Pioneer Valley Microbiology Symposium Agenda
University of Massachusetts, Amherst – March 5, 2022

9:00 - 9:30 a.m. **Registration and Coffee**

9:30 - 9:35 a.m. **Opening Remarks**: James F. Holden, Professor and Department Head, Microbiology, University of Massachusetts Amherst

9:35 - 9:55 a.m. **Celebrating Lynn Margulis** on her 84th birthday with Hummingbird Films and Emily Case

9:55 - 11:05 a.m. **Session 1**

9:55 - 10:25 a.m. ***Invited Keynote Speaker***
Small Cell Size, Big Implications: Diversity, Episymbiosis, and Disease Association of Ultrasmall Saccharibacteria
Batbileg Bor, The Forsyth Institute

10:25 - 10:45 a.m. **The Host Strikes Back! C19ORF66 restricts KSHV Lytic Replication by Targeting Viral Gene Translation**
William Rodriguez, Microbiology, UMass Amherst

10:45 - 11:05 a.m. **The King of Oils: Effect of dietary frankincense on allergic asthma through modulation of the gut microbiome**
Cassandra Suther, Food Science, UMass Amherst

11:05 a.m. - 12:00 **Poster Session 1 and Coffee Break** - *Poster # 1-30*

12:00 - 1:00 p.m. **Session 2**

12:00 - 12:20 p.m. **Comparative genomics of *Aspergillus oryzae* genomes from different clades reveals signatures of artificial selection in primary and secondary metabolism in domesticate environments**
Katherine Chacon-Vargas, Molecular and Cell Biology, UMass Amherst

12:20 - 12:40 p.m. **Multiplex imaging in living bacterial cells with fluorogenic RNAs**
Ru Zheng, Chemistry, UMass Amherst

12:40 - 1:00 p.m. **Using machine learning to understand the determinants of mRNA stability in mycobacteria**
Huaming Sun, Bioinformatics and Computational Biology, Worcester Polytechnic Institute

1:00 - 2:05 p.m. **Lunch**

2:05 - 3:15 p.m. **Session 3**

2:05 - 2:35 p.m. ***Invited Keynote Speaker***
Building the molecular genetic toolbox to probe mechanisms of RNA-protein interactions in bacteria
Katherine E. Berry, Department of Chemistry and Program in Biochemistry, Mount Holyoke College

2:35 - 2:55 p.m. **Detection and Characterization of a Novel Small Protein in *Pseudomonas aeruginosa***
Zach Jonas, Amherst College

2:55 - 3:15 p.m. **Evaluation of indole as a prospective natural agent for antimicrobial resistance management**
Xiaojing Shi, Stockbridge School of Agriculture, UMass Amherst

3:15 - 4:10 p.m. **Poster Session 2 and Coffee Break** - *Poster # 31-59*

4:10 - 5:20 p.m. **Session 4**

4:10 - 4:40 p.m. ***Invited Keynote Speaker***
Bioprospecting acid mine drainage for bioactive secondary metabolites
Lesley-Ann Giddings, Department of Chemistry, Smith College

4:40 - 5:00 p.m. **Metabarcoding analyses of animal-associated foraminifera across built and open environments produce comparable diversity using DADA2 and In-house pipeline**
Rabindra Thakur, Organismic and Evolutionary Biology, UMass Amherst

5:00 - 5:20 p.m. **FISHing for bacterial symbionts within the accessory nidamental gland of *Euprymna scolopes***
Derrick Kamp, Department of Molecular and Cell Biology, University of Connecticut

5:20 - 5:45 p.m. **Closing Remarks and Prizes**

5:45 - 7:30 p.m. **Evening Refreshments**

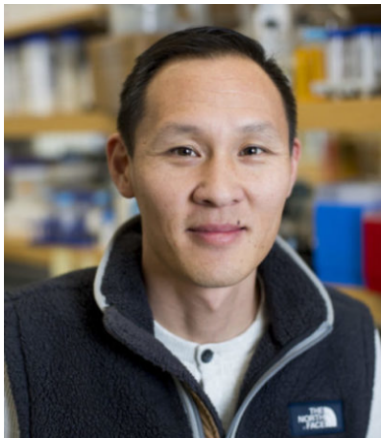
Pioneer Valley Microbiology Symposium
University of Massachusetts, Amherst – March 5, 2022

Distinguished Faculty Speakers

Medical Microbiology

Dr. Batbileg Bor
The Forsyth Institute, Cambridge, MA

Small Cell Size, Big Implications: Diversity, Episymbiosis, and Disease Association of Ultrasmall Saccharibacteria



Dr. Batbileg Bor received his Ph.D. in molecular biology and biochemistry at the University of California, Los Angeles. He studied the regulation of mammalian actin cytoskeleton and its impact on cell polarity. He became a postdoctoral fellow at UCLA school of dentistry where he changed his study field and became a microbiologist. Since then he has also been a postdoctoral researcher at Harvard School of Dental Medicine and at the Forsyth Institute. In January 2020, he became an assistant member of staff at the Forsyth Institute where he studies the recently discovered TM7 bacteria and their parasitic relationship with their respective bacterial hosts.

Fundamental

Dr. Katie Berry
Department of Chemistry and Program in Biochemistry, Mount Holyoke College

Building the molecular genetic toolbox to probe mechanisms of RNA-protein interactions in bacteria

Dr. Katie Berry obtained her bachelors at Swarthmore College and Ph.D. at the University of California, Berkeley. She completed her postdoctoral training at Harvard Medical School, where she learned tools of bacterial molecular genetics to ask questions about the mechanism of gene regulation, and the role of non-coding RNAs inside of living bacterial cells. Using an *in vivo* assay she developed, her research group at Mount Holyoke is studying how small (s)RNAs interact with proteins in disease-causing bacteria. She is the Clare Boothe Luce Assistant Professor of Biochemistry at Mount Holyoke College and teaches Biochemistry and Introductory Chemistry.

Environmental Microbiology

Dr. Lesley-Ann Giddings
Department of Chemistry, Smith College

Bioprospecting acid mine drainage for bioactive secondary metabolites

Dr. Lesley-Ann Giddings received her bachelors at Smith College and her Ph.D. in Chemistry at Massachusetts Institute of Technology. She completed her postdoctoral training in the laboratory of David Newman at the National Cancer Institute. She worked in the characterization of enzymes involved in the biosynthesis of microbial secondary metabolites as well as using microbial cocultivation as a means to identify new metabolites that are not produced under standard fermentation conditions. After her postdoctoral training, she worked at Hood College (Frederick, MD), Carleton College (Northfield, MN) and in the Department of Chemistry and Biochemistry at Middlebury College, Vermont. In July 2020, she returned to her alma mater, Smith College, as an Assistant Professor in Chemistry, where her research involves bioprospecting of extreme environments in search of bioactive agents and studying the enzymology of those agents.

Oral Presentations

9:55 – 10:25am

Small Cell Size, Big Implications: Diversity, Episymbiosis, and Disease Association of Ultrasmall Saccharibacteria

Batbileg Bor *(invited keynote speaker)*, Otari Chipashvili, Dan Utter, Jefferey McLean, Xuesong He, and Wenyuan Shi
The Forsyth Institute, Cambridge, Massachusetts, USA

Recently cultured Saccharibacteria (previously known as TM7) are members of the large lineage of bacteria named Candidate Phyla Radiation (CPR). CPR bacteria are characterized by having an extremely small cell size (200-500nm) and a reduced genome (~1 Mbp) lacking multiple essential biosynthetic pathways. Furthermore, Saccharibacteria in particular have been associated with multiple human inflammatory diseases such as periodontitis and inflammatory bowel disease. Their role in such diseases, however, remains unclear. Surprisingly, the first isolated Saccharibacteria strain, TM7x, grows on another bacteria, revealing a unique epibiont relationship that was previously thought to be extremely uncommon within bacteria. Investigating the interaction between TM7x and its host bacteria further revealed that when associated to Saccharibacteria, the host bacteria has decreased cell growth and completely inhibited cell-division, suggesting that Saccharibacteria live an epiparasitic lifestyle. This phenomenon was then observed in multiple host bacteria-TM7x pairings, demonstrating the ability of TM7x to infect multiple hosts within a phylogenetically narrow lineage. Culturing additional Saccharibacteria strains revealed a similar episymbiotic lifestyle and pattern of host range. Some of these Saccharibacteria grew on differing species of bacteria, suggesting that the majority of, if not all, Saccharibacteria may grow symbiotically on other host bacteria. Testing Saccharibacteria in a mouse inflammatory model then suggested that these ultrasmall bacteria tend to suppress, rather than increase, inflammation. Saccharibacteria elicit this anti-inflammatory effect by modulating the ability of their host bacteria to interact with eukaryotic cells. Therefore, the study of Saccharibacteria and CPR is a fascinating field with potential human health implications, and one that is expanding rapidly. These initial studies have provided us with newfound knowledge and a deeper appreciation for the vast diversity of the bacterial world.

10:25 – 10:45 am

The Host Strikes Back! C19ORF66 restricts KSHV Lytic Replication by Targeting Viral Gene Translation

William Rodriguez, Timothy Mehrmann, Mandy Muller
Department of Microbiology, University of Massachusetts Amherst, Amherst, Massachusetts, USA

During lytic reactivation, Kaposi's sarcoma-associated Herpesvirus (KSHV) seizes control of cellular gene expression by inducing a massive RNA decay event termed "host-shutoff". This host-shutoff event is orchestrated from the cytoplasm by SOX, a viral endoribonuclease, which decimates greater than 70% of cellular mRNAs (>70%). This viral takeover strategy rapidly reallocates host resources toward viral replication. Fascinatingly, we and others have shown that select host transcripts actively evade SOX cleavage via an RNA element known as the SOX-Resistance Element or SRE. We recently demonstrated that one such SRE-bearing transcript, C19ORF66, is actively protected from cleavage by multiple viral endonucleases and encodes a potent anti-KSHV factor, restricting nearly every stage of KSHV lytic replication. To better understand the role of C19 during KSHV infection, we are currently investigating the mechanism by which C19 restricts KSHV lytic replication. Here, we show that C19 inhibits expression of critical viral early genes, most notably, the latent-to-lytic master switch protein, KSHV's RTA. Several lines of evidence show that RTA is both necessary and essential for the progression of viral lytic gene expression. Using a combination of cycloheximide and MG132 assays, we found that C19 restricts the translation of RTA. To better understand the C19 mechanism, we next utilized mass spectrometry to map the C19 interactome during KSHV infection. Of the host proteins identified, many are known constituents of phase-separated RNA granules, cytoplasmic RNA-protein aggregates that regulate global gene expression. Lastly, using Immunofluorescence assays, we have confirmed that C19 expression does in-fact influence RNA granule

formation: restricting the formation of sites of RNA decay (processing bodies) while triggering the formation of sites of translational arrest (stress granules). By characterizing the interplay between C19 and KSHV, we strive to better define the complex balance struck between KSHV and its host for control of cellular gene expression.

10:45 – 11:05 am

The King of Oils: Effect of dietary frankincense on allergic asthma through modulation of the gut microbiome

Cassandra Suther[1], Liv Devon[1], Lauren Daddi[2], Suresh Bokoliya[3], Hanshu Yuan[4], Katarzyna Saar[5], Hunter Panier[6], Adam Matson[5], Matthew D. Moore[1], Yanjiao Zhou[3]

[1]Department of Food Science, University of Massachusetts Amherst, Amherst, Massachusetts, USA; [2]Department of Biology, University of Connecticut, Storrs, Connecticut, USA; [3]Department of Medicine, University of Connecticut Health Center, Farmington, Connecticut, USA; [4]Department of Genetics, University of Connecticut Health Center, Farmington, Connecticut, USA; [5]Department of Pediatrics, University of University of Connecticut Health Center, Farmington, Connecticut, USA; [6]Department of Immunology, University of Connecticut Health Center, Farmington, Connecticut, USA

The pioneer valley has one of the highest rates of asthma across the country. The continued use of steroid for treatment can lead to harmful side effects. The gut microbiome has been shown to effect asthma development and exacerbation. In this study, we used an oral supplement containing *Boswellia serrata* tree resin, which has anti-inflammatory properties, to observe the effects on both asthma conditions and gut microbiome. An OVA based allergic airway model was used and mice were given 100mg/kg of *Boswellia serrata* as a treatment throughout asthma development and reaction. Treated mice had significantly less total lung white blood cells (96223±39958 vs 49770±25833) and eosinophils counts (47342±8367.9vs 16496±4785), Th2 cytokines in lungs (IL-5, IL-4, IL-13), histology scoring and reactivity to methacholine challenged, when compared to the asthma controls. There were significant differences between starting and endpoint gut microbiome composition between the treated and asthma control mice. Asthma control mice saw a decrease in overall diversity (shannon diversity and richness p- <0.0079). *Boswellia serrata* treated mice was increases in beneficial bacteria, including *Bifidobacteria* and *Akkermansia*. *Boswellia serrata* works as an anti-asthma agent and may do so because of its positive effect on the microbiome.

12:00 – 12:20 pm

Comparative genomics of *Aspergillus oryzae* genomes from different clades reveals signatures of artificial selection in primary and secondary metabolism in domesticate environments

Katherine Chacon Vargas and John Gibbons

Program in Molecular and Cellular Biology, University of Massachusetts Amherst, Amherst, Massachusetts, USA; Department of Food Science, University of Massachusetts Amherst, Amherst, Massachusetts, USA; Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Humans domesticated different species by selecting for desired traits to enhance their benefits. Domestication is not limited to plants and animals. In parallel, microbes (bacteria, yeasts, and molds) were also domesticated for their roles in food preservation, nutrition and flavors. *Aspergillus oryzae* is a domesticated filamentous fungal species used during the fermentation of traditional Asian foods and beverages such as sake, soy sauce, and miso. The artisanal practice of continuous passage of *A. oryzae* on food substrates over thousands of years resulted in adaptation to the food environment along with genetic differentiation from its wild relative *A. flavus*, a toxin producing agricultural pest. Here, we analyzed 300 isolates of *A. oryzae* and *A. flavus* to understand how the history of domestication and how this process shaped patterns of genomic variation. Using population structure and phylogenetic analysis we identified 2 major populations of *A. oryzae* and two major lineages of *A. flavus*. Next, we used two population genomic metrics to identify regions of the *A. oryzae* genome possessing signatures of artificial selection. We identified 30 candidate genes possessing strong signatures of artificial selection, several of which have functional annotations directly related to fermentation. Specifically, alcohol dehydrogenase, fructose

transmembrane transporters and glutathione metabolism genes. Additionally, we examined differences in gene copy number variation between *A. oryzae* and *A. flavus*. Most strikingly, we found significantly more copies of the α-amylase encoding genes in *A. oryzae* compared to *A. flavus*, suggesting selection for increased carbohydrate metabolism during fermentation. Further, gene absences in *A. oryzae* compared to *A. flavus* were enriched for secondary metabolism function, suggesting selection for loss of toxicity in *A. oryzae*. Taken together, our results show the *A. oryzae* genome was significantly reshaped as a result of domestication.

12:20 – 12:40 pm

Multiplex imaging in living bacterial cells with fluorogenic RNAs

Ru Zheng, Rigumula Wu, Mingxu You
Department of Chemistry, University of Massachusetts, Amherst, Massachusetts, USA

Simultaneously monitoring different cellular targets and studying their relationships is an important goal in cell biology. However, multiplex imaging in living cells is still challenging, especially with fluorescence microscopes, due to the broad spectra and spectral overlap of available fluorophores. In this project, we will introduce a fluorogenic RNA-based sequential imaging strategy that can overcome this challenge. Fluorogenic RNA aptamers are RNA sequences that selectively bind and activate the fluorescence of corresponding dye molecules. Here, we have first explored and identified five fluorogenic RNA/dye pairs that can function orthogonally. After these fluorogenic RNAs are genetically encoded in *E. coli* cells, spectrally irresolvable dye molecules are sequentially added, imaged, and removed in several rounds of imaging cycles. A fast fluorescence activation and deactivation (in several minutes) of these RNA/dye pairs have also been demonstrated. As a result, the multiplex capacity of this novel imaging system is no longer limited by the excitation/emission spectral overlap of the fluorescent molecules. In addition, by fusing different target-binding aptamers with each fluorogenic RNA, versatile genetically encoded sensors have been developed for the imaging of various metabolites, signaling molecules, antibiotics, and RNAs at a single-cell level. The correlations among critical target molecules such as cyclic-di-GMP, (p)ppGpp, and S-adenosyl methionine can now be studied in each individual bacterial cell. This modularity of this multiplex system can be readily adapted to imaging many interesting cellular target molecules.

12:40 – 1:00 pm

Using machine learning to understand the determinants of mRNA stability in mycobacteria

Huaming Sun[1], Ying Zhou[2], Diego Vargas Blanco[3], Catherine S. Masiello[2], Jessica M. Kelly[4], Justin K. Moy[1], Dmitry Korkin[1,5], Scarlet S. Shell[1,2]
[1]Program in Bioinformatics and Computational Biology, Worcester Polytechnic Institute, Worcester, USA; [2]Department of Biology & Biotechnology, Worcester Polytechnic Institute, Worcester, USA; [3]Massachusetts General Hospital, USA; [4]Beth Israel Deaconess Medical Center, USA; [5]Department of Computer Science, Worcester Polytechnic Institute, Worcester, USA

As a highly successful pathogen, *M. tuberculosis* is able to infect, survive and proliferate within harsh microenvironments created by human host. Regulation of mRNA degradation is key for survival of the wide range of host defenses. Previous studies have shown that the variability in mRNA degradation exists not only among genes but also between conditions. A better understanding of the determinants of mRNA stability in *M. tuberculosis* will shed light on how it adapts to the harsh environments. Here we developed a computational pipeline using RNAseq and machine learning to identify the factors determine mRNA degradation in mycobacteria and to study how these factors regulate degradation. First, we performed RNAseq to quantify mRNAs degradation profiles transcriptome-wide using the non-pathogenic model *M. smegmatis* in normal and stress condition. Next, we clustered mRNAs according to their degradation patterns. Then we trained a random forest classifier to explore the mRNA features associated with different degradation patterns. Our results show that instead of one dominant feature, various types of features including nucleotide and codon content, secondary structure, ribosome occupancy and other sequence features all contribute to differentiate the degradation patterns. Our results also demonstrate that the determinants of degradation patterns are different for leadered and leaderless mRNAs and for mRNAs in

normal and stress conditions. All of these suggest that there are complex regulation mechanisms for mRNA degradation in *mycobacteria*.

2:05 – 2:35 pm

Building the molecular genetic toolbox to probe mechanisms of RNA-protein interactions in bacteria

Katherine E. Berry *(invited keynote speaker)*
Department of Chemistry and Program in Biochemistry, Mount Holyoke College, South Hadley, Massachusetts, USA

Regulatory small RNAs (sRNAs) play important roles in stress responses in nearly all bacterial organisms that have been investigated, including pathogenic bacteria. The functions of sRNAs are often supported by proteins such as the paradigmatic bacterial RNA chaperone protein, Hfq. While much is known about the mechanism of Hfq-RNA interactions, significant gaps remain in our understanding of how other bacterial RNA chaperones, such as the proteobacterial protein ProQ, interact with RNA to regulate gene expression. In addition, traditional approaches to discover RNA-binding proteins have left many bacterial species without an identified RNA chaperone protein: almost half of bacterial genomes contain neither an *hfq* nor *proQ* ortholog despite many of these organisms utilizing sRNAs. In order to study the mechanisms of known RNA-binding proteins and discover new ones, we have developed a genetic approach to probe RNA-protein interactions inside of *E. coli* cells with a transcription-based bacterial three-hybrid (B3H) assay. This assay can detect interactions in the native context of a bacterial cytoplasm and offers a genetic approach to identify novel RNA-binding proteins, as well as to identify and interrogate mutations in these proteins with molecular phenotypes of interest. We are working to optimize the assay for stronger signal-to-noise and also applying it to dissect the RNA-binding mechanisms of multiple bacterial proteins, including FinO-domain proteins such as ProQ.

2:35 – 2:55 pm

Detection and Characterization of a Novel Small Protein in *Pseudomonas aeruginosa*

Zach Jonas
Amherst College, Amherst, Massachusetts, USA

Recent studies indicate that many small open reading frames (smORFs) remain unannotated. However, small proteins, defined as less than ~50 amino acids in length, are emerging as important players in many biological processes. Using a ribosome profiling data set, we identified several novel smORFs in the opportunistic human pathogen *Pseudomonas aeruginosa*. We confirmed *in vivo* expression of one putative smORF via genomic epitope tagging and immunoblotting. The smORF is predicted to encode a 43 amino-acid-long transmembrane protein. It is not within a known operon and is located in an intergenic region antisense to its flanking genes, PA14_69040, which produces 5-formyl-tetrahydro-folate cyclo-ligase, and PA14_69050, a hypothetical protein. This sequence is highly conserved in *P. aeruginosa* strains and conserved in some halotolerant environmental bacteria. An assay of stress conditions indicates that expression of this gene is upregulated by the membrane stressors sodium dodecyl sulfate (SDS) with ethylenediaminetetraacetic acid (EDTA) and under basic conditions from sodium hydroxide (NaOH), demonstrating that the small protein may be involved in a membrane stress response. To further investigate the role of the smORF in membrane stress, an unmarked deletion mutant will be generated with an allelic exchange vector and growth in stress conditions will be compared to the WT. Additionally, because small proteins often regulate larger proteins, a co-immunoprecipitation assay will be run to identify potential proteins that interact and co-purify with the small protein. Identified co-precipitants identified with mass spectrometry would provide insight into the small protein's function. Furthermore, differential centrifugation in a sucrose gradient can confirm the subcellular membrane localization this predicted transmembrane small protein. The aim of this project is to elucidate the function of this small protein, yield important physiological insight into the stress responses of this pathogen, and provide a workflow for the characterization of additional smORFs identified by ribosome profiling.

2:55 – 3:15 pm

Evaluation of indole as a prospective natural agent for antimicrobial resistance management

Xiaojing Shi and Geunhwa Jung
Stockbridge School of Agriculture, University of Massachusetts Amherst, Amherst, Massachusetts, USA

In recent years, antimicrobial resistance (AMR) has become an important problem threatening food supply and human health. To combat the developing AMR crisis, we need to limit the evolution of microbes' resistant genes by reducing the use of antimicrobials. Therefore, developing alternative approaches is necessary. Here, we propose a combination of indole and antimicrobials to manage AMR development. Indole is an aromatic molecule having an outstanding heterocyclic compound with a wide range of pharmacological capabilities. In our previous experiment, inspired by the information provided by whole-genome analysis, indole exhibited antifungal activity at high concentrations (>1ppm) and exhibited synergistic inhibition with four fungicides classes on fungicide-sensitive and resistant strains of *Clarireedia jacksonii*, an economically important pathogenic fungus contributing to aesthetic damage and death of turfgrasses on golf courses. To determine true feasibility of our indole-fungicide combinations, it is necessary to explore their effects *in vivo* on turfgrasses maintained in the CNS Greenhouses, where an effective formula for control can be determined. An effective formula determined from greenhouse studies will be evaluated in-field at the UMass Joseph Troll Turf Research Facility in South Deerfield, MA in 2022. Additionally, the antimicrobial activity of indole against bacteria will be explored using two common bacterial models that are well-known for frequent AMR occurrences: Gram-negative bacterium *E. coli* and Gram-positive bacterium *Staphylococcus aureus*. Firstly, the effect of indole with various antibiotic combinations will be tested *in vitro*. Effective combinations will be tested on green-leaf vegetables to confirm feasibility in practice. Positive results from the above experiments will indicate indole as a promising solution for AMR management in combination with antimicrobials. At the same time, our study provides a new perspective for combatting AMR by exploring more natural products' potency as antimicrobials.

4:10 – 4:40 pm

Bioprospecting acid mine drainage for bioactive secondary metabolites

Lesley-Ann Giddings *(invited keynote speaker)*
Department of Chemistry, Smith College, Northampton, Massachusetts, USA

Most FDA-approved small molecule drugs are either natural products or inspired by natural products. In every case, microbes from all three domains of life, Archaea, Prokarya, and Eukarya, have been either identified as the producer of these secondary metabolites or speculated to be involved in their production via symbiotic associations. In the search for new microbial secondary metabolites, chemists have traditionally explored easily accessible terrestrial environments. However, by the 1990s, all of the "low hanging fruit" had been picked and natural product rediscovery rates increased. As a result, underexplored environments, such as extreme ecosystems, are now being investigated. Mining creates extreme environments by exposing metal sulfides to air and water, producing metal-rich, acidic effluent or acid mine drainage, the leading cause of surface water pollution in the mid-Atlantic. This talk will describe the characterization of a microbial community at Ely Copper Mine (Vershire, VT) and the genes involved in natural product biosynthesis. Notably, this study provides insight into how to find new therapeutics in polluted environments.

4:40 – 5:00 pm

Metabarcoding analyses of animal-associated foraminifera across built and open environments produce comparable diversity using DADA2 and In-house pipeline

Rabindra Thakur[1,2], Adena Collens[1], Elinor Sterner[1], Mattia Greco[3], Laura A. Katz[1,2]

[1]Department of Biological Sciences, Smith College, Northampton, Massachusetts, USA; [2]Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, Amherst, Massachusetts, USA; [3]Institute of Oceanology, Polish Academy of Sciences, Sopot, Poland

Foraminifera are shell-building microbial eukaryotes that support biogeochemical processes. Surveys of foraminiferal molecular diversity have hitherto been restricted to sediments/water samples and have not accounted for the full range of their ecological preferences. For example; very little is known about the diversity of foraminiferal species associated with invertebrate shells such as clams, snails, crabs. In this study, we surveyed animal-associated foraminifera at the molecular level using a metabarcoding approach. We characterize both DNA and RNA communities using foraminifera-specific PCR primers. Our analyses include 170 samples collected from the shells of clams, snails, and crabs from three environments (two 'built' aquariums, at Smith College and one 'open' salt marsh from Madison, CT). To authenticate the resulting diversity, we compared two metabarcoding survey tools: (1) DADA2 based on Amplicon Sequence Variants (ASVs) and (2) an in-house pipeline based on clustering of sequences into Operational Taxonomic Units (OTUs). We found that both OTUs and ASVs retained the same phylogeny-based taxonomy and beta-diversity. Many dominant OTUs preferred specific hosts, including these three key sister taxa: *Hemisphaerammina*, *Haynesina* and *Rosalina*. By comparing our data to published literature on microscopy studies of animal-specific foraminifera, we will be able to identify the diversity and ecology of animal-associated foraminiferal communities.

5:00 – 5:20 pm

FISHing for bacterial symbionts within the accessory nidamental gland of *Euprymna scolopes*

Derrick Kamp[1], Jessica Mark Welch[2], Spencer Nyholm[1]

[1]Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut, USA; [2]Marine Biological Laboratory, Woods Hole, Massachusetts, USA

The accessory nidamental gland (ANG) of female *Euprymna scolopes* houses a diverse yet conserved community of environmentally acquired bacteria that assist in egg defense. The bacterial community resides amid a dense network of epithelium-lined tubules dominated by Alphaproteobacteria or Verrucomicrobia, with Gammaproteobacteria and Flavobacteriia present as well. Hatchling squid lack ANGs; the nascent gland appears after approximately four weeks and develops into a superficial epithelium with numerous pores. These tissues assist in recruiting bacteria from the environment, and in the presence of sand containing native bacteria, the nascent organ develops into a mature ANG.

To understand the spatiotemporal relationship between bacteria and host throughout the course of ANG development, we used various advanced light microscopy techniques to visualize the ANG and bacteria during different stages of gland maturation. Imaging the nascent ANGs showed the ciliated pores contained sediments from the host's benthic habitat, implicating the sediment as a potential reservoir for symbiotic bacteria. Combinatorial labeling and spectral imaging fluorescence in-situ hybridization (CLASI-FISH) of sections from adult ANGs revealed specific partitioning of bacterial taxa; the Alphaproteobacteria genera *Leisingera* and *Reugeria* commonly dominate separate tubules. Although single-taxa dominance of tubules was prevalent, it was not observed to be universal. Some ANG tubules contained a mixture of Alphaproteobacteria and Verrucomicrobia, while others appeared to contain mixtures of Alphaproteobacteria and Gammaproteobacteria. Additionally, light sheet microscopy of whole-mount adult ANGs showed that individual tubules tightly bundled in confined areas of the ANG. These observations present a likely reservoir for symbiotic bacteria and suggest that symbiont distribution within ANG tubules is dynamic, composed of regions dominated by single taxa and other regions with multiple taxa. Future work will focus on understanding how these spatiotemporal relationships between microbial and host partners lead to the establishment of a functional ANG symbiosis.

Poster Presentations

Please note that underlined individuals are scheduled presenters.

Poster Session 1: 11:05 - 12:00 pm: Posters #1-30
Poster Session 2: 3:15 - 4:10 pm: Posters #31-60

1. **Temperature Sensitivity of Microbial Growth in Warming Soils**

Ashley Eng, Achala Narayanan, Kristen M. DeAngelis
Department of Microbiology, University of Massachusetts Amherst, Amherst, Massachusetts, USA

As climate change progresses, ecosystems are predicted to be impacted in new ways. Soils serve as a large carbon sink, and thus buffer against climate change. Warming may also result in adaptation of microbial traits, which would irreversibly change microbes' behavior in ecosystems; however, we do not understand this phenomenon. We studied isolates from a long-term soil warming experiment at the Harvard Forest where soils were heated 5ºC above ambient temperatures. We hypothesized that isolates from heated soil plots have adapted lower temperature sensitivities of growth in response to long-term warming. We measured OD600 of liquid cultures over time spanning temperatures from 20-37 ºC along 3 ºC increments. We used model fitting to calculate growth rate and temperature sensitivity of growth. A phylogenetic group comparison was conducted to determine whether there was a significant difference between temperature sensitivity of growth between isolates from heated and control plots. Preliminary data from the first 8 isolates suggests that bacteria from heated soil plots had lower temperature sensitivity of growths than isolates from control plots. A significant P-value of 0.001 (F = 10.34) was calculated. The group mean of the control group was 0.0050 OD1/2*min/ºC and the group mean of the warmed group was 0.0013 OD1/2*min/ºC. This suggests that microbes may have adapted traits to better tolerate increasing temperatures. We plan to continue data collection for a total of 25 isolates. Microbes may adapt or acclimate to ecosystems in response to warming due to climate change. Understanding differences between temperature sensitivities of growth among isolates from heated vs. control plots allows us to better understand soil microbial responses to warming. Determining whether growth strategies explain microbial adaptation to warming will help predict changes in microbial community and ecosystem function.

2. **Efficacy of Acetic Acid Dissolved in Oil and with W/O Emulsions against *Salmonella Enteritidis* and *Listeria monocytogenes* Desiccated on Stainless Steel Surface**

Shihyu Chuang and Lynne McLandsborough
Department of Food Science, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Exposure of bacteria to desiccation often induces cross-tolerance against environmental stresses, leading to long-term persistence in food-processing environments. In this study, an oil-based antimicrobial solution was developed for cleaning and sanitation in food plants such as peanut butter and chocolate facilities where wet cleaning needs to be avoided. A contact time of 30 min at 22 °C was used as treatment, against *Salmonella Enteritidis* and *Listeria monocytogenes* inoculated on stainless steel surface to a concentration of 7 log CFU and desiccated at 75% relatively humidity. The treatment with 200 mM acetic acid dissolved in oil reduced the desiccated *S. Enteritidis* and *L. monocytogenes* by 0.69 log and 1.43 log, respectively. Adding a small level of water to the acidified oil in the form of water-in-oil (W/O) emulsions greatly enhanced its antimicrobial efficacy. The treatments with W/O emulsions containing 0.3-9% v/v water and 200 mM acetic acid reduced both the desiccated bacteria to below the detection limit of 0.48 log. Furthermore, the water activity (aw) and the antimicrobial efficacy of the acidified W/O emulsion were found correlated. With the concentration of water fixed at 1%, a gradual decrease in the emulsion aw was accompanied by less reduction of the desiccated *S. Enteritidis* after treatment, from > 6.52-log reduction with 0.93 aw to 0.72 log with 0.28 aw. In summary, the acidified W/O emulsion exhibited efficacy against desiccated bacteria acceptable for adaptation as a sanitizing agent for food processing, and the antimicrobial efficacy of acetic acid was facilitated by osmotic downshift and retarded by osmotic upshift.

3. Uric acid-degrading bacteria in the gut: A promising Strategy to control Hyperuricemia

William Wolfe
University of Massachusetts Amherst, Amherst, Massachusetts, USA

Uric acid is a nitrogenous waste product produced through purine degradation in human metabolism. Throughout evolution, humans have lost the ability to degrade uric acid through the loss of the uricase enzyme. Therefore, when produced in excess, uric acid can build up and cause negative health outcomes including inflammation, gout, and kidney stones. About 30% of waste uric acid is removed through the large intestine and may be able to interact with the gut microbiome. We hypothesized that certain gut bacteria can degrade uric acid into less harmful water-soluble metabolites such as allantoin. In this study, strains of gut bacteria were screened for uric acid degrading activities. By utilizing uric acid's low water solubility, crystalized uric acid was used in agar plates to observe clear zone forming gut bacteria. As uric acid is degraded by bacteria, more water-soluble compounds are produced displaying clear zone formations around bacteria growing on the plates. Bacteria from Human and Mouse fecal samples were screened this way on three different types of media including De Man, Rogosa and Sharpe (MRS), liver infusion media, and tryptic soy media. Isolated strains were also cultured with soluble uric acid in liquid media to determine degradation rates of uric acid. Overall, our results demonstrated that gut bacteria may have the ability to lower the level of uric acid in the large intestine to help alleviate the negative health effects caused by elevated uric acid in humans.

4. Actinobacteria Adapt to Drought due to Long-term Soil Warming

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Climate warming alters soil microbial community composition and function, which has widespread ecosystem consequences. Long-term soil warming studies suggest that microbes are adapting to warming, but little is known about what microbial traits are adaptive to warming. As soils warm, they become increasingly dry, potentially selecting for more drought tolerant organisms. The Harvard Forest long-term soil warming experiment is ongoing for 30 years, where soils have been heated 5°C above ambient temperatures. Prior results showed that Actinobacteria are responding to warming. We aim to characterize traits underlying Actinobacterial responses to warming, and hypothesize that Actinobacteria have adapted greater tolerance to drought. To test this, we grew Actinobacteria isolated from heated and control plots in model soils along a gradient of moisture conditions, and measured cumulative carbon mineralization over three weeks as daily microbial respiration. We are also studying traits such as biofilm production, spore formation and growth rate to determine possible trade-offs to drought adaptation. We find that at low moisture availability, isolates from heated plots mineralize significantly more carbon than isolates from control plots, but that this treatment effect is obscured at higher moistures. Preliminary evidence also suggests lower moisture sensitivity of growth in isolates originating from heated plots than growth plots. We also expect to see a trade-off between growth rate and drought tolerance.This work provides evidence of drought tolerance as a trait underlying microbial adaptation to long-term soil warming. Understanding traits driving microbial adaptation to climate change is vital to predicting future shifts in ecosystem function.

5. Impact of Zooplankton Filter Feeding on Sunlight Inactivation of Viruses

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Microbial pollutants such as viruses and bacteria in aquatic systems pose risks to both human health and the ecosystem. UV disinfection is an increasingly popular method of treatment in both drinking water and wastewater treatment systems. In addition sunlight (UV) disinfection is a critical process to inactivate viruses in natural systems. While UV and sunlight inactivation are well studied, limited knowledge exists on how biotic interactions may influence these disinfection processes. Specifically, filter-feeding zooplankton can consume viruses in these

systems, but the impact of their filter-feeding and presence on disinfection rates is not understood.

The objective of our research is to quantify the impact of filter-feeding zooplankton on viral inactivation. In our experiments, we use the rotifer *Brachionus plicatilis* as a model organism and MS2 bacteriophage as a surrogate for human pathogenic viruses. We quantified the uptake rate of MS2 from water by *B. plicatilis* and showed up to a 4-log removal of MS2. However, this uptake of MS2 could cause shielding against UV disinfection rather than inactivating the virus. We quantified the effects of *B. plicatilis* on solar inactivation through a series of rooftop microcosm experiments. For each condition tested, we quantified MS2 inactivation as a function of irradiance. Our proof of principle experiments show that the presence of rotifers decreases solar inactivation of MS2.

6. **Sugar-coating persistence: metabolic stimulation and efflux pump disruption potentiates Zoliflodacin against stationary phase *Escherichia coli***

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Antibiotic treatment failure (ATF) is a pressing global health crisis that continues to worsen, with ATF-related deaths projected to reach 10 million by 2050 . A major contributor to ATF is antibiotic persistence, where a subpopulation of cells in a clonal culture displays phenotypic heterogeneity and transient tolerance to antibiotics. The conditions that lead to antibiotic persistence are still largely undetermined; however, states of slow growth have been implicated. Slow-growing cells have shown increased tolerance to drugs which target growth-related processes. Previously, we found that drugs like fluoroquinolones (FQs), which target DNA topoisomerases and gyrases can eliminate slow-growing bacteria. However, FQ-resistant strains have emerged in clinical settings, highlighting the need for additional strategies to target stationary phase cells. Zoliflodacin (Zoli), a non-FQ topoisomerase inhibitor in clinical development, can potentially bridge this gap. Although Zoli shows limited efficacy toward slow-/non-growing bacteria, data suggest that enhanced metabolic rates can increase effectiveness of many antibiotics, giving credence to the idea that the activity of Zoli can be improved. Additionally, Zoli has been implicated as a substrate of bacterial TolC-dependent efflux pumps. We hypothesize that the stimulation of bacterial metabolism and inhibition of drug efflux can increase the effectiveness of Zoli against stationary phase *E. coli*. We show the supplementation of glucose along with phenylalanine-arginine β-naphthylamide (PAβN), a chemical efflux pump inhibitor, can significantly potentiate Zoli against stationary phase cells. Additionally, our data suggest that the supplementation of glucose and PAβN can augment intracellular Zoli accumulation. Finally, the expression of genes necessary recovery from Zoli-induced DNA damage are engaged early, indicating that metabolic disruption may affect the timing of critical molecular events. Overall, this work demonstrates that glucose and PAβN have the potential as chemical adjuvants in the treatment of slow-growing cells with Zoli, expanding the weapons in our antibiotic arsenal.

7. **Is 2DUF enough? Exploring the mechanism and function of novel spore protein, 2DUF, on wet-heat resistance in *Bacillus subtilis***

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Bacterial spores are known to cause faster spoilage of food as well as cause food-borne illnesses with *Bacillus cereus* alone causing 84,000 cases a year in the United States. Bacterial spores are extremely resistant to typical forms of sterilization, but both high heat and pressure can be used to eliminate spores from food products before they are packaged. Unfortunately, some spores have been found to evolve resistance and survive temperatures that should have been adequate to kill them. Recently, wet-heat resistant spores were isolated from diverse food products and sequenced, and a novel transposon, Tn1546, was found to be directly related to this new resilience phenotype. We found that within the transposon, one protein with two domains of unknown function (named 2DUF) is necessary to trigger the wet-heat resistance. Alpha-fold has predicted that the 2DUF protein has three

transmembrane helices, and work from our lab has shown that 2DUF confers resistance to small molecules that must pass through the inner membrane to kill the spore. Based on these observations, we hypothesize that 2DUF localizes to and alters the permeability of the inner membrane. We set out to investigate if 2DUF alone is sufficient to cause the wet-heat resistance phenotype. We are constructing spores that express 2DUF fused to GFP on the C terminal end without the other proteins that are encoded in the transposon. This construct allows us to perform microscopy to track 2DUF's localization. By determining if 2DUF is sufficient to confer this wet heat resistance phenotype as well as its mechanism of action, we hope to understand more about how spores survive extreme conditions to better eliminate them.

8. Chemical mutagenesis of *Listeria monocytogenes* to investigate the genetic basis of benzalkonium chloride tolerance

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Listeria monocytogenes is a potentially fatal foodborne pathogen commonly found in the food industry, primarily in dairy products and deli meats. This bacterium is the causative agent of listeriosis, a disease that is especially dangerous for immunocompromised individuals and pregnant women. Recent studies have shown that several *L. monocytogenes* strains associated with foodborne outbreaks exhibit tolerance to benzalkonium chloride. Benzalkonium chloride (BAC) is a disinfectant that is widely used in the food industry to inhibit the growth and spread of microorganisms. In this experiment, we used chemical mutagenesis to generate *L. monocytogenes* mutants and screen them for BAC tolerant phenotypes. Ethyl methanesulfonate (EMS), a strong chemical mutagen, was used to induce random point mutations in the fully sequenced *L. monocytogenes* isolate, ALE_10_0415. The mutated bacterial cells were then grown in increasing concentrations of BAC and observed for changes in the minimum inhibitory concentrations (MIC). We found that the MIC of BAC for isolate ALE_10_0415 was 3µg/ml BAC, making it more sensitive to the sanitizer compared to other isolates. Thus, the goal of this study is to mutagenize this strain until it exhibits higher tolerance to BAC, and then identify the genes contributing to the tolerant phenotype through comparative genomic analysis. Identifying the genes responsible for *L. monocytogenes* tolerance to BAC would help create gene markers that could be used in the food industry to identify persistent strains that threaten the quality and safety of our food.

9. The Impact of Microbial Interactions and Environmental Cues on Phenotypic Heterogeneity and Response to Antibiotic Treatment

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Antibiotic treatment failure has worsened over time because of the lack of new compounds in the drug discovery pipeline along with mechanisms that bacteria can engage in to survive treatment with existing drugs. In addition to bacteria that evolve resistance to antibiotics, the phenomenon of antibiotic persistence can further compromise our battle to treat infection effectively. Bacterial persisters can undergo reversible phenotypic changes and survive drug concentrations that their genetically identical kin cannot. These changes are hard to track and can fuel rampant infection relapse and resistance development. Current research on antibiotic persistence is often studied in the context of single bacterial species and their responses to environmental cues. However, many bacterial infections are polymicrobial and the impact of these interactions on a bacterium's physiological responses to antibiotic treatment is largely unknown. We previously observed that the secreted biomolecules from the opportunistic pathogen, *Pseudomonas aeruginosa*, can inhibit the growth of *Escherichia coli* cultures. Additionally, we found that *P. aeruginosa* can induce DNA damage responses in *E. coli* similar to DNA metabolism-targeting antibiotics and increase mutagenesis in the *E. coli* population. To understand how *E. coli* reprograms its gene expression in response to these stressors, we are constructing a triple-fluorescent reporter plasmid to simultaneously track the expression of three genes of interest. These include *xseA*, which encodes an exonuclease; *umuDC*, which encodes

an error prone polymerase; and *recA*, which induces a set of survival response genes and mediates the repair of DNA. This approach will allow us to observe how *E. coli* coordinates these molecular processes in response to nutrients, secreted molecules from *P. aeruginosa*, and antibiotics in its surroundings. Through gaining a better understanding of bacterial phenotypic responses and persistence to antibiotic treatment in the context of microbial interactions, we can potentially find new ways to overcome relapsing infections.

10. Degradation of Residual Nucleic Acid on Surfaces by Commercial Disinfectants

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Polymerase chain reaction (PCR) has become widely used in recent years for a variety of diagnostic applications. However, studies have shown that PCR false positives can occur if intact nucleic acid traces are present on surfaces and equipment. Therefore, there is a need for improved surface decontamination protocols that account for nucleic acid degradation to ensure reliable PCR results. Previous studies have evaluated the efficacy of some reagents specifically targeted toward degradation of nucleic acid. Here, we conducted a similar study to compare established microbial disinfectants and determine which active ingredients can also degrade residual nucleic acid.

Four disinfectants with a range of active ingredients (one sodium hypochlorite, two improved hydrogen peroxide, and one quaternary ammonium) were provided by Diversey, LLC for testing and compared to Clorox germicidal bleach as a control. A small sample of either viral ssRNA (extracted from human coronavirus 229E) or eukaryotic DNA (from LLC-MK2 tissue cells) was dried on a stainless-steel coupon. Either disinfectant or phosphate-buffered saline (no disinfectant control) was pipetted onto the surface and incubated for the desired time point, then neutralized with tryptic soy broth. Residual nucleic acid was quantified by real-time PCR or reverse transcriptase PCR (RT-PCR).

Only 10% Clorox bleach and Diversey's sodium hypochlorite-based disinfectant showed any effect on viral ssRNA or eukaryotic DNA. The Diversey disinfectant gave a 5-log reduction in ssRNA within 2 minutes and a 4-log reduction in eukaryotic DNA within 4 minutes. Bleach could give complete degradation even faster, though results were sometimes inconsistent. No other disinfectants showed any effect on nucleic acid concentration with up to four minutes of incubation, demonstrating the importance of validating different sanitizers for this application before use. Further research will include disinfectant validation with PCR product, another potential source of surface contamination.

11. YTHDC2 protects SRE containing transcripts from KSHV endonuclease SOX

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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 influence RNA fate by directing their localization and/or stability. 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. KSHV triggers a massive RNA decay event where 70% of mRNA is degraded by 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 mRNA 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 MeRIP and mRNA sequencing, we showed that IL-6 SRE-containing reporters are m6A modified in cells, and that mutations within this putative m6A site render the transcript susceptible to SOX. Recently, we demonstrated that m6A reader YTHDC2 protects the multiple SRE containing transcripts from SOX *in*

vivo and we are in the process of characterizing the reader's method of protection. Taken together, our results reveal a novel mechanism of resistance from virally induced degradation. 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.

12. Metapangenomes reveal genomic signatures of microbial evolution to long-term soil warming

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Increasing soil temperatures due to climate change is altering below-ground microbial carbon transformations. Microbial eco-evolutionary responses to long-term warming will determine the flux of carbon between the terrestrial biosphere and the climate system. For nearly three decades, temperate forest soils have experienced in situ warming 5ºC above ambient temperatures at the Harvard Forest Long-term Ecological Research (LTER) site. Carbon quantity and quality is now lower in heated versus control plots. We propose that across generations of chronic warming, bacterial lineages evolved adaptations related to growth efficiency and carbon utilization. Further, we hypothesize that genomic signatures reflect these adaptations. From our culture collection of soil bacteria isolated from experimental heated and control plots, we sequenced genomes representing taxa sensitive to warming, including independent lineages of Alphaproteobacteria (n=2) and Actinobacteria (n=2). We investigated differences in gene content as well as genomic traits to identify genetic signatures of adaptation. Comparative pangenomics revealed differentially abundant gene clusters with functional annotations related to metabolism and carbon utilization. We also recruited reads from two metagenomic datasets (generated from heated and control plots in 2011 and 2018) onto each pangenome to compute the metapangenome, thus leveraging information from environmental gene content. Recruitment varied across taxa and genomes, ranging from approximately 0.006–0.6X of mean coverage for *Rhizobium* spp. to approximately 1–20X of mean coverage for *Bradyrhizobium* spp. Metapangenomes reveal gene clusters that are differentially abundant between core habitat-specific gene pools, which include functional annotations related to CRISPR-associated proteins. Finally, we observe differences in genome-wide codon usage bias between heated and control genomes, suggesting translational selection and potential differences in growth efficiency. Together, these data illustrate diverse lineage-specific adaptations as well as common evolutionary microbial responses to climate change.

13. Characterizing aflatoxin degradation by *Rhodococcus* species

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Aflatoxins (AFs) are fungal metabolites that ubiquitously contaminate many common food crops and contribute to major foodborne diseases in humans and animals. The ability to degrade or remove aflatoxins from common feed commodities will improve health standards and counter the economic drain inflicted by AF food contamination. Bioremediation is a promising solution to contamination by AFs since it can offer low cost, few undesired environmental side-effects, and potentially high efficiency and reliability. It has been shown that two bacterial species, *Rhodococcus erythropolis* and *Rhodococcus pyridinivorans*, possess the ability to degrade AFs through the secretion of unknown extracellular enzymes. However, a lack of knowledge about the mechanisms of degradation has hampered the efforts to improve them for applications in agriculture and the food industry. In this study, we characterize the degradation process of AFs by *R. erythropolis* and *R. pyridinivorans* to identify their AF-degrading enzymes. We found that changing the carbon source in the growth medium from glucose to starch (simple to complex carbon source) could significantly increase the degradation efficiency by cell-free filtrates, indicating a potential role of these AF-degrading enzymes in carbon scavenging. Additionally, we performed size exclusion fractionation of cell-free filtrates and determined that the size of the AF-degrading enzymes in both species is less than 10 kDa. Finally, using SignalP to predict secreted proteins from reference genomes on NCBI and applying the experimentally determined size range as a filter, we identified 30 candidate proteins between the two species as the potential AF-degrading enzymes. Future efforts focus on using proteomics to identify the

AF-degrading enzymes and testing AF-degradation performance in various conditions of pH, temperature, and metal ion availability to determine the optimal degradation conditions.

14. Concentration and Detection of Human Noroviruses from Food and Environmental Samples Using Engineered Norovirus Binding Bacteria

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Human Norovirus remains the ubiquitous cause of non-bacterial gastrointestinal infections across the world and a major cause of foodborne illness in the United States. Research in the field of Norovirus infection is potentially limited by the fact that the virus is present in very low levels in food and environmental samples. Due to this, sample concentration becomes extremely important prior to detection.

In our current study, we evaluated the use of representative bacterial strains generally found as part of the human gut microbiome and engineered strains expressing norovirus binding peptides as a tool for concentrating and detecting Human Norovirus (GII.4) from patient stool and environmental samples. *Staphylococcus aureus*, *Enterobacter cloacae*, *Bacillus* spp, *E. faecium*, *Klebsiella* spp, *Citrobacter* spp and *H. alvei* along with 7 engineered *E. coli* strains, each expressing a norovirus specific peptide were suspended with 100 µL of diluted stool sample containing norovirus. Following incubation, RT-qPCR was performed for calculating removal of input virus from the supernatant. Percentage of binding efficiency is determined by loss-to-supernatant (total input virus-virus in supernatant)/total input virus. Our preliminary results have shown us that the engineered strains were able to outperform the representative bacterial strains in norovirus capture (capture efficiency ranged from 66% to around 80%). The representative strains on the other hand were only able to capture a maximum of 60% of the input virus.

While conventional concentration methods like PEG precipitation, magnetic bead-based methods and membrane filtration successfully allow for concentration of the virus, they are limited by the carryover of certain inhibitory substances which can be detrimental to downstream detection methods. We hope to circumvent these issues by using an engineered *E. coli* strain expressing a norovirus binding peptide since it can be easily and inexpensively adopted in resource limited settings for virus concentration and detection.

15. Initial methods for an environmental health survey of *Toxoplasma gondii* in Alaskan shellfish

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Toxoplasma gondii is a eukaryotic parasite that has become widespread across terrestrial and marine ecosystems. Human health effects of toxoplasmosis infection are notable, including transient inflammation in healthy individuals and serious neurological outcomes among immunocompromised people. Exposures to *T. gondii* frequently result from food consumption; for example, the microbe's oocysts can accumulate in the filtering tissues of marine bivalves. *T. gondii* infection is of particular risk to shellfish consumers, such as American Indian and Alaska Native communities, for whom shellfish are a traditional food source. In this project, we aimed to develop a basic method for surveying *T. gondii* in bivalve tissues, which we applied to Alaskan oysters and mussels. An oocyst-specific DNA extraction and PCR protocol were modified from previous studies and assessed for efficacy. We found that a PCR primer set targeting the repetitive B1 gene paired with endpoint PCR was most useful as a basic microbial ecological surveillance tool. Initial results suggest that *T. gondii* was not present in any of 30 shellfish samples, which we plan to confirm with the development of a positive control assay utilizing oocysts shed from infected felids. Our findings indicate the absence of *T. gondii* in an as-yet unstudied population of shellfish, which better elucidates the risks associated with consumption of Alaskan shellfish. Development of a simple molecular tool for *T. gondii* identification potentiates improved environmental health surveillance of marine food sources.

16. Small protein affects *Escherichia coli* multi drug efflux pump mediated antibiotic resistance

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Multidrug efflux pumps (MDEPs) contribute to the rising global health crisis caused by antibiotic resistance in Gram-negative bacteria. AcrAB-TolC is a clinically relevant MDEP from *Escherichia coli* with AcrB as the inner membrane pump component. AcrB is part of the Resistance-Nodulation-Cell Division (RND) family which uses the proton motive force to efflux various substrates including antibiotics, dyes, and toxic organic compounds. Most AcrB studies do not take into account the effect of a 49-amino acid protein, AcrZ, which binds to the transmembrane domain of AcrB. The study reporting the discovery of AcrZ (Hobbs et al., 2012) revealed that deleting acrZ reduced resistance to three out of six AcrB antibiotic substrates assayed. We aim to further explore the effect of AcrZ on antibiotic resistance. Using minimal inhibitory concentration assays, we found that AcrZ also affects thiamphenicol and doxycycline hyclate resistance. We also further characterized the resistance profile of several AcrZ mutants identified in a preliminary screen that assayed chloramphenicol resistance. We identified a mutant that appears to decrease resistance below the ΔacrZ mutant for three additional substrates. We are also attempting to elucidate why AcrZ has a differential effect on AcrB substrates. We hypothesize that AcrZ may cause structural changes that affect substrate interaction with one portion of the AcrB distal binding site (referred to as the “cave”) while have no effect on another region (the “groove”). Preliminary experiments optimizing fluorescent efflux assays using Nile Red–a groove binding substrate–and ethidium bromide–a cave binder–are being conducted; wild type, ΔacrB, ΔacrZ strains will be assayed for changes in efflux activity. If the ΔacrZ mutant shows decreased efflux of ethidium bromide compared to the wild type while not affecting Nile Red, this would support our hypothesis. Together these studies improve our understanding of how AcrZ affects AcrB-mediated drug efflux.

17. The Fight for Viral RNA Fate: Characterizing the Interaction between the anti-viral protein C19ORF66 and the KSHV RNA-binding protein ORF57

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Kaposi’s Sarcoma Associated Herpesvirus (KSHV) is the causative agent of several B-cell lymphomas and its namesake, Kaposi’s sarcoma. KSHV infection is characterized by a biphasic life cycle consisting of a life-long latent phase broken by active lytic replication. During the lytic phase, greater than 70 % of cellular mRNA transcripts are degraded by the KSHV-encoded viral endonuclease SOX (ORF37). This “host shutoff” event frees cellular gene expression machinery to be repurposed for viral replication. We and others have shown that some host transcripts are capable of actively evading SOX cleavage via an RNA element in their 3’ UTRs known as the SOX resistant element (SRE). Through large-scale RNA-seq, we identified an SRE-bearing transcript, C19ORF66, which encodes an anti-KSHV factor, inhibiting nearly every step of KSHV lytic replication. Based on our recent evidence, the greatest impact of C19 appears to be on viral gene expression. Using mass spectrometry, we have begun mapping the interactome of C19 to investigate the mechanism by which C19 restricts KSHV replication. This analysis identified several viral proteins that interact with C19, including KSHV’s ORF57, a key regulator of viral mRNA fate. An increasing number of roles have been described for ORF57, from enhancing the stability of viral transcripts to facilitating virion production. Using co-immunoprecipitation, we’ve found that C19 interacts with ORF57 in an RNA dependent manner. We also show that overexpression of C19 restricts the expression of ORF57 protein but does not impact ORF57 mRNA levels. Lastly, using Immunofluorescence Assays, we show that C19 and ORF57 both co-localize with the human translation arrest protein TIA-1 in the cytoplasm, suggesting that C19 may restrict ORF57 translation. By characterizing C19’s interaction with ORF57, we hope to better understand the significance of this interaction to the function of C19 following its escape from viral host-shutoff.

18. Behind the scene of... albinism in *A. fumigatus*

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Aspergillus fumigatus is an opportunistic human pathogen fungus which kills 100,000 humans per year. Owing to its ability to survive at different temperature and pH condition, *A. fumigatus* disperses conidia (asexual spore) throughout air, and grows vegetatively in soil and decaying vegetation, where it plays a an important function in the cycling of carbon and nitrogen. Melanin, a natural pigment confers several functions in *A. fumigatus* that increase its pathogenicity. DHN melanin provide protection against exogenous stress (e.g. UV-radiation, elevated temperature and reactive oxygen species) and contributes to phagocytosis evasion because the compound masks antigens on the conidia surface. Interestingly, conidia of melanin knockout mutants, which are pigmentless, are phagocytoses and killed at significantly higher rates than pigmented conidia. We identified two albino strains of *A. fumigatus*, IP-23 and IP-24, which produce white conidia, rather than the typical greenish gray. We sequenced the genomes of these isolates and conducted comparative genomic analysis with two closely related isolates that possess normal spore color in an effort to determine the genetic underpinning of the albino phenotype. We identified SNPs and small indels with FreeBayes, and annotated variants with fixed differences in the albino strains compared to the wild-type strains using SnpEff. We identified several candidate variants with potential functional roles in melanin production. Because melanin can also protect the cell from host reactive oxygen species, we also measured the sensitivity of these isolates to 3% H_2O_2. This work combines comparative genomics and phenotypic characterization to better understand the genetic basis and functional effects of albinism in *A. fumigatus*.

19. Bioinformatics Lab Course for Microbiologists: Improving students' comfort and familiarity with asking and answering questions using programming and sequence analysis

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If the first great advance in microbiology was the petri dish, the second great advance was sequencing, making it possible to study microbes using cultivation-independent methods. Though our sequence databases have grown exponentially, training students to use programming and sequence analysis to study microbes has not kept pace.

To train and involve undergraduate students in analysis of genomic data, we developed a new undergraduate laboratory course entitled "MICROBIO 590B: Bioinformatics Lab". Training includes command line programming, manipulating and sorting data files, and using publicly available data to ask and answer questions about microbes. In this laboratory-style class, students and instructor work together to de novo assemble and annotate a practice genome. Next, each student is assigned a genome for their capstone project, and given raw data to de novo assemble and annotate a novel isolate from our lab culture collection. All bioinformatics are performed in the Massachusetts Green High Performance Computing Center (MGHPCC) and in the Department of Energy Knowledgebase (KBase).

The goal of this course is to improve students' comfort and familiarity with addressing questions using programming and sequence analysis. Data from the course supports our research objective of characterizing the irreversible effects of long-term soil warming on soil bacteria. Students assembled and annotated the same one genome (N=23, then adjusted the length and quality of filtered input sequence data, demonstrating how replicable our genome assembly pipeline was when conducted using different quality filtering parameters. Students then analyzed 12 novel genomes in technical duplicates. Students tested a hypothesis of their own design, and the hypothesis that genomes derived from chronically warmed soils are enriched in lignin-degradation genes. A pre- and post-course survey demonstrated the extent to which this course helped students feel more comfortable and familiar with asking and answering questions about microbes using programing and sequence data.

20. Changes in the Metatranscriptome of Acidobacteria as a Result of Long-Term Soil Warming

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As carbon and other greenhouse gas emissions increase overtime, the threat of global warming intensifies. The geographical changes are apparent, but the question remains what is happening to the microbial communities under our feet. Microbes are essential to the livelihood of the soil ecosystem as they play key roles in nutrient cycling. Thus, any changes to these microbes as a result of soil warming may have drastic effects. The relatively newly studied phylum of *Acidobacteria* is abundant in the soil microbiome. Named after its discovery in acidic conditions, this highly ubiquitous phylum has become one of the most diverse in the microbial community spanning over 26 subgroups. Due to the phylum's wide diversity and variety of glycoside hydrolase encoding genes, the group is becoming increasingly studied for its role in carbon metabolism and the soil ecosystem as a whole. In 1991, the Harvard Forest long term warming project began in an attempt to answer questions about the future of microbial communities. Plots of land are heated 5 °C above atmospheric temperatures next to control plots in an attempt to mimic climate change. In 2017, the Blanchard Lab collected 14 soil core samples from the Barre Woods location where metagenomic and metatranscriptomic analysis was performed on the samples. Using a mini-metagenomic approach, 203 high-quality metagenome assembled genomes (MAGs) were assembled, 12 of which were *Acidobacteria*. In combination with the metatranscriptomic data collected, the lab is able to observe which genomes are more active than others with a computational approach. Read Mapping of the data has provided further insight into genes that are actively expressed between the 12 genomes. With the raw read counts available, the lab is currently investigating if given genes are differentially expressed between the control and heated conditions.

21. Characterization of Viral and Bacterial Dynamics in Lake Champlain Cyanobacterial Harmful Algal Blooms

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Harmful cyanobacterial blooms (CHABs) are a growing concern for Vermont's freshwater aquatic ecosystems. These cyanobacteria produce a multitude of toxins which can be detrimental to wildlife and people including neurotoxins, hepatotoxins, and cytotoxins. In recent years the increase in CHAB prevalence has been associated with environmental changes such as eutrophication of lakes. As a result, the approaches to combating increasing CHAB frequency and severity have been largely geochemical. However, CHABs are increasing in oligotrophic aquatic environments as well, suggesting a gap in our understanding of CHAB formation and decline. Adjusting lake nutrient levels has not been sufficient enough to significantly decrease the prevalence of CHABs. With this knowledge we have decided to take a more holistic microbial community and ecological approach. Viruses present in aquatic ecosystems can often infect cyanobacteria hosts and lyse them. In summer 2021 we collected water samples from various locations in Lake Champlain in order to analyze the lake virome compositions. We took counts of both bacteria and virus-like particles via microscopy and compared these data to prior summers. We also conducted extractions of viral and bacterial DNA post filtration to generate metagenome and virome data for microbial community analyses. Additionally, we attempted to culture and isolate cyanobacterial species from the *Gloeotrichia* and *Dolichospermum* genera. With this research we hope to further understand the microbial community structure and function of Lake Champlain CHABs and identify potential CHAB mitigation strategies.

22. A High Throughput Assay for Inhibitors of the Type 3 Secretion System Translocon Assembly

Hanling Guo

Type III secretion system (T3SS) is used by many Gram-negative bacteria to colonize the host and inject effector proteins into target cells. Inhibitors targeting T3SS are important to combat the appearance of multidrug-resistant pathogens. In *Pseudomonas aeruginosa*, the T3SS secreted proteins PopB (SctE) and PopD (SctB) are required to inject effector proteins into the host cells. PopB and PopD associate with the target membrane and they are proposed to form a translocon pore by which effectors are translocated. The T3SS translocon is a good target for

inhibitors because these molecules do not need to overcome the intrinsic problems associated with cell membrane permeability and the presence of efflux pumps. In previous studies, we have reported that the PopD N-terminus is exposed to host cell cytosol. We reasoned that the PopD N-terminal exposure could be used as a reporter for properly assembled translocons. Using a split form of the small NanoLuc (NLuc) luciferase, we have developed an assay to detect the exposure of this N-terminus to the host cytosol. HeLa cells producing the large truncated version of NLuc are used as targets. These cells are incubated with a *P. aeruginosa* ΔpopD PAK strain that produces a PopD variant fused with the missing portion of NLuc at the N-terminus (Nluc10-PopD). Assembly of a functional translocon is detected by the maturation of NLuc enzyme in the HeLa cell cytosol and the production of luminescence. Failure to assemble a functional translocon did not produce luminescence. The assay has a typical signal-to-background ratio (S/B) of 6 - 10 and a Z factor of 0.5 – 0.8, constituting an excellent tool for the screening and detection of small molecule inhibitors of T3SS translocon assembly.

23. Evaluating the risks associated with utilization of modified washing machines in the processing of leafy greens

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The CDC reports that 46% of foodborne illness incidents are attributed to produce, of which leafy greens are responsible for most of these. Small and medium-sized leafy green growers commonly retrofit washing machines to dry triple-washed leaves, utilizing their spin cycle, effectively turning them into large salad spinners. However, the potential for this practice to cause microbial contamination and the degree to which it can pose a risk to cause foodborne illnesses has not been explored. This project aims to investigate the risk of cross-contamination associated with the common practice of drying leafy greens using washing machines to help inform best practices to mitigate risks. Listeria innocua was utilized as a surrogate for *L. monocytogenes*, and 103 CFU/ml was inoculated and dried onto 250 g of spinach per run to mimic natural contamination. Prior to the run and post the inoculation a wash step was introduced, and the inoculated spinach was made to run for 2 minutes. After the spin, three contact surfaces of the machine, loading basket, internal chamber and the water collection chamber were sampled using sterile microbial swabs to enumerate the relative levels of the bacterial transfer, with 3 swabs per region. The swabs were then plated and enumerated using 3M *Listeria* Petrifilms. A recovery range of 101-103 CFU/ml of from different contact surfaces was recovered from the various points, with the direct contact surface containing consistently the highest levels of *Listeria*. This suggests that use of these machines could pose a risk of contamination and validates the need for establishing cleaning and sanitation guidelines to improve the safety of processing leafy greens in this manner.

24. Characterization of drought tolerance genes of *Leifsonia poae* and *Arthrobacter bambusae*

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The Harvard Forest (HF) Long Term Ecological Research site is the location of a soil warming experiment where plots have been maintained at 5°C (9°F) over the ambient temperature since 1991. Soil CO2 flux, onset by warming, triggers the emergence of diversity in genetic adaptations to selective pressures. Two isolates, BS40 and BS71, were taken from phase 4 of the HF research site. Isolates were exposed to additional drought conditions at 30% water holding capacity for 120 days at 25℃ prior to whole genome sequencing (WGS). Genomes were then placed into the bioinformatics programs JGI, NCBI, and KBase. Using NCBI BLAST, the 16s rRNA gene sequences identified BS40 as *Arthrobacter bambusae* and BS71 as *Leifsonia poae*. KBase RAST annotations, metabolic modeling, and genome comparison applications were used to analyze genomes for drought tolerance genes. The WGS were analyzed for genes relating to osmotic pressure, osmotic stress, turgor maintenance, salinity control, heat shock, and membrane transport/permeability to identify drought tolerance genes. The genomes were compared to their closest relatives (≥98% rRNA similarity) using KBase and the JGI/IMG Genome Portal. Analysis

indicated that BS40 does not possess additional drought tolerance genes as compared to its Arthrobacter relatives. However, BS71 contains more drought tolerance associated genes in relation to its closest *Leifsonia* relatives. These genes include Aquaporin Z and glycine betaine transport proteins, which are involved in water transport and osmolyte formation, respectively. Thus, BS71 may represent a drought tolerant species, though additional analysis is required to further identify drought related genes. Additionally, BS71 produces a number of genes associated with both copper resistance and plant growth promotion. This bacterial isolate will be subject to experimental analysis to examine these characteristics and determine if this bacterium may be beneficial for crops or bioremediation.

25. Optimization of RNA Display Using GC-Clamp Modifications to Improve Genetic Detection of Bacterial RNA-Protein Interactions

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Non-coding small RNAs (sRNAs) have an important role in bacterial stress responses. In many bacteria, the binding of sRNAs to their target mRNAs at or near the ribosome binding sites is often facilitated by protein chaperones, such as Hfq or ProQ. The Berry Lab has developed a bacterial three-hybrid (B3H) assay to detect the binding of RNA with multiple RNA chaperones *in vivo*, by connecting the strength of an RNA-protein interaction to the expression of a reporter gene. The interaction between the “prey” protein fused to the α-subunit of RNA polymerase (RNAP) and the “bait” RNA tethered upstream of a test promoter stabilizes the binding of RNAP and increases transcription of the reporter gene lacZ. Despite the success in detecting many high-affinity interactions, low signal-to-noise for other RNA-protein interactions currently limits the broader utility of the assay. Secondary structure predictions by ViennaRNA suggested that the misfolding of the “bait” RNA when hybridized with other sequence elements would disrupt its interaction with the “prey” protein. To improve the breadth of detectable B3H interactions, we designed new hybrid RNA constructs with the addition of a GC-clamp flanking a region of interest to ensure proper folding and optimal display of the "bait" RNA. We hypothesized that a shorter GC-clamp (5-7 bps) would be sufficient to improve RNA display while being structurally flexible enough to allow for interaction with protein compared to a longer GC-clamp (13 bps). Preliminary results demonstrate the promise of the short GC clamp in improving B3H signal for many sRNA-Hfq interactions; however, the detection of 5’UTR-Hfq interactions is still limited. Future work aims to utilize these GC constructs to explore more sRNA interactions with protein chaperones and to modify the GC-clamp design for 5’UTRs to ultimately increase the sensitivity of the B3H assay for more RNA-protein interactions.

26. Distinguishing between structural models for RNA binding protein ProQ in *E. coli*

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Evolving research on small RNAs (sRNAs) in bacteria implicates sRNAs as a key element of gene regulation. While some sRNAs are able to act independently, many are dependent on a RNA-binding protein, such as the well-established Hfq in *Escherichia coli*. Another family of RNA binding proteins is the FinO family, including ProQ in *E. coli*. In 2017, a structure for ProQ solved through NMR was published and released onto the PDB (Gonzalez et al., 2017; PDB ID: 5nb9). In July of 2021, AlphaFold, an artificial intelligence program for protein structure prediction, launched a database which included an alternate structural model for ProQ. These two structures vary, most notably in the fold of the FinO domain which is believed to be the primary site of RNA binding by ProQ. Our lab hopes to investigate the two potential structures and offer data for determination of the most accurate structure for ProQ. Available structural models for FinO family proteins were evaluated for both structural characteristics and quality in order to develop points of comparison for the fold of the FinO domain proposed by the two ProQ

structures. Intriguing structural features of the models were investigated, most notably a potential RNA binding pocket on the concave face of the protein in the AlphaFold structure. Finally, predicted interactions between residues for each of the proposed models were probed with the use of site-directed mutagenesis, with the intention of providing experimental data in favor of one model over the other. This work hopes to provide not only indication of the appropriate structural model for study of this RNA binding protein, but also highlight the potential in modern computational protein structure prediction, such as AlphaFold.

27. Investigating the genetic determinants of *Listeria monocytogenes* stress tolerance through adaptive laboratory evolution

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Listeria monocytogenes is a foodborne bacterial pathogen that causes thousands of illness and billions in medical and food recall costs annually in the United States. *L. monocytogenes* can survive, replicate and persist in a variety of harsh environments, including conditions encountered in food processing facilities and equipment. Here, we devised an “evolve and resequence” experiment to shed light on the frequency, temporal patterns, and genetic determinants of stress resistance evolution in *L. monocytogenes*. Specifically, we will grow three replicates of three phylogenetically and phenotypically distinct strains of *L. monocytogenes* for 500 generations in sub-lethal concentrations of (i) salt-induced osmotic stress, (ii) benzalkonium chloride (BAC), a sanitizer commonly used in the food industry, and (iii) tryptic soy broth (control). We have sequenced and annotated the genomes of each of the ancestral strains. Every 50 generations, we will (i) freeze and store cultures, (ii) measure the minimum inhibitory concentration (MIC) of each stressor to track temporal changes in fitness, (iii) conduct a quantitative virulence assay to investigate the potential relationship between stress response and pathogenicity, and (iv) resequence the genome of each lineage to track changes in allele frequency and to identify candidate mutations underlying adaptive phenotypes. Lastly, we will create gene deletion mutants using the suicide plasmid pHoss1 to validate the function of candidate genes. Our results indicate that BAC-sensitive isolates, ALE_10_0415 and ALE_20_0415, exhibited a two-fold increase in MIC of BAC after ~300 generations. In comparison, BAC-tolerant isolate ALE_16_0415 showed minimal improvement at ~400 generations, which suggests that there is a physiological limit to *L. monocytogenes* BAC resistance. Our results will provide insight into the evolution and genetic mechanisms underlying *L. monocytogenes* stress-tolerance, as well as candidate genes to investigate in persistent strains isolated from food production facilities.

28. Population Genomics of *Aspergillus sojae* & *Aspergillus parasiticus*

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Like plants and animals, microbial organisms i.e. bacteria, yeasts, molds have been domesticated to enhance their utility to humans. Archeological, molecular, and genetic evidence also supports the domestication of fungi for their utility in food preservation, flavoring, aroma, and texture. For example, *Aspergillus sojae* is a filamentous fungi used for its flavor enhancing properties and enzymatic production to produce several Asian fermented foods (e.g. soy sauce, miso, and mirin). Little is known about structural and genomic variation of *A. sojae* isolates from different *A. parasiticus* isolates. Whole-genome sequence data was generated using short-read illumia sequences from a diverse collection of 12 isolates of *A. sojae* and nine isolates of *A. parasiticus* in an effort to illuminate the evolutionary relationship of these species. 658,329 SNPs were identified using Freebayes1.3.5 across the 21 genomes, which were used to perform phylogenetic and population structure analyses. Phylogenetic analysis suggests the existence of two genetically distinct populations as well as a single domestication event of *A. sojae* followed by a clonal expansion. Population genomic analysis of *Aspergillus* isolates also suggest two major populations present with little nucleotide diversity and recombination amongst the *A.sojae* isolates. Structural variation using CNV analysis was done to assess presence and absence of genes amongst the *A.sojae* samples and *A.parasiticus* samples. CNV profiles were generated for each sample to investigate the prevalence and function

of CNV in *A.parasiticus*. A total of 12,370 genes were annotated in *A.parasiticus*. Of the genes annotated, 8% represented secondary metabolite genes. Further analysis of genes with a VST value greater than 0.5, indicated 20.6% of these genes were secondary metabolites. In sum, these analyses shed light on the population structure and genomics of *A. sojae* species and *A. parasiticus* species.

29. Discovering New sRNA-Binding Protein in *Chlamydia trachomatis*

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sRNAs (small, non-coding RNAs) are essential for the bacteria in various ways such as stress response, metabolism, and virulence as they regulate the mRNA expression in microorganisms. And, there are several identified chaperones that help the function of sRNAs. Hfq and ProQ, which originated in the *E. coli* strain, have been predominantly studied because they are generic sRNA chaperones encoded in many different bacterial strains. However, there are other bacterial strains that do not encode either of those proteins, so the studies on discovering new sRNA-binding proteins can provide a new perspective on understanding bacterial behaviors and pathogenesis. Among those strains, *Chlamydia trachomatis* was selected for further research because it is a pathogen that causes chlamydia, one of the most common sexually transmitted infections, and has its own identified sRNAs. Therefore, to discover new sRNA-binding proteins encoded in the genomes of this pathogenic strain, this study employs a bacterial three-hybrid (B3H) assay, developed by Berry and Hochschild (2018) to measure RNA-protein interactions. To screen sRNA-protein interactions in *C. trachomatis*, four sRNAs (ctrR1, ctrR2, ctrR0332, and IhtA), previously identified by Albrecht et al. (2010), were cloned into pBait (RNA moiety in the B3H system) and transformed into reporter cells with the pPrey (protein moiety) library of *C. trachomatis*. Through this process, some candidate sRNA-binding proteins have been selected, and a β-galactosidase assay was used to quantify their interactions with sRNAs. As a next step, the mechanisms of the candidate proteins working as sRNA chaperones will be studied through structural analysis and site-directed mutagenesis. The screening of the *Mycobacterium tuberculosis* strain to further the research spectrum is also undergoing. This study will not only provide new pharmaceutical targets for chlamydia treatment by finding novel sRNA-binding proteins in *C. trachomatis* but also bring focus to a newly uncovered microbial research field.

30. Evaluating the structure of the FinO domain of the *Escherichia coli* RNA chaperone protein ProQ

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ProQ is a global RNA chaperone protein in *Escherichia coli* (*E. coli*) that interacts with RNA for gene regulation. Its N-terminal FinO domain (NTD) is essential for RNA-binding activity and several residues from this domain are identified indispensable for the binding. In particular, Arg80 is completely conserved across FinO domain proteins. Even a very conservative mutation (R80K) completely abrogates RNA binding. There are two published structures of this domain of *E. coli* ProQ: an NMR structure reported in 2017 and an AlphaFold predicted model in 2021. As these two structures differ in the positions of some residues including Arg80, this study aims to investigate the *in vivo* position of Arg80 to evaluate the two models. Because R80K eliminates RNA binding through a subtle substitution, it should be possible to find compensatory substitutions in nearby residues that could “rescue” the RNA-binding activity of R80K. Sequencing of such compensatory mutations would allow us to map them to the two structures to see which is most consistent with the residues that can compensate for an R80K substitution. To generate a mutagenesis library from which to screen for compensatory mutations, we took the *E. coli* ProQ NTD carrying R80K and introduced substitutions to residues close to Arg80. We then used an *in vivo* RNA-binding assay — the bacterial three-hybrid system — to identify mutations from the library that restored RNA binding activity. The preliminary results show that most of these mutations are clustered nearby to one another and to the location of Arg80 in the structural model generated by AlphaFold, which supports the AlphaFold model. The evaluation of the

position of Arg80 and the structure of ProQ NTD provides more accurate information to investigate how ProQ interacts with RNA to achieve the gene regulation in *E. coli*.

31. Host phylogeny shapes the microbiome of the female reproductive organ in cephalopods

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Some species of squid have a light organ symbiosis with bioluminescent species of *Vibrio* often used as a model system to study many aspects of host-bacteria interactions. Female cephalopods have a less studied reproductive organ called the accessory nidamental gland (ANG), which hosts a consortium of bacteria. The ANG acquires the bacteria environmentally, and the absence of bacteria results in the lack of ANG development. The ANG is made up of many intertwined tubules that are filled with bacteria. This symbiotic consortium of bacteria is deposited in the eggs before laying and provides defense against fouling/pathogenic microorganisms through the production of antimicrobial compounds. Thus, bacteria in the ANG may serve as new sources for antimicrobial compound discovery. Sequencing and analysis of the V4 region of the 16S ribosomal gene revealed that the ANG microbiomes of members of four different cephalopod families were significantly different ($p < 0.001$). However, members of closely related Sepiolidae and Idiosepiidae, collected from five distinct geographic regions, had similar ANG microbiomes, consisting of bacteria from the Class Verrucomicrobiae, Alphaproteobacteria, and Gammaproteobacteria. Similar ANG communities shared among these cephalopod groups may indicate a conserved antimicrobial function in egg defense. Also, the overall microbiome from different cephalopod species correlated with host phylogeny. This pattern of microbial community relationship recapitulating host phylogeny may reflect phylosymbiosis. We observed evidence for phylosymbiosis using Robinson-Foulds metric and Mantel test with Weighted Unifrac. Understanding ANG bacterial diversity will assist with the development of tractable cephalopod species that can be used as model organisms to study host-microbe interactions.

32. Coronavirus Conundrum: Exploring the differences between severe and non-severe coronaviruses

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Viruses and their hosts are in a constant battle to control the infected cell and gene expression machinery. Coronaviruses, with their cytoplasmic RNA genome, are particularly subject to the host anti-viral defenses. Yet, as demonstrated by the current COVID19 pandemic, some coronaviruses can be very successful. How the virus manipulates the host cellular machinery to facilitate infection and how it evades immune sensing remains unclear. Here we focused on the first protein expressed during coronavirus infection: nsp1. Nsp1 is a non-structural protein that drastically affects the gene expression environment to prepare the host for complete viral takeover. Nsp1 has been shown to bind ribosomes and block translation of host transcripts. Nsp1 has also been shown to trigger widespread RNA decay, another mechanism that leads to a global shut down of host gene expression. Nsp1 is thus believed to be a major contributor to coronavirus pathogenesis. Yet not all coronaviruses lead to worldwide pandemic or cause severe respiratory symptoms. Many coronaviruses are referred to as “common cold” viruses and circulate yearly. We hypothesized that this difference in host takeover may be driven by nsp1 and that nsp1 from highly pathogenic coronaviruses may have a distinctive range of targets compared to the common coronaviruses. To address this possibility, we generated a library of nsp1-inducible cells from an array of coronaviruses and explored the extent of nsp1 effect on the host transcriptome by RNAseq. Moreover, we investigated the interactome of these nsp1 proteins by mass spectrometry to identified common and divergent interactors. This work will shed light on the mechanism of action of a crucial coronavirus protein as well as providing important insights into the fundamental differences between highly pathogenic and common coronaviruses.

33. Elucidation of genetic targets and cellular-physicochemical interactions for prevention of

catheter-associated bacterial biofilm infections using a genome-wide approach

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Treatment and prevention of bacterial biofilm infections largely rely on broad-spectrum antibiotics and anti-biofilm agents that either contribute to the rise of antimicrobial resistance or target one phenotype, yet we increasingly understand that biofilm infections are typically multi-phenotypic. Here we propose a new strategy to engineer the biomaterial properties and targeted cellular response in unison for the robust treatment and prevention of biofilm infections. In this project, we aim to demonstrate the approach for clinical bacteria using catheter-associated urinary tract infections (CAUTIs) caused by uropathogenic *E. coli* as a clinically relevant biofilm infection with well-studied bacteria. Notably, CAUTIs are a major global concern for public health and are the most prevalent healthcare-associated infection in the US, accounting for more than 30% of acute hospital infections. The most frequent pathogen associated with CAUTIs is *E. coli* (18% of cases), and these uropathogenic *E. coli* (UPEC) are increasingly antimicrobial-resistant. We aim to identify the genetic-biomaterial interactions that contribute to the formation of catheter biofilm infections on physicochemically diverse catheter coatings by phylogenetically distant UPEC bacteria. To do this, we developed and tested a set of three different CRISPR inhibition (CRISPRi) systems to control transcriptional repression in two clinical UPEC strains (CFT073 and UMN026). From these results, we determined rules to inform the design of our pooled genome-wide CRISPRi libraries for the UPEC strains. Using these design rules, the CRISPRi systems developed here achieved gene repression of 4- to 1000-fold for genomic DNA in the CFT073 and UMN026 strains. In ongoing work, we will identify and quantify genetic targets that confer biofilm formation on varied polymer biomaterial surfaces with different chemistries and stiffnesses by performing quantitative genomic mapping using these new genomic tools for clinical UPEC bacteria.

34. The Hunt for Forest Giant Viruses

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Our understanding of microbes inhabiting forest soil is limited, as more than 99% of them have never been cultured in the laboratory. Giant viruses, which refer to a phylum of single eukaryote-infecting DNA viruses called *Nucleocytoviricota* (NCVs), have large genomes and virions exceeding the size of some bacteria. Our project focuses on ‘giant viruses’ identified from forest soil using metagenome-based technologies at our lab’s Harvard Forest location. This collection includes representatives of several new genera and families as well as the world’s second largest genome recorded (to date). The large genome size is due in part to virus-encoded genes that manipulate host metabolism. Our lab visualized virus-like particles in the environment at Harvard Forest using transmission electron microscopy (TEM). There is remarkable diversity observed, including strange morphologies. In order to better characterize these viruses, infection with a known amoebal host is essential to purifying the viral samples for further sequencing and analysis. Our project aims to develop a protocol to successfully cultivate soil giant viruses found in Harvard forest samples using the known amoebal hosts *Acanthamoeba castellanii* and *Vermamoeba vermiformis* (*Harmannella vermiformis*). These host systems have been optimized over the past two decades, but to our knowledge, never been used to isolate viruses from terrestrial soil. Challenges to create this novel system in our lab includes combating contamination with an array of antibiotics, maintaining lysate cultures once infection has taken over cultures, and upscaling the protocol to produce sufficient data.

35. The Exchangeability of the GerI Spore Germinant Receptor from *B.cereus* to *B.subtilis*

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The *Bacillus* genus is unique in that it is one of few bacterial genera able to sporulate, transforming into a highly resilient structure able to withstand extreme environmental conditions. This survival mechanism occurs when nutrient levels are low or other stress factors are applied and the spores can then survive for many years. Although spores are metabolically dormant, they contain highly specialized germinant receptors (GRs) that detect their corresponding germinant. This recognition of nutrients initiates the process of germination as it indicates that the conditions are more favorable for growth.

The exact mechanism of nutrient binding and triggering of the germination cascade remains largely unclear. It is known that the GR structure is generally composed of three subunits located in the inner membrane of the spore. A notable distinction between the receptors is the stark differences in their corresponding germinants and how they are specific to their *Bacillus* spp. What this experiment hopes to explore is whether the GR specificity alone is the sole factor determining which nutrient will induce germination through the GR-germinant interaction. This was accomplished by exchanging the species-specific GerI receptor from *B. cereus* and transferring it into a *B.subtilis* strain.

The GerI receptor was fused with a GFP marker into a plasmid in order to confirm the successful transfer into the recipient inner membrane. When in *B.cereus*, the GerI induces germination after binding to inosine. The actions of the recipient strain when exposed to inosine may give a better understanding of germinant recognition and the outcomes of the GR-germinant relationship.

36. *Naegleria*'s mitotic spindles are built from unique tubulins

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The "brain-eating amoeba" *Naegleria fowleri* causes a disease with a 97% fatality rate. Current treatments are not reliable and risk significant side effects, including brain damage. Because cell proliferation is essential for disease progression, targeting the mitotic spindle is a promising strategy to develop effective therapeutics. However, we lack key information about the evolutionary conservation of basic cell biological mechanisms that organize the *Naegleria* mitotic spindle, so it is not yet possible to rationally design precise antimitotic therapies with limited side effects on human spindles. To this end, we have investigated the evolutionarily divergent features of *Naegleria* tubulins, the fundamental building blocks of the mitotic spindle. Previous studies showed that *Naegleria* amoebae express a divergent α-tubulin during mitosis, and we now show that *Naegleria* amoebae express a second mitotic α- and two mitotic β-tubulins. Sequence analysis of the mitotic tubulins reveals that they are at most 58% identical to human α- and β- tubulins, and contain residues that suggest distinct microtubule properties. Consistent with this, we find that standard microtubule inhibiting drugs do not affect the growth of *Naegleria*, suggesting that these inhibitors may not bind to the divergent mitotic tubulins. Furthermore, *Naegleria* mitotic tubulins lack several post-translational modifications that control microtubule dynamics and stability in animal cells, further underscoring their distinct regulation in *Naegleria*. These divergent properties raise the possibility that *Naegleria* tubulins could be pharmacologically disrupted without significant effects on human tubulin, suggesting that the mitotic spindle will be a promising target for future treatments for the devastating disease caused by *Naegleria*.

37. Triplet repeats mediated RNA phase transitions in live cells

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Living cells contain many membrane-less organelles that are assembled based on liquid–liquid phase separation.

Different proteins and RNAs can be recruited into these cellular granules to facilitate spatiotemporal regulation. However, the effect of these granule formation on the cellular functions of specific RNAs or proteins is still largely unknown. Herein, we introduce a genetically encoded RNA tag that could be used to recruit almost “any” RNA of interest into membrane-less granules both in vitro and in living bacterial cells. In our system, different lengths of CAG triple-ribonucleotide repeats were used as a scaffold to form multivalent interactions and drive granule formation. Our results indicated that after conjugating with these CAG repeat tags, target RNAs (including small noncoding RNAs and mRNAs) with lengths even >3,000 nucleotides can be recruited into granules in the absence of any proteins. With the help of a fluorogenic RNA aptamer Broccoli, we have further systematically studied the dependence of the granule size and density on the RNA and magnesium ion concentration and the length of CAG repeats and target RNA. The kinetics and cellular distributions of granule formation have also been monitored in live *E. coli* cells. We believe these modular and genetically encodable CAG-repeat tags can be widely used to study the regulation function of granules on the gene expression, RNA-RNA and RNA-protein interactions.

38. Inducible production of Lipid A by therapeutic *Salmonella* generates innate immune cell activation

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Immunotherapies are a promising new avenue for the treatment of cancer; however, only 15-30 percent of patients respond positively to immune checkpoint blockades, the current gold standard therapy. This failure is due to low initial immune activation. *Salmonella* are an ideal tumor delivery vehicle due to their targeting of tumor microenvironments and controllable release of therapeutics. Anti-cancer *Salmonella* therapies are often attenuated by the deletion of the gene msbB, which is a key enzyme in the production of Lipid A. Lipid A is a critical moiety of LPS which stimulates the innate immune system through toll-like receptor (TLR)-4. We have engineered the attenuated strain of *Salmonella* to express msbB (Sal-msbB) under the control of an arabinose-based promotor and various ribosomal binding sites (RBS) for controllable LPS production. Expression of the msbB protein was 7-times higher when a strong RBS was used as compared with a weak RBS. Incubating innate immune cells, including immature monocytes and dendritic cells, with Sal-msbB causes cell activation. Monocytes cocultured with induced Sal-msbB exhibited TNFα expression proportional to RBS strength. TNFα expression caused by msbB under a strong RBS was comparable to that of wild-type bacteria, while the TNFα levels from cells cocultured with uninduced bacteria was similar to those of the attenuated bacteria. Induction of Sal-msbB generates dendritic cell activation. Induced bacteria generate 5 percent more activated cells than uninduced, but both improve activation over no treatment. Together, this data demonstrates that induced production of LPS by engineered *Salmonella* generates a robust innate immune response. Activating innate immune cells in tumors and changing the tumor cytokine profile is critical to overcoming immunotherapeutic limits. This engineered system will allow *Salmonella* to retain key attenuation during delivery, and to generate a localized pro-inflammatory immune response providing a promising avenue to enhance tumor immunotherapies.

39. The Impact of Public Versus Private Metabolism on the Stability of Microbial Communities

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To survive and thrive, microbes must obtain nutrients from their environment through cooperative and competitive actions. A common strategy to obtain nutrients involves secreting enzymes into the external, “public” environment to break down or capture resources before they are taken up into the cell. The metabolic products are cooperative public goods as they are generated externally and so benefit other cells in the shared environment. This seemingly successful strategy termed “public metabolism” is used by a wide range of microbial species that inhabit diverse habitats, yet it has two obvious drawbacks. First, the public goods can easily be lost into the environment before they are successfully taken up by the cell that generated them. Second, the public goods can be exploited by microbes not contributing to their production but still reap the rewards. These shortcomings can threaten the success of public metabolism and the stability of microbial communities. An exploitation-free strategy exists

whereby microbes can secure nutrients by taking them directly into the cell, with digestion taking place “privately” inside the cell, instead of “publicly” in the environment. We have developed a well-defined and tractable synthetic yeast communities to probe the fitness of different metabolic strategies experimentally and computationally to assess community stability and function. We have developed models for public, private and cheat and use *in silico* methods to analyze the growth rates under co-culture conditions. Uptake kinetics for the three strains were built and the metabolic modeling with dynamic flux balance analysis (DFBA) method was applied in this research. The model simulations predicted that private metabolizers would dominate in pairwise competitions between public and private metabolizers and in three-strain communities including cheats, while the total cell density declined because of a diminished growth rate.

40. Genetically encoded RNA-based bioluminescent sensors

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Genetically encodable bioluminescent sensors are important tools in bioanalysis. To achieve real-time quantitative measurement, a powerful type of bioluminescent sensor has been designed based on bioluminescence resonance energy transfer (BRET) between a donor and a fluorescent acceptor (e.g., fluorescent protein). However, the broad applications of these protein-based BRET sensors have been hindered by the availability of target-recognition protein regions and limited signal-to-noise ratio. In this project, by replacing the fluorescent protein-based BRET acceptors with fluorogenic RNA aptamers, we have developed the first genetically encoded RNA-based bioluminescent sensors that can be used to target analytes both *in vitro* and in living bacterial cells. Compared to fluorescent proteins-based sensors, the design of these fluorescent RNA sensors is much easier and modular. Target-binding RNA modules (i.e., aptamer) can be rapidly identified for almost “any” target analyte of interest. After optimizing the RNA-protein interaction strength, energy transfer efficiency, and linker length, we have generated the first RNA-based BRET sensors by coupling a NanoLuc (donor) with a fluorogenic RNA Pepper aptamer (acceptor). These BRET sensors have been further engineered to detect target molecules including metabolites, signaling molecules, and antibiotics. With future optimization, we believe these novel bioluminescent sensors can be potentially applied for high-throughput screening, as well as quantitative and sensitive *in vivo* imaging.

41. Converting to a CURE: MCC community supporting faculty to introduce research into their laboratory courses

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Course-Based Undergraduate Research Experiences (CUREs) provides undergraduates opportunities to gain research experience and practice scientific thinking in their coursework. Studies show that undergraduates doing scientific research report a higher likelihood of persistence in STEM and greater sense of independence. By developing research projects that can be conducted in laboratory courses, more students can be exposed to this high-impact practice without increasing faculty workload. Converting a course from standard teaching lab to a CURE is time consuming, our NSF-funded (RCN-UBE 2119918) network of faculty, focused on research projects involving the protein Malate Dehydrogenase (MDH), the MDH CUREs Community (MCC), supports faculty developing such projects with collaborations and resources. In so doing, we are lowering barriers to adoption of this important practice.

Students in my CURE explore interactions between Malate Dehydrogenase (MDH) with enzymes from related metabolic pathways, to promote flux of intermediates via substrate channeling. We are interested in substrate channeling in *Trypanosoma brucei*, the protozoan that causes African sleeping sickness. This unicellular eukaryote depends on metabolic regulation to transition from growth in insect to mammalian hosts. Insect-stage growth utilizes oxidative phosphorylation of proline, whereas mammalian-stage growth is strictly glycolytic. Three MDH isoforms exist in *T. brucei*, localized to different compartments. We want to study how these different *T. brucei*

MDHs interact with other enzymes. Students have to learn background information, develop a good hypothesis and perform experiments in cloning and expression, interaction assays and enzymatic analyses, often they choose to continue a project from a previous semester. Teams present their work in class and also share their projects with outside colleagues from the MCC who are working on similar projects with other organisms. MCC enables comparative studies and offers the class access to a scientific community beyond the students and instructors in the course, facilitating this research.

42. Season influences long-term warming's impact on ecosystem multifunctionality and microbial diversity

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Soils harbor some of the most diverse microbial communities, in addition to providing critical ecosystem services. While the positive relationship between microbial diversity and ecosystem function has been validated across ecosystems, less is known about how increasing temperatures due to climate change will impact this relationship. We have previously observed increases in microbial diversity in plots that had been warmed 5 degrees C above ambient temperature, and other studies have observed increases in ecosystem function under simulated climate warming. We hypothesized that warming would increase ecosystem multifunctionality and microbial diversity, resulting in a similar relationship between the two with higher overall functioning. Using soils from the Harvard Forest long-term soil warming experiment, we assessed how long-term warming impacts the relationship between ecosystem multifunctionality and microbial diversity. We measured seven different ecosystem functions and sequenced the bacterial community from two soil horizons, two different seasons, and two field experiments that had been warmed for different durations (28 years and 13 years). We found that warming decreased ecosystem multifunctionality in both field experiments, but only in summer. We also found that evenness, Shannon diversity, and Simpson diversity were not influenced by season, warming, or soil horizon. Despite this, warmed plots had a weaker relationship between microbial diversity and ecosystem multifunctionality compared to the control plots. Results from this study suggest that warming's impact on EMF and microbial diversity are dependent on season and emphasize the importance of repeated sampling across time. Additional analyses will examine the impact that warming has on the relationship between fungal diversity and ecosystem multifunctionality.

43. Lipid-DNA conjugate for selective and efficient modification on bacterial membranes

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Decorating bacterial membrane is imperative to investigate their fundamental functions. Current methods of introducing functional moieties onto bacterial membranes still suffer from time consuming processes and limited efficiency and specificity. Herein, we developed a highly simple, fast, and efficient approach to modify bacterial membranes, with the help of lipid-DNA conjugates. DNA owns great programmability, and after conjugating with lipids, these functional lipid-DNA conjugates exhibit high insertion efficacy onto cell membranes via hydrophobic interaction. Lipid-DNA conjugates have been widely applied for eukaryotic cell membrane analysis and regulations. In this project, for the first time, we have further showed that the lipid-DNA conjugates can also be used for a wide range of applications on live bacterial cell membranes. We first constructed a synthetic lipid-DNA library and investigated the insertion kinetics and efficiency of different lipid-DNA conjugates onto several types of Gram-positive and Gram-negative bacteria. Very interestingly, our results indicated that different lipid-DNA conjugates exhibit quite different selectivity pattern on bacterial membranes. Taking advantage of these membrane selectivity, we have applied these lipid-DNA conjugates to differentiate various bacterial species, such as methicillin-resistant *Staphylococcus aureus* (MRSA). We believe these lipid-DNA conjugates can function as a very useful platform in the field of bacterial membrane engineering, bioanalysis, functionalization, and therapy.

44. Finding a Small Gene in *P. aeurgonisa*: How Do Small Gene Discovery Algorithms Perform?

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There remain many genes yet to be discovered, even in model organisms. Small open reading frames (smORFs) in bacteria encode for small proteins of ~50 amino acids long. Historically, smORFs have been ignored due to their small size since they were assumed to be “noise,” or randomly occurring sequences that coincidentally have an in-frame start and stop codon. However, the small proteins characterized to date have been demonstrated to serve significant biological roles and may even be expressed at high levels under certain conditions, which has led to researchers becoming interested in small proteins. By using a combination of dRNA-Seq, Term-seq, and Ribo-Seq, we were able to detect a putative smORF in *Pseudomonas aeruginosa* antisense to a Type VI lipase adaptor. To identify gene expression, we will use epitope tagging to attach an SPA tag. *P. aeruginosa* itself is significant due to its role as a model organism, while also being a major nosocomial pathogen. We intend to investigate the algorithms smORF Finder, sPepFinder, and smORFer for their intended ability of discovering putative smORFs while evaluating how well they identify smORFs that may contain noncanonical start and stop sites, or are internal to annotated genes by critiquing what biological information they use as evidence. By comparing each “smORF Hunter” to each other we can determine which tool might be the best at providing auxiliary data for finding putative smORFs, which can increase our efficiency at discovering novel genes.

45. Optimization of the Pegylation assay to study the topology of PopD translocon from *Pseudomonas aeruginosa* in native membranes

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The type III secretion system (T3SS) is employed by several pathogens to promote infection of host cells. A phylogenetic analysis of T3SS based on conservation of their basal body ATPase indicates the presence of at least 7 families. In the case of *Pseudomonas aeruginosa*, a single T3SS is encoded in the genome grouped within the Ysc family. Central to the function of the T3SS is the injectisome, a membrane protein complex that allows translocation of cytotoxic effector proteins into host cell cytosol. In addition, two other proteins called translocators (PopB and PopD, in *P. aeruginosa*) insert into the target membrane and form a pore through which proteins are injected. Although the translocon pore is essential for T3SS-mediated infection, little is known about the structure and topology of PopB and PopD when associated with membranes *in vivo*. In this work, I try to optimize a cysteine specific labeling assay to study the transmembrane topology of PopD upon insertion into host cell (HeLa cells) membranes. The transmembrane orientation of these cysteines was study based on their accessibility to PEG5K-maleimide (PEG), a cysteine specific labeling reagent. For this purpose, we first try to optimize the pegylation assay using cysteine containing proteins (rPFOE167C and PopDH284C). PEG was added to different final concentrations and samples were incubated at diverse temperatures. In addition, urea, SDS, DTT or TCEP were added. PEGylated proteins were visualized using a coomassie blue method or by western blotting. The best condition for protein labelling was 2 mM of PEG at 37°C (1 hour). However, the reaction was not complete (<70%). Using the PEGylation assay, the topology of PopD inserted in membranes of HeLa cells after incubation with *P. aeruginosa* PAK was next studied. Preliminary results showed that a loop domain of PopD is accessible to host cytosol upon translocon formation.

46. Soil Respiration Over Seasons, Across Depths, and In Response to Soil Warming and Nitrogen Addition

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The Soil Warming and Nitrogen Addition experiment at the Harvard Forest Long-Term Ecological Research (LTER) site has been examining ecological responses to simulated global change since its establishment in 2007. Increased soil respiration is an especially concerning response to warming that hinders carbon storage and potentially amplifies climatic change. Our study measured apparent respiration quotients (ARQ) from 2018-2021 to gauge how soil CO_2 production changes in response to warming and nitrogen enrichment. ARQ is the ratio between CO_2 and O_2 concentrations, making it an integrative proxy of biogeochemistry and microbial metabolism because it reflects oxygen consumption and the oxidation state of organic substrates. Our main questions were: (1) How does ARQ vary seasonally and across depths? (2) Does CO_2 concentration change in response to soil warming and nitrogen enrichment? We took seasonal gas well measurements from four depths: organic-mineral layer interface (OA); and 10, 30, and 50 cm below the organic horizon; within four experimental treatments: 5°C above ambient temperature (H), 50 kg N/ha/yr enrichment (N), combined heating and enrichment (HN), and control (C). ARQ tended to reach an annual maximum >1 in July. All m easurement years had wintertime minimums <<1 around February/March, which suggests that substrate availability may be a critical limiting factor that forces the microbial community to use more reduced substrates like lignin. Furthermore, both N and HN plots showed increased CO_2 concentrations over the past year and a half. This difference was the greatest in HN plots which implies that there is an important thermodynamic interaction effect on microbial metabolism.

47. Single-celled transcriptomics to uncover links between morphology, phylogeny, and behavior in test-building Arcellinida

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Shell-building amoeba in the order Arcellinida (Amoebozoa) are abundant in freshwater ecosystems such as bogs and fens, where they serve as important bioindicators in these fragile environments. Arcellinida microfossils play an important role in paleoecological studies, and have been used to identify changes in climate over fine geological scales. . This study aims to use single-cell transcriptomes isolated from multiple genera of Arcellinida collected in bogs in the Eastern US to further understand Arcellinida morphology and phylogeny. We are pairing these ‘omics data with observation at the microscope to understand behaviors such as shell-building and interactions between Arcellinida and other microeukaryotes. Our dataset includes over 100 Arcellinida transcriptomes, both from cells isolated in the Katzlab and sequences sourced from GenBank from 11 different genera. We are analyzing these data with our in house phylogenomic pipeline PhyloToL to examine phylogenetic relationships within Arcellinida by estimating species trees using over 1,300 gene families. For cases in which we have multiple transcriptomes per species, we will combine these data with the tree reconciliation softwares GeneRax and SpeciesRax to examine possible cryptic species. Additionally, we will examine the transcriptomes of different Arcellinida genera that were isolated in the process of cell division to understand which gene families are differentially expressed during this process as compared to non-dividing cells. Finally, we hope to investigate a novel interaction between Arcellinida and an unknown genus of flagellate found living within the tests of multiple genera isolated at Acadia National Park, ME. As a whole, we hope that these investigations will reveal more about the biodiversity, phylogeny, and life history of the important, but poorly understood, group of the test building amoeba.

48. Exploring the diversity of microbial eukaryotes living inside *Nepenthes* pitcher plants

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The majority of eukaryotic diversity is microbial, with estimates claiming these single-celled microeukaryotes (i.e.

protists) represent more than 80% of all eukaryotic diversity. However, many of these lineages remain understudied because they are currently uncultivable. Here, we survey protist diversity within phytotelmata (water cavities; e.g. pitchers on a pitcher plant) of Nepenthes tropical pitcher plants. We focus our efforts on exploring the diversity of the SAR (Stramenopiles, Alveolates, and Rhizaria) major clade that includes representative photosynthetic lineages (e.g. diatoms, dinoflagellates), parasites (e.g. apicomplexans, oomycetes), heterotrophs (e.g. ciliates, most Cercozoa). We are using a metabarcoding approach that relies on SAR-specific primers designed to amplify a portion of the SSU-rRNA gene; analyses of the resulting amplicons will allow us to characterize community diversity in pitchers sampled from Nepenthes pitcher plants at the Smith College Lyman Plant House and Conservatory. Pitchers were sampled from different life stages (closed juvenile, recently-opened adults, and open mature pitchers) to investigate whether unopened pitchers are microbially sterile or, instead, are seeded with a microbial community from their parent plant or environment. In addition, we have launched a pilot metatranscriptomic study to identify the function of the eukaryotic lineages, including those SAR members that inhabit adult pitchers. Our preliminary amplicon analyses suggest that juvenile unopened pitchers harbor a less abundant and sometimes undetectable SAR community while open adult and mature pitchers harbor well-established and diverse SAR communities.

49. Deploying microscopy and molecular tools to illuminate the nuclear nature of ciliate species and their associated microbiomes

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Ciliates, a ~1 billion year old clade of eukaryotes, have long been a model organism for genomic study. Ciliates are characterized by two main morphological features: one is hair-like cilia that surround their body and the another one is nuclear dimorphism (the presence of both somatic macronuclei and germline micronuclei within the same individual). These nuclear features are connected with their genomic features. During the development of the somatic macronucleus from the zygotic nucleus, DNA fragmentation, elimination, and genome rearrangement occurs on a large scale throughout the ciliate life cycle, which is also connected with molecular evolution. However, the relationship between nuclear structure and molecular evolution is not yet well explored, particularly in uncultivable lineages. Similarly, little is known about the diversity and function of microbes living within ciliates (e.g. symbionts). We are combining microscopy and single-cell 'omics to understand both the evolution of nuclear features and the diversity of associated microbiomes among the ciliate genera *Chilodonella* and *Halteria*.

50. Discovering freshwater foraminifera biodiversity using a metabarcoding approach

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Foraminifera are a diverse, ~550 million-year-old clade of amoeboid single-celled eukaryotes (i.e. protists). Characterized by their intricate spiderweb-like network of pseudopods that stream cytoplasm bi-directionally, foraminifera are considered ecosystem engineers that contribute significantly to oceanic carbon cycling. Despite traditional, textbook definitions of foraminifera as marine protists, freshwater foraminifera were discovered over 150 years ago. Yet, to date, very few freshwater foraminifera have been described in part because these fragile organisms are currently uncultivable. Today, there are fewer than ten freshwater foraminifera species that have been described both morphologically and molecularly, and only one species has had its whole genome sequenced.

In this study, we use foraminifera-specific primers designed to amplify a portion of the SSU-rRNA gene as a means of characterizing community diversity. Our focus is on exploring trends in the species richness of freshwater foraminifera in low-pH bogs and fens located in New England, USA. Our initial results indicate the existence of a low-diversity of freshwater foraminifera in the environment, which is in striking contrast to the tremendous diversity of marine lineages. Taken together, these preliminary data illuminate the biodiversity of freshwater protists in threatened environments such as peatlands on a rapidly evolving planet.

51. Meiosis-related gene search in foraminifera: an evolutionary viewpoint of meiosis in an early eukaryote

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Meiosis is the process by which diploid nuclei (2N) reduce to haploid (N). The origin of meiosis is argued to have occurred with the origin of eukaryotes. The so-called meiosis toolkit has been studied extensively in lineages across the eukaryotic tree of life. The presence of most of the meiosis genes confirms sex, but the presence of only some of these genes may lead to the speculation of facultative or cryptic sex. Cryptic sex is a process where organisms are supposedly asexual but occasionally undergo sexual reproduction. This is likely to be the case in microbial eukaryotes since many of them have both sexual and asexual life cycles and possess fewer meiosis genes. To test this hypothesis, we use foraminifera, a clade of shell-building microbial eukaryotes. Foraminifera are a good model organism in this study for at least two reasons: 1) they are an ancient lineage of eukaryotes dating back more than 550 million years and, 2) they have both sexual and asexual life cycles. Here, we used the power of single-cell transcriptomics to search for candidate meiosis genes in foraminifera. In this study, we expanded the “meiosis detection toolkit” to a total of 64 meiotic genes, among which 11 are meiosis-specific. We focus on 30 newly sequenced foraminiferal single-cell transcriptomes representing diverse lineages. We anticipate that this study will help us understand the nature of sex in foraminifera, and perhaps also in early eukaryotes.

52. Evidence of alternative splicing complexity in the ciliate transcriptome with A case study of the ciliate class Heterotrichea

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Intron retention is a type of alternative splicing where introns are sometimes retained rather than spliced out in mature mRNAs, which results in multiple protein isoforms with different functions. This phenomenon is well documented in animals, plants, fungi, and some microbial eukaryotes, but little is known about this process in ciliates. Ciliates are one of the diverse clades of unicellular eukaryotes characterized by their nuclear dimorphism where chromosomal rearrangement occurs during the development of the transcriptionally active somatic macronuclear genome (MAC) from the germline micronuclear genome (MIC). In this study, we analyzed single-cell transcriptomes from multiple individual cells from Heterotrich ciliates. Comprehensive analysis of multiple data sets indicate that Heterotrich species have small size introns (15 bp) and that these introns are alternatively processed to generate protein diversity. Therefore, our current findings broaden the view of ciliate biology and the evolution of intron retention across the eukaryotic tree of life.

53. Mycorrhizal and Rhizosphere Characterization of Tundra Plants

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Plant communities in the North American Arctic are shifting from graminoid-dominated to shrub-dominated in a

process known as shrubification. The change in foliage is associated with shifts in belowground microbial ecology such as mycorrhizal colonization, rhizosphere community composition and biogeochemical functions such as soil carbon sequestration and decomposition. The variation in mycorrhizal species colonization across different tundra plant types remains a knowledge gap. Likewise, it is unclear the extent to which mycorrhizal colonization and plant species affect the community composition of root associated microbes. Since rhizosphere microorganisms can have substantial impacts on carbon fluxes between soil organic matter and the atmosphere, we wanted to examine if mycorrhizal fungi influence the rhizosphere community composition of various plants throughout the tundra. It has been shown that different tundra plant species will harbor different compositions of mycorrhizal fungi and rhizosphere communities; however, less is known about mycorrhizal and rhizosphere variation within a plant species. Since most tundra plants are generalists and can harbor many different species of mycorrhizal symbiotes, it was expected that within plant species variation of mycorrhizal composition would be high. To that end, we hypothesized that the mycorrhizal fungal composition would be an explanatory variable of rhizosphere community composition. Roots and rhizosphere samples belonging to various tundra plants were collected in August of 2021 near Toolik Lake Field Station, Alaska. The primary purpose of this study was to explore the variation between the mycorrhizal and rhizosphere community compositions across different tundra plant species, as well as to explore the link between mycorrhizal and rhizosphere communities. We hope this work will serve as foundational information for other researchers involved in tundra biogeochemistry and mycorrhizal fungi.

54. The role of tuberculostearic acid in the integrity of mycobacterial plasma membrane

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In *mycobacteria*, the plasma membrane is compartmentalized to spatiotemporally coordinate biochemical reactions. Dibucaine is a membrane fluidizer that disrupts the compartmentalization of mycobacterial plasma membrane and arrest the cell growth. We devised a Tn-seq approach to identify genes that are important for the recovery of mycobacterial cell from dibucaine treatment. Through the genetic screening, we identified cfa as a gene underrepresented in the cell population treated with dibucaine compared with vehicle-treated control population. The cfa gene encodes cyclopropane-fatty-acyl-phospholipid synthase and is proposed to be involved in the synthesis of tuberculostearic acid. Tuberculostearic acid is a characteristic C-19 mono-methyl branched stearic acid found abundantly in mycobacterial membrane phospholipids. Lipidomics indicated that the cfa deletion mutant was deficient in lipids containing tuberculostearic acid and accumulated lipids containing C18:1 monounsaturated fatty acid oleic acid, a precursor to tuberculostearic acid. This analysis further validated the role of cfa in tuberculostearic acid synthesis. The cfa deletion mutant showed decreased fitness and delayed recovery from dibucaine treatment. We are currently testing if the cfa mutant is defective in plasma membrane compartmentalization and displays increased membrane permeability. Cfa could serve as a potential drug target that could help increase the efficacy of existing first line antibiotics and facilitate the development of novel membrane active drugs.

55. The role of the chaperone Hsp104 in connecting the amyloid state to its prion phenotype

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The protein-only hypothesis proposes that an infectious protein can be inherited by other cells through the cytoplasm and can seed the conversion of the native protein into the infectious aggregated state leading to changes

in cellular physiology. 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. Any changes to the ratio of soluble protein to aggregated protein either by overexpression of the native protein or the decrease in activity of molecular chaperones leads to a change in associated toxicity. These instances of toxic phenotype due to sequestration are observed under conditions where protein interaction with the cellular environment is impacted. 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, a clearer understanding of how phenotype is determined under conditions that are associated with the accumulation of amyloid is lacking. This study aims to elucidate the link between chaperone activity and the phenotype of the cell. Our observations reveal an interesting connection between phenotype and chaperone limitation that may be reversed with an increase in chaperone activity with increased temperature.

56. Visualizing the life cycle of *Allogromia laticollaris*, a single-chambered foraminiferan, through light and confocal microscopy

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Despite their ecological ubiquity, the life cycles and genome dynamics of Foraminifera are poorly understood. Here we study the nuclear dynamics of the single-chambered foraminiferan, *Allogromia laticollaris*, which we are able to maintain in laboratory cultures. In our pilot experiments, we have been able to track individual cells throughout their life cycles using light microscopy and subsequently stain DNA with Hoechst 33342 and RNA using Click-iT technology. In addition, we derived *Allogromia*-specific probes to locate ribosomal DNA and RNA using FISH (Fluorescence in situ hybridization). By overlaying these stains and probes, we are elucidating the life cycle stages and nuclear structure of *Allogromia*. These techniques have rarely been applied to this eukaryotic clade, making this pilot study especially important for understanding the diversity of eukaryotic genome structure and life cycles. While still in the early stages of image collection, data so far indicate non-canonical eukaryotic nuclear architecture including “adult” cells whose nuclei have distinguishable chromosomes in the otherwise DNA-poor center, plus a dense lattice of DNA around the periphery of each cell.

57. A metatranscriptomic analysis of the long-term effects of warming on the Harvard Forest soil microbiome

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2020 marked one of the hottest years on record to date, with the average global temperature reaching 1.2 °C above pre-Industrial era (1880) temperatures. Rising temperatures are largely attributed to increasing CO_2 levels from the widespread burning of fossil fuels. Terrestrial ecosystems are the largest global carbon reservoir. In the soil, microorganisms play major roles in carbon and nutrient cycling, decomposition, and mediation of plant health, among others. Involvement in such processes makes soil microbial communities incredibly insightful for understanding earth’s changing climate. The Harvard Forest Long-Term Ecological Research site in Petersham, MA implements belowground heating cables to warm experimental soils 5°C above the ambient soil temperature. With this dramatic temperature difference, researchers intend to simulate a worst-case scenario for earth’s climate. However, upward trends in global warming make this projection not as far out of reach as originally thought. After 15 years of heating, the cables were turned off and the soil was allowed to re-equilibrate to the ambient temperature. Significant changes in soil respiration levels and moisture were observed, leaving the question as to whether the genes expressed by the soil microbial community changed as well. Here I present a metatranscriptomic analysis of gene expression following soil temperature re-equilibration and resumption of heating. As the earth continues to warm, humanity faces the consequences of high emissions and unsustainable practices dating back over a century. Since terrestrial soil microbial communities are a major driving force of biogeochemical cycling, this

endeavor will uncover changes in expression for genes involved in these processes, and reveal the long-term effects of warming.

58. Improving specificity of the LasR homoserine lactone quorum sensor in bacterial consortia using site-directed and saturation mutagenesis

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The availability of genetic parts to control intercellular communication, such as quorum sensing, in bacterial consortia have provided the foundation for industrial biomanufacturing applications. Homoserine lactone quorum sensing is comprised of the synthesis of homoserine lactone (HSL) biochemical signals by an HSL synthase and the binding of those HSL signal molecules to quorum sensing regulators for transcriptional regulation of gene expression. These HSL quorum sensors commonly exhibit some degree of crosstalk with other LuxR-type quorum sensors natively, and this signal interference is often detrimental to applications where specific intercellular signaling allows for more tightly controlled cell-cell communication. In this project, we apply targeted protein engineering strategies to elucidate the sequence-function relationship for quorum sensing regulators and aim to identify variants with improved signaling specificity across a set of commonly used quorum sensors. Here we target the LasR quorum sensor, which is among the highest level of crosstalk observed and for which the protein-ligand interactions and protein structure have been well studied. Mutations of ligand binding residues were generated for LasR using site directed mutagenesis. Using modular cloning and flow cytometry, a LasR mutation V76T has been identified to increase selectivity towards its cognate 3-oxo-C12-HSL signal relative to induction with the set of noncognate signals. A pooled saturation mutagenesis library of LasR variants (10,120 mutants) was designed by mutating a ligand binding pocket region (LasR L125 – L131) by custom scripts and then constructed using an oligo pool to build the LasR sensor library. In ongoing work, a “sort-seq” approach using fluorescence-activated cell sorting (FACS) and next-generation sequencing (NGS) is being employed to quantitatively screen the pooled library. Further analysis of these protein libraries will inform how this class of quorum sensors can be engineered to mitigate crosstalk and allow for investigation of the tradeoff of sensitivity and specificity for this class of sensors.

59. Resistance Variation To Necrotrophic And Biotrophic Diseases Caused By Fungi on Grape

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Understanding how resistances to different diseases interact within the same host is key to designing agro-systems that are naturally more resilient to diseases. More specifically, little is known about how resistances to necrotrophic and biotrophic fungi interact. We used grape as our model system because grapes have multiple species in the Northeast and harbor a myriad of endemic foliar diseases. In the laboratory, we inoculated three different necrotrophic fungi on six grape varieties and evaluated the level of resistance of each variety based on the diameter of the leaf necrosis caused by each fungus. In the field, we evaluated the level of resistance to the biotrophic downy mildew by quantifying the percentage of the leaf surface covered by the disease. We will present and discuss how disease resistances vary across hosts and the relationship between different resistance types.

Carolina Santamaria, Harita Sistu, Eileen Black, Irene Lepori, Briana Kubik, Kiserian Jackson, Eddy Hernandez, Gema Garcia, Yajaira Bermudez, and Stefanos Stravoravdis
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We would like to thank our microbial community of the Pioneer Valley.
We are Valley Microbes.

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