function, Lon is also known to bind to DNA. Recent studies have shown that when Lon is prevented from binding, *C. crescentus* is observed to have increased sensitivity to DNA damage. While in vivo assays have provided a strong understanding of Lon's physiological relevance as both a protease and DNA-binding protein, however little is understood of how Lon's activity is impacted in the presence of allosteric regulators. Through the application of in vitro biochemical assays, allosteric regulation of Lon in the presence of both DNA and other select small molecules can be measured. In understanding this regulation and the role of each regulator within the cell the physiological importance of Lon can be better understood.

26. The grape microbiome in wild and domesticated grapes

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The cold climate wine industry has recently boomed in the Northeastern America after the successful breeding of cold-tolerant grape varieties. Vineyards harbor a wide variety of microorganisms that play a pivotal role in grape quality and will contribute significantly to the final aromatic properties of wine and vine health. If essential beneficial microorganisms have been identified in traditional wine cultivars, in contrast little is known about cold-climate cultivars. This project investigates how microbial communities vary between wild native grapes and agrosystems such as vineyards of Northeastern America. This will help in the discovery of yeast and bacterial species essential to wine quality and vine health and guide future sustainable farming practices.

27. The Microbial Community of Kitchen Sponges: Experimental Study Investigating Bacterial Number, Composition and Resistance

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In the United States alone, there are approximately 9.4 million episodes of foodborne illnesses each year, 3,000 of which result in death. Many cases may be prevented with improved sanitation techniques. Studies suggest that kitchen sponges are reservoirs for pathogens and can serve as vehicles of cross-contamination. The goal of this research study was to quantify bacterial number in typical household kitchen sponges. Five sponge were distributed to participants. Every 7 days for a total of 28 days, a square centimeter from each sponge was removed and the bacterial load was quantified using colony forming units (CFU) per cm^3 on nutrient agar media. Interestingly, a significant correlation between the bacterial load and the amount of time the sponge was used was not observed for every sponge. Some sponges reached a quantity of 8 Log CFU/cm^3. To determine the most effective strategies for decontamination, sponges that had been used for 28 days were subjected to 10% bleach, 70% ethanol, hot soapy water, dishwasher, and microwave. These sanitized sponges were quantified for bacterial content, and the bacterial abundance appeared to be significantly reduced with the use of all sanitation methods. This was especially true for chemically treated sponges, which showed no culturable bacterial growth. These data support previous studies which suggest that kitchen sponges provide a hospitable environment for microbial growth, and our data provides better information about proper sanitation techniques to eliminate bacterial contamination in kitchen sponges. Future studies aimed at using multipyrosequencing will give us a better insight to the microbiome, as well as the true number, diversity, and identity of microbes living in kitchen sponges.

28. Urea nitrogen utilization is restored by complementing a mutation in the *Bifidobacterium suis* UMA399 *ureC* gene

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Urea accounts for 15% of total nitrogen in human milk. Our hypothesis is this urea is salvaged by urease$^+$ bifidobacteria in the infant gut to provide a secondary nitrogen source. A urease deficient mutant was generated in *Bifidobacterium suis* UMA399 via chemical mutagenesis (i.e. ethyl methanesulfonate). Genome sequencing identified a lesion in the *ureC* gene in a mutant strain deficient for urease activity which cannot utilize urea as a nitrogen source. If the appropriate genetic tools were developed and electroporation protocols adapted, urease activity of the mutant could be restored through complementing the mutation. Bifidobacterial genetic research is currently hindered by the absence of these tools. Accordingly, a wildtype *ureC* was cloned into a shuttle vector adapted (i.e. pDOJHR) to transform the mutant strain. Segregation stability of pDOJHR in bifidobacteria was tested during 30 generations of growth by sub-culturing without selection. To check if urease activity was restored, a modified phenol- hypochlorite assay was performed and the growth in 2% of urea as a primary nitrogen source was monitored anaerobically. Results indicate that the shuttle vector pDOJHR was successfully transformed into *B. infantis* UMA272 and *B. suis* UMA399 with an efficiency ranging from 7.5 X 10^1 to 3.3 x 10^3 to remain stable after

30 generations. Moreover, urease function was restored in the mutant which resulted in the capacity to grow in 2% of urea.

29. Phylogenomic reconstruction of the phylum Ciliophora using single-cell 'omics'

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Ciliates are a large group of unicellular protists with high diversities. They play important roles in many biological and ecological studies. However, the phylogenetic relationships within ciliates mostly depend on single marker gene, like SSU rDNA, mtSSU rDNA or α-tubulin gene. In this study, we isolated 140 ciliate individuals representing 10 classes, performed single-cell genome and transcriptome amplifications and high-throughput sequencing. We used the resulting data to reconstruct the phylogeny of ciliates, with the intent of gaining a better understanding of ciliate evolution in 'omics'. Besides, gene-size chromosomes, which are extensive fragmented through the macronuclear differentiation, are found in species belonging to three classes, Spirotrichea, Armophorea and Phyllopharyngea. We provide more evidence for this finding by performing statistics analyses of contigs with two telomeres in our data. What's more, we also assess the stop codon reassignments of these species.

30. Role of ORF20 During KSHV Infection

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Viruses are evolutionarily constraint to hijack the host gene expression machinery for their own benefit and for proper progression of the viral life cycle. This is particularly true for viruses like herpesviruses that need to establish a good balance with their host to maintain decade-long infections. We focus on KSHV (Kaposi Sarcoma Associated Herpesvirus) and oncogenic herpesvirus linked with the development of several malignancies in immunocompromised individuals. While some of the KSHV proteins that contribute to viral and host gene regulation are known, it is clear that there are additional uncharacterized contributing viral factors. Identifying these proteins and their role in gene regulation is important to determine the mechanistic underpinnings of the complex replication cycle of KSHV. We set out to characterize KSHV ORF20, a member of the widely conserved herpesviral core UL24 family. Little is known about ORF20 but its conservation among all herpesviruses suggests a fundamental role for this viral protein during infection. By qPCR, we observed a burst of ORF20 expression at 72h post viral reactivation, suggesting that ORF20 is a Delayed Early (DE) gene, narrowing down its possible function to helping in viral replication. ORF20 ortholog in Herpes Simplex was shown to induce the dispersal of the host protein Nucleolin in the nucleus, a process crucial to liberate space in replication compartments. Ribosomal footprinting has previously identified 3 distinct isoforms for ORF20: ORF20FL (full-length), and two shorter isoforms (ORF20A and ORF20B) starting at internal start codons. Our data suggest that ORF20 isoform B is the only isoform with nuclear expression. We are in the process of testing whether ORF20B is responsible for Nucleolin dispersion during KSHV infection. By studying the mechanism by which ORF20 contributes to the progression of KSHV infection, we hope to further our understanding of the complex interplay between KSHV and its host.

31. Effects of Extracts from *Scutellaria barbata* and *Hedyotis diffusa* on Regeneration in Planarian Flatworms

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The extracts from herbs such as *Scutellaria barbata* and *Hedyotis diffusa* will be used to study the effect on regeneration in planarian worms. Planaria (*Dugesia*) are flatworms (Platyhelminthes) with an extraordinary ability to regenerate. Extracts from *S. barbata* and *H. diffusa* introduced by eastern medicine and culture has indicated that there are positive effects on cancer patients who have implemented these herbs into their diet. Although some

mechanisms have been proposed (apoptosis, increase of activity by the immune system) the actual mechanism by which these herbs exert on cancer cells is not known. Use of such herbs might potentially inhibit planarian worm regeneration. Extracts obtained from *S. barbata* and *H. diffusa* will be made in serial dilutions against spring water controls to determine planarian regeneration from tail cuts.

32. Chemical-genetic interactions of essential *Mycobacterium tuberculosis* systems

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Tuberculosis (TB) is one of the most prevalent infectious diseases in human history. Approximately a third of the world's population has been exposed to *Mycobacterium tuberculosis* (*Mtb*), the causative agent of TB, and this infection is responsible for more than 1 million deaths annually. TB must be treated with combinations of antibiotics to minimize the emergence of resistance but despite this complex regimen, TB cure requires months of therapy and multiple drug resistant (MDR) strains continue to emerge. Therefore new strategies are needed to both accelerate cure and treat MDR infections. The requirement for multi-drug therapy represents both a limitation and an opportunity. While combining agents can dramatically increase efficacy, predicting which potential new drugs will produce a synergistic effect in the relevant environmental conditions remains a challenge. Here we utilize conditional *Mtb* mutants of essential genes to investigate chemical-genetic interactions that occur during TB infection to identify synergistic interactions relevant *in vivo*, and compare with relevant *in vitro* conditions. Synergies were identified with antibiotics during growth in different carbon sources in both context independent and dependent manner, with specific synergies found during cholesterol growth. Of note, depletion of different components in the *Mtb* peptidoglycan biosynthesis pathway showed condition- independent synergies with multiple antibiotics, suggesting permeability and cell wall integrity could be targeted to improve antibiotic treatment. By creating an atlas of chemical-genetic interactions of essential genes during TB infection, we can identify new synergies to be exploited for drug development that will improve current antibiotic efficacies and treatment timelines for patients.

33. *Bifidobacterium infantis* utilization of human milk oligosaccharides as a primary nitrogen source

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Human milk represents the first food introduced to the breast-fed infant, containing high concentrations of human milk oligosaccharides (HMOs). The amino sugar N-acetyl-glucosamine (NAG) is one of the five monosaccharide building blocks of HMOs. Interestingly, NAG catabolism could provide microbes with both a carbon and a nitrogen source. While utilization of HMOs and NAG as a carbon source by infant associated bifidobacteria has been well characterized, the utilization of NAG as a nitrogen source has not received scientific scrutiny. In this study, we evaluated the ability of *Bifidobacterium infantis* to utilize HMOs and NAG as a primary nitrogen source. In addition, we utilized RNAseq to characterize the global gene expression of three *B. infantis* strains while these strains subsist on HMO and NAG as their primary nitrogen source. *B. infantis* utilizes HMOs and NAG as a primary nitrogen source by significantly increasing the expression of NAG catabolism genes including *nagA* and *nagB*. For all *B. infantis* strains, ammonia assimilation genes were upregulated while grown on NAG. Strain variation in growth phenotype was evident while subsisting on NAG. Distinct global transcriptomic responses of *B. infantis* occurred in response to different nitrogen sources for genes involved in cell wall biogenesis and central carbon metabolism. For certain strains, adhesion rates to Caco-2 cells were significantly increased by 4.5-4.8% while *B. infantis* utilizes NAG as a primary nitrogen source. This study provides evidence for infant-associated bifidobacteria to utilize HMO-borne nitrogen in addition to a carbohydrate source.

34. Trehalose recycling promotes redox and metabolic homeostasis in carbon-starved mycobacteria

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We have determined that mycomembrane reorganization triggered by glucose deprivation occurs via recycling of trehalose, a non-mammalian glycan that shuttles fatty acid mycolates to the mycomembrane. Trehalose recycling is essential for *M. tuberculosis* survival in a mouse model of infection. It has been widely hypothesized that the *in vivo* growth defect of trehalose recycling mutants stems from carbon starvation. Our work suggests a new model, namely that *de novo* trehalose synthesis induced in the absence of recycling consumes ATP, increases respiration and produces excess reactive oxygen species (ROS). A long-standing goal in the field is to identify pathways in non-growing *M. tuberculosis* that, when inhibited, potentiate killing by the host. Accordingly, we will test whether redox alterations contribute to the *in vivo* defect of the trehalose recycling mutant by perturbing ROS levels.

35. Testing Sesquiterpene Lactones from *Neurolaena lobata* as Potential Drugs for Treating Lymphatic Filariasis

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Lymphatic filariasis is a neglected tropical disease (NTD) infecting over 120 million people worldwide. Current treatment and elimination efforts rely on mass drug administration (MDA) guided by the World Health Organization. These drugs, however, do not target adult nematodes, leading to a need for new anti-filarial drug candidates that are more effective in eliminating adult nematodes already present in the human body. This is particularly important since it is the adult stages of the parasite that cause most pathogenicity in the human host and are responsible for production of microfilariae that transmit the disease via mosquito vectors. We are investigating the anti-adult killing activity of sesquiterpene lactones from the medicinal plant *Neurolaena lobata*. These compounds were tested on adult *Brugia pahangi* nematodes and the most effective derivatives were found to be neurolenin B, acetylated neurolenin C, and propionic esterified neurolenin B which all reached complete mortality of both adult female and adult male parasites within 96 hours of treatment. After determining significant antifilarial activity, the next steps in developing a potential drug candidate is to evaluate both the relative toxicity and mutagenic effects of neurolenin B and other effective neurolenin derivatives. The Ames test is the most universally accepted biological assay used to determine mutagenicity for a given compound and has demonstrated that neurolenin has low mutagenic effects. Additionally, work is being done to culture hepatic HepaRG cells and perform the MTT assay to identify if neurolenin derivatives exhibit cellular toxicity. With the demonstrated activity of these neurolenin derivatives against *B. pahangi*, and the molecules' low level of cellular mutagenicity and toxicity, we have identified a potential drug candidate for treating lymphatic filariasis and other filarial neglected tropical diseases which has greater biological activity against adult parasites.

36. Fecal Microbiomes of Saltmarsh Sparrows (*Ammodramus caudacutus*)

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Saltmarsh sparrows (*Ammodramus caudacutus*) live on the eastern coast of the United States. Because they live on the coast, they experience flooding events that cause nest failure and bird death. Due to sea level rise and climate change, the species is expected to be extinct by 2035. Previous studies show decreased amount of ectoparasites on the feathers during the flooding events. However, no previous microbiome analyses of the saltmarsh sparrow have been completed. In this project, the saltmarsh sparrow fecal microbiome was studied and

characterized for the first time. Two locations were chosen in Connecticut for sampling in the summer of 2017: Hammonasset State Park and Barn Island Wildlife Management Area. These two sites were far enough apart to prohibit the same bird to visit both sites. Mist nets were used to capture birds and the birds were put into a clean box to collect a fecal sample. The samples were collected and the DNA was extracted. The DNA was sequenced, processed in Mothur, and analyzed in R. The abundance of each phyla was observed in each sample with no clear dominant phylum in the samples and the samples appeared distinct from each other. A Principle Coordinate Analysis was made of the beta-diversity and showed no clustering of the samples based on location, sex, or age. A Shannon Diversity metric was also performed, which measures both abundance and evenness of the samples, but showed no consistency between the samples. In this study, the Saltmarsh Sparrow fecal microbiome is shown to be highly variable and not correlated to age, sex, or location.

37. A Novel Role for cAMP in the Regulation of the Acetate Switch in the *Euprymna scolopes – Vibrio fischeri* Model System?

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The gram-negative, bioluminescent marine bacterium *Vibrio fischeri*, enters into a mutualistic monospecific relationship with the Hawaiian bobtail squid, *Euprymna scolopes*. *V. fischeri* colonize the light organ on the ventral surface of the squid, and at night, projects light to mask the squid's shadow. In return, the squid provides the bacteria with shelter and nutrients. In order to colonize the squid, the bacteria must regulate gene expression so as to best respond to the changing nutrient conditions of the light organ. This involves regulation of the acetate switch, during which *V. fischeri* halt net excretion of acetate and begin assimilating this compound from the environment. This shift requires activation of the gene, *acs*, which encodes the enzyme acetyl-CoA synthetase. In *E. coli*, the global regulator cAMP-CRP is known to regulate the acetate switch. While investigating CRP and cAMP as regulators of *acs* in *V. fischeri*, we unexpectedly observed that addition of cAMP to the media lowered expression of *acs*. *V. fischeri* can consume cAMP as a nutrient source, and we hypothesize that catabolism of cAMP may reduce *acs* transcription via an unknown mechanism. We have confirmed that *V. fischeri* can grow on minimal media supplemented with only cAMP as a carbon and nitrogen source. We are currently testing whether mutations in genes required for cAMP catabolism interfere with the ability of exogenous cAMP to repress *acs*. We hope to reveal novel mechanisms through which CRP and cAMP may control *acs* expression in *V. fischeri*.

38. Characterizing Hfq-RNA interactions using a bacterial three-hybrid assay

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Both proteins and non-coding RNAs play a role in gene regulation across all domains of life. Bacteria use non-coding small RNAs (sRNAs) to regulate expression of target mRNAs, often with the help of protein chaperones. The bacterial protein Hfq is a well-characterized chaperone of sRNA-mediated gene regulation. Here we use a recently developed bacterial three-hybrid (B3H) assay to further elucidate the interactions between Hfq and its RNA substrates. This assay connects the interaction between a DNA-bound RNA and a RNA polymerase (RNAP)-bound protein to the expression of a reporter gene, providing a genetic route to the analysis of protein-RNA interactions. Here we present data collected using the B3H assay to understand the interactions of Hfq with RNAs in multiple capacities. One area of our work suggests that differences in residue charge in non-conserved regions of Hfq result in species-specific binding preferences for different sRNAs. This finding suggests a model where RNAs have evolved distinct mechanisms for Hfq binding that take advantage of differing electrostatic character of rim-surface residues in protein chaperone Hfq. Single-sited and chimeric mutants are being made to test aspects of this model. We also demonstrate the potential of this assay to detect Hfq's interactions with its mRNA targets in addition to its established capacity to detect Hfq-sRNA interactions, and show preliminary data suggesting that the results of B3H assays correlate with *in vitro* binding affinities of Hfq-RNA interactions. Using these approaches, we hope to gain a better understanding of the mechanisms of Hfq interacting with its RNA targets.

39. Authentic research as a capstone course in the teaching laboratory: comparative studies of malate dehydrogenase isoforms in trypanosomes

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Offering authentic research experiences to all undergraduates at a large university can be challenging. We are using Course-Based Undergraduate Research Experiences (CUREs), to make these experiences available to all Biochemistry and Molecular Biology (BMB) majors. One CURE focuses on Malate dehydrogenase (MDH) isoforms in *Trypanosoma brucei*, a protozoan parasite that causes African sleeping sickness. This organism utilizes oxidative phosphorylation of amino acids during growth in its Tsetse fly host, but is strictly glycolytic during growth in the mammalian bloodstream. Metabolic regulation in *T. brucei* is important to elucidating how the parasite proliferates in the host. *T. brucei* has MDH isoforms in mitochondria, cytoplasm and glycosomes. MDH catalyzes the oxidation of oxaloacetate to malate, and reduction of NAD+, is highly conserved and important for many processes including redox balance. We have generated clones for recombinant expression of all three trypanosome MDHs and have begun kinetic analyses. Students can design a projects *de novo* or choose to continue a project from students in a previous semester. A consortium of faculty from various colleges and universities in the protein-centric MDH CURE community (MCC), give faculty and students access to scientific information and community beyond the students and instructors in the course, assisting development of hypotheses and sharing of reagents. During the term, students give presentations and submit written assignments, with opportunities for peer review and revision. Teams present their results in a department-wide poster session at the end of the semester. Projects a have included comparisons between recombinant forms with C- or N-terminal tags and between different isoforms, or orthologs of different organisms, function at different temperature, pH or ionic strength. These studies provide tools for further analysis of biochemical and kinetic properties of the *T. brucei* enzymes and to perform comparative analyses with well characterized mammalian and plant MDH enzymes.

40. Inducible Peptidoglycan Cross-linking by D-amino Acid Chemical Reporters

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The peptidoglycan (PG) layer is a polymer consisting of sugar components and short chains of l- and d-amino acids. The peptide portions can be cross-linked to one another, forming a mesh-like layer. This structure is essential for bacterial viability as it provides resistance to turgor pressure. However, the limited approaches to study its full potential about it structure integrity and the ability to control macromolecular structures results in a gap that acts as a challenge. We used metabolic labeling and biorthogonal chemistry to produced synthetic cross-links in *Escherichia coli* and *Listeria monocytogenes*. More specifically, by incorporating two probes, an azide and alkyne d-alanines, then applying a biocompatible copper click (CuAAC) reaction to promote triazole formation between the two probes integrated to the cell wall. Visualization of hypothesized synthetic cross-links have been done by fluorescent microscopy and quantification of fluorescence signal by FACS. Additionally, HPLC-MS of peptidoglycan confirmed crosslinks in *E. coli*. To analyze the stability of the peptidoglycan at cross-linking conditions, we are using different antibiotics and check viability of bacteria. We found that cross-links bacteria are less susceptible to β-lactams.

41. Analysis of a Putative *Brugia malayi* POU-homeodomain Transcription Factor

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Neglected tropical diseases (NTDs) mainly afflict individuals living predominantly in developing countries. One of the most impactful NTDs is lymphatic filariasis (LF), a parasitic disease that results in the development of elephantiasis and is caused by the parasitic nematodes: *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori*. With an increasing prevalence of drug resistant human filarial nematodes, the need for novel therapeutics to eradicate LF has become crucial. Despite the urgent necessity to understand gene expression in filarial parasites for identification of new therapeutic targets, little is known about promoters and transcription factors in these organisms. The aim of this study is to identify possible transcription factors in *B. malayi*, using molecular and biochemical techniques, that could serve as potential drug targets to help combat filariasis. A comparative analysis between the protein sequence datasets of *B. malayi* and *C. elegans* identified a putative transcription factor Bma-UNC-86 and its cognate promoter *mec-3*. Electrophoretic mobility shift assay (EMSA) are being used to confirm the specific binding of the protein to the promoter, while a DNA binding affinity chromatography assay will allow us to capture the Bma-UNC-86 transcription factor from total protein extracted from *B. malayi* as well as other putative transcription factors that recognize the *mec-3* promoter.

42. Optimization of a Bacterial-Three Hybrid Assay for Hfq-sRNA Interactions

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Non-coding RNAs regulate gene expression in every domain of life. In bacteria, non- coding small RNAs (sRNAs) regulate gene expression in response to stress and are often assisted by protein chaperones. Hfq is an RNA chaperone protein that, through RNA interactions on distinct surfaces of its hexameric structure, supports many sRNAs to regulate translation and stability of their mRNA targets. Though Hfq is well characterized in *E. coli* and some additional model bacteria, Hfq homologs have only been identified in ~50% of sequenced bacteria, leading to speculation that additional RNA chaperone proteins may play important roles in sRNA function in other bacteria. The Berry Lab has recently developed a bacterial three-hybrid (B3H) assay that detects the binding of *E. coli* sRNAs with multiple RNA chaperone proteins. This assay couples the transcription of a genetic reporter to the interaction of a DNA bound “bait” RNA (*e.g.* an sRNA) and an RNAP-fused “prey” protein (*e.g.* Hfq). Successful interaction between RNA and protein stabilizes RNA polymerase at a test promoter and activates reporter gene expression. Current efforts are focused on optimizing the signal of the B3H assay so that weaker RNA-protein interactions can be reliably detected to lay the foundation to search for new bacterial RNA-binding proteins. Here, we show results from our efforts to improve the assay’s signal-to-noise ratio by re-examining the sequences, spacing, and the concentrations of the B3H assay components. We have seen an improvement in the assay’s signal in response to system altercations of the assay components. We continue to improve the B3H assay we aim to produce an assay that is able to discover new sRNA binding proteins and uncover the regulatory roles sRNAs and these proteins play in biology.

43. Investigation of putative virulence factors in *Chlamydia trachomatis* serovar D

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Chlamydia trachomatis is a sexually transmitted obligate intracellular bacterial pathogen that is classified into multiple serovars. When infections with *C. trachomatis* serovar D (Ct-D) in the female genital tract are left untreated, they can cause immunopathology that drives severe disease sequelae including pelvic inflammatory disease and ectopic pregnancy. We and others have previously shown that Ct-D is also more virulent in cell culture and in mouse models of genital tract infection, especially when compared to serovar L2 (Ct-L2). We hypothesize that Ct-D expresses virulence factors that are lacking in Ct-L2. We identified putative virulence factors found specifically in the Ct-D genome through sequence analysis. One of these putative virulence factors has significant sequence and predicted structural homology to the *Clostridium difficile* virulence factors TcdA/B. We are in the process of creating targeted mutants in these putative Ct-D virulence factors using the TargeTron™ Gene

Knockout system. The TargeTron™ system, which employs a group II intron, has been shown to be one of the few methods for producing targeted genetic mutations in *Chlamydia*. We are modifying this method for use in Ct- D with the goal of producing stable mutant which will be used in ongoing work to test whether these virulence factors are necessary to drive the increases in immunopathology observed in cell culture and mouse models of infection.

44. Effect of Depo-Provera Administration and *Chlamydia trachomatis* Infection on Antimicrobial Peptide Expression in the Female Reproductive Tract

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Depot-medroxyprogesterone acetate (brand name: Depo-Provera) is a highly effective hormonal contraceptive used by 30 million people worldwide. However, epidemiological studies suggest that Depo-Provera use may increase susceptibility to sexually transmitted infections (STIs). Susceptibility to microbial infection may be due to decreased activity of immune defenses, like β-defensins, in the female reproductive tract. β-defensins are a family of antimicrobial peptides that fight skin and mucosal tissue infection and inactivate STI-causing pathogens in the female reproductive tract. Here, we examine the expression of β-defensins in the female mouse reproductive tract after Depo-Provera administration and after infection with *Chlamydia trachomatis*. In particular, we focus on the basally-expressed β-defensin 1 and infection-induced β-defensins 2 and 3. We hypothesize that expression of β-defensin 1 will decrease in Depo-treated mice compared to PBS-treated control mice. We used RT-qPCR to measure the amount of β-defensin 1 mRNA in the mouse female reproductive tract in Depo- treated mice and PBS-treated control mice. Preliminary RT-qPCR data show a 5-fold reduction in β-defensin 1 expression in the female reproductive tract after Depo-administration, supporting our hypothesis. In ongoing studies, we are also testing the hypothesis that expression of β-defensins 2 and 3 will not change in Depo-treated mice and will increase in *Chlamydia trachomatis*-infected mice.

45. Environmental influences on microbial community structure in fungus-growing ant gardens

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The fungus-growing ant *Trachymyrmex septentrionalis* cultivates and maintains gardens of a specific cultivar fungus in underground nest chambers. These fungus gardens are the ants' primary food source. Each fungus garden hosts a microbial community that has been shown to provide nutrients to the cultivar fungus in other fungus-growing ant species. The microbial community in *T. septentrionalis* fungus gardens is less well studied. We therefore characterized *T. septentrionalis* fungus-garden and nest soil microbial communities across these ant's geographic range and during different parts of their active season using community 16S rRNA gene sequencing. The fungus-garden microbial communities had higher alpha- (within sample) and beta- (between sample) diversity than expected when compared to previously studied species. Only a few genera of bacteria are prevalent in most fungus gardens but not nest soil, and therefore represent core members of the fungus garden microbiome. We hypothesize that the fungus garden microbial community is instead largely transient, driven by both dispersal limitation and the seasonal availability of forage materials. Fungus garden microbial community composition correlates with both geographic distance between colonies and the season when colonies were sampled. There is no evidence that distance and season co-vary, indicating that geographic location and season independently influence the microbial community. Collectively, our findings suggest that *T. septentrionalis* fungus-garden microbial communities are likely composed largely of a highly variable and transient consortia of microbes that originate from immigration and may not provide any function in the symbiosis. The remainder is a relatively small core of

conserved microbes which represent the true symbionts in the fungus garden microbial community.

46. Studying gene family evolution and genome diversity of ciliates using single-cell 'omics

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Ciliates, a diverse group of single-cell protists, are featured by the presence of cilia and nuclear dimorphism. For decades, research in ciliate genomics was limited to a few cultivable lineages, e.g. *Tetrahymena*, *Paramecium*, and *Euplotes*. Genome architecture and gene family evolution in the bulk of ciliates (currently uncultivable) however remains unknown. Karyorelictea is one of these uncultivable groups and was long assumed to be 'primitive'. This is mainly because they are the only ciliates whose somatic nuclei are not able to divide. Instead, their somatic nuclei are differentiated and developed anew from germline nuclei during each cell cycle. Despite their unusual features, very limited data are available for Karyorelictea (e.g. only eight protein sequences are available on GenBank), as well as the majority of other ciliates. We performed single-cell transcriptomes from 56 ciliate individuals covering 10 out of 14 ciliate classes, and assessed transcript diversity among the focal groups as a proxy of paralog diversity. We also characterized single-cell genomes from members in three other understudied ciliate clades. Analyses from the resulting data show:1) paralog diversity is lower in karyorelictids compared to other ciliate groups given their nature of non-dividing soma; 2) the "primitive" description of the karyorelictids is inaccurate because their somatic macronuclei contain loci of varying copy number; 3) gene-sized somatic chromosomes exist in the class Litostomatea. Overall, we found single-cell 'omics techniques as a powerful tool to gain insights into genome architecture of uncultivable lineages and evaluate long-held questions in genome biology.

47. Does Oxygen Contribute to *acs* Regulation? Searching for Environmental Regulators of *acs* Expression in *Vibrio fischeri*

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Vibrio fischeri colonizes the light organ of the Hawaiian bobtail squid *Euprymna scolopes* due in part to its expression of acetyl-CoA synthetase (Acs). Acs, which converts acetate into acetyl-CoA, is regulated CrbS/CrbR two-component system. In order to determine mechanisms through which the host environment regulates bacterial metabolism, we are investigating the effects of oxygen on *acs* expression in *V. fischeri*. We grew *V. fischeri* carrying a transcriptional fusion of the *acs* promoter to the *lacZ* gene under conditions with varied oxygen availability. We then performed beta-galactosidase assays in order to measure *acs* expression. Preliminary results suggest that *V. fischeri* grown under oxygen rich conditions highly express *acs*, and cultures grown in oxygen deficient conditions demonstrated lower levels of *acs* expression. Our goal is to understand mechanisms through which a variety of environmental factors, including oxygen, impact *acs* expression.

48. Pathogen Weather Mapping: Metagenomics sequencing, geospatial technology and public health data mining

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According to the United Nations, contaminated water is the leading cause of death around the world, contributing to more deaths than all violence, including war. A major contaminant of water contributing to illnesses and deaths are microbial pathogens, which are invisible to the naked eye. This project investigates pathogens in various water sources locally and in Haiti. This work contributes to a project to optimize the Oxford Nanopore Technologies MinION platform for field diagnostics, particularly for environmental samples in developing countries. Our lab is funded to work in Haiti on improving cholera surveillance using whole genome sequence (WGS) analysis. Upon investigating specific genes of concern, such as the shigatoxin converting phage in *E. coli*, samples from the Mill River and Lake Warner were looked using PCR. Point sources polluters were identified such as agricultural runoff, a major contributor of toxigenic *E. coli* to water sources. PCR and multiplexing MinIon data revealed a wealth of information on multiple pathogens, virulence genes, converting phages and antimicrobial resistance genes. By combining the metagenomics sequencing data with geospatial technology I have created a google maps style interface, a user friendly resource that reveals the pathogens found in a water source. In an effort to advance the frontlines of preventative medicine through a public health perspective, this “weather mapping” of pathogens and antibiotic resistance in combination with bio-statistical algorithms designed for disease forecasting allows for major implications in the fields of natural disaster response, global public health, epidemiology, and disease threat analysis.

49. Hidden relationships: searching for bacterial symbionts in free-living testate amoebae

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Vector-borne diseases account for more than 17% of all infectious diseases, causing more than 700,000 deaths annually. So far, most investigations into these illnesses have focused on animals as vectors, despite the fact that protists can also serve as extremely potent vectors of disease transmission. However, most protist lineages, and especially free-living groups, remain largely understudied and knowledge on their association with prokaryotes is scarce. In order to deepen our understanding of protist vectors, our research focuses on free living amoebozoa (FLAs), which have been shown to harbor pathogenic bacterial symbionts from their environment. As a first step, we are trying to characterize the community of prokaryotes associated with free-living amoebae, focusing on two species of uncultivable testate amoebae, *Hyalosphenia papilio* and *Hyalosphenia elegans*. These amoebae are among the most abundant protists in *Sphagnum* moss communities in bogs and fens. While carrying out single-cell ‘omics on these species to study their biodiversity and phylogenies, we obtained prokaryotic sequences as by-product which may stem from potential endobionts of the amoebae. We are using bioinformatic approaches to identify these potential bacterial symbionts within *H. papilio* and *H. elegans*. So far, we have identified one possible candidate, *Sphingomonas* spp., which occured in multiple amoeba samples. Future work will entail designing a species-specific probe to use in conjunction with fluorescent- in-situ-hybridization (FISH) to locate the symbionts within the cell. We hope by finding these possible candidates, we will understand more about the genetic and ecological relationships between amoebae hosts and their symbionts.

Please note that underlined individuals are scheduled presenters of the presentations. In addition, posters with even numbers will be presented from 2:55-3:50pm and odd numbers will be presented from 3:50-4:45pm.

Emily Melzer, Srishti Kashyap, Gina Chaput, Carolyn Anderson, Caralyn Sein, Masroque Musa, Melissa Shinfuku, Alam Garcia-Heredia, Samar Mahmoud and Ian Sparks
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