CORNELL CENTER FOR
IMMUNOLOGY

CORNELL INSTITUTE OF HOST-MICROBE INTERACTIONS AND DISEASE

RESEACH SYMPOSIUM 2022

August 10, 2022

9:00 – 5:00

coffee at 8:30, lunch provided

Stocking Hall's PepsiCo Auditorium

Cornell University

8:30 – 9:05 **Coffee and Pastries, poster set up**

9:05 – 9:15 Opening Remarks (Brian Lazzaro, CIHMID Director)

9:15 – 9:30 Scott Keith (CIHMID Postdoc)
"Endocrine regulation of microbe-dependent Drosophila physiology"

9:30 – 9:45 David W. Gludish (Microbiology and Immunology, CVM)
"Not the usual suspects: modeling myeloid tissue reservoirs of HIV using the right lineage"

9:45 – 10:00 Sarah Caddy (Baker Institute for Animal Health, CVM)
"What is the point of non-neutralizing antibodies?"

10:00 – 10:30 Courtney Murdock (Department of Entomology, CALS)
"Predicting vector-borne disease transmission in a human-modified world"

10:30 – 11:15 **Coffee Break and presentation of ODD posters**

11:15 – 11:30 Amanuel Asras (Biology and Society, CALS)
"Understanding Bacterial Chemotaxis: Cloning, Expression and Characterization of the Oxygen Receptor (Aer)"

11:30 – 11:45 Nicole K. Reynolds (School of Integrative Plant Science, CALS)
"Mucoromycota fungal and associated bacterial communities in the rhizosphere of two different plants in the Sonoran Desert, California"

11:45 – 12:15 Brian Lazzaro (CIHMID Director, Department of Entomology, CALS)
"Gram-negative bacterial infection in Drosophila: a model for infection kinetics and outcome"

12:15 – 12:20 Announcements (Brian Lazzaro, CIHMID Director; Melanie Smee, CIHMID Postdoc Association)

12:20 – 1:45 **LUNCH**

1:45 – 2:15 Ilana Brito (Biomedical Engineering, CoE)
"Mechanisms linking gut bacteria to human cellular physiology and disease"

2:15 – 2:30 Connor Loy (Biomedical Engineering, CoE)
"Cell-free nucleic acid profiles in pediatric COVID-19 and MIS-C"

2:30 – 2:45 Sohel Ahmed (Cornell Wildlife Health Lab, CVM)
"Evaluation of SARS-CoV-2 infection in white-tailed deer in New York State 2020-2021"

2:45 – 3:30 **Coffee Break and presentation of EVEN posters**

3:30 – 3:45 Andrea Darby (Department of Entomology, CALS)
"Feeding on high sugar post-development increases susceptibility to infection in Drosophila melanogaster"

3:45 – 4:00 María Teresa Reinoso Pérez (Cornell Lab of Ornithology, CALS)
"Haemosporidia circulating in Mexican house finches and their potential interaction with M. gallisepticum"

4:05 – 4:30 Michelle Heck (Boyce Thompson Institute and USDA)
"Using molecular biology to develop novel vector management tools "

SUBMITTED ABSTRACTS – TALKS:

Endocrine regulation of microbe-dependent *Drosophila* physiology

Scott Keith

Department of Entomology, Cornell University

Circulating hormones can simultaneously impact varied physiological functions through effects on gene expression and cell biology. In *Drosophila*, multiple, broadly conserved endocrine signaling pathways modulate growth, metabolism, behavior, immunity, and other traits subsequent to interactions with a range of microbes spanning the beneficial microbiota to lethal pathogens. Two such hormones, ecdysone (20E) and juvenile hormone (JH), regulate development, metabolism, and reproduction across insect taxa, and their actions in *Drosophila* have long served as a highly tractable system for studying endocrine control of complex animal physiology. These hormones and their cognate receptors also have reciprocal effects on immunity, with 20E potentiating and JH suppressing innate immune responses. Further evidence suggests these hormones, particularly JH, can mediate physiological tradeoffs between immunity and other processes like reproduction during bacterial infection. Yet we know little about the molecular mechanisms of this regulation, the scope of additional microbe-responsive physiologies controlled by 20E and JH, or how they interact as a functional endocrine network in the context of host-microbe dynamics. To address these questions, we are using a combination of classical genetics and genomic approaches to discover how 20E and JH signaling drive infection-induced molecular changes specifically in the *Drosophila* fat body, a highly versatile tissue with immune, metabolic, and reproductive functions. Our long-term goal is to define the gene regulatory networks activated by 20E and JH in the fat body in response to extrinsic microbial stimuli, and to determine the consequences of these GRNs on organism-level physiology. Through this work, we will ultimately better understand the mechanistic principles of hormone-mediated regulation of development, metabolism, reproduction, and immunity in the context of host-microbe interactions.

Not the usual suspects: modeling myeloid tissue reservoirs of HIV using the right lineage

David W. Gludish

Department of Microbiology and Immunology, Cornell University College of Veterinary Medicine

HIV infects a diversity of immune cells during acute and chronic infection, and has been extensively studied in T-lymphocytes as key reservoirs for the virus during long-term antiretroviral therapy. These reservoir cells are considered the current barrier to a durable cure. However, the case for viral persistence in alternative reservoirs such as myeloid lineage cells remains unresolved. Tissue-resident macrophage populations are known to be susceptible to HIV infection in vitro and in both humans and non-human primates, but these cells in humans are more challenging to study. It has become clear that tissue-resident macrophage populations derive from fetal liver hematopoietic precursors, and not from bone-marrow monocytes as had been assumed during most research performed during the HIV pandemic. To model these tissue-resident cells more closely, we have studied HIV infection in fetal liver-derived macrophages compared to adult monocyte-derived cells. We find embryonic cells are much more susceptible to HIV infection in vitro, but are unable to shed virus in co-culture. Leveraging novel reporter cell tools to quantify viral outgrowth, we are exploring the mechanisms for complete viral restriction in fetal liver-derived macrophages, and are defining a new model of in vitro macrophage culture to study tissue-resident macrophage populations.

What is the point of non-neutralizing antibodies?

Dr Sarah Caddy

Assistant Professor, Baker Institute for Animal Health, Cornell University

A wide range of antibodies are made following virus infection. Many antibodies have been characterized that can bind to viruses, but do not prevent infection *in vitro*. Consequently, the role of these 'non-neutralizing' antibodies is unclear. However, animal studies have shown that certain non-neutralizing antibodies are protective *in vivo*. This shows they are valuable but must be functioning via mechanisms that are not tested by cell culture infections. We sought to determine how such antibodies targeting the middle capsid protein (VP6) of rotaviruses and the nucleoprotein (N) of enveloped viruses could be protective.

We discovered that the intracellular antibody receptor TRIM21 was mediating a key role in the activity of non-neutralizing antibodies. Using *in vitro* and *in vivo* models of rotavirus infection, we showed that intracellular IgG bound to VP6 could be targeted by TRIM21 for proteasomal degradation. This rapidly blocked viral replication.

We next demonstrated that the same TRIM21-dependant pathway was active for N-protein specific antibodies induced by LCMV infection. Unexpectedly, viral replication was not reduced until day 7 post infection in mice, and it was found that CD8 T cells were essential for this phenotype. Investigation of this mechanism revealed that anti-N antibodies in tandem with TRIM21 were driving enhanced antigen presentation. This resulted in a more robust anti-N CD8 T cell response.

Future work will evaluate the significance of non-neutralizing antibodies for immunity to a range of different viruses. We aim to develop ways to harness these mechanisms to result in improved vaccine design.

Understanding Bacterial Chemotaxis: Cloning, Expression and Characterization of the Oxygen Receptor (Aer)

Amanuel E. Asras[1], Dr. Brian R. Crane[2]

[1]Senior, Biology & Society, Cornell University
[2]Department of Chemistry and Chemical Biology, Cornell University

Bacterial chemotaxis is form of locomotion for bacteria in response to a chemical gradient. Aer, *taxis to oxygen and certain repellents*, a bacterial protein plays a critical role in bacterial chemotaxis. Bacteria that contain Aer can interact with the environment, by sensing the relative concentration of oxygen, and moving towards optimum levels of oxygen. How bacteria can sense oxygen and relay signals through Aer are currently unknown. Aer is known to be a relatively large membrane protein that belongs to class of proteins called methyl accepting chemoreceptors (MCPs) with its structure currently unsolved. We show how bacteria are able to recombinantly express Aer and will then discuss various steps to obtain purified Aer. Protein purification occurs by the use of diagnostic biochemistry machinery, which is then evaluated. The characterization of Aer is performed by using a variety of spectroscopic techniques such as florescence imaging, small angle X-ray scattering and cryoEM. The importance of such research lies in the potential of understanding bacterial movement and the possibility of inhibiting disease-causing bacteria, reducing pathogenicity of some sicknesses.

Biography: Amanuel Asras is a Senior Biology & Society (BSOC) major at Cornell University. He chose to study BSOC because he is interested in the dynamics of human health and its effects on health. His research in the Crane group, focuses on protein signals and networks in bacteria. His research involvements led him in joining the McNair scholars, a program that aims to bridge the gap between underrepresented minorities and their goal of achieving a higher graduate degree.

He has an interest of obtaining the dual MD/PhD degree with the goal of studying cardiovascular diseases in the future. He hopes that his work will benefit the communities similar to the ones he is from.

Acknowledgements: Amanuel would like to thank Cornell University's McNair Scholars program for funding and providing resources that were critical to his research success. Amanuel would also like to thank faculty research advisor, Dr. Brian R. Crane and the Crane group, for guiding and supporting his project this summer. Amanuel's research work would not be possible without the Crane group. Amanuel would also like to thank the Douglas Foundation for granting the Douglas Scholarship that provided funding for his research.

Mucoromycota fungal and associated bacterial communities in the rhizosphere of two different plants in the Sonoran Desert, California

Nicole K. Reynolds[1]*, Kevin Amses[2], Jessie Uehling[2], Rasheed Adeleke[3], Teresa E. Pawlowska[1]

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The Mucoromycota comprises a diverse group of filamentous fungi including mycorrhizal symbionts (Glomeromycetes, Endogonomycetes) and rhizosphere-associated and soil saprotrophs (Mortierellomycetes, Mucoromycetes). Recent and ongoing discoveries about the endosymbiotic bacteria (EB) that many Mucoromycota species harbor have generated new questions regarding the effect of EB on host biology and ecology. Depending on the species, EB can influence host asexual and sexual reproduction and metabolic functioning, but for some species the host effects remain unknown. To investigate the potential role of EB in structuring Mucoromycota communities, we are using a combination of culture-based and metabarcoding methods to analyze soils collected from *Ambrosia dumosa* (Asteraceae) and *Larrea tridentata* (Zygophyllaceae) plants in the Sonoran Desert (California, USA) using both bacterial and fungal primers. We utilized network analyses to visualize associations between fungal and bacterial amplicons to explore possibly novel fungal-bacterial interactions. Our results indicate that both biotic filtering influenced by host plant and dispersal filtering based on geographic distance influence microbial community structure. Network analyses suggest several different bacterial and fungal amplicons are uniquely important between the two plant rhizosphere communities.

Cell-free nucleic acid profiles in pediatric COVID-19 and MIS-C

Conor Loy

Department of Molecular Biology and Genetics, Cornell University

COVID-19 was initially described as a mild disease in children, however these initial observations have been challenged by reports of severe cases and a rare hyper-inflammatory syndrome associated with COVID-19: multi-system inflammatory syndrome in children (MIS-C). In collaboration with the NIH PreVAIL consortium, we have characterized cell-free DNA (n=67), cell-free RNA (n=132), and whole blood RNA (n=217) from patients with pediatric COVID-19, MIS-C, and good health to probe cellular death and immune dynamics. Using nucleic acid sequencing, we have discovered distinct cell-free DNA, cell-free RNA, and whole blood RNA profiles, providing insights into pathology and targets for diagnostic assays. Most strikingly, we observe markers of endothelial cell and Schwann cell damage in MIS-C as compared to COVID-19 and healthy controls. We also observed skewed levels of T cell receptor genes in the whole blood RNA of MIS-C patients. Lastly, analysis of matched whole blood RNA profiles highlights the similarities and differences between plasma and whole blood RNA. This work provides a systems-level analysis of tissue damage and immune changes during MIS-C and pediatric COVID-19.

Evaluation of SARS-CoV-2 infection in white-tailed deer in New York State 2020-2021

M.S. Ahmed[1†], R. C. Abbott[1], B.J. Hanley[1], N. Hollingshead[1], G. Diego[2], C. Leonardo[2], M. Martins[2], S.L. Buttand[2], K.L. Schuler[1].

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Recent research has identified widespread SARS-CoV-2 infection in free-ranging white-tailed deer (*Odocoileus virginianus*; WTD) in the United States (US), where the novel virus spilled over from humans into wildlife. Free-ranging WTD presents a risk as a new wildlife reservoir for the SARS-CoV-2 pathogen because of the potential for virus recombination that would produce new strains of coronaviruses with the potential for spillback into humans. Wildlife officials in New York State (NYS) have previously identified a cluster of infection in WTD in Onondaga County, but the spatial extent of the sampling was limited, rendering inferences from those data constrained. To better understand the degree to which WTD were infected in NYS, we conducted a comprehensive evaluation of deer infection and variant type in the retropharyngeal lymph nodes of 5,426 hunter-harvested WTD over the past two years (2020-2021, 2021-2022) using PCR and genetic sequencing. Our objective was to identify "hotspots" in infection (areas with higher than expected disease prevalence) of WTD and to explore why those hotspots occurred in those locations. We used the SaTScan spatial analysis software to identify statistically significant clusters of infection. We found 19 clusters in WTD across NYS and "hotspots" observed in Allegany, Livingston, Chemung, Tioga, Tompkins, Orange, Sullivan, and Lewis counties. These hotspots are far away from urban centers where humans have high densities. Additionally, this research revealed that males were more likely to test positive for SARS-CoV-2 than females, and adult males were more likely to be associated with positive cases than younger animals. Our analysis is a critical first step to understanding the risks of virus spillover and spillback between humans and wild WTD. Moreover, we are also conducting further research to examine the relationship between environmental variables to ascertain how deer may be exposed and spread the virus to their population.

Feeding on high sugar post-development increases susceptibility to infection in *Drosophila melanogaster*

Andrea M. Darby[1], Brian P. Lazzaro[2]

[1]Department of Entomology, Cornell University
[2]Cornell Institute of Host-Microbe Interactions and Disease

An organism's diet is a critical factor for its ability to survive an infection, and overnutrition of dietary sugar in particular has been demonstrated to increase susceptibility to infection across animal models and humans. Adult *Drosophila melanogaster* reared on high sugar diets experience higher bacterial burdens and higher mortality post infection, although the genetic and physiological mechanisms that lead to this outcome are not well understood. Prior studies that have investigated the impact of high dietary sugar on survival of infection have been performed using flies that fed on high-sugar diets throughout their entire life, thus making it difficult to distinguish developmental consequences from acute metabolic effects on immunity. We hypothesized that even transient exposure to high-sugar diet in the adult stage might cause metabolic dysregulation with adverse consequence for immune function, and that pathogens may use excess sugar in its host to proliferate faster leading to increase host death. To test this hypothesis, we first reared *D. melanogaster* on a standard 4% (w/v) sucrose diet, then transferred them to one of six experimental diets varying from 0% - 24% sucrose upon eclosion. After 3-5 days of feeding, we assayed their infection survival over five days against systemic infection with two Gram-negative bacteria, *Providencia rettgeri* and *Serratia marcescens*, and two Gram-positive bacteria, *Enterococcus faecalis* and *Lactococcus lactis*. I found that even three days of exposure to elevated sugar in the diet was sufficient to significantly reduce the probability of surviving infection. However, this effect was specific to the Gram-negative bacteria assayed, suggesting that the immediate metabolic effects of high sugar may have disproportionate impact on the immune pathways that are required for combating Gram-negative infection. High sugar also causes higher pathogen load for both *Serratia marcescens* and *Providencia rettgeri* infections. Further, we used immune compromised flies to test *in vivo* whether high sugar increases pathogen growth in the two Gram-negative bacteria tested. I found that high sugar leads to increased proliferation of *S. marcescens* in the first two to eight hours of infection compared to *P. rettgeri*, which did not have higher pathogen load on high sugar until 12-hours into the infection. This suggests that *Serratia* is carbohydrate limited, and could be utilizing the excess sugar to proliferate faster in the early stages of infection. I am currently measuring antimicrobial peptide (AMP) expression to determine whether high sugar diets may impair immune function by impairing transcription of immune effectors like AMPs. Collectively, these results are foundational for further understanding of the genetic and physiological mechanisms by which high sugar impacts infection survival.

Haemosporidia circulating in Mexican house finches and their potential interaction with *M. gallisepticum*

María Teresa Reinoso Pérez[1], Doriane Anglade[1], Andrés Yanez[2], Víctor Manuel Rodríguez-García[2], André Dhondt[1]

[1]Cornell University
[2]Tec de Monterrey

Haemosporidia often coinfect hosts which can influence the virulence of one or more of the parasites (Dhondt and Dobson, 2017). Recent experimental infections of house finches with the bacterium *Mycoplasma gallisepticum* [which causes conjunctivitis], showed that birds coinfected with *Plasmodium* spp. developed more severe conjunctivitis than the control birds (Dhondt et al., 2017). Moreover, in individuals infected with *M. gallisepticum, Plasmodium* spp. parasitaemia increased while *Leucocytozoon* spp. parasitaemia does not seem to be affected (Reinoso-Pérez et al., 2020). American house finch populations have been dealing with mycoplasmal conjunctivitis, an emerging disease caused by the bacterium *M. gallisepticum*, that expanded across North America. Here we report on the results of a survey of house finch in its native range in Mexico to identify the Haemosporidia parasites circulating in those populations and the extent to which they interact with *M. gallisepticum*. Their presence could facilitate the course of the mycoplasmal conjunctivitis epizootic into Mexican house finch populations.

SUBMITTED ABSTRACTS - POSTERS:

Are Mobile Genetic Elements More Abundant in Freshwater Particle-Associated Bacterial Communities?

Michael Bai

Cornell University

Mobile genetic elements (MGE) are the major driver of horizontal gene transfer (HGT), which allows bacteria to acquire new traits that enable them to adapt to their environment. In aquatic systems, heterotrophic bacteria strongly influence the carbon cycle as they can consume up to 50% of the carbon fixed by phytoplankton. The composition of freshwater bacterial communities consists of two interconnected groups: particle-associated bacteria and free-living bacteria, each having their own unique lifestyle and metabolic functions. Since particle environments are rich in nutrients compared to the free water, particle-associated bacteria grow by attaching to the particle surface and forming dense, multi-species communities with high rates per-capita growth and likely increased cell-cell interaction. However, the extent of the presence of MGEs among particle-associated and free-living bacterial communities still remains unclear. Here, we searched for MGEs from freshwater microbial communities within a drowned river mouth Muskegon Lake (Muskegon, MI), which connects to Lake Michigan, one of the world's largest freshwater bodies. We assembled and annotated high throughput metagenomic sequencing data to identify mobile genetic elements within these two contrasting communities. We predict there are higher frequencies of MGEs in particle-associated communities in comparison to their free-living counterparts due to increased cell density that allow for more opportunities for HGT. These results will add to our understanding of the impact of MGEs towards the acquisition of adaptive traits such as antibiotic resistance among freshwater bacteria.

Increased cytokine storm and altered signaling in a 'humanized' murine CD28 model

Alex Brady, Mandy McGeachy,

Department of Microbiology and Immunology, Cornell University

Animal models do not always reflect the human condition, which complicates translation of laboratory findings to the clinic. One such example was the phase one clinical trial of a CD28 super agonist antibody (CD28SA). In mice, CD28SA treatment expanded regulatory T cells (Tregs) and reduced autoimmune disease. However, when healthy human volunteers received CD28SA, they experienced life-threatening cytokine storm and multiorgan failure requiring intensive care hospitalization. CD28 is constitutively expressed on T cells and provides the canonical co-stimulatory "signal 2" needed for T cell activation. The proline residues in the CD28 PYAP signaling domain are required for downstream signaling and this motif is conserved across species. However, the human PYAP domain is adjacent to an additional proline, while murine PYAP is followed by an inactive alanine residue. Previous in vitro studies have suggested this single amino acid variation modulates downstream signaling leading to interspecies differences in T cell activation. We have developed a 'humanized' CD28 mouse with a CD28 A to P substitution at residue 210 adjacent to the C-terminal proline rich domain ($CD28^{A210P}$). In vitro, $CD28^{A210P}$ T cells had increased Akt phosphorylation, JunB nuclear localization, and produced more IL-2 compared to WT T cells when stimulated with anti-CD3 and CD28.In addition, we assessed the *in vivo* response of the "humanized" CD28 mice to the CD28 super agonist antibody that induced a regulatory response in wild type mice and a cytokine storm in human patients. After injection with CD28 super agonist, $CD28^{A210P}$ mice had significantly increased weight loss, elevated pro-inflammatory cytokine production, and less Treg expansion compared to WT mice. Overall, this study suggests interspecies variation in CD28 signaling due to differences outside of the well-studied signaling domains can alter outcomes of CD28 ligation and promote divergent outcomes when targeted *in vivo.*

Staphylococcus aureus supernatant causes metastasis of zebrafish melanoma cells

Jerome Dovan[1,3], Morgan Giese[1], Gayathri Ramakrishnan[1], Anna Huttenlocher[1,2]

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[2]Department of Pediatrics, University of Wisconsin-Madison, Madison, WI
[3]Department of Microbiology, Cornell University, Ithaca, NY

Staphylococcus aureus is a gram-positive bacterium that has recently been found to colonize and proliferate in human tumors, particularly cutaneous melanoma. Using a zebrafish model, it has been shown that melanoma cells grown in the presence of *S. aureus* supernatant are more prone to dissemination, or metastasis, in vivo. Further, zebrafish melanoma cell states known to metastasize have been observed to exhibit remarkably large cluster formation in vitro. Consistent with this phenotype, melanoma cells exposed to *S. aureus* supernatant form large clusters in vitro. We looked to investigate, in vitro, the factor found in *S. aureus* supernatant that is responsible for this upsurge in clustering. We show that this supernatant-derived factor is exclusive to the *S. aureus* species rather than the *Staphylococcus* genus or gram-positive bacteria. A preliminary transposon mutagenesis screen reveals three genes that are potentially involved in clustering. It is crucial to characterize the factor driving this clustering phenotype to gain insight into potential mechanisms underlying metastasis of melanoma in vivo.

Identification of antileukoproteinase in the mucosal immune response to Equine herpesvirus type 1 (EHV-1) infection

Camille M. Holmes, Susanna Babasyan, Bettina Wagner

Cornell University, College of Veterinary Medicine, Population Medicine and Diagnostic Sciences

Equine herpesvirus type 1 (EHV-1) is an alphaherpesvirus that infects through the upper respiratory tract (URT) of the horse, leading to respiratory disease. Infection of local lymphoid tissues allows for the establishment of cell-associated viremia, which can lead to devastating disease outcomes including abortion or equine herpesvirus myeloencephalopathy (EHM). A robust mucosal immune response can prevent viremia, limiting the occurrence of severe disease manifestations. Nasal transcriptomic profiling of immune and susceptible horses over the course of EHV-1 infection was used to identify genes that differ based on immune status and found differential expression of antileukoproteinase (SLPI) between groups. To further characterize SLPI in the horse, novel equine-specific monoclonal antibodies (mAbs) were developed by mouse hybridoma technology and used for the establishment of a Luminex assay to quantify SLPI secretion and for FACS to identify $SLPI^+$ cell populations. Horses at healthy state were found to have a basal level of SLPI secretion in the oral cavity, URT, female reproductive tract, and periphery. Within the periphery the primary producer of SLPI is $CD14^+$ monocyte-derived cells. Cell specific production of SLPI is also detectable by primary nasal epithelial cells through *in vitro* secretion and immune-fluorescent staining of tissue biopsies. During EHV-1 infection, horses show a significant increase in the secretion of SLPI, which differs based on the immune state of the animal. Work is ongoing to further investigate the role SLPI plays in respiratory viral infection. Here we developed novel equine-specific mAbs, which allow for SLPI detection for the first time in the horse.

Analysis of Bacterial Community Assembly Around Chitin Microparticles Through Cultivation

Sam Katz, Marian Schmidt

Department of Microbiology, Cornell University

For a long time microbiologists believed that aquatic bacteria were roughly evenly distributed throughout freshwater systems, but with the advancement of technology, scientists are beginning to learn more about nutrient hotspots that form in the water column. Zooplankton and other particulate organic matter (POM) are said to be drivers of bacterial diversity and dynamics yet, we still lack understanding of the bacterial communities that form around particulate carbon supplies. In this experiment we set out to increase our understanding of the individual roles played by primary degraders and secondary consumers, with an inoculum from Beebe Lake, focusing on the timeline in which communities of bacteria are assembled to break down particulate organic matter. To do this we used magnetic chitin beads, an abundant polysaccharide in freshwater ecosystems, to culture bacteria that associate with chitin particles over a 96 hour timeframe. We predict that there will be a group of highly specific primary degrading bacteria that first attach to the model particle, which will later be followed by secondary consuming bacteria surviving off the carbon supplied from the bacterial community rather than the original particle itself. This study will produce a culture collection of primary degraders or particle-associated bacteria, which will later be taxonomically identified with Sanger sequencing of the 16S gene and available for genetic manipulations to evaluate their role in the environment. The results of this experiment will help shape our understanding of how freshwater microhabitats develop over time and assist in our understanding of individual taxa's roles within these microbial communities.

Floating solar photovoltaics and their effects on aquatic microbial ecology

Julia Meyer

Department of Microbiology, Cornell University

Floating solar photovoltaic (FPV) energy has been proposed to expand terrestrial solar energy development to meet energy demands while freeing up land for other uses. While FPV reduces evaporative water loss, there are potential unknown risks. FPV may adversely affect the microbial ecology of the ecosystem, which is reliant on the very sunlight that FPV will block. Previous studies have shown that the richness of bacterial groups has different responses to modifications in primary productivity, indicating that shading of bacteria will likely vary by taxonomic group. Therefore, we predict that shading will affect the abundance and richness of phytoplankton populations, because of the different intensities of sunlight that disparate taxa require to photosynthesize optimally. Phytoplankton with low light intensity requirements will be superior competitors underneath FPVs and may persist even as other species populations are adversely affected by the lack of sunlight. To test the effect of shading on the microbial environment, we will monitor ten ponds from the Cornell Experimental Ponds in 2022 to see which are the most similar in their microbial community composition. In 2023, we will begin the experiment on 6 ponds: two ponds with 25% FPV coverage, two ponds with 75% FPV coverage, and two ponds with no coverage. We hypothesize that shading the ponds will decrease richness of heterotrophic bacteria and phytoplankton and increase the abundance of phytoplankton with a low sunlight intensity requirement. If there is a significant decrease in the overall phytoplankton population, we expect to see a decrease in dissolved oxygen and DOM, an increase in carbon export to depth from phytoplankton biomass aggregation, as well as a possible decrease in pH due to increased heterotrophic bacterial respiration. Understanding the effect floating solar panels may have on microbial communities, and wildlife ecology as a whole, is vital to prevent possible adverse effects of this new source of renewable energy.

Lymphocyte proliferation in neonatal foals following EHV-1 vaccination

Abigail Reid, Elisabeth Simonin, Bettina Wagner

Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University

Neonatal foal immune system development challenges the effectiveness of traditional vaccines. Little is known about the immune cell types and their activation and subsequent proliferation during early life. The aims of this study were to determine which cell types proliferate, if any, and how cell proliferation changes over time as the equine foal immune system matures. Peripheral blood mononuclear cells (PBMC) were collected from foals before and after Equine Herpesvirus 1 (EHV-1) vaccination. Cells were stimulated in vitro and allowed to incubate before being immunophenotyped and traced for proliferation using flow cytometry. Results demonstrated that foal IgM+ B cells, CD4+ T cells, and CD8+ T cells proliferate after 3 days in culture during the first weeks of life. An increased percentage of cells proliferate following vaccination at 2 days of age. Understanding lymphocyte proliferation during early foal immune system development can assist the future establishment of more effective neonatal vaccines to target specific mechanisms of these active cell types.

Research Funding: Harry M. Zweig Memorial Fund for Equine Research
Student Support: Cornell University College of Veterinary Medicine

Coordinated downregulation of CXCR3 upon entry into inflamed tissue is important for Th1 effector function

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Pathogen-specific naïve T cells must complete a series of choreographed steps within the draining lymph node to gain effector functions required for migration to the site of infection and for proper pathogen control. However, little is known about how T cells search vast areas of inflamed tissues to locate and deliver their effector function for effective pathogen control. To gain insight into the events occurring after tissue entry, OT-II Th1 cells were generated in vitro and adoptively transferred to mice followed by immunization with OVA/CFA, for synchronize delivery of antigen-specific Th1 cells. Transferred Th1 cells were generated from mice expressing a green to red photoconvertible protein, Kaede, which allowed for spatial–temporal marking of cells within inflamed tissues after exposure to violet light. Cells that remained in the tissue 24 hours after photoconversion (Kaede-red, >24hrs in tissue) were transcriptionally and phenotypically distinct from Th1 cells that entered after the photoconversion (Kaede-green, <24hrs in tissue). Specifically, CXCR3 was highly expressed on Th1 cells that had recently entered the tissue but was found to be downregulated on Th1 cells 24 hours after tissue entry. This was paired with an upregulation of CCR8 and maintained expression of CCR2 and CCR5. To determine the functional significance of receptor modulation in the tissue, CXCR3 down-regulation was disrupted by ectopic expression of CXCR3. Th1 cells unable to downregulate CXCR3 entered the inflamed tissue, but production of IFNγ was significantly reduced. This defect in effector function was not observed prior to transfer into hosts, suggesting that downregulation of CXCR3 in the inflamed tissue is essential for optimizing effector function. Current studies will more precisely follow the temporal changes in chemokine receptor and effector molecules and will be complemented by intravital microscopy to visualize spatial organization and migratory behavior of Th1 as they enter inflamed tissues and deliver effector function. Understanding the spatial–temporal steps involved in effector T cell activation at inflamed sites is likely to identify novel molecular targets that could be exploited to modulate T cell effector function.

Optimization of spatial transcriptomics to characterize the transcriptional landscape of arbuscular mycorrhizal symbiosis in *Medicago truncatula*

Trevor R. Tivey[1], Iwijn De Vlaminck[2], Maria J. Harrison[1]

[1]Boyce Thompson Institute
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Arbuscular mycorrhizal symbiosis is an association that forms between host plant roots and soil fungi. The underground relationship takes place within plant root cells, and the colonization of these plant cells enables both plants and fungi to exchange and acquire critical nutrients. Through the use of model plant species amenable to genetic manipulation, key plant genes central to the symbiotic program have been identified; however, the broader regulation and timing of the transcriptional landscape requires further characterization. To detect differences between colonized and noncolonized plant roots, we adopted a spatial transcriptomic approach with the objective to map the plant and fungal transcriptomes along a spatiotemporal gradient of colonization. Optimization for plant root tissue was necessary prior to constructing a spatial RNAseq library using the 10X Genomics Visium platform. A modified tissue optimization protocol was used which incorporated fluorophore-conjugated nucleotides during reverse transcription to visualize the cDNA footprints prior to sequencing. Tissue optimization experiments show that complete removal of plant root tissue is necessary to prevent strong autofluorescent signal from root vascular tissue. After complete removal of tissue, the cDNA footprint can be seen best after 30 minutes of pre-permeabilization and 18 minutes of permeabilization. The spatial capture of plant and fungal transcripts will enable a higher-resolution understanding of the colonized plant root gene expression landscape.

The following abstracts are from the CIHMID URE (Undergraduate Research Experience) students, and this summer's cohort of the NSF-funded REU (Research Experience for Undergraduates), "Microbial Friends & Foes."

***URE student**
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Oyster Farming Increases DNA Concentration of Marine Sediment Bacterial Communities

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Oysters are ecosystem engineers and are crucial organisms to aquatic environments due to their ability to act as habitats for other marine organisms and aid in the removal of nutrient and sediment loads. However, negligent actions taken by humanity have led to the decimation of wild oyster populations and their reef structure. Today only 1% of wild oyster populations have survived human activity and overharvesting, and some coral reefs around the globe are functionally extinct. The interaction between oysters and microbial organisms found in marine sediment is a particular area of scientific interest, raising the question of how these ecosystem engineers can influence the biodiversity, composition, and biogeochemistry of sediments and marine bacterial communities. Here, we carried out bacterial DNA extractions on marine sediment samples taken underneath four oyster farms located between Rhode Island and Massachusetts, where oyster aquaculture is widespread across the coast. Control samples were also collected 10 meters away from the oyster beds. The DNA samples were later quantified with a Qubit, amplified via PCR, and submitted for 16S rRNA sequencing, where future analysis will explain bacterial biodiversity and composition found in oyster bed sediments. Based on Qubit results, oysters actively participate in increasing bacterial DNA concentrations in marine sediments compared with bare (i.e., control) sediments. However, Ninigret Pond oyster sediment samples possessed less bacterial DNA concentrations compared to control sediment samples from the same location. Since oysters possibly increase the abundance of bacteria, the oyster sediment microbial community composition may be more complex and diverse than control microbial communities. Understanding these interactions could explain the possibility of species interdependence between oysters and the microbial community.

The Role of TLR Activation in Cell Recruitment to T-Cell Activation Niches

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The immune system relies on many different signals and pathways to relay an effective, orchestrated response. Although it is known that T cells rely on chemical signals provided by other immune cell subsets to navigate towards infection, how this occurs in peripherally inflamed tissues is still poorly understood. Antigen Presenting Cells (APCs) express Toll-like receptors (TLRs) that are able to detect pathogen-associated molecular patterns (PAMPS). Our lab has found that one possible way T cells are directed to these APCs is through immune cell clusters enriched for chemokines that are induced via Complete Freund's Adjuvant (CFA) immunization. These activation niches are believed to be the consequence of a number of downstream signaling events leading up to the lymphocyte immune response. The activation of TLRs begins a signaling cascade that is crucial to inducing an inflammatory response both locally in tissue and distally in lymph nodes. The mechanism behind TLR induced immune responses is well understood, but how this leads to T cell -activating niches requires further study. In order to determine whether or not specific TLRs require activation to produce an activation niche, different adjuvants were injected into control and experimental groups of mice. Adjuvants are substances that contain ligands that target and activate specific TLRs by mimicking the PAMPs of an infection. The first part of determining which receptor is the most effective involved quantifying and comparing the immune response between CFA and three other adjuvants: Poly(I:C), Pam3CSK4, and LPS. CFA contains chewed up mycobacteria which are able to activate TLRs 2, 4, and 9, while the other adjuvants target TLRs 3, 2, and 4 respectively. CFA stands as the lab's control adjuvant, while Incomplete Freund's Adjuvant (IFA) does not trigger any TLRs. Five days post-immunization, the ears and draining lymph nodes were harvested to be physically and chemically digested. The cells were then stained with fluorescence antibody in preparation for flow cytometry. Flow was utilized to compare and analyze cell recruitment between the adjuvants, allowing for the conclusion that there were differences in myeloid cell recruitment. The most stark contrast between adjuvants was that TLR 4 activation alone is able to recruit a higher frequency of MHCII+ cells, markers of APCs, compared to activation of TLRs 2, 4, and 9 combined. Additionally, TLR 4 activation was determined to not be sufficient in full recruitment of neutrophils, which are innate cells that make up the early immune response. Enhanced knowledge of the signaling process and its correlation to specific cell recruitment when compared to CFA will allow for future insight into optimizing TLR activation to produce T cell activation niches. Understanding the start to finish mechanism of forming T cell activation niches could provide potential therapeutic targets for boosting or hindering immune response.

Investigating the Effects of a High Sugar Diet on the Gut Microbiome and Immunity in *Drosophila melanogaster*

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Diet not only heavily influences the host's immune function but also shapes the composition and abundance of gut microbes. Utilizing the model insect, *Drosophila melanogaster*, we can study how diet can impact bacterial communities and the resulting impact of those shifts on immune function. Drastic changes in microbe composition and abundance can result in dysbiosis or susceptibility to diseases. Previous studies have found that a high sugar diet (HSD) can cause a shift within the gut microbiota of *D. melanogaster* throughout development, however, it is not understood if switching the diet post-development in the adult stage will also lead to dysbiosis in conventionally reared flies. We have also demonstrated in the lab that feeding flies high sugar in the adult stage increases mortality and bacteria load after infection, but to what extent the gut microbiome may have on this phenotype is unknown. Thus, we hypothesize that if flies are fed a HSD post-development, it can cause gut dysbiosis and result in adverse immune phenotypes such as higher mortality. The objectives of this study are to 1) analyze the effect that feeding adult Drosophila a HSD has on gut microbiome abundance and 2) determine whether the absence of the gut microbiome yields similar infection survival phenotypes already observed in conventionally reared flies. To achieve this, flies were reared on a lab standard 4% (w/v) sucrose, yeast and cornmeal diet and then transferred to either a low (2% w/v sucrose) or high (16% w/v sucrose) sugar diets upon emerging. After 3-5 days of eclosion, females from both diets were sampled for gut bacteria load across four selective bacteria agar media that select for *Acetobacter* and *Lactobacillus* genera, which comprise the bacteria found in the *Drosophila* gut community. For a more precise measurement of *Acetobacter* and *Lactobacillus* counts, we used qPCR analysis to assay their abundance in high and low sugar flies. To assay whether the absence of the microbiome impacts infection survival, we generated germ-free flies by bleaching embryos with a series of washes to deprive the flies of their microbiota. Germ-free flies were similarly transferred to 2% and 16% diets and then challenged with a systemic bacterial infection with *Providencia rettgeri* over a five-day survivorship period. We found that 2% flies overall have a higher bacterial load in comparison to 16% flies. Specifically, they have higher *Lactobacillus*, demonstrating that there is a difference in the abundance and composition of species. However, for both 2% and 16% diets there is a lower bacterial load than expected in literature suggesting that the preparation of flies causes a significantly reduced load of bacteria. Despite the reduced bacterial load and shift in the diets, there is no microbiome effect in the survivorship of germ-free flies which indicates that the survivorship in conventionally reared flies is not due in part to the microbiome.

Transcriptomics of *Medicago truncatula* when in symbiosis with arbuscular mycorrhizal fungi.

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Mutualistic symbioses between plants and mycorrhizal fungi are essential to plant growth. Mycorrhizal fungi provide limiting nutrients like phosphate to plants, making this symbiosis important for sustainable crop production (Choi et al., 2018). Further research of specific mycorrhizal symbioses can help develop ways to facilitate these interactions to replace synthetic crop management practices. One clade of mycorrhizal fungi, Arbuscular mycorrhizal fungi (AMF), is characterized by how they penetrate plant cells and dramatically rearrange their contents to form tree-like structures known as arbuscules. Arbuscules increase surface area for nutrient exchange between both symbionts (Ivanov and Harrison, 2014). By evaluating the patterns of gene expression induced specifically in colonized plant cells during this symbiosis, we can better understand the cellular interactions occurring during this interaction. My objective was to develop a protocol to isolate nuclei into colonized and noncolonized plant populations from inoculated *Medicago truncatula* plants for further RNA isolation and qPCR to quantify gene expression. These developing protocols are novel because they aim to evaluate transcriptomics of pure nuclei populations as opposed to full tissue samples, observing how gene expression can vary in a single individual between cells. To isolate nuclei, I optimized the yield of healthy nuclei extracted from plant roots with the least background debris. I experimented with different manual homogenization and filtering techniques to produce replicate nuclei isolates. I found that chopping with a razor array and filtering sequentially through Miracloth, 40-, and 30-um filters gave the highest nuclei yields with little debris. To sort nuclei using flow cytometry into colonized plant nuclei, noncolonized plant nuclei, and fungal nuclei, I used the *M. truncatula* line BCP1p::NLS-mCherry, which produces a fluorescent protein in the nuclei of colonized plant cells. I used DAPI to mark all nuclei, allowing mCherry-negative nuclei to be sorted into a noncolonized population. RNA was isolated from the sorted populations and converted into cDNA for qPCR to quantify expression of certain marker genes. I used the housekeeping gene *MtEF1* to validate the plant nuclei populations, *MtBCP1* and *MtPT4* to validate AM-symbiosis interaction, and *Rhizophagus irregularis (Ri) alpha-tubulin* to identify fungal populations. Quantitative PCR results showed the gene *MtBCP1* amplified at higher levels in colonized plant nuclei as compared to mock, indicative of proper sorting of this population. The gene MtPT4, however, had inconsistent and lower amplification across replicates than predicted, and fungal nuclei went undetected by the *Ri* marker gene. From my sorting experiments, I determined that single-nuclei sequencing is attainable with increased plant colonization. Although sorting for total plant nuclei collected 40,000 nuclei within minutes, sorting 10,000 nuclei from colonized plant nuclei took hours. Due to the small population of colonized nuclei, in many sorted samples cDNA did not amplify effectively during qPCR, likely due to low cDNA concentration (< 5 ng). Optimized AMF colonization with nurse plants could produce higher nuclei yields from colonized plant cells for successive qPCR amplification and single-nuclei sequencing.

Phylogenetics of *Wolbachia* endosymbiont in the harvester ant genus *Pogonomyrmex*

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Wolbachia bacteria are the most common endosymbiont in all arthropods and have been shown to induce a range of unique morphological and behavioral changes in their hosts. *Wolbachia* infection of ants is very common, but although ants are one of the most important insects in ecosystems around the world, Wolbachia interaction with ants has historically been poorly understood. For example, the mechanism of transmission through colonies, how closely *Wolbachia* evolution mirrors that of its host's, and how *Wolbachia* impacts ant reproduction are all standing questions. I worked with *Pogonomyrmex* (Harvester ant) samples to determine the scale of *Wolbachia* infection in native ant populations, the genetic diversity of these infections, and their geographic distribution. This project utilized preserved genetic samples extracted from *Pogonomyrmex* harvester ants collected from across the American continents. Of the 276 samples tested, 19 were found to be positive, and 4 of those samples only had one strain of *Wolbachia* present rather than multiple infections. The origins positive samples were roughly split between north and south America, although all four singly infected samples were from Argentina. This is likely the first time Wolbachia has been found in many of these *Pogonomyrmex* species, for example in *P. lagunabravensis* and *P. inermis*. Mapping the spread of bacterial infections will hopefully lay the groundwork for future investigations into *Wolbachia* and ants, and successfully isolating single strains of *Wolbachia* may aid research into the genetics and evolution of this bacteria.

Does Asteraceae Pollen Reduce *Crithidia* Presence in Wild Bees?

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Pathogens, such as the gut parasite *Crithidia bombi*, are known to contribute to population declines of certain bee species, potentially threatening the health of ecosystems. Understanding factors that mediate or potentially alleviate disease is thus of great importance. Asteraceae pollen, which includes pollen from sunflowers, asters, and other common species, has been experimentally found to reduce *Crithidia bombi* infection intensity in lab-raised colonies of the bumble bee species *Bombus impatiens* (Locasio et al., 2019). However, it is not known if this effect occurs in wild bee species. By screening the gut pollen content of wild-caught bees from three field sites in the Ithaca area, I assessed if there was a negative association between Asteraceae pollen presence and *Crithidia* presence in bee guts. I prepared and screened 87 pollen slides and identified pollen based on morphology, counting each grain of Asteraceae pollen and non-Asteraceae pollen. There was a negative association between *Crithidia* load and the presence of Asteraceae pollen in the gut. However, this trend was not statistically significant ($p = 0.366$), probably due to the low sample size. Instead, bee genus was a stronger predicter of *Crithidia* presence ($p = 0.009$) than Asteraceae pollen presence. Further sampling of the gut contents of a wider range of species is needed to confirm the negative association and to show species specific trends.

Effects of Temperature-Humidity Interactions on *Aedes aegypti* Larvae

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Aedes aegypti mosquitoes are notable vectors of arboviruses with severe impacts on human health, including dengue virus, yellow fever virus, Zika virus, and chikungunya (Tesla). Temperature has been shown to affect developmental traits (e.g., larval development rate) but there is limited data on how other key environmental variables like relative humidity impact these traits (Delatte, Tesla). We tested if variation in relative humidity altered the relationship between temperature and larval development of Ae. aegypti in a broad range of conditions. Using incubators set at unique temperature-humidity combinations, we tested three temperatures (20, 28, and 35C) and three humidity treatments (30%, 60%, and 90% relative humidity), with 6 trays of 100 larvae in each incubator. 3 trays were allowed to evaporate, and 3 trays were topped up to 1L of RO water daily. We measured larval survival, pupation rate, probability of egg to adult, and evaporation rates of water from each control tray. This data informed analysis on the proportions of emerged larvae and pupae, pupation rate, probability of adult survival, and evaporation rate. As is consistent with existing literature on temperature, the mean development rate between larval trays in the same incubator significantly rose from those in the lower temperature to those in the middle and high temperature blocks. However, when looking at humidity variations within same temperature (eg., 28C-30% RH, 28C-60% RH), variation was observed between the three humidity groups, particularly at 20 and 28C. We identified a positive relationship between relative humidity level and larval development rate, which was distinct in the 20C and 28C temperature treatments. In the 35C treatment, larval development had a shared maximum rate in the 60 and 90% humidity conditions. This indicates how humidity might affect the thermal maximum of larval development rate. Given the prevalence of *A. aegypti*, it is imperative to develop a deeper understanding of how mosquito development may be affected by a suite of interacting environmental factors so we can anticipate how risks of vector-borne diseases like dengue fever may shift or intensify (Liu-Helmersson).

Uncharacterized Phosphatases and Kinases in the Osmolarity Response and Nutrient Sensing Pathways of *Neurospora crassa*

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Nutrient sensing is an essential process in fungi that allows them to identify possible sources of food and prepare the necessary enzymes to ingest them. Osmolarity sensing is used by fungi to regulate internal processes and increase the likelihood of survival. A previous study on the filamentous fungus *Neurospora crassa* supported that increased environmental osmolarity resulted in a decrease in the production of enzymes necessary to utilize complex carbohydrates, meaning that the fungal cells could have some overlap in the genetic regulation of the nutrient sensing and osmolarity response pathways (Huberman, 2017). This project investigated the role of uncharacterized phosphatases and kinases in *Neurospora crassa* in regulating the osmotic response pathway and nutrient sensing processes or both. This research was conducted using the *N. crassa* deletion collection, which contains targeted deletions of nearly all genes in the *N. crassa* genome (Color HV et al., 2006). Mutants missing a gene encoding for a phosphatase or kinase were chosen for the potential role of phosphatases and kinases in one or either of the respective response pathways in question. These mutants were grown under experimental conditions designed to determine whether the respective gene impacts that strain's ability to utilize complex versus simple sugars during hyperosmotic stress. The four different conditions of the culturing media were sucrose, sucrose and 1M sodium chloride, xylan, and xylan and 1M sodium chloride. The biomass was then measured to determine which strains were able to best utilize the available nutrients. Multiple deletion mutant strains were identified as potentially missing a gene that encodes for a protein involved in either nutrient sensing or osmolarity sensing, including **Δ**NCU01767 and **Δ**NCU05364, which experienced difficulty growing in media containing sucrose and 1M NaCl. Having a better understanding of *N. crassa's* nutrient sensing and signaling response pathways is applicable to pathogenic fungi that secrete cell-wall degrading enzymes in a similar manner. Lignocellulolytic enzymes produced by certain filamentous fungi are also used to produce biofuels. A better understanding of filamentous fungi's metabolic networks and nutrient sensing regulation will provide additional insight into how to improve the quality and yields of these enzymes (Wu et al., 2020).

Investigating the Essentiality of Ribosome Recycling Factor (RRF) in *Bacillus subtilis*

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Ribosome recycling factor (RRF) catalyzes the splitting between the ribosome, mRNA, and tRNA. RRF, and other factors can disassemble the ribosome and release the subsequent mRNA and tRNA. Ribosome recycling factor is an essential gene, meaning that the cell cannot function or survive without it. Without RRF, the mRNA and tRNA will not be released from the ribosome, which decreases the pool of free ribosomes for reuse in translation. To test the essentiality of RRF, the gene was depleted using a CRISPRi/dcas9 system where the promoter in the chromosome of *Bacillus subtilis* is xylose inducible. When xylose is present, the sgRNA binds to dcas9 protein and interacts. This complex binds near the promoter and blocks the RRF gene from being transcribed. Growth of the model organism, Bacillus subtilis with and without this gene depletion was monitored using cultures in liquid LB with xylose followed by a plating assay with varying concentrations of xylose to induce the xylose promoter. Our results show that there was indeed breakthrough growth and potential suppressor mutations indicating that the bacteria have overcome the RRF knockdown by means that need to be further investigated.

Pathogenicity of Human Breast Milk Isolates using the *Galleria mellonella* Infection Model

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We recently identified gram negative bacteria as a large component of the milk microbiome when pumped at home, but do not know how pathogenic these organisms are. We hope to use *Galleria mellonella* wax moth as an invertebrate infection model of virulence to study these bacteria. Galleria larvae are inexpensive, easy to obtain, and easy to handle. It has an innate immune system that is functionally similar to humans, which offers insight to human response to pathogens. We are interested in using it to test the pathogenicity of *Acinetobacter* and other gram-negative bacteria isolated from human milk. One challenge to the method is the lack of easily comparable standards between labs since even similar bacterial species can have different pathogenicity. This has created a need for consistent controls between laboratories. The lethal concentration of boric acid using *G. mellonella* has been noted in previous immunotoxicity analyses, but this is the first approach to use it as a positive abiotic control. Additionally, I will use a widely studied and accessible pathogenic *E.coli* as a positive biotic control. I used Kaplan-Meier plots to model mortality. These results have demonstrated the efficacy of *E.coli* and boric acid so they will be used as controls in experiments with *Acinetobacter* strains isolated from human breast milk samples. Little is known regarding the ability of *Acinetobacter* to alter human milk and the overall effect on infant health. Genomic analyses will also be used to determine genetic differences in virulence between isolates. This work will help us establish a range of lethality for milk-derived bacteria and lay the foundation for further investigations into the mechanism of lethality.

Investigating the Role of the Gut Microbiome in Hummingbird Physiology

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The intestinal microbiome is an important factor into overall host health and fitness. Studies on humans and model organisms have revealed the variation in the gut microbiome and its effects on host physiology. The potential benefits of gut microbiota in wild vertebrates is poorly understood. Here, we investigate the composition and potential function of the gut microbiome of hummingbirds in different levels of torpor and how the gut microbiome changes as the hummingbirds transition from the breeding season to the fall migratory period. Specifically, we are doing a shotgun metagenomics analysis on the DNA from previously collected ruby-throated hummingbird fecal samples to investigate associations between host physiology and the gut microbiome. In characterizing the gut microbiome of hummingbirds, we can also determine trends in how the microbiome changes and contribute to our understanding of the ecology and evolution of migratory birds.

The effect of 1% starch in subSHI media on human oral bacterial yield in vitro

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One of the most intricate bacterial communities in the human body is the oral microbiota, and its composition is affected by the host's genetic diversity (Poole et al. 2019). The AMY1 locus is encoded with salivary amylase and its copy number (CN) variation is associated with the ability of starch digestion. The digestion of dietary starch begins in the mouth with the release of an abundant source of carbohydrates for oral bacterial nutrition. Thus, considering the abundance of salivary amylase contributes to the establishment of the oral microbiome (Nikitkova et al. 2013). Our goal is to optimize an in vitro model system to be the most representative of the human oral microbiome. A subSHI media, which was previously proposed to support the growth of the microbiome in the original inoculum saliva samples obtained from human (Tian et al. 2010; Edlund et al. 2013). In this phase of research, we aim to investigate how an extra 1% starch in subSHI media affects bacterial culture yield in vitro. Microbial DNA was extracted using the Qiagen DNeasy PowerSoil Pro Kit and the concentration was measured using PicoGreen. R studio was applied for data analysis. By running at-test, it was shown that subSHI media samples had higher DNA concentrations compared to subSHI with 1% starch ($p<0.01$). Further work using 16S rRNA sequencing to determine microbial composition and shotgun metagenomics will be employed to figure out the functions of the bacterial species present.

Investigating the horizontal mobility of an antibiotic resistance transposon in *Acinetobacter baumannii*

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Acinetobacter baumannii is a multidrug resistant bacteria that has been identified by the CDC as an urgent threat to humans (1). A. baumannii causes various nosocomial infections; predominantly pneumonia, as well as as urinary tract infection and bloodstream infection (2). One of the main reasons for concern regarding A. baumannii is the ability for its antibiotic genes to be horizontally transferred between strains and possibly other species through the movement of a transposon family named Tn6022(3). Transposons are mobile elements of DNA that can move between positions in the genome. They generally encode genes involved in transposition and genes that are carried along with the element called cargo genes which can be reservoirs for antibiotic resistance genes, as is the case with Tn6022 (4). Based on homology with a well classified transposon, Tn7, we predict that Tn6022 has two distinct targeting pathways: one that targets a specific site, the comM gene, to promote vertical transmission and another that targets conjugal plasmids to promote horizontal transmission of the element (3). Recently, orf4 within Tn6022 was predicted to have homology to the target site selecting protein TnsE from Tn7, which is responsible for horizontal mobilization. We set out to investigate if orf4 was involved in horizontal mobilization of Tn6022 in order to understand acquisition of antibiotic resistance genes in A. baumannii. Preliminary evidence suggests that the transposition pathway is active in the presence of the target selector protein, having a higher transposition frequency when the gene is being expressed. Further research should be conducted to identify what Tn6022 is targeting.

T cell development is regulated by high fidelity replication of mitochondrial DNA

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T cells are important for host defense and tolerance. For these cells to develop, mature, and function, they require energy, which depends on proteins made by mitochondria. Each T cell carries multiple mitochondria, and when they divide the mitochondrial DNA (mtDNA) can accumulate mutations that can impair proper mitochondrial function, and fully dysfunctional mitochondria are typically degraded. Therefore, the fidelity of copying effectiveness of mtDNA, carried out by polymerase (PolG), is especially important. This has the potential to negatively affect highly proliferative cells that depend on the mitochondria for energy. T cells are highly proliferative during their development in the thymus, specifically, right before T cells develop their T cell receptor, known as the double negative (DN) stage. This DN stage is further divided into stages DN1, 2, 3, and 4, where the highest proliferation rate is in the DN3-4 stages. We hypothesize that mice expressing a dysfunctional PolG will generate many mtDNA mutations during the highly proliferation stages of T cells, and therefore reduce the mitochondrial density in these cells. We used antigen specific OTII transgenic mice carrying a mutant PolG that results in increased mutations in mtDNA, to investigate this. We quantified mitochondria in T cells from the thymus of mature aged mice in DN3-4 stages via microscopy. Preliminary results indicate that mitochondrial density and proliferation are impaired in adult mice with increased mutations in mtDNA.

Exploring Mucoromycotina and their bacterial symbionts through Deserts and Xeric Shrubland.

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Mucoromycotina are an understudied group of early divergent fungi, which are best known for their plant associations and pathogenicity. Mucoromycotina are involved in ancient symbiosis with endosymbiotic bacteria: Mycoplasma-related endobacteria (MRE) and Burkholderiales-related endobacteria (BRE). However, these relationships are not well described, so the significance of these symbionts is often unclear. This study had two objectives: (i) to isolate and identify Mucoromycotina fungi associated with the rhizosphere of Ambrosia dumosa (Asteraceae) from Deserts and Xeric Shrublands, and (ii) to survey these fungi for the presence of bacterial endosymbionts. Soil samples were collected by The Pawlowska Lab from Sweeney Granite Mountains Reserve and the Boyd Deep Canyon Reserve in California in the Spring of 2022. Ten soil samples per site were plated on microbiological medium WG10 and replicated with Benomyl. Benomyl is a fungicide that selective inhibits the growth of ascomycete fungi to allow a more fruitful recovery of Mucoromycotina. Fungi displaying Mucromycotina mycology were selected for further subculturing to obtain pure cultures. The fungi and their endosymbiotic bacteria were identified by PCR with fungal ITS rDNA genes primers and bacterial 16S rRNA gene primers followed by sequencing. Phylogenic analysis enabled identification of fungal and bacterial taxa including Cumminghamella, Mucor, and Rhizopus. Bacterial sequencing revealed some Burkholderia-related bacteria. Mucor racemosus possibly hosts Achromobacter spanius, additionally, Rhizopus microspores had a Mycetohabitans rhizoxinica. These findings contribute to a better understanding of early divergent fungi diversity in desert habitats, which can be used for microfungi conservation efforts. They also add additional documentation to the evolution of the fungal-bacterial symbiosis.

Effect of Different Iron Concentrations on Pyoverdine Production of *Pseudomonas syringae*

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Pseudomonas syringae is a bacteria that can live epiphytically on the aerial parts of plants, an environment called the phyllosphere. Because microbes are subject to harsh conditions in this environment such as temperature fluctuations, exposure to high levels of UV, and sparse nutrients, microbes tend to be more resilient than their rhizomal counterparts and have mechanisms to obtain nutrients from their environments. *Pseudomonas syringae* employ fluorescent siderophores to scavenge iron from the phyllosphere environment. Siderophores are molecules secreted by producing bacteria which bind to environmentally available iron molecules. Once bound, the siderophore and iron are taken up by bacteria, allowing survival in iron-limited environments. Fluorescent siderophores, such as pyoverdine (PVD), can be detected and avoided by the insect pest *Acyrthosiphon pisum* (pea aphid) under UV light. Here, I test how siderophore fluorescence is affected by different iron conditions and how pea aphids react to this difference, focusing on pyoverdine, the primary siderophore of *P. syringae* and the strain B728A, as well as a pyoverdine deficient B728A mutant (B728AΔPVD). Low iron conditions are simulated by adding various concentrations of iron chelator (tannic acid) to a solution of *P. syringae* and high iron conditions are simulated by adding various amounts of Iron chloride to *P. syringae*. These mixtures are then sprayed on fava bean plants and put in a growth chamber. Bacteria are then washed off the surface of leaves and measured for fluorescence. We find that different iron conditions do not result in significant differences of fluorescence in leaf washes. As pea aphids may be more sensitive to differences in fluorescence, we tested aphid choice between leaves painted with B728A and our PVD-deficient mutant under different iron conditions. Our results show that Pea aphids prefer feeding on leaves painted with iron-supplemented bacteria compared to controls of bacteria with no supplemental iron added. Interestingly enough, pea aphids prefer leaves painted with high iron condition to leaves to mutants with the pyoverdine gene knocked out, and did not have a preference when choosing to feed on a leaf with high iron condition to low iron conditions. These results suggest that the pyoverdine mutants may have a different siderophore that makes it more fluorescent, and that either pea aphids may detect fluorescence differently than how fluorescence was measured in the experiment or there is another factor at play that causes aphids to choose what leaves they avoid.

Neonatal and Adult CD8+ T Cells Respond Differently to T Cell Receptor and Proinflammatory Signals

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T cells are members of the adaptive immune system. In early life, T cell progenitors originate in the fetal liver. Post birth, they originate in the bone marrow before migrating to the thymus to mature. After maturation, exposure to an infectious agent causes the naïve CD8+ T cells to differentiate into subsets of effector and memory T cells. The exposure to an infectious agent causes the CD8+ T cells to differentiate into effector T cells to eliminate the infection and a subset will differentiate into memory cells which provides the host long term immunity upon repeat exposure of the same antigen. Cytokines are messengers that relay information to the immune system, which instructs naïve T cells to differentiate into the required T cell needed for that specific immune function. The aim of this research is to better understand what signal 3 cytokines are doing in the presence of high or low signal 1 peptide and to explore the differences in signal 3 function between neonatal and adult mice. This was accomplished by researching the optimal peptide concentration for T cell activation and then treating the T cells with various cytokines such as IL-12, IL-18, IL-6, and IFNa. This research is significant because skewing cells towards effector cells has the potential to help boost immune therapies and skewing towards memory cells may help enhance vaccine treatments. This study concluded that signal 1 alone promotes maximum division in adult and neonatal CD8+ T cells, but the addition of signal 3 is needed to promote maximum GranB and IFNy production. In the absence of signal 1, signal 3 cytokines are needed to reach maximum division, GranB and IFNy production. In addition, synergy between IFNa and IL-6, with IL-12 upregulates the production of GranB while synergy between IL-18 and IL-12 upregulates the production of IFNy.

Self-cleavage of NIaPro affects aphid fecundity and interactions with viral proteins

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Potyviruses are single-stranded RNA viruses infectious to plants that negatively affects crop yields and are transmitted through insect vectors such as aphids. NIa-Pro (Nuclear inclusion a-protease) is a viral protease that has been shown to increase aphid growth on host plants. In some species of potyvirus such as *Turnip mosaic virus* (TuMV), NIa-Pro can self-cleave a 20 amino acid peptide off of itself. However, NIaPro from other potyvirus species such as *Soybean mosaic virus* (SMV), do not self-cleave and transient expression of SMV NIaPro does not increase aphid fecundity. Therefore, understanding the importance of NIaPro self-cleavage for protein interactions in the plant and for its impact on aphid fecundity will give insight on how viruses affect plant defenses. In this project we ectopically expressed wild type TuMV NIaPro and a mutant of TuMV NIaPro that cannot self-cleave using agrobacterium. Three-week-old *Nicotiana benthamiana* plants were agroinfiltrated with the construct separately and then the plants were used in aphid fecundity bioassays with *Myzus persicae*. Aphids had higher fecundity on plants expressing the wild type TuMV NIaPro compared to plants expressing the self-cleavage mutant of TuMV NIaPro. However, plants expressing TuMV NIaPro self-cleavage mutant did not show significant difference in aphid fecundity compared to the control. We verified the cleavage ability of NIa-Pro mutant by transiently expressing a two viral proteins, NIb-CP (Nuclear inclusion protein b and the viral coat protein), that are separated by a NIa-Pro cleavage site. Immunoblot analyses showed that wild type TuMV NIaPro does cleave NIb-CP into the two proteins, while the self-cleaving mutant of NIa-Pro showed weaker cleavage of NIb-CP compared to the wild type. Our results suggests that the ability to self-cleavage the 20 amino acid peptide from itself affects NIaPros interaction with other proteins and these differences may be mediating changes in aphid fecundity.

Investigating the effects of bile salts on inflammatory bacteria's biofilm formation

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Colorectal cancer (CRC) is currently the third most common form of diagnosed cancer in the United States. Recent studies have shown biofilm formation in the inner colonic mucosal layer not only to be a distinctive feature of proximal CRC, but also to be a prerequisite for the instigation of CRC development. In CRC, biofilms are made of clusters of hundreds, if not thousands of different species of microorganisms that are embedded in the mucus that covers the intestinal epithelium, and it is thought that their formation can be modulated by a variety of factors such as the presence of bile salt. Within these inner colonic biofilms, there have been several genre of bacteria that have been reported such as *Ruminococci* and *Bacteroides*. Within *Bacteroides*, *Bacteroides fragilis* specifically has been linked to CRC progression, while with *Ruminococci* species such as *Ruminococcus gnavus* has been linked with Crohn's disease and inflammatory bowel diseases. Since we currently do not understand much about the mechanisms by which biofilms form in the human intestinal track, the goal of my summer research has been to test the affect of bile salts on *R. gnavus* and *B. fragilis* in human intestinal mucus. In order to accurately compare the amount of biofilm formed in our assays between samples, we first determined the respective colony forming units (CFUs). After determining the CFUs, we then proceeded with a Crystal Violet assay to form a baseline and gain a better understanding of how the bacteria respond to bile salt alone before assaying the bacteria in the intestinal mucus. From these assays, we have found that bile salt increases *S. mutans'* and *B. fragilis'* biofilm formation and impairs *R. gnavus* and *P. aeruginosa's* abilities to form biofilms. Interestingly, *R. gnavus* grew more in the bile salt treated conditions.

Identifying Suppressor Mutants in Depletion Strains of Bacillus Subtilis

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Bacillus subtilis is a gram-positive, non-pathogenic aerobic bacterial model species that has a unique molecular machinery for its survival. There are also a multitude of essential genes encoded that help with cell growth and metabolism. A tool for depleting essential genes in B. subtilis is the CRISPRi/dCas9 system. This system encodes the single guide RNA and the dCas9 protein which interact and block the promoter upstream of the peptidyl-tRNA hydrolase (Pth) gene. The guide RNA is under the control of a xylose inducible promoter. When xylose is added to the cells, the guide RNA will be expressed and bind the dCas9 protein. The genes within the open reading frame aren't expressed as the RNA polymerase has nowhere to bind to start transcription. We used a strain of B. subtilis containing a guide RNA specific to PTH and looked for suppressor mutants that could survive PTH depletion. We were interested in finding what kinds of mutations occurred in the suppressors that grew on varying concentrations of xylose. We completed a series of serial dilutions on the parent strain of PTH, on various concentrations of xylose to deplete the cells of PTH. The colonies were then spot plated and grew at 30 degrees C. Colonies that could survive the xylose depletion were stored at -80˚C as potential suppressor mutants. The suppressor colonies were cultured overnight and gDNA was later extracted. We found that at higher concentrations of xylose (1%) the PTH depletion strains with suppressors grew while the PTH-depletion parent strain with the dCas9 did not. On low concentrations of xylose, the PTH with the Cas9 survived at 0.025% and 0.5% and while the parent strain didn't survive.

Diversity of the Gut Microbiome in Rodents: An Evolutionary or Ecological Phenomenon?

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All mammals have bacterial symbionts, but in most cases it is not known which bacteria represent lineages that have persisted over evolutionary time and which are transient or environmentally acquired. There is evidence to suggest that bacterial lineages have codiversified with primate hosts, but it is currently unknown if the same trend occurs in rodents. This raises the question of whether the pattern of more closely related rodent hosts possessing more similar gut microbiomes is the result of codiversifcation, an evolutionary phenomenon, or habitat filtering, an ecological phenomenon. In order to test this question, 5085 bacterial metagenome-assembled genomes (MAGs) were isolated from 16 different rodent host species and annotated to observe the prevalence of certain genes and proteins. Predicted gene and protein functions within the bacterial cell were also displayed in the annotations. Analysis of the bacterial MAGs revealed that Firmicutes and Bacteriodota were the most abundant bacterial phyla present in the sampled rodent hosts. A bacterial phylogeny containing 5081 lineages was subsequently generated to display the dominant phyla. A host phylogeny including all 16 rodent host species was assembled and annotated to indicate the different rodent families within the phylogeny including *Cricetidae, Muridae, Castor, Cavies,* and *Erethizontidae*. Lastly, a bioinformatic tool was developed in Bash to automatically detect and count specific gene or protein families from established databases such as Pfam. The tool allows for the quick analysis of gene and protein prevalence in large quantities of bacterial MAGs and can be utilized in future studies. This work has shown that Firmicutes and Bacteriodota are the dominant bacterial phyla within the studied rodent hosts. It has also resulted in the generation of a list of unique protein families that were detected in the 5085 annotated bacterial genomes and the creation of a bioinformatic tool to automatically measure gene and protein prevalence.

Translational Capacity of the Fat Body in Mated *Drosophila melanogaster* Females

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We used *Drosophila melanogaster* as a model organism to further understand the mechanisms behind immunity, in this case, immunity-reproduction tradeoffs. Past research in the lab showed that unmated females have higher susceptibility to survival than mated females but the transcription levels for AMPs don't very much. We studied what was the translational capacity of D. melanogaster's fat body and how does it affect the immune response of mated females. We found high mortality on flies that express the protein GAL4 in the fat body under the promoter yp1 when infected with bacterium *Providencia rettgeri*. Also, we saw that one or two copies of the transgene in the fat body has the same result of high sensitivity to infection. In addition, as an extent of our project we compared the effects of GAL4 using different types of promoters (yp1, C564 and r4) where we found that yp1 caused most deaths in comparison to the other promoters, while C564 caused the least. Furthermore, we wanted to see if expressing a different protein in the fat body affected survival. We observed that flies expressing RFP have higher mortality than flies that don't overexpress a protein. We predicted that if the high mortality observed in yp1-GAL4 mated females is due to overexpression of GAL4, then there would be evidence of E.R. stress levels in the fat body after infection. For this, we used qPCR to quantify production of E.R. stress genes six hours post-infection. Our data supports our hypothesis that overexpressing a protein in the fat body causes a declined immune response in flies, which may suggest that the fat body has a low translational capacity. For future directions, we will measure translation levels for GAL4, and AMPs expressed in the fat body, quantify levels of E.R. stress in the fat body with more time points post-infection, and use fly lines with yp1 expressing different proteins.

Defining inhibitory function of peptides with integrin binding motifs to obstruct enveloped virus entry

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Hendra virus and Nipah virus are enveloped negative-sense single-stranded RNA viruses and cause severe respiratory and encephalitic infections, with case fatality rates from 40-100%. With no approved vaccines, research is crucial to understand how HeV and NiV facilitate viral entry and membrane fusion. An important molecule involved with entry is integrin—transmembrane receptors that mediate cell adhesion and immune responses. Many enveloped viruses take advantage of integrin molecules expressed on the host cell surface as attachment factors or primary receptors by displaying short and distinct amino acid motifs on their glycoproteins which integrins bind. Thus, we have hypothesized that integrins are a promising target for the creation of antiviral compounds against HeV and NiV that would block interactions of the viral glycoproteins with host integrins. We created free-body peptides consisting of integrin-binding motifs to antagonize the active site on the integrin to determine if these compounds display inhibitory functions and decrease virus infectivity. Our research suggests that HeV and NiV do not recruit cellular integrins as part of their entry mechanism.

The Mechanism of Disease: How The Upregulation of OsSULTR3;6 Increases *Xanthomonas oryzae pv. oryzicola* Virulence in Rice

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Xanthomonas species are common plant-infecting bacteria. Different species of Xanthomonas are able to infect different types of crops, and collectively they cause a wide variety of diseases that cause yield losses around the world. Xanthomonas pathogens inject Transcriptional Activator-Like Effectors (TALEs) into plant cells and upregulate host susceptibility (S) genes to facilitate disease onset and progression. *Xanthomonas oryzae pv. oryzicola*(Xoc) infects rice plants, causing Bacterial Leaf Streak (BLS). BLS causes symptoms of water soaked lesions but the mechanism underlying watersoaking is unknown. Tal2g, a TALE from Xoc, is a known virulence factor that upregulates the *OsSULTR3;6 S* gene. We hypothesized that tal2g would increase water into the apoplast by targeting the OsSULTR3;6 gene, stomata will close to prevent more bacteria from entering the plant and water will move due to osmotic pressure to cause longer lesions. Virulence assays showed that Nipponbare rice had longer lesions as compared to the EBE-edit line (with a promoter mutation that inhibits the binding of tal2g) when inoculated with WT and Δtal2g Xoc. RT-qPCR confirms that OsSULTR3;6 has increased upregulation in plants inoculated with WT compared to those inoculated with Δtal2g. Stomatal conductance assays showed that stomates of the plant are more open in leaves inoculated with WT Xoc compared to those inoculated with Δtal2g starting at 72 hours post inoculation when watersoaking begins, nullifying our hypothesis.These results raise more questions on how watersoaking directly contributes to the mechanism of infection. A better understanding of watersoaking in BLS by looking at lesion length, gene expression and stomatal conductance could potentially lead to disease prevention in the future.

Phylogenetic relationships within the fungal genus *Cunninghamella*

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The main goal of this project was to examine the diversity and phylogenetics of the fungal genus *Cunninghamella* using soil culturing techniques, PCR, and DNA sequencing. In order to produce DNA sequences for identification, ITS and 28S rDNA sequences were used, of which, only 28S rDNA was successful. *Cunninghamella* is a member of the order Mucorales, a group of fungi that are known to associate with bacterial endosymbionts. To assess endosymbiotic bacteria, a 16S rDNA primer was also used on these samples to identify bacterial signatures within the fungi. Reference sequences were used to identify a collection of unknown isolates. The final phylogenetic tree shows species relationships within this group of fungi, species variation across physical space, and the evolutionary history of *Cunninghamella* compared with related taxa. The 16S PCR primer produced the sequence of an uncultured Burkholderiaceae, a family of bacteria often involved in endosymbiosis with *Mucoromycota*, which requires further investigation. Importantly, several isolates that were tentatively identified as species of *Cunninghamella* were determined to be members of a morphologically similar genus, *Actinomucor*. These two genera of fungi share only relatively distant common ancestors but can be easily, and incorrectly, conflated based on purely visual features. The analysis of these samples ultimately led to an exploration of the limitations of visual and morphological based identification compared with molecular identification. The misidentification of fungi species is far from uncommon, due to the countless number of known and unknown species, and the fact that many of these organisms are microscopic. Before the advent of high magnification microscopy and DNA sequencing, the distinctions within genera of fungi were performed based solely on morphology. This can be particularly misleading and damaging to databases and studies, because many fungi that share morphological characteristics do not necessarily share close ancestry. This sentiment holds significance within mycological studies and the field of medicine, as *Actinomucor* and *Cunninghamella* contain known opportunistic human pathogens, and correct identification is required for proper medical treatment.

Characterization of Cell Wall Defects Caused by the Deletion of RcsBCD Proteins in response to Antibiotic Stress in *Serratia marcescens*

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Serratia marcescens is an environmental, opportunistic, gram-negative bacterium known for being multi-drug resistant pathogen. Antibiotic resistant pathogens continue to be an increasing problem and it is important to gain an understanding of how this resistance works and the mechanisms involved. *S. marcescens* employs a variety of mechanisms to combat antibiotics and not all of these tools are well understood. The regulator of capsule synthesis (Rcs) pathway is believed to be a system involved in *S. marcescens'* antibiotic resistance. The Rcs pathway is a stress response pathway that is activated when the cell envelope experiences stress from an outside source such as an antibiotic. Therefore, the goal of this study is to determine if Rcs pathway affects *S. marcescens'* ability to resist antibiotics. To answer this question the RcsBCD proteins were deleted from the wild-type strain 2698B of *S. marcescens* to create *S. marcescens* 2698B ΔRcsBCD. RcsC and RcsD regulate to the phosphorylation of RcsB in response to stress. RcsB uses a variety of ways to combat this stress. The Kirby-Bauer Disk Diffusion Assay was completed to determine if the deletion of the RcsBCD proteins affects antibiotic resistance. The antibiotics used were ampicillin, carbenicillin, meropenem, cephalexin, polymyxin B, colistin, bacitracin, and vancomycin because they attack and affect the cell envelope of bacteria. Kanamycin was used as a control as it targets to 30S subunit of ribosomes within the bacteria. LB and BHI agar were used. Both the wild-type and ΔRcsBCD strains were completely resistant to bacitracin. Besides in the control, ΔRcsBCD showed an increase in susceptibility to each antibiotic when compared to the wild-type. On LB agar, cephalexin, colistin, and vancomycin provided a significant difference ($p < 0.05$) between the wild-type and mutant strain, and on BHI agar, carbenicillin, cephalexin, colistin, vancomycin and the reduced growth zone for meropenem provided a significant difference; for polymyxin B on the BHI agar ΔRcsBCD's increased susceptibility was slightly significant ($p < 0.1$). Microscope imaging of the reduced growth zone cells from meropenem shows that the mutant strain cells have a deformed and irregular cell shape when compared to the spherocytes formed by the wild-type strain. This data shows that the Rcs pathway does play a role in *Serratia marcescens'* antibiotic resistance.

Developing a Pipeline to Perform Spatial Transcriptomics (ST) to Map the Immunological Landscape of the Inflamed Dermis

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T cells are initially activated and differentiated in the lymph nodes, but must be reactivated by Antigen Presenting Cells (APCs) at the site of peripheral infection for pathogen control. Chemokines are a type of signaling molecule that play a role in coordinating the interaction between T cells and APCs, but how they precisely coordinate T:APC interactions at peripheral sites for pathogen control is not well understood. Through the use of Reporter for the EXpression of CXCR3 Ligands (REX3) mice, our lab has previously identified perivascular cellular niches enriched with the chemokine CXCL10 within the inflamed dermis. Type 1 helper T (Th1) cells make prolonged contact with these niches upon entry into the dermis. These CXCL-10 Peripheral Activation (PAC-10) niches are likely early activation sites for Th1 cells before they move on to clear intracellular infection within the inflamed tissue. Given their spatially restricted nature, PAC-10 niches may be a mechanism by which T-cells are activated while maintaining homeostasis in other parts of the tissue where exposure to effector cytokines may cause unnecessary damage. To understand how the inflamed dermis is organized between sites of activation (PAC-10 niches) and sites of non-activation, we seek to employ spatial transcriptomics (ST). ST is a novel technology that relays information on gene expression with respect to its spatial organization within tissue. Applying ST to the dermis, where immune cell infiltration exists during inflammation, will inform us on how effector and activation programs are spatially balanced to maintain tissue homeostasis. In previous experiments, we identified PAC-10 niches from a frontal view of the tissue via multiphoton microscopy. To view the dermal regions with respect to the niches, we sought to isolate frontal dermal sections for ST instead of the traditional transverse section of the inflamed skin. We first optimized two sets of antibody conditions for identification of fibroblasts (indicative of the dermis) and BFP expression (indicative of the PAC-10 niches) in transverse sections to get a clearer understanding of the morphology of the cells. We successfully applied these staining conditions to identify PAC-10 niches in dermal frontal sections. In addition to isolating frontal dermal sections, we sought to identify a model pathogen to use for ST analysis. We intradermally infected REX3 mice ears with fluorescent vaccinia virus (VV) and found that CXCL10 expression was spatially restricted much like the originally identified PAC-10 niches. Therefore, VV is a candidate model for ST analysis due to both its fluorescent nature and induction of spatially restricted activation niches. Taken together, these tissue staining and infection conditions provide us with a platform to characterize the immunological landscape of the inflamed dermis via ST.

Roles of the different lamins on the sensitivity of cultured human neuronal progenitor cells to Zika virus-induced mitotic catastrophe

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Zika virus (ZIKV) is transmitted by mosquitoes, sexually, and vertically from mother to offspring. A surge in microcephaly in babies born to ZIKV infected mothers was first identified during the 2015 Brazil outbreak. ZIKV invades the fetus and targets embryonic neural progenitor cells resulting in a loss of these cell populations, which leads to the impaired neurogenesis that results in microcephaly. However, the mechanisms of ZIKV toxicity to the neural progenitors are not entirely understood. Our group has found that ZIKV induces mitotic catastrophe (MC) in cultured human neural progenitor cells. MC results from the unscheduled entry of cells into mitosis in the presence of DNA damage or incompletely replicated DNA. It presents morphologically as abnormal multilobular nuclei connected by chromosomal bridges, together with micro and macronuclei. Mitotic catastrophe induced by ZIKV is far more prevalent and severe in neural progenitor cells than in differentiated somatic cells such as U2-OS, an osteosarcoma-derived cell line. We aim to understand the mechanisms that render neural progenitor cells particularly susceptible to ZIKV-induced MC. Lamins are structural proteins that support the nuclear membrane, thus maintaining nuclear morphology. Lamin A/C provides strong mechanical support to the integrity of the nuclear envelope and anchors chromosomes, increasing nuclear stiffness, whereas B-type lamins provide more flexibility to the nuclear structure. Different lamins are expressed in embryonic cells during development than in somatic differentiated cells. Lamin B is preferentially expressed in the former, and A/C in the latter. Our hypothesis is that neural progenitor cells are particularly sensitive to ZIKV induced MC because they express lamin B but not A/C. To test the hypothesis, we need to evaluate the expression of lamins A/C and B in the neuronal progenitors and overexpress lamin A/C to evaluate how they respond to ZIKV infection. My role has been to construct the lentivirus vectors that will be used to transduce the neural progenitor cells to overexpress human lamin A/C. To achieve this goal, I used PCR to add the restriction sites Sal1 and Nhe1 to the cDNA of lamins A and C. I then digested the lentiviral transfer vector, pLJM1, and the PCR products to ligate them together to construct the lentiviral transfer plasmids. The lentiviral plasmids were sequenced, confirming successful cloning of lamins A and C into pLMJ1. HEK-293T cells will be co-transfected with the transfer plasmids and the third-generation packaging vectors necessary to produce third-generation lentiviral vectors to transduce the neural progenitor cells. I also aimed at testing the levels of expression of A/C and B-types lamins in neural progenitor and somatic differentiated U2-OS cells through Western blotting, but have not succeeded at getting the antibodies to detect their target protein yet.