# Pioneer Valley Microbiology Symposium
# UMass Amherst – January 25, 2020

## Distinguished Faculty Speakers

Dr. Wendy Mok
Department of Molecular Biology and Biophysics, University of Connecticut

**Deciphering the survival strategies of bacterial persisters**

Dr. Wendy Mok obtained her H.BSc. and completed her Ph.D. in the Department of Biochemistry and Biomedical Sciences at McMaster University in Canada. From 2013 to 2018, she trained in Dr. Mark Brynildsen's lab in the Department of Chemical and Biological Engineering at Princeton University as a Charles H. Revson Senior Fellow, where she studied the physiology and metabolism of bacterial persisters. Since January of 2019, she has been an Assistant Professor in the Department of Molecular Biology and Biophysics at UConn Health, where her lab focuses on bacterial responses that contribute to antibiotic failure and infection relapse.

Dr. John Gibbons
Department of Food Science, UMass Amherst

**Genomics of *Aspergillus*: the Dr. Jekyll and Mr. Hyde of Fungi**

Dr. John Gibbons received his B.S. in Biology at Keene State College and his Ph.D. in Biological Sciences from Vanderbilt University. John was a Postdoctoral Fellow at the Harvard T.H. Chan School of Public Health. John spent four years as an Assistant Professor of Biology at Clark University before joining the UMass Department Food Science as an Assistant Professor in 2018. John is broadly interested in using evolutionary genomics as a tool identify the genetic basis of traits relevant to food fermentation fungi and pathogenic fungi.

Dr. Peter Chien
Department of Biochemistry and Molecular Biology, UMass Amherst

**AAA+ proteases control bacterial growth and stress responses**

Dr. Peter Chien received his Ph.D. from the University of California, San Francisco. He completed postdoctoral training at the Massachusetts Institute of Technology as a Jane Coffin Childs Postdoctoral Fellow in Tania Baker's lab, where he worked on biochemical reconstitution of proteolytic pathways in *Caulobacter crescentus*. His research group at UMass Amherst, which he started in 2010, is focused on how regulated protein degradation shapes biological responses, with a particular interest in how energy dependent proteases impact bacterial cell cycle, development, and stress responses.

# Talks

*9:35 – 10:05am*

**Deciphering the survival strategies of bacterial persisters** *(invited keynote)*

Wendy Mok
Dept. of Molecular Biology and Biophysics, UConn, Farmington, CT

Bacterial persisters are phenotypic variants in clonal bacterial cultures that can survive lethal doses of antibiotics. Consequently, they are important contributors of antibiotic treatment failure, relapsing infections, and resistance development. To devise more effective strategies for eradicating persisters and combating the rise of antibiotic resistance, a better understanding of persister physiology, metabolism, and response to different classes of antibiotics is needed. Despite their remarkable ability to survive lethal doses of DNA topoisomerase-targeting fluoroquinolones (FQs), FQ persisters originating from non-growing cultures still suffer DNA damage and depend on DNA repair after antibiotic removal to remain culturable. We recently discovered that the nutrient environment following antibiotic removal can modulate the timing of molecular events pertaining to DNA damage repair and growth resumption, impacting whether a cell persists or succumbs to treatment. These findings suggest that the post-antibiotic treatment period offers a window of opportunity to manipulate the infection environment and molecular events to enhance persister killing. In this talk, I will discuss our group's efforts in investigating the impact of environmental triggers on the orchestration of molecular events affecting persistence and resistance development following fluoroquinolone treatment and their potential therapeutic implications.

*10:05 – 10:25am*

**Investigating the Mechanisms of Antifungal Resistance in Invasive Plant Pathogens using Genomics Tools**

Stefanos Stravoravdis[1], Nicholas R. LeBlanc[2,3], Robert E. Marra[4], Jo Anne Crouch[2], Jonathan P. Hulvey[1]
[1]Biology Dept., Eastern Connecticut State University, Willimantic, CT, [2]Mycology and Nematology Genetic Diversity Laboratory, USDA-ARS, Beltsville, MD, [3]ARS Research Participation Program, Oak Ridge Institute for Science and Education, Oak Ridge, TN, [4]Dept. of Plant Pathology and Ecology, The Connecticut Agricultural Experiment Station, New Haven, CT

*Calonectria pseudonaviculata* and *C. henricotiae* are two closely related fungal species responsible for boxwood blight disease of ornamental shrubs (*Buxus spp.*) in the U.S. and Europe. A previous study has shown isolates of the latter species, which is restricted to Europe, to be less sensitive to tetraconazole, an azole antifungal. In this study, we have analyzed the CYP51 paralogs for polymorphism in 26 genomes, representing geographically disparate populations of *C. pseudonaviculata* (n = 19) and *C. henricotiae* (n = 7), from the U.S., Europe, Asia, and New Zealand. Additionally, RNA-Seq analyses were performed for control and tetraconazole-treated isolates of *C. pseudonaviculata* and *C. henricotiae*. The gene expression levels of the CYP51A and CYP51B paralogs were compared as well as candidate detoxification genes overexpressed in *C. henricotiae*. The presence of a CYP51A pseudogene and lack of a functional CYP51A paralog in all *C. pseudonaviculata* genomes examined is a novel discovery for fungi and could have implications for the evolution of resistance to antifungal chemicals. Further, the identification of potential detoxification genes can inform future research into antifungal resistance and treatment of resistant pathogens.

*10:25 – 10:45 am*

**Chemical and mechanical properties of polymer thin films affect the initial adhesion of *Staphylococcus aureus***

Irene Kurtz and Jessica D. Schiffman
Dept. of Chemical Engineering, UMass Amherst, Amherst, MA

Bacterial adhesion and subsequent biofilm formation 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. The growing prevalence of infections caused by methicillin-resistant *Staphylococcus aureus* (MSRA) has directed our work to better understand the initial adhesion of *S. aureus* to medically relevant surfaces. In this study, we systematically test soft, thick polymer films made from polydimethylsiloxane (PDMS), which is commonly used for medical devices and coatings. PDMS was prepared with varying stiffnesses. Materials properties such as tackiness, viscoelasticity, homogeneity, and hydration were quantified using rheology, Fourier-transform infrared spectroscopy, and contact angle measurements. Two strains of *S. aureus* were tested to explore the universality of their response to the varying chemical and mechanical properties of PDMS. Both *S. aureus* ATCC 12600 (a genome-sequenced strain) and *S. aureus* SH1000 (a clinically-isolated strain) adhered the most to the softest PDMS. PDMS was coated with fibrinogen to explore the mechanism of adhesion for *S. aureus*. A thin coating of fibrinogen caused a rapid formation of a young biofilm regardless of the mechanical properties of the underlying PDMS. This work provides insight into the mechanism of adhesion for *S. aureus* and the future design of bacteria-resistant surfaces with broad implications for applications including medical implants and equipment.

*11:25 – 11:45 am*

**Microbial diversity drives carbon use efficiency in a model soil**

Luiz A. Domeignoz-Horta[1], Grace Pold[2], Xiao-Jun Allen Liu[1], Serita Frey[3], Jerry M. Melillo[4], Kristen DeAngelis[1]
[1]Microbiology Dept, UMass Amherst, Amherst, MA, [2]Graduate Program in Organismic and Evolutionary Biology, UMass Amherst, Amherst, MA, [3]School of Natural Resources and the Environment, University of New Hampshire, Durham, NH, [4]The Ecosystems Center, Marine Biological Laboratories, Woods Hole, MA

Empirical evidence for the response of soil carbon cycling to the combined effects of warming, drought and diversity loss is scarce. Microbial carbon use efficiency (CUE) plays a central role in regulating the flow of carbon through soil, yet how biotic and abiotic factors interact to drive it remains unclear. Here, we manipulated microbial diversity and measured CUE under different temperature and moisture in a model soil system. Moisture and temperature did not directly affect CUE, and instead acted through the biotic components. This was epitomized by bacterial diversity being positively correlated to CUE, but not under low moisture. Our results suggest that CUE in soils with declining diversity is more likely to be less efficient under abiotic stress. Diversity may therefore be even more important than previously foreseen for soil carbon cycling, and is crucial considering the various aspects of climate change.

*11:45 am – 12:05 pm*

**Implementation of engineered *Dysgonomonas* to study microbial interactions in the termite hindgut**

Charles M. Bridges and Daniel J. Gage
Dept. of Molecular and Cell Biology, UConn, Storrs, CT

The lower termite *Reticulitermes flavipes* harbors a complex microbial community in its hindgut consisting of Bacteria, Archaea and Eukaryotic fungi & protozoa. Despite this diversity, members of the genus *Dysgonomonas* (Phylum Bacteroidetes) are routinely found within *R. flavipes* hindguts. *Dysgonomonas* are facultative anaerobes found in soil, higher & lower termites, xylophagous cockroaches, *Drosophila*, as nematode ectosymbionts, and importantly, in three vector genera of mosquito (*Aedes*, *Anopheles* & *Culex*). To elucidate their putative functions, we isolated novel strains of *Dysgonomonas* from *R. flavipes* hindguts and sequenced their genomes. Genomes contained pathways involved in polysaccharide degradation, utilization of diverse glycans, production of short-chain fatty acids, and amino acid & vitamin biosynthesis. We also found genes encoding leucine-rich repeat (LRR) proteins, known to mediate inter-kingdom interactions. These results suggest that members of *Dysgonomonas* likely engage in mutualistic interactions by degrading and growing on lignocellulose- and host-derived glycans, while providing metabolites, vitamins, amino acids and other cofactors to community members and the termite. We developed a chemically defined medium and genetic system to directly test hypotheses regarding microbial interactions and function in the hindgut. Our genetic system permits transformation by electroporation or conjugation of replicative, recombinatorial or transposon-harboring plasmids with functional gene expression cassettes. GFP-expressing *Dysgonomonas* isolate BGC7 was fed to worker termites and the termites observed by fluorescence microscopy. Although some colonization was seen, much of the inoculum underwent predation by resident protists. GFP signal was observed inside protist phagosomes, although the fate of ingested *Dysgonomonas* cells was unclear. The phenomenon of mass phagocytosis by resident termite protists combined with our genetic system could permit *Dysgonomonas* to deliver DNA, RNA or protein to other community members including eukaryotic protists. Additionally, our system uniquely positions *Dysgonomonas* as candidates for paratransgenesis, allowing delivery of molecular payloads to pathogenic protozoa, nematodes and mosquitos.

*12:05 – 12:25 pm*

**Investigating the Role of ArcAB and Redox in Regulating Acetate Metabolism in the *Vibrio fischeri*-Hawaiian Bobtail Squid Symbiosis**

Kyra K. Raines and Alexandra E. Purdy
Dept. of Biology, Amherst College, Amherst, MA

A number of genetic and environmental factors determine the ability of *Vibrio fischeri*, a beneficial symbiont to the Hawaiian bobtail squid, to colonize and thrive inside the squid's light organ. One requirement for this symbiosis is the ability of *V. fischeri* to uptake and process acetate (Studer *et al*., 2008), a byproduct of fermentation. We previously performed a transposon mutagenesis screen in *V. fischeri* investigating regulation of acetate metabolism, using acetyl-CoA synthetase (Acs) expression as a genetic marker for acetate uptake and processing. We found that acs transcription is regulated by ArcAB (Flores-Ramos *et al*., in prep), a conserved two-component system that responds to the redox state of quinones to control the shift from aerobic to anaerobic metabolism (Malpica *et al*. 2004). The influence of ArcAB on acs indicates that redox environments regulate the timing of acetate uptake and metabolism. We are investigating the effect of ArcAB and redox environment on acetate metabolism in *Vibrio fischeri*, in order to understand factors that control this important metabolic process. We tested acs expression in multiple redox environments and in ΔarcA and ΔarcB mutants. We found that ΔarcA and ΔarcB mutants demonstrate reduced acs expression, indicating that the ArcAB system activates acs and triggers acetate uptake and metabolism. We have observed that ArcAB control of acs decreases as bacteria progress from exponential phase to stationary phase. Therefore, we hypothesize that ArcAB regulation of acs is growth phase-dependent, and other systems may control acs at different periods. In a symbiotic context, ΔarcA mutants exhibit a competitive defect during colonization of the light organ (Bose *et al*., 2007), indicating that redox response inside the squid is important for symbiosis. Our research shows that this response to redox includes regulation of acetate metabolism, indicating that redox environments influence acetate processing by *V. fischeri* in symbiosis.

*1:30 – 2:00 pm*

**Genomics of *Aspergillus*: the Dr. Jekyll and Mr. Hyde of Fungi**

John Gibbons
Dept. of Food Science, UMass Amherst, Amherst, MA

Examining genome variability is a critical step in understanding the phenotypic differences within and between species. In this light, our research interests center on understanding the genomic processes and evolutionary forces underlying phenotypic variation. To address this fundamental topic, we concentrate our work primarily on fungi because of their remarkable diversity, ecological importance, and societal impact. In addition, fungi offer ideal systems to study comparative and functional genomics because of their compact genomes, experimental tractability, and ease of genetic manipulation. We pursue our research theme by combining computational genomic analysis with complementary molecular and experimental approaches. Specifically, we study the genetic basis of *Aspergillus fumigatus* pathogenicity. *A. fumigatus* is an opportunistic fungal pathogen that is responsible for the deaths of ~100,000 people annually. *A. fumigatus* pathogenicity is a complex polygenic trait involving host immune evasion, host tissue degradation, and nutrient acquisition occurring over conidial and hyphal stages of development. We combine phenotypic screening with genomic, statistical, and molecular genetic approaches to identify novel genetic variants and genes involved in pathogenicity. Second, we study effect of domestication on microbial genome structure and function. Similar to plants and animals, bacteria, yeasts, and molds were domesticated by humans for food fermentation. We primarily focus our work on *Aspergillus oryzae*, which has been utilized for millennia in the production of sake, miso, and soy sauce. We use population genomics and laboratory experiments to compare the effects of domestication on *A. oryzae*, and other domesticated microbes, to patterns observed in plants and animals.

*2:00 – 2:20 pm*

**Human gut bifidobacteria that encode the gadB and gadC genes produce the inhibitory neurotransmitter γ-aminobutryic acid**

Michelle Rozycki[1], Asha Rani[1], David A. Sela[1,2]
[1]Dept. of Food Science, UMass Amherst, Amherst, MA, [2]Dept. of Microbiology and Physiological Systems, UMass Medical School, Worcester, MA

Bifidobacteria are the predominant members of the infant gut, colonize adults to a lesser extent, and are recognized as beneficial microbes. Various bifidobacterial species produce γ-aminobutryic acid (GABA), the chief inhibitory neurotransmitter in the mammalian central nervous system. It is postulated that in order to produce GABA, the bifidobacterial genome must contain the gadB and gadC genes which encode a glutamate decarboxylase and a glutamate/GABA antiporter, respectively. Once exported by GadC, GABA is absorbed and transported systemically throughout the host. We hypothesize that specific dietary oligosaccharides will modulate bifidobacterial production of GABA due to varying intracellular concentrations of glutamate. To test this, 22 bifidobacterial strains were screened for GABA production via reverse phase HPLC and a phylogenetic gene tree was assembled to determine sequence divergence of gad genes. Of those screened, 4 strains contained both gadB and gadC genes, and one strain only encodes gadB (*Bifidobacterium adolescentis* JCM 1275T). Interestingly, the strains that possess both gad genes produced detectable GABA *in vitro*. *Bifidobacterium moukalabense* JCM 18751 produced the highest concentrations of GABA (126.38±7.56 mM) whereas *Bifidobacterium angulatum* JCM 7096 produced the least (37.62±0.76 mM). Although the *B. adolescentis* type strain did not produce GABA, *B. adolescentis* JCM 15918 produced 69.60±1.02 mM of GABA, the most for a human-derived isolate. To further elucidate the extrinsic factors influencing GABA production, strains will be subjected to various environmental conditions and dietary components. Specifically, dietary oligosaccharides will be evaluated for their ability to promote biosynthesis of intracellular glutamate and thus potentially GABA. Understanding the relationship between diet, bifidobacterial physiology, and GABA production may inform dietary interventions to modulate this neurotransmitter *in vivo*.

*2:20 – 2:40 pm*

**Identification and characterization of curcumin-metabolizing gut bacteria**

Ermin Zhao[1], Katherine Chacon-Vargas[2], Zhengze Li[1], Yukun Sun[1], John Gibbons[1], Hang Xiao[1]
[1]Dept. of Food Science, [2]Graduate Program in Molecular and Cellular Biology, UMass Amherst, Amherst, MA

Gut bacteria can help the human body to metabolize curcumin, a dietary compound that is widely used as a yellow pigment. The microbial mediated curcumin metabolites from this process have a significant influence on the host health and may be the reason of different individual responses on curcumin supplements. Although increasing evidence implicates that the gut microbiome contributes to the curcumin metabolism, the identities of the bacteria and the bacterial genes involved in curcumin metabolism have been poorly studied. In this study, we surveyed the interactions between curcumin and pure strains from human fecal bacteria, and cell-free supernatants were profiled for targeted metabolites using high performance liquid chromatography. For the first time, we identified three strains that were capable of metabolizing curcumin to produce its hydrogenated products and ferulic acid. Next, we sequenced the whole genome of each bacterium using Illumina HiSeq platform, and ran the large-scale blast score ratio pipeline and bacterial comparative genomic analysis to identify the unique genes that were potentially involved in curcumin metabolism. In conclusion, a combination of metabolic and genetics profiling is developed to identify and characterize three gut symbionts *Lactobacillus* spp. that generate bioactive curcumin-metabolites. These results emphasize the importance of gut microbial genomes in biotransformation of food components and its impact on human health.

*4:00 – 4:20 pm*

**Is it a GTA or not?: A machine learning approach to distinguish Gene Transfer Agents (GTAs) from integrated viruses**

Roman Kogay, Taylor Neely, Daniel Birnbaum, Camille Hankel, Migun Shakya, Olga Zhaxybayeva
Dept. of Biological Sciences, Dartmouth College, Hanover, NH

Gene Transfer Agents (GTAs) are virus-like particles that are produced by some bacteria and archaea. However, unlike viruses, GTAs are too small to package all of its own DNA but instead usually packages random pieces of host's genome. Similar to viral infection, GTA production lyses the host cell and can deliver these DNA fragments to other cells in a population. The best studied GTA belongs to the Alphaproteobacterium *Rhodobacter capsulatus* (RcGTA). While there is a cost of RcGTA-production (lysis), maintenance of RcGTAs may be selected for due to fitness benefits associated with DNA exchange within a population. RcGTA is encoded by at least 24 genes that are distributed across five different loci in the *R. capsulatus* genome. Seventeen of the 24 genes are situated at one locus and encode proteins involved in the head and tail morphogenesis of the RcGTA particle. Due to the high structural similarity of GTA with tailed viruses, most of 'head-tail' genes have corresponding viral homologs. Additionally, the overall structure of the locus resembles a genome of a small siphovirus. Since many sequenced bacterial and archaeal genomes contain integrated viruses, there is a possibility that some are unrecognized GTAs. We developed a machine-learning approach that allows us to distinguish GTAs from integrated viruses with high degree of precision. Specifically, we used weighted support vector machine approach to differentiate GTAs from viruses based on their k-mer composition. We evaluated the quality of our model via the five-fold cross-validation and achieved weighted accuracies ranging from 95.6% to 100% for the examined 'head-tail' genes. Using our program, we surveyed 1423 alphaproteobacterial genomes for presence of GTAs. We found that 58% of the genomes likely encode GTAs, suggesting that GTAs and GTA-mediated horizontal gene transfer may be more ubiquitous than was previously recognized.

*4:20 – 4:50 pm*

**AAA+ proteases control bacterial growth and stress responses** *(invited keynote)*

Peter Chien

Dept. of Biochemistry and Molecular Biology, UMass Amherst, Amherst, MA

Protein degradation is required in all kingdoms of life. In bacteria, different ATP dependent proteases are responsible for degradation of different substrates at different times. For example, during cell cycle progression in *Caulobacter crescentus* the essential ClpXP protease selectively degrades critical replication factors. By contrast, the Lon protease is primarily responsible for degrading unfolded proteins during stress conditions. Using systems approaches, genetic screens, and biochemical reconstitution we have recently uncovered fundamental principles of how these proteases work. We found that subtle mutations in proteases can dramatically affect their specificity, how protein misfolding stress can be sensed to elicit changes in metabolism, and how novel properties of these proteases afford them an ability to selectively respond to different stresses.

# Posters

**Please note that underlined individuals are scheduled presenters.**

Poster Session 1: 10:45 – 11:25 am: Posters #1-20
Poster Session 2: 2:40 - 3:20 pm: Posters #21-40
Poster Session 3: 3:20 - 4:00 pm: Posters #41-62

1. **Investigation of a putative virulence factor, CT166, in *Chlamydia trachomatis***

Hannah R. Knapp-Broas, June Graham, Kyra Sieger, Everett Webster, and Rebeccah S. Lijek
Dept. of Biological Sciences, Mount Holyoke College, South Hadley, MA

*Chlamydia trachomatis* is an obligate, intracellular bacterial pathogen responsible for genital, ocular, and lymphatic infections in humans. Many but not all serovars of *C. trachomatis* are capable of causing chronic immunopathology in the uterus and ovaries, which can lead to pelvic inflammatory disease and ectopic pregnancy. *C. trachomatis* serovars capable of inducing mucosal immunopathology contain a gene called CT166 that is lacking in serovars that do not cause mucosal immunopathology. The predicted protein product CT166 is homologous to *Clostridium difficile* cytotoxins TcdA/B, and the ectopic expression of CT166 in HeLa cells is sufficient to induce cytotoxicity. We hypothesize that CT166 is expressed during *C. trachomatis* infection of the upper genital tract and acts as a virulence factor necessary for mucosal immunopathology. Using a novel RT-qPCR assay, we detected CT166 transcription in the uteri of mice infected with *C. trachomatis*. We then generated rabbit antisera against CT166 and are currently determining whether CT166 protein can also be detected in infected tissue by Western blot. To determine the necessity of CT166 in inducing mucosal immunopathology and cytotoxicity, we created plasmids with functional knockouts of this gene using TargeTron, which interrupts gene expression through the insertion of group II introns. *C. trachomatis* mutants containing these plasmids will be used for further research in cell culture and mouse models. In parallel, we are also measuring whether vaccine-induced antibodies against CT166 are sufficient to reduce *C. trachomatis* burden and immunopathology. Together, these experiments will elucidate the role of CT166 as potential virulence factor during *C. trachomatis* infection.

2. **The effect of *Lactobacillus*-inhibiting-isolates on *Crithidia bombi* levels in *Bombus impatiens***

Jane Milcetic[1], Chris Vriezen[1], Lynn Adler[2]
[1]Dept. of Biological Sciences, Smith College, Northampton, MA, [2]Biology Dept., UMass Amherst, Amherst, MA

Pollinating over a third of the world's crops, bees sustain globally cultivated and wild ecosystems, yet many bee species' survival rates have steadily decreased amidst environmental shifts and spreading disease (1,2). Antagonized by the gut pathogen *Crithidia bombi*, bumblebees suffer from altered foraging ability, increased mortality, and queens are prevented from founding hives (3). Yet, gut microbiome composition alters *C. bombi*'s growth in bumblebees, and abundant *Lactobacillus*, *Apibacter*, and *Gilliamella* correlate with low *C. bombi* amounts (3). Considering bumblebees' ground nesting behavior, *Lactobacilli* may be encountered in the soil, and contribute to the gut microbiome alongside floral microbial contributions. My experiment aims to correlate different levels of *Lactobacillus* with *C. bombi* amount, and I will use a novel approach to alter the amount of *Lactobacillus* in bumblebees by feeding them antagonistic soil bacteria. My objectives are (i) identify soil bacteria able to inhibit or kill *Lactobacillus in vitro*, (ii) determine the effect of co-inoculation of killer bacteria and *Lactobacillus* on *C. bombi* levels, and (iii) determine the effect of co-inoculation of killer and *Lactobacillus* on the bumblebee gut microbiome. I expect that bees with highly abundant *Lactobacillus* will correlate with low *C. bombi* levels, although bees that receive killer strains will have decreased amounts of *Lactobacillus*.

References: 1."FAO - News Article: Declining Bee Populations Pose Threat to Global Food Security and Nutrition." Accessed October 1, 2019. http://www.fao.org/news/story/en/item/1194910/icode/. 2. Kulhanek, K. et al. 2017. Journal of Apicultural Research 56, no. 4 https://doi.org/10.1080/00218839.2017.1344496. 3. Mockler, B. K., W. K. Kwong, N. A. Moran, and H. Koch. 2019. Applied and Environmental Microbiology 84, no. 7. https://doi.org/10.1128/aem.02335-17.

**3. N6-Methyladenosine (m6A) protects key mRNAs from viral induced mRNA degradation**

Daniel MacVeigh-Fierro, Mandy Muller, Angelina Cicerchia
Microbiology Dept., UMass Amherst, Amherst, MA

Chemical modifications are critical to guiding mRNA processing as well as the fate of mRNA. N6-methyladenosine (m6A) is one of the most abundant internal RNA modifications of cellular mRNAs. This modification recruits reader proteins that can guide RNA fate in the cell from P-bodies for decay or stress granules for storage. Our lab focuses on viruses that co-opt cell pathways to control RNA stability. We work with Kaposi sarcoma-associated herpesvirus (KSHV), a gamma-herpesvirus associated with the development of several cancers. This virus triggers a massive RNA decay event where 70% of RNA is degraded after the expression of SOX, a virally-encoded endonuclease. This process is believed to allow the virus to free up cellular machinery to promote viral gene expression while dampening immune sensing at the same time. Of the 30% of RNA that escape viral-induced decay, we found a class of mRNA that are protected from degradation by a novel type of RNA element named SRE for “SOX Resistant Element”. To better understand how this SRE mediates protection from SOX, we bioinformatically looked for possible motif in this RNA element and found potential m6A sites. Using a combination of MeRIP and qPCR, we have demonstrated that the SRE is m6A modified *in vivo* and that mutating the putative m6A site is enough to partially restore degradation viral endonucleases. We are currently investigating if this m6A modification allows for the recruitment of m6A reader proteins and whether that triggers the protection from viral endonucleases. Taken together, our results reveal a novel mechanism of resistance from viral-induced degradation. Better characterization of this protection phenotype could provide insights into RNA stability regulation during stress such as during viral infection but also in non-pathogenic settings.

**4. Testing Mushroom Extracts and Plant Essential Oils for their Effect on Biofilms in *Pseudomonas fluorescens* and *Chromobacterium violaceum***

Mitchell R. Cristofori, Johnathan Hulvey
Biology Dept., Eastern Connecticut State University, Willimantic, CT

The rapid development of antibiotic resistance heightens the need of new treatment methods that target novel components of microbial metabolism. One such avenue is the inhibition of quorum sensing: a universal communication pathway used by bacteria. Quorum sensing is crucial in promoting the expression of specific genes for biofilm formation, as well as virulence factors that promote infection. In this study, mushroom tinctures derived from Reishi and Chaga mushrooms were compared to essential oils including Peppermint, Spearmint, Eucalyptus, and Lavender, for their efficacy in disrupting biofilms. Both the essential oils and tinctures contain lactone based molecules which may mimic autoinducers and disrupt biofilms. Compounds were tested on *Chromobacterium violaceum* (CV1) and *Pseudomonas fluorescens* (PF) isolates cultured by Microbiology students at Eastern Connecticut State University during Fall ’18 and Spring ’19 semesters as part of the Tiny Earth project to student-source antibiotic discovery. A disk diffusion assay was employed to test the ability of serially diluted extracts and essential oils to disrupt biofilms, and also to obtain qualitative observations on their effects on biofilm morphology. Development of a quantitative spectrophotometric assay is in progress, with preliminary results presented here.

**5. Discovery of inhibitors of T3SS translocon assembly**

Hanling Guo[1], Mariana Breña[2], Alejandro Heuck[2]
[1]Molecular and Cellular Biology Graduate Program, [2]Dept. of Biochemistry and Molecular Biology, UMass Amherst, Amherst, MA

To deal with multidrug resistant bacterial infection, a new target is required. We focus on Type 3 secretion system (T3SS) in Gram negative bacteria that inject effector proteins into host cells. We use Gram negative, multidrug resistant opportunistic pathogen *Pseudomonas aeruginosa* as our model to study the T3SS. In *P. aeruginosa*, the T3SS secreted proteins PopB and PopD are found on the target cell membrane. In previous studies, PopB and PopD are believed to form a translocon, open transmembrane channel on host cell membrane and thus allow other four effector proteins, ExoT, ExoY, ExoS, or ExoU translocate into host cells. In previous study in our lab, we found PopD N-terminus expose to host cell cytosol only when the translocon is functional via GSK-tag phosphorylation assay and western blot. Inspire by this, we decided to develop a high throughput screening assay for small molecules inhibitors of T3SS translocon assembly. To detect whether the N-terminus of PopD expose to the host cell cytosol, we decided to use NanoLuc or Nluc as our reporter. NanoLuc is a luciferase that catalyze the Furimazine reacting with Oxygen, to produce light at 460 nm. It has a structure of 10 stranded beta sheets, and the last13 amino acids, including the 10th beta barrel, can be split and fused to the N-terminus of PopD. Using *P. aeruginosa* strains with fusion proteins of NLuc 10 located at PopD N-terminus to infect HeLa cells expressing NLuc 1-9, and incubate in media contain Furimazine. If the translocon is functional, the NLuc 10 on PopD N-terminus will expose to HeLa cell cytosol, and bind with NLuc 1-9 to catalyze the reaction mentioned above. Thus we can tell whether the translocon is functional or not by detecting luminance.

**6. Bacteria Identified from an Athletic Turf Field at MCLA**

Issa Jalloh, Elizabeth Billetz
Biology Dept., Massachusetts College of Liberal Arts, North Adams, MA

There is a growing concern for athletes contracting skin infections from bacteria residing on the turf field. This research project will evaluate the variety of bacteria present and look specifically for the presence of *Staphylococcus aureus*. Samples were collected from a variety of locations on the MCLA turf field by collecting small amounts of the rubberized granules and placing them in sterile water. 100 L of water, from each sample site, were plated on nutrient agar and brain heart infusion agar and incubated at 37°C for 24-48 hrs. A large amount of bacterial growth was obtained. Pure cultures were isolated and individual bacteria are being characterized.

**7. The effects of nutrient source and oxygen availability on gene expression in uropathogenic *Escherichia coli***

Grace M. Moore, Christine White-Ziegler
Dept. of Biological Sciences, Smith College, Northampton, MA

*Escherichia coli* strains reside in and move through a variety of environments that require them to adapt to the challenges that each of the niches present. Looking specifically at pathogenic strains, they can sense several environmental cues that can trigger a large suite of virulence genes to turn on and stimulate infection. Previous work in the White-Ziegler lab has modeled the effects of temperature on gene expression in enteropathogenic (EPEC), uropathogenic (UPEC), and commensal K-12 strains of *E. coli*. Using temperatures of 37°C and 23°C to represent inside and outside of a human host, the White-Ziegler lab has found that there are significant changes to gene expression between these two temperatures and that at 37°C, there is increased expression of virulence and virulence-related genes. UPEC strains which cause approximately 80% of community-acquired urinary tract infections (UTIs) worldwide are highly pathogenic in the bladder environment but act as commensals in the gut. This indicates that the transfer of UPEC from the gut to the bladder provides signals that initiate colonization. To test this hypothesis, we are assessing the impact of two cues that differ between the gut and the bladder. The gut is an anaerobic/microaerophilic environment with nutrients of all kinds whereas the bladder is a low oxygen environment with higher amino acid content and low carbohydrates. To mimic conditions of these environments, UPEC is grown in minimal medium containing glycerol (“gut”) or casamino acids (“bladder”) as the carbon source under microaerobic oxygen levels at 37°C to be compared to growth under aerobic conditions. By isolating the RNA under these conditions and analyzing the transcriptome derived by RNA-Seq, we aim to understand how bacteria sense and respond to the varying carbon source and oxygen levels they experience in these host niches and whether these impact virulence.

**8. Surveying microbial eukaryote diversity in phytotelmata habitats**

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Microbial eukaryotes represent the majority of eukaryotic diversity, encompassing a spectrum of microorganisms such as fungi, protists, and algae. These microbes are important for ecosystem functioning, essential for monitoring 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 these previously under-sampled lineages to be explored. In this study we survey microbial eukaryote diversity in the freshwater habitats of pitcher plant and bromeliad phytotelmata. 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), 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. Phytotelmata were sampled from natural and built environments allowing the factors that structure microbial communities to be examined. The microbial communities inhabiting the fluid in pitchers and bromeliads may be, in large part, the result of niche availability and chance events. The purpose of this study was to compare the individual communities within these ecological units in order to assess the similarities and differences of species richness and abundance. Our analyses indicate that bromeliad communities were dominated by Alveolate taxa, specifically ciliates, while pitcher plant communities had more representation from Rhizarian taxa.

**9. Transfection in *Brugia Malayi*: Transgenesis to Cure LF**

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*Brugia malayi*, a parasitic nematode, is most prominently known for its role in the transmission of the Neglected Tropical Disease known Lymphatic Filariasis (LF). Transmitted by mosquitoes to its human host, this parasite causes one of the world's leading disability-related diseases (CDC). There is a global call for a novel therapeutic treatment to be quickly and readily available. In response, we utilized a previously developed preliminary protocol for the general transfection of *B. malayi* (Liu et al.) with the integration of our own plasmids that were generated in-house to stabilize a method for transgenesis. This particular transfection method is centralized around a set of plasmids that encode for a transposon system: a plasmid with a non-native promoter region, a reporter gene (CHR), and a luciferase coding region flanked by two ITR regions, and a plasmid that encodes for the transposase enzyme. Transfection of these worms was done over an 8-day period with collection of media each day for a luciferase assay, where the bioluminescence response from the resulting secreted luciferase enzyme concentration was collected in order to characterize the efficiency of the transfection. Live worms were also image using fluorescence microscopy to visualize the distribution of the secreted luciferase throughout the organism. As a future goal, this protocol is to be applied to CRISPR technology to target specific developmental and molting genes in *B. malayi*. The usage of targeted transgenesis with CRISPR can provide a more specialized basis for the precursors to a treatment for LF.

**10. Iron Corrosion via Direct Metal-Microbe Electron Transfer by *Geobacter sulfurreducens***

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The anaerobic corrosion of iron structures is expensive to repair and can be a safety and environmental concern. It has been known for over 100 years that the presence of anaerobic microorganisms can accelerate iron corrosion. Microorganisms that can directly accept electrons from conductive surfaces have been receiving a considerable amount of attention. Previously, we used *Geobacter sulfurreducens* autotrophic strain ACL (possessing ATP-citrate lyase genes), and strain ACLHF (lacking genes for the uptake of hydrogenase and formate dehydrogenases from strain ACL), to grow with Fe(0) as the sole electron donor and fumarate as the electron acceptor. All results suggest that direct metal-microbe electron transfer is the most likely option for Fe(0) serving as an electron donor. Most microbiologically influenced corrosion (MIC) research has focused on electron uptake from Fe(0), however, corrosion studies done with stainless steel (SS) have significantly more environmental relevance. In our newest study, we chose 316L SS, which been used in many oilfield devices, as the electron donor. The corrosive ability of the different strains was tested through electrochemical tests and microscopy observations of the SS surface. Both strains had incredible corrosive capabilities, with strain ACLHF being the most corrosive. The results provide strong evidence that hydrogenases may not be necessary for MIC, which contradicts the well-accepted theory in MIC research that hydrogenase is vital. The ACLHF mutants with deletion and complement of the omcS gene confirmed that outer-membrane c-type cytochrome OmcS regulated direct electron transfer-MIC on 316L SS. These results confirm the role of direct electron transfer in MIC, and give a better understanding to the mechanisms involved in MIC and in the role played by OmcS in the direct electron transfer process of *G. sulfurreducens*.

**11. Ecological Distribution of Testate Amoeba in New England bogs**

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The bulk of eukaryotic diversity is made up of microorganisms that are vastly understudied in comparison to macroorganisms. One such group of microbial eukaryotes are testate (shell-building) amoeba of the order Arcellinida. Arcellinida are widely distributed in freshwater ecosystems, such as bogs and lakes, where they play important roles as predators and in nutrient cycling. Arcellinida are highly sensitive to environmental parameters, such as pH and moisture level, and are therefore used extensively as bioindicators for ecosystem health. These bioindicator studies are generally based on the identification of morphospecies for the characterization of Arcellinida communities. However, this approach is prone to error, due to the existence of cryptic diversity within morphospecies and the challenge of correctly identifying morphospecies. To address the problem of cryptic diversity, we developed a molecular approach to identify Arcellinida communities by environmental amplicon sequencing. In this current project we apply this amplicon sequencing approach to study the distribution of Arcellinida on small scales within local bogs and on larger scales between bogs of Massachusetts and Maine. In detail, we are investigating how Arcellinida community structure changes within those sites in response to environmental conditions, such as along a transect from forest to open bog. With this study, we aim to elucidate the ecological distribution of Arcellinida to gain a better understanding of their associations with environmental factors and how they may be affected by environmental changes.

**12. Optimization of a bacterial-three hybrid assay for discovery and characterization of sRNA-binding proteins**

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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 using Hfq-sRNA interactions as a model system, so that weaker RNA-protein interactions can be reliably detected to lay the foundation to search for new bacterial RNA-binding proteins. Here we will present our efforts to improve the assay’s signal-to-noise ratio by adjusting the sequence, concentration, and spacing of different components within the B3H assay. Our aim is to optimize the B3H assay so that it is able to identify novel protein-RNA interactions, as well as dissect the binding mechanisms of these interactions.

**13. The mechanism of prion loss by excess Hsp104**

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In contrast to our classical view of one-sequence-one-fold, some proteins can adopt a natively folded conformation and multiple alternative conformations under physiological conditions. The most well-known examples of this phenomenon are the prion proteins, which adopt alternative conformations that assemble into ordered, linear aggregates known as amyloid. The ends of these complexes template the conversion of natively folded monomers into the alternative conformation by binding to and incorporating these species into the amyloid aggregate. Conversion to the amyloid conformation is associated with not only disease states as in the case of the transmissible amyloidopathies of mammals but also novel, transmissible phenotypes as found across many species. In yeast, the transmissibility of prion-associated phenotypes depends on the molecular disaggregase Hsp104, which fragments amyloid to create new ends for templating and smaller aggregates for partitioning into daughter cells. In the case of the [PSI+] prion, the self-replicating prion form of the Sup35 protein, both inhibition and overexpression of Hsp104 causes amyloid loss (i.e. curing); however, the mechanism by which this curing occurs is unclear. In this study we investigate the effect of increased Hsp104 levels on Sup35 amyloid structures using GFP fusions and microscopy. Our observations reveal a difference in the mechanism by which curing occurs between Hsp104 inactivation and overexpression.

**14. Characterizing nuclear architecture and genome size in uncultivable ciliates through fluorescent microscopy**

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In the ancient ciliate clade (~1 billion years old), the majority of members are yet uncultivable and poorly understood. *Loxodes* is a group of uncultivable ciliates and hosts unique nuclear features, such as non-dividing somatic nuclei. Each *Loxodes* cell possesses multiple nuclei and even multiple nuclear groups consisting of both somatic nuclei and germline nuclei. Due to their distinct nuclear characteristics, and rather basal position in ciliate phylogeny, they are key members for understanding ciliate nuclear and genome evolution. Thus, we carried out experiments to characterize the nuclear architecture and genome size of *Loxodes*. As they are not yet cultivable in lab, they are sensitive to a variety of commonly used fixatives. We have successfully adapted fluorescent staining protocols for uncultivable cells, and are using fluorescent confocal microscopy to investigate the size of the *Loxodes* genome and relative DNA content of the somatic nucleus and germline nucleus. In addition, since, unlike the other ciliates, the somatic nuclei of *Loxodes* (and other members in class Karyorelictea) do not undergo division, we are also exploring the effect of “age” differences between somatic nuclei (i.e. different somatic nuclei in the same cell that have survived different number of generations) on their relative DNA content. Overall, we provide methods to explore the DNA content of uncultivable cells with no reference genomes, and our data contribute to our understanding of the diverse nuclear architectures present in microbial eukaryotes.

**15. Extremely short introns in *Loxodes* (Protist, Ciliophora)**

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Ciliates, a >1billion old clade of microbial eukaryotes, are defined by their division of genome function between transcriptionally inactive germline micronuclei and functional somatic macronuclei. For decades, research in ciliate genomics was limited to a few cultivable lineages, e.g. *Tetrahymena thermophila*, *Paramecium tetraurelia*, and *Oxytricha trifallax*. Nuclear and genome architecture in the bulk of ciliates (currently uncultivable) however remains unknown. Karyorelictea is one of these uncultivable groups and unique among ciliates mainly because they are the only lineages whose somatic nuclei are not able to divide. Despite their unusual features, very limited data are available for Karyorelictea. We isolated cells from a local low pH pond (pH ~ 4.5) and performed single-cell genome amplification on 8 *Loxodes* individuals that belong to the same species according to small subunit rDNA. Resulting data from high-throughput sequencing are then analyzed with a variety of bioinformatic tools. Our analyses reveal that *Loxodes* possess a range of extremely short introns. The majority of introns have a length between 16 nt and 25 nt. In addition, the tiny introns have the canonical exon-intron boundaries 'GT/AG' which indicates that they might still be spliceosomal. We are comparing intron size distribution as well as other characteristics among other ciliates with genome annotation available and a few other protist lineages for more insights into the splicing mechanisms. Overall, we demonstrate the power of single cell transcriptomics as a tool to investigate genome architecture and evolution in uncultivable microbial lineages.

**16. A refined approach to assess Foraminifera diversity at different habitats using High-throughput sequencing**

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Foraminifera are a clade of mostly marine shell-building amoebae that act as biomarkers of past and present climatic conditions. Yet, their diversity assessment previously relied on morphological assessment, which requires specialists, can be impacted by cryptic morphologies, and can be time-consuming. Furthermore, molecular marker-based (SSU-rDNA) diversity assessments using 'universal' eukaryotic primers have limited resolution for Foraminifera because of its rapid rates of evolution with many insertions and considerable length variation. Thus, many inserts in foraminiferan SSU-rDNAs require a customized amplicon approach to characterize their diversity. Therefore, we designed Foraminifera specific primers to characterize two hypervariable regions of their SSU-rRNA genes and deployed them in a pilot study to assess diverse foraminiferan communities. We apply our methods across several ecological scales, including the intertidal habitats of the North Atlantic Ocean, open ocean habitats, and desiccated freshwater vernal pools. This study reveals considerable genetic diversity within foraminiferan, much of which comes from morphospecies whose sequences are not yet deposited on GenBank. In addition, though there is considerable heterogeneity among habitats, open ocean communities are more similar to communities in the intertidal region. Furthermore, analyses of most abundant community members show that single-chambered lineages (i.e., OTUs) are abundant everywhere. This pilot study indicates the potential of a refined amplicon approach to elucidate the diversity of Foraminifera communities in diverse habitats.

**17. The chytrid fungus *Batrachochytirum dendrobatidis* has both animal-like and yeast-like actin networks**

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The frog parasite *Batrachochytirum dendrobatidis* (Bd) is a chytrid fungus with two main life stages, a motile zoospore stage lacking a cell wall, and an immotile zoosporangia stage possessing a cell wall. We have previously shown that Bd zoospores move on surfaces using actin and Arp2/3 dependent cell protrusions called pseudopods. Because this motility is likely involved in pathogenesis, it is important to understand how pseudopods and other actin structures are regulated in Bd. To understand the possible mechanisms Bd may be using to control its actin networks, we used BLAST searches to determine the conserved set of actin regulatory proteins present in Bd and other chytrid fungi. We found that chytrids have an actin regulatory network intermediate to that of animals and that of other fungi. For instance, chytrids encode the canonical actin nucleators Arp2/3 complex and formins, and, unlike other fungi, the Arp2/3 regulator, SCAR/WAVE complex. Given chytrids' divergence before the diversification of fungi, this finding provides fundamental insight into the evolution of actin regulatory networks. Through a combination of phalloidin staining and small molecule inhibitors, we found that Bd has temporally and phenotypically distinct actin structures that resemble either those of animals or of yeast. The finding of animal-like and yeast-like actin structures present in one organism proves useful in uniting the two main model systems of actin study. Furthermore, expanding the knowledge of actin-based motility in Bd may provide an avenue for combating the devastating effects this pathogen has on amphibian populations worldwide.

**18. Evolution of DNA Packaging Machinery in Gene Transfer Agents**

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Gene transfer agents (GTA) are virus-like particles encoded and produced by many bacteria and archaea. Unlike viruses, GTAs package fragments of the host's genome instead of the genes that encode the GTA itself. GTAs are hypothesized to serve as a mechanism of horizontal gene transfer in prokaryotic populations. The maintenance of GTA genes in many prokaryotic genomes could be a result of natural selection due to advantages associated with gene exchange. The origin of GTAs and their evolution from their presumed viral ancestors are still unknown. The most well-studied GTA is produced by an alphaproteobacterium *Rhodobacter capsulatus* (RcGTA), and its homologs are widespread in other alphaproteobacteria. RcGTA packages random portions of the host's genome. To shed light on the differences between GTA and viral packaging, we reconstructed an evolutionary history of the large subunit of the terminase (TerL), an enzyme used by viruses and GTAs to package DNA. Distinct methods of viral DNA packaging correspond to differences in TerL amino acid sequence, forming separate groups on a TerL phylogeny. We found that RcGTA-like TerLs grouped within viruses that use a "headful" packaging strategy, suggesting that RcGTA evolved from a virus that used headful packaging. Headful packaging is the least sequence-specific mode of DNA packaging, and thus evolution of the TerL in the RcGTA ancestor could have resulted in a switch from packaging of the GTA genome to packaging of random pieces of the host's genome. The exact changes to the GTA TerL that led to this transition remain to be elucidated.

**19. The Human Microbiome in Asthma during *Boswellia serrata* and Bacterial Regimens**

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The human microbiome is a collection of bacteria, virus and fungi that colonize within the human body. Asthma is a chronic inflammatory disease which affects the airways of the lungs. Bacterial infection plays a significant role in asthma exacerbation. However, not all bacteria are pathogenic. Many of their beneficial host interactions are known, including digestive help, protection from pathogens, modulation of host immune response and having pro- or anti-inflammatory properties. In clinic, oral steroid treatment is commonly used to treat severe asthma exacerbation, despite a wide range of side effects. *Boswellia serrata*, a natural resin, has been shown as a potent anti-inflammatory agent in asthma. To date, it is unknown whether the *Boswellia serrata* administration orally affects the gut microbiome composition, and if so, whether the altered gut microbiome from the treatment contributes to the protection of asthma development and symptoms. Additionally, in our preliminary studies, we found the nasal microbiome to be associated with future asthma exacerbation. We further identified groups of bacteria (*Staphylococcus*, *Streptococcus*, *Moraxella* and *Corynebacterium*/*Dolosigradulum*) that are potentially protective or detrimental in future asthma control in those who have asthma. However, it is unclear whether these bacteria have any causal role in asthma development. Therefore, the goal of this study is to determine new techniques to treat asthma using both the nasal and gut microbiome.

**20. Can global warming impact the genomes of soil microbial communities?**

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Climate warming, due to rising levels of carbon dioxide (CO2) in the atmosphere is heating soil and impacting soil microbial communities. Soils are the major source of carbon, and soil microbial communities are crucial for carbon (C) cycling processes, important for regulating greenhouse gas fluxes of CO2 in soils and the atmosphere. Despite their important role, it is still uncertain whether global warming could produce alterations in the genomes of soil communities which could imply changes in soil carbon cycling, potentially reinforcing global warming. To determine this, we will use the next-generation sequencing (NGS) of Nanopore MinION device to sequence isolates from a 27-year chronic soil-warming experiment at the Harvard Forest and detect if long term warming can impact the genomes of soil microbial communities and its potential effect on carbon cycling. We hypothesize that genomes of soil bacteria have changed in response to chronic warming and these modifications may favor soil carbon loss. This study will reveal if long-term warming changes gene frequency in genomes of soil microbial communities which would be a response to long-term warming. Completion of this research will help get a better understanding of how climate warming is affecting soil microbial communities.

**21. Impact of Post Treatment Conditions on *E. coli* Persistence and Resistance Development**

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Antibiotic resistance and the lesser known phenomenon of antibiotic persistence pose a huge threat to the future of infectious disease treatment with antibiotics. Resistance refers to the ability for bacteria to grow in the presence of an antibiotic due to heritable genetic mutations that have been acquired. Persistence, on the other hand, refers to a non-inherited phenotypic transient state that enables bacteria to cease growing in the presence of an antibiotic and survive treatment. Upon removal of the drug, a bacterial persister is able to resume growth, giving rise to a progeny with both persisters and non-persisters. Additionally, bacterial responses and environmental factors after antibiotic removal can impact persister and resistance development. It was recently observed that persisters do in fact experience DNA damage during antibiotic treatment, similar to their sensitive cell kin. Furthermore, it was shown that certain stresses could modulate the timing of DNA repair and growth resumption following antibiotic treatment, which influences whether a cell survives or dies. Our preliminary data also suggest that nutrient availability during the post antibiotic treatment period can alter the expression of genes involved in error-prone DNA repair processes, which affects resistance development. In this project, we are interested in testing other post treatment conditions that influence persister survival. Some treatment conditions we will examine include the use of efflux pump inhibitors, as well as peroxides and other compounds to modulate oxidative stress and thus ROS accumulation. By achieving a greater understanding of the conditions that impact persister survival, we will gain further insight into how best to target these cells and prevent antibiotic treatment failure.

**22. Assessing the mode of metabolic regulation by glycerol-3-phosphate in *Vibrio fischeri***

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Carbon sources play a crucial role in regulating bacterial responses to environmental conditions, which may in turn affect microbial interactions with hosts. *Vibrio fischeri* with a mutation in acetyl-CoA synthase (acs), an enzyme that facilitates the transition from releasing to assimilating acetate, show a competitive disadvantage in colonizing the Hawaiian bobtail squid *Euprymna scolopes*. Previous work has shown that the presence of glycerol in the media represses expression of acs, and glycerol is one of several carbon sources provided to *V. fischeri* by *E. scolopes*. In *V. fischeri*, glycerol from the environment is transported into the cell and converted to glycerol-3-phosphate (G3P). Here, we demonstrate that a mutation in glpD, which prevents the bacteria from further metabolizing G3P, restores high acs expression in glycerol. We also find that the addition of G3P represses acs in a glpD mutant, suggesting that G3P itself is responsible for mediating the repression of acs. In *Escherichia coli*, G3P has been proposed to inhibit the production of cyclic AMP (cAMP) by interfering with adenylate cyclase (cyaA) activity. The cAMP-CRP complex has further been shown to activate acs expression in *V. fischeri* in our lab. To test whether G3P acts through cyaA to repress acs expression in *V. fischeri*, we tested acs expression in a cyaA mutant. Supplementing the cyaA mutant with cAMP and G3P would dissociate the hypothesized connection between G3P and cAMP production. We found that G3P still repressed acs expression, suggesting *V. fischeri* utilizes an alternative mechanism. Altogether, this indicates that *V. fischeri* employs a unique system to regulate a metabolic pathway important for symbiosis.

**23. *In vivo* Testing of *Borrelia burgdorferi* Strains and Development of a Multiplex qPCR Assay for Detection of Infectivity-Associated Plasmids**

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*Borrelia burgdorferi*, the causative agent of Lyme disease, has a genome consisting of one linear chromosome and up to 23 linear and circular plasmids. Evidence suggests that proteins encoded on certain plasmids, (e.g. lp25, lp28-1, and lp36) are associated with mammalian infectivity. After an unsuccessful attempt to infect C3H/HeJ mice with *B. burgdorferi* strain B31, we screened our strain library using conventional PCR and discovered strain B31 was missing lp25, but a B31-derived clone (5A1) contained all three plasmids. Repeat *in vivo* testing confirmed that strain B31 was not able to infect mice. However, strain 5A1 was infectious to mice as determined by qPCR and culture analyses of blood and tissue samples. Subsequently, a high-throughput qPCR assay was designed that allows simultaneous detection of all three plasmids associated with infectivity. This work illustrates that commercially available *B. burgdorferi* strains may be lacking key plasmids necessary for infectivity, confirms the importance of lp25 for infectivity, and provides a valuable and efficient assay for screening *B. burgdorferi* strain libraries.

**24. *Bifidobacterium infantis* Metabolizes Free and HMO-Derived Fucose to Produce 1,2-Propanediol in the Infant Gut**

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While indigestible by the infant host, human milk oligosaccharides (HMOs) contribute to overall infant nutrition by causing a shift in the gut microbiome to favor beneficial microbes that metabolize HMOs. 2´-fucosyllactose (2´FL) is one of the most abundant fucosylated HMOs and is added to commercial infant formula. *Bifidobacterium infantis*, often the dominant microbe in the infant gut, exhibits rapid utilization of fucosylated HMOs and possesses a core gene cluster dedicated to HMO metabolism. Despite these adaptions, it is not well understood if *B. infantis* utilizes fucose, much less the underlying mechanism. The *B. infantis* chromosome encodes genes that may enable partial metabolism of fucose following a canonical pathway exhibited by other bacteria. Thus, we have hypothesized an alternate pathway based on the *B. infantis* genome. Accordingly, *B. infantis* grows during co-fermentation of fucose with glucose or lactose and on sole carbon sources 2´FL and soluble fucose. Furthermore, *B. infantis* consumes fucose (-44.0±0.5 µmoles) to produce 1,2-propanediol (+14.06±0.07 µmoles) in co-fermentation with glucose (-39.0±0.9 µmoles fucose, +58.0±0.3 µmoles 1,2-PD) or lactose (-15±5 µmoles fucose, +8.3±0.5 µmoles 1,2-PD). Proteomic analysis of *B. infantis* growth on fucose identified complementary proteins supporting the alternate pathway. Interestingly, *B. infantis* growth shows an unexpected inverse trend with decreased 2´FL concentration. Increased growth reaches a maximum OD600 1.17±0.06 at 0.6% (w/v) 2´FL. Fucose-specific metabolite 1,2-PD also follows this parabolic relationship with 2´FL. Determining the molecular mechanisms of fucose utilization and 2´FL's unexpected effects on *B. infantis* physiology will provide insights into the role of fucosylated HMOs in supporting infant nutrition, development, and health.

**25. The impact of overexpression of small regulatory RNAs on *Escherichia coli* biofilm formation**

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The formation of biofilms is an important adaptive strategy in the survival of *Escherichia coli* as biofilms provide protection from environmental changes such as the introduction of antibiotics or a host immune system. For this reason, biofilms are an important part of the pathogenesis of *E. coli* and many other microorganisms. *E. coli*'s ability to produce biofilms is tied to the production of small regulatory RNAs (sRNA) which post-transcriptionally regulate the production of proteins and molecules that are required to produce biofilms. In the White-Ziegler lab, we have found that genes associated with biofilms are preferentially expressed at low temperature (23°C) as compared to 37°C in nonpathogenic *E. coli* K-12. Many of these genes are regulated by sRNAs so we seek to understand if the temperature regulation is mediated through these molecules. To test this hypothesis, we will overexpress individual sRNAs that are cloned on plasmids under the control of IPTG induction. We then aim to molecularly and phenotypically analyze the impact of these sRNAs on the ability of *E. coli* to produce biofilms at both 37°C and 23°C. The primary technique of our phenotypic analysis is the binding of *E. coli* surface proteins to congo red dye. The ability of cells to uptake this dye is dependent on the presence of curli proteins and cellulose, which are expressed in the top layer of biofilms and result in characteristic morphologies. Using this strategy, we will be able to determine if overexpression of a specific sRNA disrupts the normal thermoregulatory phenotype. Molecularly, we will use QRT-PCR to confirm the expression of the sRNA and assess whether biofilm genes targeted by the sRNA are altered in expression.

**26. Developing a Sensitive, Species Specific qPCR Diagnostic Assay for *Schistosoma Japonicum***

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The goal of the project is to develop a sensitive, species specific qPCR assay for the detection of *Schistosoma Japonicum*, a nematode that causes Schistosomiasis. A highly repetitive sequence in the genome of *S. Japonicum* was identified through bioinformatic analysis using Repeat Explorer. The sequence and the primers and probe designed for it were blasted against other specifies in NCBI to ensure species specificity. The only other species hit by the primer blasts search in NCBI was *Schistosoma Mansoni*, but alignment of the primer and probe sequences in the *S. Mansoni* sequences showed a significant difference between the primer probe sequence and that of *S. Mansoni* so the next step in the project is testing the sensitivity, specificity of the assay on *S. Japonicum* as well as optimizing the assay.

**27. A Shift in the Right Direction?: The Role of C19ORF66 in KSHV Lytic Infection**

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Eukaryotic cells rely on a dynamic equilibrium between transcription and translation to regulate gene expression. To quickly react and adapt to environmental challenges, the cells often modulate mRNA stability as an energy-efficient way of impacting gene expression. Perhaps not surprisingly, viruses have evolved to co-opt RNA regulatory pathways, re-purposing them towards their own needs. We are interested in Kaposi's sarcoma-associated herpesvirus (KSHV), a herpesvirus that triggers a massive cellular RNA decay event using a virally encoded endoribonuclease, SOX. While over 70% of the host transcriptome is decimated, roughly 30% evades degradation during viral infection. We showed that one particular host transcript, C19ORF66, is actively protected from viral-mediated cleavage. To determine whether this escape from degradation is beneficial for viral infection, or on the contrary, participates in an anti-viral defense, we are interested in deciphering the role of C19 during infection. We first demonstrated that C19 encodes a potent antiviral factor, and upon further investigation, we found that this restriction of KSHV results from the inhibition of KSHV lytic cycle, most notably restriction of key viral gene expression. Over-expression and knockdown experiments have revealed that C19 selectively curbs KSHV transcript levels. Thus, we hypothesize that C19 selectively triggers decay of viral RNA during herpesviral lytic reactivation. We are currently investigating how C19 is brought to its target RNA, looking into the interaction between C19 and Poly-A binding protein cytoplasmic 1 (PABPC1) as well as extending the search for C19 interaction partners by mass spectrometry. Furthermore, past literature has identified C19 as a regulator of a unique ribosomal frameshift, which could be another way of recruiting C19 to viral mRNA. Overall, deciphering the role of C19 during KSHV infection will give us insights into the complex battle between KSHV and its host for the control of the gene expression machinery.

**28. Development of a Novel Quantitative Real-Time PCR Diagnostic Assay for Harbor Seal Lungworm (*Otostrongylus circumlitus*)**

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Marine mammal populations are negatively impacted by climate change as rising global temperatures correlate with increases in the geographic distribution, prevalence, and virulence of aquatic parasites. With harmful parasites threatening the biodiversity of many marine mammal species, we aim to develop novel molecular tools to quickly and reliably identify these infections. *Otostrongylus circumlitus* (harbor seal lungworm) causes bronchitis-like symptoms in adult harbor seals, eventually decreasing their overall fitness, and is lethal in seal pups. In addition, *O. circumlitus* migrates to the heart of its incidental host, the northern elephant seal, where it is immediately responsible for fatal conditions. Current PCR diagnostics lack the sensitivity to detect DNA from parasite eggs or larvae in samples collected from live, infected marine mammals. Thus, to optimize sensitivity and specificity, we are developing a quantitative real-time PCR (qRT-PCR) assay to target highly divergent, high copy-number repetitive DNA sequences. To be practical, this assay needs to be easily accessible to marine mammal rescue centers and be capable of detecting *O. circumlitus* infection in non-invasive samples, such as blood, feces and/or sputum. Currently, treatment of *O. circumlitus* infections is initiated based on clinical pathology and presentation. The standard diagnostic tool for *O. circumlitus* infections detects elevated levels of Serum amyloid A (SAA) proteins in the blood of these infected animals due to increased inflammation. However, the sensitivity and specificity of the SAA assay for *O. circumlitus* infections in marine mammals is unknown. Additionally, since there is no current antemortem diagnostic test for *O. circumlitus*, animals that are assumed to be infected with this nematode are given a general anthelmintic, such as ivermectin. Therefore, this qRT-PCR assay will also be used to evaluate the efficacy of these current diagnostics and therapies through the analysis of pre- and post-treatment samples from infected marine mammals.

**29. Analyses of germline/soma distinctions allows for species delineation among four diverse ciliates species**

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As the initial sole criteria for species delineation, morphology cannot distinguish cryptic species well, especially in microbial eukaryotes, and need to combine with molecular information. Ciliates are a large and diverse group of unicellular microorganisms. Due to their characteristic nuclear dimorphism (i.e. germline micronucleus and somatic macronucleus within each cell/individual) and somatic genome rearrangement from zygotic nucleus during sexual reproduction (including genome fragment, internal eliminated sequences excision, telomeres added and genome scramble in some classes), ciliates serve as model organisms in genome studies. Despite the few cultivable model ciliates, species boundary in most ciliates have not yet been extensively discussed. Nowadays, with the development of single cell genome/transcriptome technique, studies on uncultivable ciliates become practicable. According to previous studies, germline-limited sequences evolve more quickly than sequences retained in somatic genome and have the potential to be a good marker to delineate species in ciliates. Here, we focus on four uncultivable ciliates species belong to different classes with >1 billion years divergence time and sample different populations in various sites. We're currently analyzing germline-limited sequences between different populations combining single-cell transcriptome/genome data with bioinformatic approaches. Together, we aim to 1) define species boundaries through germline-limited sequences and develop species-specific IES primers for community analyses; and 2) elucidate germline and somatic genome evolution in cryptic ciliates species, and speculate on broader application of our approach in general in microbial eukaryotes.

**30. Preliminary analyses in potential bacterial symbionts in free-living protists using single-cell 'omics**

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Symbiotic relationships between eukaryotes and bacteria are found in nearly every ecosystem on Earth. Studies of these symbiotic interactions focus mainly on plant and animal hosts, while relationships between bacteria and microbial eukaryotes (i.e. protists), especially free-living groups, remain largely understudied. Endosymbiotic bacteria have been reported to increase the fitness of both the host organisms and themselves by altering the gene expression of their hosts or manipulating the host cell cycles. Given that protists represent the majority of diversity in eukaryotes, understanding their associations with bacteria will expand our knowledge of adaptation and genome evolution. After gathering and analyzing single-cell transcriptomes and genomes from uncultivable free-living protist *Loxodes* sp. (Ciliophora), we identified bacterial sequences as candidate symbionts. We are now using fluorescent in situ hybridization (FISH) on samples with both general bacterial and candidate-specific probes to confirm the presence and explore the physical location of associating bacteria. These data provide a first step towards understanding the microbiomes of uncultivable free-living protists.

**31. Isolation and Characterization of Plant Based Antibacterial Molecules**

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A consistent rise in the number of drug-resistant bacteria has led to an increased demand for the discovery and development of new antibacterial agents. We are seeking to characterize specific antibacterial compounds found within common foods, herbs, and spices. Currently the main targets of our work are cinnamon and pomegranate. Cinnamon species are known to have broad spectrum antimicrobial properties, being active against gram positive and gram-negative bacteria, but the differences between the individual species remain unclear. The pomegranate extracts appeared to be only effective against gram positive bacteria. Extracts were collected from a Soxhlet extractor using various solvents to get a range of compounds. The samples were analyzed by disk diffusion assay against *E. coli* (gram negative) along with *B. subtilis* and *M. luteus* (gram positive). LC-MS analysis is currently underway to identify compounds within each cinnamon and pomegranate extracts.

**32. A cell envelope protein, which facilitates lipomannan maturation in mycobacteria, is critical for cell envelope maintenance and antibiotic resistance during stress exposure**

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The rise of antibiotic resistance in *Mycobacterium tuberculosis*, the causative agent of tuberculosis, is a global concern. The cell envelope is critical for the survival and virulence of the pathogen during infection, and its biosynthesis has been a proven drug target. *Mycobacterium smegmatis* is a model organism for the study of this devastating pathogen. We identified LmeA as a cell envelope protein that is critical for the control of mannan chain length of lipomannan (LM) and lipoarabinomannan (LAM), essential lipoglycan components of the cell envelope. The deletion mutant, ΔlmeA, accumulates abnormal LM/LAM with fewer mannan residues. LmeA deletion leads to increased sensitivities to antibiotics, and lmeA overexpression leads to increased antibiotic resistance. ΔlmeA is also more efficient in taking up ethidium bromide in the cell, indicating a defective permeability barrier. LmeA is important for protein stabilization under stress conditions. During stress conditions in ΔlmeA, levels of a protein involved in elongation of the LM and LAM mannan chain decrease significantly relative to wild-type. To help elucidate the role of LmeA at the molecular level, we are currently investigating how it binds to membrane. In a subcellular fractionation analysis, we have previously shown that LmeA localizes to fractions containing plasma membrane (PM), which is tightly bound to cell wall layers. To test the binding of LmeA to membrane further, LmeA was heterologously expressed in *Escherichia coli*, purified, and mixed with PM fraction. LmeA could still bind to the membrane and cofractionated with the membrane in a density gradient fractionation, indicating that LmeA is capable of localizing to fractions containing only PM. LmeA also binds phospholipids and associates with spheroplasts. We are currently investigating LmeA binding behavior, if LmeA is involved in the stabilization of other proteins in the LM/LAM biosynthesis pathway, and the mechanism through which this stabilization occurs.

**33. A Worm's Tale: A study of microbial travels through the digestive tract of the earthworm**

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We propose to follow a bacterial and/or fungal organism through the digestive tract of the earthworm (*Eisenia fetida*) using GFP (Green Fluorescent Protein) to track the organism on its journey. The earthworm has been long known to be associated with healthy and fertile soil. So much so that plant and crop growers often provide earthworm casts or raise earthworms to enhance their soil fertility. The simple and easily accessible digestive tract of the earthworm makes it conducive for histological evaluation of the potential sites of colonization or transient localization along the earthworm's digestive tract.

**34. Genome Wide Association Study (GWAS) for antifungal sensitivity in the opportunistic pathogen *Aspergillus fumigatus* *(invited keynote)***

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*Aspergillus fumigatus* is a filamentous fungus that is typically found in soil, compost and other decaying organic matters. This species is also an opportunistic human pathogen that kills an estimated 100,000 people annually. Unfortunately, the frequency of *A. fumigatus* antifungal resistance has rapidly risen making treatment particularly challenging. Candidate gene approaches have yielded insight into a number of different antifungal resistance mechanisms including: target overexpression, target alteration, efflux overexpression and intake control. However, candidate gene approaches are inherently biased because they can overlook genes with minor but additive effects, or genes with functions that are unknown or seemingly unrelated. To avoid this bias, we applied Genome-Wide Association (GWA) to identify genetic variants associated with Itraconazole (ITCZ) sensitivity in *A. fumigatus*. Three different statistical approaches (RoadTrips, plink_permutation and plink_PCA) were performed to conduct GWA using 43,967 single nucleotide polymorphism (SNPs) in 69 isolates with matched minimal inhibitory concentrations (MIC) of ITCZ. We identified 26 SNPs, overlapping 14 genes, associated with ITCZ sensitivity. We used CRISPR to knockout the top seven candidate genes then tested the growth rate of knockouts in the presence of ITCZ. Three of the seven genes (Afu2g02010, Afu2g02020, and Afu3g13670) showed significant effects on growth in the presence of ITCZ. Our results suggest that ITCZ sensitivity is regulated by a collection of genes, all of which had not been implicated previously. In addition, this study demonstrates the power of GWA paired with molecular genetics to better understand pathogenicity related traits in *A. fumigatus*.

**35. Cranberry proanthocyanidins and various dietary oligosaccharides synergistically interact with *Lactobacillus plantarum* ATCC BAA-793**

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Plant-based foods contain bioactive compounds such as polyphenols that resist digestion and potentially benefit the host through interactions with their gut microbiome. Accordingly, probiotic *Lactobacillus plantarum* uses dietary oligosaccharides and plant polyphenols to secrete metabolites that interact with host tissue. Thus, we hypothesize cranberry polyphenols and oligosaccharides synergistically impact *L. plantarum* physiology. In this study, *L. plantarum* ATCC BAA-793 was grown on proanthocyanidins (PACs) extracted from cranberry in the presence of dietary oligosaccharides including cranberry xyloglucans, fructooligosaccharides (FOS), and human milk oligosaccharides (HMOs). The strain exhibits a differential metabolic response to cranberry PACs dependent on the carbohydrate source and polyphenol fraction. Accordingly, the PAC1 fraction increases growth regardless of oligosaccharide whereas PAC2 only positively modulates growth during xyloglucan metabolism. RNA-seq transcriptomics indicate cellular macromolecular biosynthetic processes were enriched while interacting with PAC1 alone and PAC1 with HMO. Moreover, PAC1 induces nitrogen-related metabolic pathways during HMO fermentation. PAC2 induces transcription of carboxylic acid and organic acid biosynthetic pathways. In terms of metabolites, PAC2 increases lactate when introduced alone and during FOS fermentation, and prompts acetate and formate production during xyloglucan fermentations. Thus, cranberry PACs and dietary oligosaccharides interact synergistically with lactobacilli and represent a potential nutritive strategy to enhance probiotic or in situ microbiome function.

**36. Actin nucleation drives pathogenic behaviors and cell survival in a model for the "brain-eating amoeba"**

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Infection with the "brain-eating amoeba" *Naegleria fowleri* causes a devastating form of encephalitis that is almost always fatal. While little is known about *N. fowleri* pathogenesis, its migration to and within the brain and the consumption of host tissue through a phagocytosis-like process undoubtedly contribute to disease. While the molecular underpinnings of these behaviors have been studied in several model organisms, *Naegleria* diverged from these species over a billion years ago. Moreover, unlike nearly every other known cell type, *Naegleria* amoebae lack cytoplasmic microtubules. Therefore, we hypothesized that cell motility and phagocytosis, as well as other processes required for cell survival and pathogenesis, are heavily dependent on the actin cytoskeleton. Consistent with this, the genomes of *N. fowleri* and *Naegleria gruberi*, a nonpathogenic model for *N. fowleri*, each encode an extensive actin cytoskeletal repertoire. Using *N. gruberi*, we show that actin nucleation by the Arp2/3 complex and formin family proteins is critical for both cell motility and phagocytosis. Further, the inhibition of formins, which did not cause any global defects in F-actin content, resulted in the enlargement of contractile vacuoles (organelles critical for withstanding osmotic stress in many protists), and eventually promoted cell rupture in certain environments. Collectively, these findings highlight the importance of actin dynamics to basic cellular processes that are critical for cell survival and pathogenesis of *Naegleria*.

**37. Characterization of a *Brugia Malayi* POU-homeodomain Transcription Factor Binding to its Cognate Promoter and Throughout the Genome**

McKayla Ford[1], Nicole Frumento[2], Hafsa Mire[3], Sophie Chertock[4], Grace Mosley[4], Lauren Cho[1], Hannah Snell[4,5], Sanna Muhammad[4], Steven Williams[1,4]
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*B. malayi*, a parasitic nematode, causes lymphatic filariasis in many developing countries in Asia. Despite the disease burden caused by lymphatic filariasis, which threatens more than 886 million people in 52 countries, and is economically and medically devastating, little is known about the genetic regulation of the parasites that cause it. Current predictions are based on the distantly related *C. elegans* free-living nematode, potentially not capturing nematode adaptations to the parasitic lifestyle. Previously, we identified a conserved transcription factor - DNA promoter binding pair, UNC-86::mec-3, which in *C. elegans* regulates neuronal development. We have successfully expressed the recombinant UNC-86 protein in *E. coli* and have produced mec-3 promoter DNA *in vitro*. Recent results have demonstrated the binding of UNC-86 to multiple mec-3 promoter binding sites using a modified electrophoretic mobility shift assay (EMSA). In addition, we are initiating a ChIP-Seq project to characterize all binding sites of UNC-86 throughout the *B. malayi* genome. These new data will further explore the genetic regulation of *B. malayi* neuronal development and will identify proteins able to act as potential drug targets against lymphatic filariasis.

**38. A Target Genome-wide Approach to Elucidate Biofilm Formation and Antibiotic Resistance Genes and Investigate Their Genetic Interactions**

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Biofilm infections on indwelling biomedical devices are the leading cause of hospital infections around the world. The most common are catheter-associated infections caused by *Escherichia coli* and *Staphylococcus aureus*. Current methods try to prevent biofilm formation by using imbedded broad-spectrum antibiotics, but these antibiotics promote antibiotic resistance and do not fully prevent biofilm formation. Elucidating the genes behind biofilm formation and the enhanced antibiotic resistance of biofilms and investigating their genetic interactions could be used in the rational design of targeted antimicrobials. However, the genes and genetic mechanisms behind these cellular processes have not been thoroughly studied. Past genome-wide studies on biofilm formation have not used comprehensive libraries, and studies on the antibiotic resistance of biofilms have not used genome-wide approaches. We aim to design genome-wide CRIPSRi libraries in *E. coli* and *S. aureus* to uncover genes associated with biofilm formation and antibiotic resistance through a competitive attachment assay. CRISPRi provides efficient and targeted repression of every gene in each species' genome and, due to its simple design, easily scales for high-throughput, genome-wide assays. We have established CRISPRi in *E. coli* and are characterizing a DNA part library to establish CRISPRi in *S. aureus*. The attachment assay will be quantified by calculating the Malthusian fitness of each guide RNA and will use a platform of biomaterials of varying hydrophilicity, stiffness, and composition since these properties have been shown to affect biofilm formation. From a selection of the genes found to be associated with biofilm formation and antibiotic resistance, we will create comprehensive combinatorial CRISPRi libraries to study their genetic interactions by performing the attachment assay using the combinatorial libraries. The gene combinations that show the lowest fitness may provide gene targets for the design of targeted antimicrobials, such as peptide nucleic acids, that could ultimately treat and prevent biofilm infections.

**39. Structural and Functional Characterization of the Interaction Between the Protease Chaperone ClpC and a Putative Sporulation-Specific Adaptor Protein**

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*Bacillus subtilis* sporulation is a developmental process that culminates in the production of an environmentally resistant, metabolically dormant cell type called a spore. However, the molecular mechanisms that drive metabolic dormancy are poorly understood. We recently identified a previously uncharacterized gene, micA (for "metabolic inhibitor candidate A"), that is expressed in the developing spore and whose knockout displays phenotypes consistent with increased metabolic capacity. Conversely, overexpression of micA during vegetative growth is highly toxic. Genetic and biochemical approaches revealed that the MicA protein mediates these effects in collaboration with the AAA+-ATPase chaperone-protease ClpCP. We are currently testing a working model in which MicA functions as a novel adaptor for the ClpCP chaperone-protease to target the degradation of key metabolic enzymes in the forespore, in turn driving metabolic dormancy. Toward that end, we report here a co-crystal structure between MicA and the ClpC N-domain. This structure indicates that MicA interacts with an interface of the ClpC N-domain that is unique from that bound by the well-characterized adaptor protein MecA. We further report results of site directed mutagenesis to confirm the functional importance of the identified MicA-ClpC interaction interface *in vivo*. All together, we expect that these studies will reveal the molecular mechanisms by which regulated, adaptor-driven proteolysis drives metabolic shutdown during bacterial development.

**40. Probing the molecular mechanism of ProQ-sRNA interactions using a bacterial three-hybrid assay**

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In bacteria, small RNAs (sRNAs) are known to play important roles in gene regulation. The interactions between sRNAs and mRNAs are often facilitated by protein chaperones. Recently, ProQ has been shown to bind to dozens of *E. coli* sRNAs and mRNAs, and to facilitate base pairing between the mRNA hupA and sRNA RaiZ with the effect of regulating hupA. These observations have led to the proposal that ProQ may act as a widespread regulator of bacterial gene expression. Our goal has been to understand the molecular mechanisms of ProQ's interaction with RNAs, mapping the amino acids on ProQ's surface and nucleotides of RNAs that contribute to binding and regulation. To do this, we have used 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 a sRNA or mRNA 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, lacZ. We have detected B3H interactions of ProQ with several RNA partners, and have looked closely at its interactions with SibB and cspE. Using domain truncations, an unbiased forward genetic screen, and site-directed mutagenesis, we have identified a surface on ProQ's NTD that we believe to be the primary surface for recognition of these two RNAs *in vivo*. We have used our genetic data to create a model for ProQ binding to an A-form RNA duplex. We are currently working to probe whether ProQ uses the same binding site(s) to bind to all RNAs, or if there are unique surfaces that contribute to binding of certain of sRNAs and/or mRNAs.

**41. Investigating the Role of Post-Antibiotic Molecular Events on Fluoroquinolone Persistence and Resistance Development**

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Antibiotic resistance is a rising issue in modern healthcare. Besides resistance development, antibiotics can also fail to treat an infection due to bacterial persistence. Unlike resistant bacteria, bacterial persisters are phenotypic variants of the population that are able to survive lethal concentrations of antibiotics without acquiring heritable genetic mutations. The tolerance exhibited by persisters is often attributed to decreased activity in cellular processes, which stall growth and decrease metabolic capacity, seemingly allowing them to evade damage from antibiotics. However, research has shown that persisters are not necessarily undamaged from treatment. The environment following antibiotic treatment removal has the potential to affect molecular processes involved in repair and growth, which in turn influences whether a cell persists or dies. The opportunity to recover from treatment can also enhance development of mutants resistant to multiple antibiotics. To gain a better understanding of how bacteria cope with and recover from antibiotic treatment, we are investigating the expression of genes that can potentially lead to resistance enhancement, including those encoding error-prone polymerases and antibiotic efflux pumps, following antibiotic removal. We also determined the impact of stresses during this critical period on gene expression. We found that a subset of bacteria induced the expression of these genes during recovery from antibiotic treatment. We discovered that when cells were starved for a period of time after antibiotic removal, expression of genes that can contribute to resistance enhancement is dampened compared with cells that were fed immediately after antibiotic treatment. In a health system where drug recalcitrant bacteria is becoming a growing threat, better understanding bacterial tolerance, persistence, and resistance could provide novel advances in combating antibiotic treatment failure.

**42. Have your microbes and eat them too: *Drosophila* gut bacteria as fermenters and food**

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In nature, fermentative growth of fly-associated microbes on food facilitates its breakdown into digestible substrates, as adequate nutrition is not readily extracted by *Drosophila* from fresh food sources. While the impact of microbes on fly physiology has been studied extensively, little has been done to assess the direct effect fly gut bacteria have on food and how this relationship impacts fly health. In this study, we determined the impact of gut bacteria on the nutritional content of the food source and the resulting effect on host life history. We observed increases in protein and moisture, and decreases in carbohydrates due to bacterial growth across diets tested, and additionally found that bacteria increased levels of tryptophan in the fly food. These results, in addition to aiding in our understanding of how fermentation of the fly food source affects physiology, shed light on processes that occur during digestion within the gut, leading to insights into how the gut microbiome can affect host health across model systems.

**43. Isolation, Comparison, and Identification of Bacteria Isolated from Campus Water Bottle Filler and Associated Drinking Fountain**

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With expanding efforts to decrease single-use plastic water bottles, colleges across the nation are installing motion sensor water bottle fillers. A sterile swab was used to collect bacteria from the dispensing spout of a water bottle filler as well as the associated drinking fountain at the base of the system. The bacteria were then cultured on nutrient agar plates, isolated, and tested. One colony was found on the water bottle filler and four colonies were found on the drinking fountain. The bacteria from the water bottle filler are gram negative rods arranged in chains and clusters. Gram results, morphology, and arrangement are still being determined for the bacteria on the drinking fountain. Further tests are being performed to determine bacterial properties and optimum environments in hopes of identifying these bacteria.

**44. Assessing linkages between soil conditions and denitrification rates during the spring snowmelt period in Vermont**

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The spring snowmelt period could be a ‘hot moment’ (i.e. period of elevated process rates) for biological soil denitrification (i.e. the microbial reduction of nitrate or nitrite to gaseous $N_2$, or an oxide of N). During this period, key regulators of denitrification (e.g. soil moisture, soil temperature, and substrate availability) fluctuate to levels that are supportive for this process. Riparian areas, which are situated at the interface between the terrestrial and aquatic environment, represent ‘hot spots’ for denitrification. Since the regulators of denitrification act through the biotic community, the connections between changes in soil conditions and denitrification rates remain poorly understood (Dandie et al., 2011). As such, in addition to process rates and soil conditions, changes to the biotic community (i.e. activity and abundance of denitrifying bacteria) must be monitored to gain a better understanding of denitrification dynamics during spring snowmelt. To address this knowledge gap, we studied riparian soils of different adjacent land use (agricultural and forested) and landscape position (upland and wetland) throughout the spring snowmelt period in Vermont, USA. We paired data on denitrification rates with surface nitrous oxide ($N_2O$) fluxes, soil and soil water carbon (C) and N concentrations, and high frequency data from an in-situ soil sensor network that provides continuous data on soil conditions. We collected soil samples to isolate functional denitrification genes in order to measure denitrifier gene abundance and expression. These data will be used to assess linkages between soil conditions and the capacity of the riparian soils to denitrify. Preliminary results indicate higher denitrification rates in the agricultural riparian area, compared to the forested site, suggesting that substrate availability is a major driver of denitrification rates during the snowmelt period.

**45. Investigation of Phenotypic Responses in Gram-Positive and Gram-Negative following Antifolate Treatment**

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The relatively recent introduction of antibiotics produced a monumental impact on modern medicine, but the use of antibacterial agents propagated an evolutionary arms race. Antibiotic treatment inevitably promotes the emergence and spread of organisms harboring genetic mutations that confer resistance. As of 2013, the CDC roughly estimated two million annual cases of antibiotic-resistant infections. In addition to resistant mutants, antibiotics can also fail to cure an infection due to the presence of bacterial persisters, which harbor no mutations but have adopted reversible phenotypic states that enable them to survive adverse conditions. Bacterial persisters pose a looming health threat and may underlie recurrent infections or resistance development. Persistence exists as a heterogenous phenotype, where bacteria may employ various survival strategies to overcome the lethality of different classes of antibiotics. Trimethoprim (TMP) and Sulfamethoxazole (SMX)—antifolate drugs that are often used in combination—are routinely prescribed for urinary tract infections and Methicillin-resistant *Staphylococcus aureus* (MRSA)-associated skin infections. The mechanisms of persistence in response to TMP-SMX and other antifolates are not well understood. Our preliminary data demonstrated altered killing kinetics in *Escherichia coli* and MRSA treated with TMP/SMX. The most notable finding was the observation of a growth stasis period prior to killing. To achieve a more complete understanding of the phenotypic response of bacterial populations toward TMP-SMX, we are investigating metabolic and gene expression changes in these two organisms following antibiotic administration. Identifying biochemical pathways that may be perturbed as a result of antifolate treatment can potentially lead to the development of novel therapeutic strategies to eradicate bacterial persisters and preserve the efficacy of this important drug class.

**46. Investigation of a *Chlamydia trachomatis* virus-like particle (VLP) vaccine candidate**

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*Chlamydia trachomatis* is an obligate intracellular pathogen and so relies on infection of epithelial cells to proliferate. *C. trachomatis* uses a type III secretion system (T3SS) to facilitate infection. We hypothesize that a vaccine targeting the T3SS protein Ct584 will block *C. trachomatis* infection in the female genital tract. To test this, we designed vaccines against two predicted surface-exposed B cell epitopes of Ct584: residues 70-77 and 154-164. These epitopes were used to create 3 virus-like particle (VLP) vaccines, two constructed with residues 70-77 or 154-164, and a third with a mixture of the two, each conjugated to a qbeta capsid. A negative control vaccine consisted of a bare qbeta capsid. Vaccines were administered intramuscularly in female C57Bl6 mice 3 times at 3 week intervals, followed by transcervical Ct challenge. Sera was collected after immunization and before *C. trachomatis* challenge to measure antibody responses by ELISA. Bacterial burden was measured in the uterus 3 days after challenge. Two independent vaccine trials, n=5 mice/vaccine and n=20 mice/vaccine, were performed. We found a significant decrease in *C. trachomatis* burden between vaccine and control groups in the first but not the second trial. Both trials elicited high titer antibody to Ct584 epitopes in the serum and uterus. These results suggest that Ct584 VLPs are capable of stimulating robust antibody production but do not provide reproducible defense against *C. trachomatis* infection in the uterus. Future studies will use whole cell ELISA to assess the ability of anti-Ct584 antibody to bind to whole *C. trachomatis* bacteria.

**47. Drug-resistant *Candida albicans* biofilms are susceptible to killing by medium-chain fatty acids**

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*Candida albicans* is a normal part of the healthy human microbiota, but in immunocompromised patients, it can cause life-threatening systemic infections. It is the fourth leading cause of bloodstream infections in American hospitals; these infections have a mortality rate of ~40%. One reason these infections can be so hard to treat is that *C. albicans* readily forms biofilms on indwelling medical devices, such as catheters, artificial heart valves, or artificial joints. *C. albicans* biofilms tend to be highly resistant to treatment with fluconazole and other antifungal drugs, regardless of whether the cells are susceptible or resistant to antifungal drugs during planktonic growth. The medium-chain fatty acids found in coconut oil, particularly decanoic and dodecanoic acids, have been shown to have antimicrobial properties against bacteria, fungi, and enveloped viruses. We have previously found that both octanoic and decanoic acids can kill *C. albicans in vitro*, and that dietary supplementation with medium-chain fatty acids can reduce *Candida* gastrointestinal colonization in both mice and humans. The Centers for Disease Control and Prevention (CDC) have classified fluconazole-resistant *C. albicans* as a “serious threat” to public health. We tested the susceptibility of *C. albicans* biofilms to octanoic and decanoic acid, and found that both have antibiofilm activities; decanoic acid is especially effective at killing biofilms. We further compared the susceptibility of fluconazole-susceptible and fluconazole-resistant strains; the latter included both lab-derived strains and clinical isolates, and several different mechanisms of fluconazole resistance. Both octanoic and decanoic acid reduced the number of viable cells in *C. albicans* biofilms; all strains showed similar susceptibility to the medium-chain fatty acids, regardless of whether the strain was fluconazole-susceptible or resistant, or the mechanism of fluconazole resistance. These natural products are safe for both topical application and ingestion, and therefore have great potential as possible prophylactics or therapeutics.

**48. Investigation of RNA Secondary Structure Involved in Escape from Viral Nuclease Cleavage**

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Viruses are evolutionarily constraint to co-opt their host resources in particular the host gene expression machinery. We study Kaposi’s Sarcoma Associated Herpesvirus (KSHV) which triggers lifelong infections. To access the host translation machinery, KSHV triggers a process known as host shutoff that results in widespread RNA decay. During this event the virally expressed endonuclease SOX cleaves mRNA, exposing them to degradation by host factors. It is estimated that this process leads to downregulation of up to 70% of total mRNA in the cell. We showed that in the 3’UTR of spared mRNA, a protective structural motif coined the SOX Resistant Element (SRE) is responsible for providing protection against SOX decay. The SRE forms a hairpin-like secondary structure that is sufficient to promote protection against cleavage by SOX as well as several other viral nucleases. We used predictive modelling to investigate this oligo structure, further exploring whether or not the escape capability can be correlated to the secondary structure. Now we are experimentally testing our prediction model, in collaboration with Dr. Sztuba-Solinska, using a newly developed RNA structure method called SHAPE-MaP to generate quantitative views of these structures. SHAPE-MaP uses RNA binding dynamics to replace the free hydroxy group on unbound RNA to actively map out nucleotides that belong in bulges or loops. This modified RNA is deep sequenced and processed to determine the likelihood of each nucleotide to engage in interactions and thus providing us with a high confident map of the secondary structure. We expect that this quantitative model can help us explore the functionality of the SRE and determine if structure is the key to escape. Host shutoff is crucial for progression of viral infection, therefore designing small molecule inhibitors specifically targeting the SRE secondary structure could lead to the development of novel anti-viral therapeutics.

**49. An Algorithmic Approach to Program Sequential Logic in Probiotic Bacterial Strains for *In Vivo* diagnostics**

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Through the implementation of designable genetic circuits, probiotic strains of bacteria can be used as non-invasive diagnostic tools for the gastrointestinal tract. In order for these programmed cells to be able to detect and report disease biomarkers after exiting the gut, the genetic circuits need to be able to record these signals it received within the gut environment using genetically-encoded memory. Complex memory register circuits would allow for multiplex interrogation and detection of disease biomarkers. We have developed a computational approach for the scalable design of genetic circuits that contain memory, which are known as sequential logic circuits. Importantly, these sequential logic circuits can also be implemented for temporal programming of cells. The theory-based approach to design sequential circuits from simple NOT gate responses is robust and makes accurate predictions for standard cell growth conditions. However, the way in which circuit component performance varies for different bacterial strains and gut-relevant environments is poorly understood and could lead to loss of performance of the circuit. Here we aim to develop a computational approach for the design of robust sequential logic circuits for *in vivo* diagnostic and therapeutic applications. In this work, we use a TetR family of repressors to build NOT and NOR logic gates that can be composed into complex sequential circuits. The NOT gates were characterized in the probiotic strain *Escherichia coli* Nissle 1917. Using this data, we designed and predicted the behavior of larger circuit designs. We present a set of genetic circuits that encode combinational logic and sequential logic and show that the circuit outputs are in close agreement with our quantitative predictions from the design algorithm.

**50. Controlling the developmental switch between a human-like to a yeast-like cell in a chytrid fungus**

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Actin is a core and highly conserved component in every eukaryotic lineage. Despite this conservation, actin networks drive a vast array of behaviors, often within the same cell. Our previous work shows that the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* (Bd) exhibits temporally and phenotypically distinct actin networks. During its juvenile stage, Bd uses a single posterior flagellum to swim through water and actin-filled pseudopods and an actin cortex to crawl across surfaces, resembling amoeboid animal cells. As the chytrid matures, Bd switches to a sessile stage utilizing actin patches and cables to facilitate the growth of a hyphal-like rhizoid and an exit tube for the internally replicated juveniles, resembling filamentous fungal lineages. We discovered that mucin, a signature of Bd's host environment, acts as an external switch to trigger the transition between the two life stages within minutes. Further, we have employed small molecule inhibitors of actin assembly to impair actin pseudopods and filopodia-like protrusions prior to this transition. With these tools, we can control both Bd’s transition and ability to assemble distinct actin networks, to ultimately characterize the role of actin during development. Moreover, we are currently determining the mechanisms of Bd’s reaction to mucin by assessing its substrate trigger specificity, its characteristic cell polarity markers, and potential developmental pathways through drug inhibition. In addition to answering questions about actin evolution and network regulation, a better understanding of Bd’s cell biology will aid in our efforts to combat chytridiomycosis – skin infections causing a dramatic decline in amphibian populations worldwide.

**51. High-throughput Sequencing for Arcellinida Identification and Theories of Distribution**

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The mechanisms for global dispersal of testate amoebae (Arcellinida, Amoebozoa) is not understood and has long been absent in studies. These single-celled eukaryotes have been identified in the paleontological record, and appear in bogs and fens around the world. They are considered effective bioindicators, which are important in developing a better understanding of current global climate change. Despite recognizing that there is much that can be learned from these organisms, studies have only recently begun to use genetic data rather than only morphological data. Many Arcellinida species are considered “cryptic” species, and are very difficult to differentiate correctly based only on morphology. Current studies that combine morphological and molecular data have vastly increased knowledge about these organisms, but there are many species for which there are no reference genomes. In addition to the lack of genetic information, there have been no studies to attempt to explain how these unicellular organisms have come to be distributed worldwide. In order to fill in some of the missing genetic data on species we observe morphologically, this study uses high-throughput sequencing techniques to sequence individual cells from bog samples. In addition, we are working to test an initial hypothesis that birds may be involved in the dispersal of Arcellinida. We are using amplicon sequencing techniques with Arcellinida-specific primers, to test bird feces and swabs from their feet to determine whether testate amoebae might be dispersed over long distances by birds.

**52. Detection of genetic regulators of acetyl-CoA synthetase expression in *Vibrio fischeri* grown in a host-relevant carbon source**

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In the context of a symbiotic relationship, bacterial populations in a host organism are established and maintained by available nutrient sources. Glycerol is a readily available carbon source in the light organ of *Euprymna scolopes*, the host species of symbiont *Vibrio fischeri* bacteria. Acetyl-CoA synthetase (acs) is a central metabolic regulator of this symbiosis. Transcriptional regulation of acs contributes, in part, to the metabolism of acetate in *V. fischeri*, a key requirement for colonization of the *E. scolopes* light organ. However, the presence of glycerol in growth media inhibits acs expression. Using a transposon mutagenesis screen, we identified mutants which permitted acs expression on plates containing glycerol. We screened over 20,000 mutants grown on plates with added 10 mM glycerol and found thirteen mutants with reproducibly high acs expression phenotype on plates. One mutant of interest has a mutation in the gene glpX, which codes for a type II fructose-1,6-bisphosphate, and it shows high acs expression on plates. In order to quantitate and confirm high acs expression levels in liquid culture, we further performed beta-galactosidase assays. Five of the thirteen identified mutants exhibited high acs expression in culture, and three of these carry a transposon insertion in glpK. The gene glpK codes for a glycerol kinase, an enzyme critical for glycerol metabolism. Our results demonstrate a link between glycerol metabolism and acetate metabolism in *V. fischeri* grown in culture which may be relevant in the *V. fischeri-E. Scolopes* symbiosis.

**53. Exploring the role of low molecular weight metabolites in the formation of host brain lesions during *C. neoformans* infection**

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The pathogenic fungal yeast *Cryptococcus neoformans* infects about 220,000 people every year and is known to cause 181,000 deaths in immunocompromised people annually. The mechanisms leading to lesion (cryptococcoma) formation in host brain tissue during *C. neoformans* infection are yet to be completely understood. We propose that non-enzymatic generation of hydroxyl radicals (•OH) mediated by 3-hydroxyanthranilic acid (3-HAA), an intermediate in the tryptophan degradation pathway, plays an important role in lesion formation. 3-HAA is a known iron chelator for *C. neoformans* and has the potential to participate in "chelator-mediated Fenton" chemistry to reduce ferric iron in host brain tissue. The reduced iron can then react with hydrogen peroxide produced both by host immune cells and by redox cycling of 3-HAA, leading to the generation of •OH. This mechanism for •OH generation has not been previously reported in *C. neoformans*, and it is possible that the generation of a sustained stream •OH may be involved in cryptococcoma development. Electron paramagnetic resonance spectroscopy suggests that although 3-HAA is an antioxidant at physiological pH (7.4), it is a pro-oxidant at pH 5.5, leading to a sustained production of •OH. Using mouse organotypic brain slice (OBS) cultures, we observed that the pH of infected OBS after 10 days of infection is lowered from a physiological pH of 7.4 to ~5.5. *C. neoformans* also demonstrated a significant increase in the amount of 3-HAA produced at pH 5.5 compared to pH 7.4 *in-vitro*. This indicates that pH may be an important driver of 3-HAA production by *C. neoformans* and that 3-HAA production can potentially be co-opted to cause oxidative cellular damage. Exploring the role of 3-HAA in triggering hydroxyl radical production at reduced pH leading to cryptococcoma formation will broaden our understanding of *C. neoformans* virulence and may ultimately provide targets for future gene therapy.

**54. Impact of commensal bacteria exoproducts on *Staphylococcus aureus* antibiotic persistence**

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*Staphylococcus aureus* is part of the human commensal microbiota, but when the cutaneous and mucosal barriers are breached, *S. aureus* can act as an opportunistic pathogen. *S. aureus* can cause skin and soft tissue infections, and biofilm-associated infections, such as endocarditis. Antibiotic treatment failure of S. aureus infections is not only linked to antibiotic resistance. Bacterial persisters, which are subpopulations of cells in a genetically clonal culture that are able to survive doses of antibiotics lethal to their kin, are thought to be a source of infection relapse. At *S. aureus* infections sites, other bacteria that are part of the host microbiome can colocalize with the pathogen. These host-colonizing bacteria can secrete metabolites and proteins that affect *S. aureus* physiology and virulence. The impact of interspecies interactions on *S. aureus* antibiotic persistence and the nature of the exoproducts involved in crosstalk at infections site remain unknown. In this study, we will first evaluate the impact of secreted products from commensal bacteria on *S. aureus* antibiotic persistence by challenging *S. aureus* against different types classes of antibiotics, including fluoroquinolones and antifolates. Secondly, we will identify the nature of the commensal exoproducts using biochemical techniques. Our preliminary results suggest that cell-free conditioned media from the skin commensal bacterium *Staphylococcus epidermidis* can decrease persisters when the population is treated with levofloxacin, a DNA topoisomerase-targeting fluoroquinolone. The results of this study will contribute new knowledge of how secreted metabolites from commensal species can impact *S. aureus* antibiotic persistence and can potentially lead to the development of new therapeutic strategies to treat chronic and recurrent infections.

**55. Getting the message: expansion of a bacterial three-hybrid assay to mRNA-protein interactions**

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Non-coding small RNAs (sRNAs) contribute to bacterial biofilm formation, antibiotic resistance, and virulence, but there is much we do not understand about their molecular mechanisms. Hfq is the best-studied bacterial RNA chaperone protein and has become the paradigmatic example for how RNA-binding proteins facilitate mRNA regulation by sRNAs. Here we present progress on establishing and broadening the capabilities of a bacterial three-hybrid (B3H) assay for genetic detection of interactions between Hfq and its RNA targets. In addition to well-established detection of sRNA-Hfq interactions, we have demonstrated the assay's capacity to detect interactions between mRNA mimics and Hfq. In order to expand from mimics of mRNA sequences to native 5' UTRs, current efforts to improve mRNA-Hfq detection involve probing the effects of RNA structure, ribosomal translation, and RNA turnover. The results of these efforts highlight the complex challenges associated with detecting interactions *in vivo* with 5' UTRs and coding sequences. We have refined the B3H assay so that it detects the interaction *Escherichia coli* Hfq and its target mRNA sodB, demonstrating the potential of the assay to detect a wider variety of RNA-protein interactions than previously possible. Our progress in overcoming these challenges is exciting in light of recent evidence that other major RNA binding proteins such as the proteobacterial ProQ bind extensively to 5' UTRs and coding sequences. In order to better understand the relationship between *in vivo* and *in vitro* binding, we are also exploring the relationship between the *in vivo* B3H data and the binding energetics of RNA protein interactions measured *in vitro*. By expanding the capabilities of this bacterial three-hybrid assay and our understanding of the data it provides, we hope to broaden its utility in studying bacterial gene regulation and RNA-protein interactions.

**56. The Role of ORF20 in KSHV Lytic Replication**

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Viruses are evolutionarily constrained 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), an 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. Herpesviruses utilize the rolling circle mechanisms during lytic replication. However, the endonuclease responsible for the initial nick in the viral dsDNA episome is unknown. It is known that ORF20 encodes a highly conserved endonuclease domain, making ORF20 an ideal candidate for the missing replication endonuclease. Moreover, we observed that ORF20 localizes to the viral replication compartment, further suggesting a role for ORF20 in this fundamental process. We are currently purifying ORF20 in *E. coli* to biochemically assess its putative endonuclease properties on the viral lytic origin of replication. We are also using biotin proximity labeling (BioID) to map ORF20 direct and indirect interaction network by mass spectrometry. Using this information, we will be able to better characterize the role of ORF20 during lytic replication, which will provide insights into the complex regulation of KSHV infection.

**57. Model-Guided Metabolic Engineering of Porphyran Utilization in Gut Microbial Communities**

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Probiotic bacteria often struggle to effectively colonize the human gut when administered as a medical intervention. Studies have shown that the best predictive factor for whether a probiotic bacterial species will be able to effectively colonize a gut is whether or not it can occupy a carbon-utilization niche that is not already taken by a preexisting species. The carbohydrate porphyran found in red seaweed, such as nori, represents such a unique carbon source that is largely absent from the western diet. Research has shown that the colonization of porphyran-digesting gut microbes can be finely controlled by feeding porphyran to the host animal. This ability to tune colonization of a gut microbe could be extremely useful for many medical applications if it could be transferred to a genetically tractable species. The goal of this project is to engineer *Escherichia coli* (*E. coli*) to digest and utilize the carbohydrates found in red seaweed. *E. coli* is an easily cultured and genetically tractable model organism, which can be used as a probiotic. Our approach to engineering *E. coli* to metabolize porphyran has been to transgenically express and secrete novel porphyranases and agarases originally found in the human gut bacterium *Bacteroides plebeius* (*B. plebeius*). After successfully expressing the necessary enzymes, we will modulate the expression of native metabolism genes to optimize *E. coli*'s physiology for the consumption of porphyran. This optimization process will be informed by genome scale metabolic modeling and computational flux balance analysis. In this work, we report initial results characterizing the enzymatic activity of the cloned porphyranases and agarases from *B. plebeius*. Enzyme activity was assayed through polyacrylamide carbohydrate gel electrophoresis (PACE) to identify oligomeric carbohydrate breakdown products. We also report on use of the hemolysin Type I Secretion System to secrete the porphyranase and agarase enzymes in *E. coli*.

**58. Pigment secretion in a laboratory-adapted strain of *Bacillus subtilis***

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Wild type *Bacillus subtilis* communities have the ability to come together to form complex communities called biofilms. Biofilm formation is favorable for *B. subtilis* in the wild because it allows the microbe to form symbiotic relationships with plants and improves resistance against antibiotics. When *B. subtilis* was brought into the laboratory it lost its ability to form biofilms over time thereby domesticating the bacterium. Two months of laboratory culture of 10 populations of a *B. subtilis* strain that forms biofilms revealed diverse biofilm forming capabilities. At the end of that two-month experiment, many cells in the 10 populations had partially or completely lost the ability to form biofilm. SH1 is one strain of *B. subtilis* with reduced biofilm complexity and additionally, it secretes a pink pigment. We hypothesized that the pink pigment could be pulcherrimin/pulcherriminic acid or coproporphyrin III. We used NMR to examine the structure of the pigment, and observed evidence supporting the presence of coproporphyrin III in our samples. However, we also ran genetic tests by deleting either a key gene required for pulcherrimin biosynthesis (yvmC) or a repressor of pulcherrimin production (yvmB aka. pchR) in SH1 and ancestral strain 3610, and this genetic analysis suggests that the pink pigment is pulcherriminic acid. Interestingly, the media used alters the color of the secreted pigment, so to test the possibility that our pigment could bind other metals besides iron, we grew SH1 on media with one or more of various metal ions present, including Co, Cu, Fe, Mn and Zn, to see if the metals included alter the pigment or biofilm phenotypes of pigment producing and pigment non-producing strains. We hope to definitively prove that this pink pigment is pulcherrimin and further, to learn about the role it plays within the cell.

**59. Design and Optimization of a Quantitative PCR Assay for the Diagnosis of Ross River Virus: Mosquito Xenomonitoring of Samoa and American Samoa Mosquito Samples**

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Ross River virus (RRV) is a mosquito-borne disease endemic to islands in the South Pacific, including Samoa and American Samoa. Screening endemic regions for RRV can predict where future outbreaks of the virus may emerge, highlighting the need for a sensitive and specific surveillance system. Therefore, a quantitative polymerase chain reaction (qPCR) assay was developed to successfully amplify 13 RRV strains and differentiate them from other alphaviruses. Mosquito samples were obtained from Samoa and American Samoa through xenomonitoring at multiple locations and mosquito samples were collected in site-specific pools. After mosquito RNA isolation, samples were tested using qPCR and the previously designed primers. 1500 pools from Samoa and American Samoa were tested; one positive pool has been identified. After testing the remainder of the RNA samples, mapping positive results to mosquito collection locations will inform where future RRV outbreaks may originate.

**60. Decreased biofilm complexity and reduced motility in laboratory adapted strains of *Bacillus subtilis***

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Biofilms are communities of bacteria that are surrounded by an extracellular matrix. Depending on the bacterial strain and growth conditions, biofilm formation varies. In previous work, the biofilm forming strain of *Bacillus subtilis* was grown for approximately 300 generations in the laboratory medium LB, and lab-adapted strains with altered biofilm forming abilities were isolated. The goal of these experiments was to identify specific mutations in the laboratory-adapted strains SH1 and SH2 relative to strain 3610, the ancestral strain. Linkage analysis by phage transduction for the Eps operon showed no linkage between the SH1 biofilm phenotype and the Eps operon. We identified mutations in the bacteria's genome by sequencing genomic DNA from SH1, SH2, and 3610. Strains SH1 and SH2 possess mutations in motility genes when compared to the wildtype strain. Motility genes and the regulation of motility control the organism's ability to swim in liquid media, swarm on moist semi-solid surfaces, or stay in one location and form a biofilm. Our current experiments include transforming wildtype motility genes into SH1 and SH2 to determine if after transformation, motility is restored and a wildtype biofilm phenotype is observed. Additionally, we are testing the swarming and swimming ability of phenotypically variable colonies isolated from the evolved populations, as after 300 generations, each community contained cells that form differently sized and structured colony biofilms. By subjecting the different colonies to swarming assays, we seek to determine if all lab-evolved strains have a difference in their motility when compared to 3610, which would suggest the laboratory environment selects against maintenance of motility in *B. subtilis*. Further experiments will include transforming mutated versions of candidate genes into the wildtype strain to see if these mutations recapitulate the SH1 or SH2 phenotypes, and further analyzing the influences of accumulated mutations in the lab-evolved strains.

**61. Piecing together a function: Mutational analysis of C19ORF66, a suppressor of KSHV Reactivation**

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KSHV (Kaposi's sarcoma-associated herpesvirus) is a herpesvirus associated with the development of several carcinomas, including its namesake Kaposi Sarcoma (KS). KS is the second most frequently occurring cancer of HIV/AIDS patients worldwide. During KSHV infection, the virus hijacks host resources for its own benefit, in particular by remodeling the host RNA regulatory pathways. Recently, we identified a host protein, C19ORF66, that stringently restricts KSHV infection. To better the effect of this protein on KSHV, we are taking on a mutational approach to determine identify which domain(s) of C19ORF66 are necessary and sufficient for its antiviral properties. We first deleted 50 amino acids increment portions of C19ORF66 from both the N- and C-terminal ends of the full-length protein. These mutants were then transfected into KSHV-infected cells and viral infection was monitored by immunofluorescence. We found that while all of the forward deletion mutants had antiviral properties similar to that of full length C19ORF66, only two of the reverse deletion mutants restricted KSHV. This suggested to us that there must be complementary domains responsible for C19ORF66's activity. Since C19ORF66 shuttles to and from the nucleus, one possibility is that its subcellular localization is important for its potent antiviral activity. We are currently studying the localization of each C19ORF66 mutant during KSHV infection using immunofluorescence, and directly quantifying the effect of the mutants on specific viral genes by PCR. Overall, by teasing apart the necessity of each domain, we hope to better characterize the functional role of C19ORF66 during the complex interplay between KSHV and its host.

**62. Fats vs. Fungi: Susceptibility of *Candida auris* to medium chain fatty acids**

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*Candida auris* is a fungal pathogen that has caused hospital outbreaks around the world. Most strains of *C. auris* are resistant to multiple classes of antifungal drugs, and some are resistant to all currently available drugs. *C. auris* is impervious to standard hospital disinfectants, and can survive on surfaces for weeks. It is a persistent skin colonizer, and can be spread from person to person or via contact with contaminated surfaces. New strategies are urgently needed to fight this growing threat to public health. We investigated the antifungal activities of the medium chain fatty acids octanoic and decanoic acids, found in coconut oil, against *Candida auris*. Both showed fungistatic, and decanoic acid fungicidal, activity against a panel of *C. auris* strains, including strains from all four geographic clades and strains resistant to several classes of antifungal drugs, during both planktonic and biofilm growth. These safe, natural products could provide a new strategy for skin decolonization and environmental decontamination.

**Melissa Shinfuku, Carolyn Anderson, RoseAnn Vik, Alam Garcia Heredia, Caralyn Sein, Katherine Chacon-Vargas, Malavika Prithviraj, Rebecca Gordon, Rabindra Thakur, Rachel Bell**

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