2022 Northwest Branch Meeting Abstracts

American Society for Microbiology
November 4-5th, 2022

Antiactivators prevent self-sensing in *Pseudomonas aeruginosa* quorum sensing

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Quorum sensing is described as a widespread cell density-dependent signaling mechanism in bacteria. Groups of cells coordinate gene expression by secreting and responding to diffusible signal molecules. Theory, however, predicts that individual cells may short-circuit this mechanism by directly responding to the signals they produce irrespective of cell density. In this study, we characterize this self-sensing effect in the acyl-homoserine lactone quorum sensing system of *Pseudomonas aeruginosa*. We show that antiactivators, a set of proteins known to affect signal sensitivity, function to prevent self-sensing. Measuring quorum-sensing gene expression in individual cells at very low densities, we find that successive deletion of antiactivator genes qteE and qslA produces a bimodal response pattern, in which increasing proportions of constitutively induced cells coexist with uninduced cells. Comparing responses of signal-proficient and -deficient cells in cocultures, we find that signal-proficient cells show a much higher response in the antiactivator mutant background but not in the wild-type background. Our results experimentally demonstrate the antiactivator-dependent transition from group- to self-sensing in the quorum-sensing circuitry of *P. aeruginosa*. Taken together, these findings extend our understanding of the functional capacity of quorum sensing. They highlight the functional significance of antiactivators in the maintenance of group-level signaling and experimentally prove long-standing theoretical predictions.
(This work has just been published in PNAS 2022, e2201242119)

The cell membrane protein Kre1 as a receptor for the K1 killer toxin in pathogenic yeasts

Julia Major, Dr. Paul Rowley, University of Idaho

Killer yeasts are single-celled members of the Fungal kingdom that possess the ability to create antifungal proteins called killer toxins. These killer toxins compete with other yeasts in their environment to inhibit growth and kill fungal cells. Due to these functions, killer yeasts are studied for their biomedical potential. Statistically speaking,1 in 3 women will get a yeast infection at one point in their lives. To treat vaginal yeast infections, azoles are the most commonly prescribed class of drugs, and are thought to be as much as 32% ineffective for treating these infections. *Candida glabrata* is a yeast responsible for vulvovaginal candidiasis and is highly susceptible to a killer toxin named K1, produced by baker's yeast (*Saccharomyces cerevisiae*). Kre1 is believed to be the secondary cell receptor for K1, potentially playing a role in yeast cell sensitivity. I hypothesize that Kre1 is the primary determinant of K1 sensitivity in diverse *Ascomycota* yeasts. This hypothesis is supported by findings from Breinig et al., among others, as their research found kre1Δ cells are completely resistant to K1, and expression of KRE1 restored sensitivity. I will be further testing this hypothesis by extracting, cloning, and modifying KRE1s from a variety of *Candida* yeasts, expressing them in kre1Δ S. cerevisiae, and observing their levels of sensitivity to K1. I expect to find that successful expression of KRE1 in kre1Δ *S. cerevisiae* cells will result in sensitivity to K1. Understanding the susceptibility determinants of pathogenic fungi will enable the potential future application of K1 as a novel therapeutic.

An investigation of the association of killer yeasts with insects

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Insects and yeasts interact with mutual benefit outcomes. For yeasts, this allows mating and hybridization, and enhances vectoring and distribution. Competition between yeasts in many environments can be driven by the production of antifungal toxins that leads to the death of sensitive cells. The prevalence of these "killer yeasts" and their association with different insect species is poorly understood. In some species, toxins are encoded by double-stranded RNA (dsRNA) satellites maintained by dsRNA totiviruses. Considering the paucity of knowledge regarding insect-yeast interactions, and the diversity of killer yeasts and their viruses/satellites, we profiled yeasts isolated from different insect orders in Brazil and US, for the presence of dsRNA viruses and killer toxin production. A total of 380 insects belonging to 12 orders, and 49 other sources (mainly fruits and culture collections) were used for yeast isolation, by gut dissection or suspension in saline-buffer solution. A total of 869 yeast and yeast-like microorganisms were obtained, mainly from *Hymenoptera* (i.e., bees, wasps, and ants). The presence of dsRNA viruses was observed in 14% of isolates and 19% produced killer toxins. Importantly, most killer yeasts were devoid of dsRNA viruses and satellites. The killer yeasts were mainly obtained from fungus-growing ants and bees' guts (64 out of 119 positive killer yeasts; 54%). Our results indicate that insects can be a diverse source of killer yeasts, with a low prevalence of dsRNA viruses. The lack of dsRNAs would suggest that the observed killer phenotypes are related to genome-encoded toxins. Future efforts in the isolation and killer phenotype assays will provide clues on the nature and ecology of killer yeasts associated with insects, and also shed light on the prevalence, diversity, and evolutionary origins of dsRNA viruses and satellites infecting different fungal lineages.

A Consortium of Soil Bacteria Utilizes PVA and LDPE as Sole Carbon Sources

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Plastic waste and pollution is an all-consuming problem facing the world's ecosystems and human health. Bacterial species have been discovered to have abilities to enzymatically degrade plastics. Previous research has discovered that a consortium of two *Bacillus* and three *Pseudomonas* strains were able to use polyethylene terephthalate (PET) plastic as their sole carbon source. Using the consortium, we investigated whether other types of plastics, namely polyvinyl alcohol (PVA) and low-density polyethylene (LDPE) were able to be degraded. To test this, the full consortium and single isolates were placed in a liquid carbon-free base medium, and I monitored bacterial growth throughout the process by reading absorbance at 600nm and determining colony forming units (CFU/ml) for experimental flasks. Bacterial isolates and species were able to utilize PVA and LDPE as their sole carbon sources for growth indicated by increasing absorbances at 600nm and statistically significant bacterial growth measuring CFU/ml values as compared to the control ($p=.0001$). Different isolates were more effective with different plastics, suggesting that the degradation process is different between PVA and LDPE. All experimental flasks had some extent of growth, signifying that every bacterial strain in the consortium has the ability to degrade these plastics, albeit with different degrees of efficacy. The utilization of more plastic polymers by these bacterial strains may start allowing the possibility of bacterial plastic degradation to reach public recycling plants, providing another option to lessen plastic pollution around the world.

Exploring relations between killer toxin K62 from *Saccharomyces paradoxus* and aerolysin family proteins

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Pore-forming proteins allow ions and macromolecules to diffuse across membranes and are found in various functional roles, from aquaporins and ion channels to lethal toxins. Pore-forming toxins, such as members of the aerolysin family, assemble multimeric protein pores in cell membranes causing ion leakage and cell death. Aerolysin-like toxins are found across many domains of life and have shared structural motifs despite low sequence similarity. Using a homology modeling and AlphaFold2, we have found that an antifungal killer toxin named K62 from the yeast *Saccharomyces paradoxus* has structural homology to an aerolysin family toxin called parasporin-2 found in bacteria, with only 13% amino acid sequence identity. Molecular dynamics validated the stability and conformational state of monomeric and oligomeric modeling predictions. Superimposing the K62 model with parasporin-2 resulted in an average root mean squared deviation (RMSD) of 6.10 √Ö. K62 causes zones of inhibition against other yeast strains at an optimal pH of 4.0 at 25¬∞C, which is typical of other yeast toxins. We have epitope-tagged K62 and are working to ectopically express the protein using *S. cerevisiae* or *Pichia pastoris*. This will enable the purification of K62 to confirm steps in post-translational modification and whether it oligomerizes like other aerolysin proteins. To validate our modeling predictions, we will use site-directed mutagenesis to disrupt the oligomerization and the membrane insertion mechanisms of K62. We have also identified homologs of K62 in diverse yeasts, including opportunistic fungal pathogens of humans (*Candida sp.),* and pathogens of plants (*Fusarium sp*.), and have confirmed that several are cytotoxic to yeasts. Gaining a better understanding of K62 and its homologs will allow us to confirm that they are aerolysin-like toxins and further explore their potential role in fungal disease.

FoxR is an AraC-like-transcriptional regulator of ferrioxamine uptake in *Salmonella enterica*

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Salmonella enterica spp. are Gram-negative bacteria that occupy diverse environments. There is a constant tug-of-war between *Salmonella*, other microbes and/or the mammalian host for acquisition of limited nutrients in all these environments. Iron is an essential nutrient for nearly all bacterial species but is toxic in excess; therefore, its uptake and export must be tightly regulated. During iron limitation, microbes release siderophores into the environment to capture iron. *Salmonella enterica spp.* produce two siderophores, enterobactin and salmochelin, and also use three xenosiderophores secreted by other microorganisms, ferrichrome, coprogen, and ferrioxamine. Here we focused on FoxA, a ferrioxamine transporter. In *S. Typhimurium*, adjacent to foxA is SL0358 (foxR), a gene annotated as a helix-turn-helix (HTH) domain-containing protein. FoxR shares homology with transcriptional regulators of the AraC/XylS family. foxR is syntenic with foxA in the *Enterobacteriacea*e family, suggesting their functional relatedness. Both foxA and foxR are repressed by the ferric uptake regulator (Fur) under iron-rich conditions. When iron is scarce, FoxR acts as a transcriptional activator of foxA by directly binding to its upstream regulatory region. A point mutation in the HTH domain of FoxR abolished this binding, as did mutation of a direct repeat motif in the foxA upstream regulatory region. Desferrioxamine enhanced FoxR stability and foxA transcription but did not affect the affinity of FoxR binding to the foxA regulatory region. In summary, we have identified FoxR as a new member of the AraC/XylS family that regulates xenosiderophore-mediated iron uptake *by S. Typhimurium* and likely other *Enterobacteriaceae* members.

Investigating the role of the *Candida glabrata* cell wall in killer toxin resistance

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Candida glabrata is an opportunistic pathogenic yeast associated with candidiasis in humans. While *C. glabrata* is intrinsically resistant to antifungal pharmaceuticals, it is uniquely susceptible to killer toxins, antifungal proteins produced by the Brewer's yeast *Saccharomyces cerevisiae*. Potential use of killer toxin-derived pharmaceuticals for treating *C. glabrata* encouraged the investigation of evolved killer toxin resistance. Populations of *C. glabrata* were challenged with killer toxin K2 resulting in the emergence of resistant clones. After whole genome sequencing analysis, it was found that nearly every clone contained mutations in genes associated with cell wall biosynthesis. The loss of cell wall-associated genes in the resistant clones prompted the investigation of killer toxin resistance by screening the effect of killer toxins on *C. glabrata* spheroplasts, cells that lack their cell wall. If killer toxin resistance is dependent on the presence of the cell wall, it is expected that *C. glabrata* spheroplasts would be susceptible to killer toxins. In this study, it was observed that many of the K2 resistant mutants of C. glabrata were also resistant to a related killer toxin, K1. This resistance appeared to be independent of the cell wall for many of the mutants as spheroplasts remained partially resistant to K1. Maintenance of K1 killer toxin resistance in the absence of a cell wall suggests that alterations to the yeast cell wall that impart K2 resistance are not as protective against K1. Overall, this suggests that the mechanism of cell wall interaction of K1 and K2 are somehow distinct even though both toxins are thought to bind the same cell wall glucans as their primary receptor. This study prompts continued characterization of the role of the *C. glabrata* cell wall in killer toxin intoxication and resistance.

The prevention of diastatic contamination using killer yeast

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Diastatic yeast contamination is a problem that many craft-brewing companies struggle with as it can prolong fermentation after packaging, increasing gravity, off flavors, and the explosion of cans and bottles. Diastatic yeasts are a strain of Saccharomyces cerevisiae that contain the STA1 gene that allows these strains to hydrolyze residual carbohydrate polymers of dextrin and starch. Killer yeasts have the unique ability to release protein toxins that can inhibit the growth of competing yeast. In this study, we tested 8 canonical killer yeast against 38 diastatic strains and discovered that the killer toxin K1 was effective at inhibiting 89.7% and K2 inhibited 55% of these spoilage yeasts. Four killer toxin-resistant strains of diastatic yeasts were found to secrete killer toxins that were identified as K2 by reverse transcriptase PCR. The production of K2 by diastatic yeasts would possibly explain the reason why these strains were resistant to K2. These four resistant strains were tested against a collection of 200 unknown killer yeast and were susceptible to ten or more of the novel killer yeast. Based on their spectrum of activity it appears these unknown killer toxins spectrum of activity are similar to K2 toxin, this indicates that these novel killer yeast strains may produce a variant K2 that is effective against strains that are resistant to the canonical K2. Fermentation trials have shown that K1 killer yeasts are also effective in inhibiting the growth diastatic yeast in a simulated contamination event, without affecting the gravity of the beer. Therefore, killer yeasts appear to be an effective intervention to prevent the spoilage of craft beers by diastatic yeasts. Continued collaboration with Rhinegeist breweries will enable the further commercialization of this novel biological control.

Structural Exploration of Killer Toxin K1

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Fungal diseases cause millions of deaths every year and are responsible for a significant portion of crop loss and spoilage around the world. There is a need to find new, more effective methods to combat harmful fungi. Killer yeasts, which produce antifungal killer toxins, are a novel solution to this problem because of the diversity of the mode of action of these toxins. The most well-studied killer toxin is K1 produced by Saccharomyces cerevisiae. This toxin is an ionophore that attacks membranes and has three structural domains - Œ± for toxicity, Œ≥ for immunity, and Œ≤ for target cell specificity. This research is aimed at gaining a structural understanding of K1 and its homologs to enable a connection to its mode of action. I have generated eleven structural models of K1, and its homologs from the *Saccharomycotina* subphylum of yeasts using the cutting-edge neural network AlphaFold2 and optimized them using GROMACS, a molecular dynamics software. These models have a predicted LDDT score of up to 60% confidence. The model of K1 suggests a role for Œ≥ and Œ≤ as chaperones that embrace the toxic Œ± domain, cupping it like a baseball glove. This likely prevents membrane attack during toxin synthesis. We find that disulfide bonding predicted by previous empirical studies fits with the placement and orientation of cysteine residues in our models. Confidence in the model is augmented by similar structural models of the K1 homologs as RMSD (Root Mean Squared Deviation) values compared to K1 are between 6.1√Ö to 6.7√Ö, indicating a similar tertiary structure, despite less than 20% amino acid identity. The current aim of the project is to test the predicted interaction between Œ≥/Œ≤ and Œ± using the yeast-2-hybrid system. This approach will give us an understanding of important structural features of these antifungal proteins.

Characterization of novel dsRNA viruses found in *Euglena mutabilis*

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Pathogenic protozoans harbor dsRNAs (double-stranded RNA) viruses that play a role in parasite physiology, i.e., by increasing pathogenicity or fecundity. *Euglena* species are non-pathogenic, photosynthetic protozoa related to human pathogens of the genus *Leishmania*. *Euglena sp*. is often found in toxic environments such as mine run-offs and volcanic springs and is used in life support systems for space exploration. To determine whether *Euglena* hosts viruses, phenol-chloroform extraction, and gel electrophoresis were used to screen different species of *Euglena*. Five strains of *Euglena mutabilis* isolated from abandoned mines in Canada and the U.K, pristine bogs and lakes in Idaho and Canada, and a polluted river in Germany was found to contain dsRNAs. The size of dsRNAs ranged from 3-4 kilobase pairs and were all degraded by RNase III but was resistant to DNase. dsRNA sequences from *E. mutabilis* strain SG6 were determined using reverse transcriptase PCR (rt-PCR) and random hexamer primer sequencing with Illumina next-generation sequencing. Assembled contigs were confirmed by rt-PCR and were identified to encode an RNA-dependent RNA polymerase with homology to viruses of the family *Partitiviridae* (identity of 29%). The low amino acid identity suggested that the dsRNA virus represents a new genus of viruses. In addition, we found dsRNAs within the cytoplasm of *Euglena* through cellular fractionation, which is consistent with other protozoan dsRNA viruses. *Euglena* viruses were isolated in strains found in environments with high acidity, heavy metals, or evidence of past mining activities, suggesting a correlation between the presence of viruses and toxic environments. Future work will include the chemical curing of viruses cycloheximide and comparing the physiology and transcriptomes of infected and uninfected *Euglena* using RNA-seq.

Shaping the understanding of the Unfolded Protein Response and Vector Competency in *Ixodes scapularis*

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Of the U.S. vector-borne disease cases reported to the CDC in 2019, 91% of them were attributable to ticks. Ixodes scapularis, the North American deer tick, can transmit up to 7 different pathogens that effect human health including *Borrelia burgdorferi,* the causative agent of Lyme disease, and *Anaplasma phagocytophilum*, the causative agent of human granulocytic anaplasmosis. We have limited knowledge about what factors influence vector competency, which is defined as the ability of an arthropod to harbor and transmit pathogens. Recent work has shown that arthropod immunity impacts vector competence. However, little is known about the immune system of non-insect arthropods as arthropod immunity has primarily modeled in *Drosophila*. Cellular stress responses, like the unfolded protein response (UPR), are increasingly being linked to innate immunity. Therefore, in this project we asked if the *I. scapularis* UPR cross-talks with innate immune pathways in the tick to limit bacterial colonization. The UPR is a highly conserved mechanism across species and is regulated by three transmembrane receptors, with IRE1Œ± being the most conserved. In mammals, upon activation, IRE1Œ± recruits TRAF2 leading to NF-–∫B signaling. We first determined an up regulation of key UPR genes in infected *Ixodes.* Using protein modeling and immunoprecipitation, we show that IRE1Œ± and TRAF2 interact in *I. scapularis.* In vitro and in vivo findings show that both IRE1Œ± and TRAF2 limit bacterial colonization of A. phagocytophilum and *B. burgdorferi.* Upon further investigation, activation of IRE1Œ± and TRAF2 signaling lead to the induction of the Immune Deficiency (IMD) pathway's NF-–∫B-like factor, Relish, indicating a linkage to arthropod immunity. To our knowledge, this is the first time that cellular stress responses have been implicated in influencing vector competency. Overall, by characterizing the interplay between stress responses and immunity, we can understand how ticks regulate acquisition, maintenance, and transmission of tick-borne diseases.

Innate Immune Suppression by West Nile virus NY-99 in *Drosophila melanogaster*

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West Nile virus (WNV) is the leading cause of arboviral diseases in the United States and is one of the most common mosquito-borne pathogens in the world. Two strains of the virus, New York-99 (NY-99) and Kunjin virus (KUNV), share high sequence identity. However, the two strains differ in that NY-99 is able to inhibit the mammalian antiviral interferon response, while a point mutation in KUNV NS5 renders it unable to do the same. Whether NS5 inhibits innate immunity in insect hosts remains unknown. The goal of this study is to investigate the ability of NY-99 to inhibit the innate immune response, using *Drosophila melanogaster* as an insect model. To accomplish this goal, we measured gene expression of two anti-viral cytokines (upd2 and upd3) in infected *Drosophila* S2 cells and adult animals. We hypothesized that expression of these cytokines would be significantly downregulated in NY-99 infected cells and would be relatively unaffected by KUNV infection. Preliminary results show that expression of upd 2 is significantly downregulated in NY-99 infected cells as compared to KUNV and uninfected cells. Additionally, in NY-99 infected *Drosophila*, upd3 expression is significantly downregulated compared to KUNV infected flies and uninfected flies. The results obtained from this study will help us understand host-pathogen interactions involved in pathogenicity and transmission of WNV.

A Study of the Cytotoxic Mechanism of Killer Protein 4-Like proteins in *Saccharomyces cerevisiae*

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Crop loss and spoilage caused by fungi cost the global economy over $60 billion annually, with $21 billion in the United States alone. *Ustilago maydis* is known to produce an antifungal protein known as Killer Protein 4 (KP4). Using KP4 in transgenic plants resulted in increased resistance to multiple crop pathogens , including corn smut, head blight in wheat, stinking smut, and black rot. KP4 has been shown to inhibit fungal growth by blocking calcium channels. Homologs of KP4 (KP4-like; KP4L) have been identified, but the functions of the proteins remain unknown. These proteins could be a potential resource for creating new antifungals. A survey of KP4L proteins showed increased diversity and strongly conserved primary and secondary structures. Eight KP4L proteins were used to transform the model yeast Saccharomyces cerevisiae to study their expression and antifungal activities. However, the induction of KP4L genes caused growth arrest in *S. cerevisiae*, but with the resumption of growth when arrested cells were plated on non-inductive media. To identify if this toxicity was due to KP4L export outside of the cell, an N- terminal truncation mutant lacking an extracellular signal sequence was created and did not induce growth arrest. To confirm the extracellular export, a C-tag was fused to the KP4L C-terminus, and extracellular KP4L was detected by Western blotting. Importantly, the N-terminal truncation mutant that lacked a signal sequence did not traffic outside of the cell. These results indicate that KP4L proteins are targeting the cell surface for toxicity like KP4 and could be utilized as novel antifungals in crops. Further experiments will be needed to confirm the KP4L mechanism.

Sporulation Patterns in a PET Plastic Degrading Bacterial Consortium

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Our lab has previously isolated an environmental consortium containing 5 bacterial strains, 3 *Pseudomonas spp*. and 2 *Bacillus spp.*, that have the ability to degrade polyethylene terephthalate (PET) plastic. The goal of this research was to look at the synergy between Pseudomonas and Bacillus by looking at the sporulation of Bacillus while cultured with PET being the only carbon source in hopes of identifying a way of increasing plastic degradation. Spore quantification was done by microscopy and spore staining using Malachite green and safranin throughout a growth curve. Semi-Quantitative RT-PCR was also conducted to measure expression of VgrG, the tip protein in the T6SS of the *Pseudomonas,* and the expression of Spo0A, a master regulator for sporulation in *Bacillus*. There is very little sporulation occuring in cultures containing the consortium in LB, but when grown with PET as the only carbon source, there is an increase in sporulation for the whole consortium between days 5 and 10 before the *Bacillus* population decreases by the end of week 2. RT-PCR results also show that expression is the strongest for week two and decreases again as time passes. Plastic degradation requires biofilm formation and we know that the bacteria form stronger biofilms in consortia than individually. By characterizing the sporulation patterns of *Bacillus* in consortia, we hope to find ways to increase *Bacillus* longevity and increase biofilm strength on plastic.

Optimizing non-*Saccharomyces* yeast temperature and sulfure dioxide conditions for lowering alcohol in wines

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Introduction: Changing viticultural practices have resulted in increased grape sugar levels, resulting in higher alcohol wines that may reduce sensory quality or negatively affect consumer health. Non-*Saccharomyces* yeasts can reduce ethanol yields when sequentially inoculated with *Saccharomyces cerevisiae,* but winemaking conditions, including temperature and sulfite tolerance, must be determined. This research aims to establish SO2 tolerance and optimal temperatures for previously isolated non-*Saccharomyces* strains. Material & Methods: Bottles containing synthetic grape juice medium (pH 3.70, 300 mg/L YAN, 240 g/L fermentable sugars) were inoculated with *Mt. pulcherrima* P01A016, *My. guilliermondii* P40D002, or *S. cerevisiae* D254 (control). Ferments were subjected to 0.0, 0.2, 0.4, 0.6, or 0.8 mg/L molecular (m)SO2 and held at 10, 15, 20, 25, or 28¬∞C for 10 days to measure population and sugar consumption. Results: All ferments with >0.4 mg/L mSO2 inhibited non-*Saccharomyces* yeasts growth regardless of temperature. However, non-Saccharomyces growth and sugar consumption at 0.0, 0.2, or 0.4 mg/L mSO2 were temperature-influenced. Both yeasts consumed the most sugar in ferments containing 0.4 mg/L mSO2 between 20 and 25¬∞C. Conclusion: *Mt. pulcherrima* and *My. guilliermondii* are most efficient at typical winemaking temperatures when added sulfites are present, increasing their potential to produce healthier wines through ethanol reduction.

Differential Restriction of Viral Lifecycles by the Type VI CRISPR System

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While bacteria can benefit from both temperate phages and effective immune systems, the interactions of the two remain poorly understood. Here we demonstrate that the type VI CRISPR adaptive immune system can restrict temperate phage lytic replication while allowing lysogeny.

Characterization of a *Helicobacter pylori* cytotoxin-associated gene pathogenicity island-located small RNA by RT-PCR suggests multiple transcripts are present

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The human gastric bacterial pathogen *Helicobacter pylori* infects approximately 50% of humankind, producing disease outcomes ranging from asymptomatic gastritis to severe gastritis, gastric and duodenal ulcers, gastric adenocarcinoma, and MALT lymphoma. Surviving the inhospitable stomach and producing variable outcomes suggest tightly-regulated gene expression. However, *H. pylori* lacks many bacterial regulatory proteins, including the RNA chaperone Hfq. At least 900 small noncoding RNAs have been identified in *H. pylori*, over 200 of which are classed as small RNAs (sRNAs; noncoding regulatory RNAs shorter than 300 nucleotides). Few have been fully characterized, and others not included in the 200 referenced above have been identified in previous work from our lab. One such, called HPnc2665 (following the *Helicobacter pylori* noncoding RNA designation from Sharma et al. 2010), occurs antisense to cagE in the cytotoxin-associated gene pathogenicity island (cagPAI). This region of virulence genes associated with strains producing severe disease encodes a type IV secretion system and associated toxins. To initiate investigation of regulatory role(s) of sRNAs from the cagPAI, we sought to characterize the exact sequence location and transcript length of HPnc2665 by RT-PCR primer walking. Unexpectedly, numerous transcripts substantially larger than 300 nucleotides were identified (up to 724 nt), with results suggesting the possibility of multiple transcripts. We hypothesize that HPnc2665 may overlap an upstream sRNA, HPnc2660, and HPnc2660's terminator may exhibit variable efficiency potentially leading to read-through transcription. Additionally, one or both transcripts may be much larger than expected. Northern blotting of total RNA hybridized with a probe complementary to the suspected overlap region is being performed to untangle these unclear preliminary RT-PCR results by identifying the number and size of transcript(s) present. Characterizing the location, size, targets, and mechanisms of sRNAs in this prevalent pathogen can explicate regulation of the *H. pylori* transcriptome and what role(s) sRNAs from the cagPAI might play in severe disease.

A Tale of Two Opps: Characterizing the Roles of OppA1 and OppA2 in the *Borrelia burgdorferi* Enzootic Cycle

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The Lyme disease spirochete, *Borrelia burgdorferi (*Bb), is an extreme auxotroph and must acquire many nutrients (such as amino acids) from the environment for survival. To overcome this challenge, Bb uses the essential oligopeptide (Opp) ABC transporter system to acquire peptides in the tick vector and mammalian host. Recent studies have shown distinct structure and functionality of Bb's oligopeptide binding proteins (OppA1-5) during its enzootic cycle. Using available Bb transposon mutants for oppA1 (bb0328) and oppA2 (bb0329), OppA1 and OppA2 have recently been shown to play dissimilar roles. OppA1 contributes to Bb survival during the larval blood meal. In contrast, OppA2 has been shown to play a role in Bb dissemination in a murine model. Furthermore, Bb oppA2tn-infected mice failed to establish robust antibody responses to infection. While OppA1 and OppA2 play distinct roles in the Bb infection cycle, further studies are needed to understand the mechanism by which loss of OppA1 and OppA2 result in disparate phenotypes. In this study, we test the hypothesis that OppA1 aids Bb adaptation to osmotic stress during larval feeding using an in vitro bacterial culture approach using modified osmolytes (betaine, sodium chloride, sorbitol). We show the Bb oppA1tn mutant exhibits a growth defect compared to wild type Bb when cultured in 450 mOsm BSK-II supplemented with sorbitol. Furthermore, we characterize host responses to wild type and Bb oppA2tn infection and show differing host responses in blood, lymph nodes, and meninges that may contribute to or are a result of lack of Bb oppA2tn dissemination. Together, these data further characterize the roles of OppA1 and OppA2 in the Bb enzootic cycle.

Host-pathogen ubiquitin modifications

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The function of a protein is heavily dictated by its structure. Small changes to protein structure through covalent post-translational modifications (PTMs) such as acetylation, phosphorylation, and ubiquitination (among many others) control protein localization or stability, modify binding partners, or change protein activation state. In particular, protein ubiquitination has vast implications in cellular events including proteostasis, DNA repair, macromolecular trafficking, and numerous signal transduction pathways. Ubiquitination occurs through a cascade of three enzymes that activate, conjugate, and subsequently ligate the small protein ubiquitin (Ub) onto a target substrate. The ubiquitination cascade is tightly controlled by enzyme specificity, localization, and natural fluctuations in protein levels. Further post-translational modifications to any of the enzymes involved in the cascade or even to Ub itself serve additional regulatory roles. Human PTMs to Ub include acetylation, phosphorylation, and ubiquitination at sites covering 25% of the total sequence, constituting a complex ,PTM of a PTM that alters Ub regulation and/or signaling outcomes. Owing to its roles in regulating our immune response, numerous human pathogens such as *Shigella flexneri*, *Legionella pneumophila*, and *Salmonella Typhimurium* have adapted sophisticated mechanisms to combat host Ub signaling. I will discuss some bacterial effector proteins secreted during infection that redirect host ubiquitination through their activities in Ub ligation, Ub removal, and Ub modification. I compare bacterially induced Ub modifications to those that are host-derived and provide rationale as to why bacteria benefit from controlling host Ub machinery.

Detection of a SUMO E3 ligase secreted by *Mycobacterium tuberculosis*

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Small ubiquitin-like modifier (SUMO) is a member of the ubiquitin-like protein family. Members of this family serve as post-translational modifications to a variety of cellular substrates. SUMO in particular is localized largely to the nucleus, where it regulates processes like the DNA damage response, nuclear transport, and transcriptional regulation. Manipulation of the ubiquitin system by invading pathogens is well documented, but whether the SUMO system is also hijacked remains unclear. Previous research has unearthed some cases where the SUMO system is manipulated by pathogenic bacteria: LLO in *Lysteria monocytogenes*, XopD in *Xanthamonas euvesicatoria*, Ca influx during *Shigella flexneri* infection, and miRNAs in *Salmonella Typhimurium*. The aforementioned effectors either alter the level of SUMO conjugating enzymes SAE1/2 and Ubc9 or cleave SUMO from target substrates via deSUMOylase activity. To date no bacterial E3 ligase has been found for SUMO, although many have been for ubiquitin. Chemical warheads like Ub-DHA can be employed in the discovery of new E3 ligases by forming irreversible covalent bonds with cysteine-based conjugating enzymes. Using SUMO-DHA probes, we have uncovered the presence of a SUMO E3 ligase within the effectors secreted by *Mycobacterium tuberculosis*. Beyond expanding what we know about the SUMO system and its manipulation by invading bacteria, it sheds light on a new class of SUMO E3 ligase that, unlike analogous enzymes in Eukaryotes, employs a catalytic cysteine dependent mechanism.

Tracking Aeromonas Phage Throughout the Mosquito Life Cycle

Raegan Bowyer, Emma Horstkamp, Christy Andrade

The composition of the mosquito microbiome can influence host metabolism, behavior, immunity, and transmission of specific pathogens including arboviruses. While the role of bacteria in the mosquito microbiome is gaining in popularity as a focus in research, phages (bacterial viruses) in mosquitoes have not been well studied. Previously, when screening urban mosquitoes for culturable bacteria, we discovered a high proportion of *Aeromonas* bacteria and therefore chose *Aeromonas* as a host for phage hunting. We have successfully discovered and characterized phages in mosquitoes that utilize natural isolates of *Aeromonas salmonicida.* In the current study, we developed an in vivo time course to track *Aeromonas* phage throughout the mosquito life cycle. Mosquito larvae were offered a diet of media, *Aeromonas*, phage or *Aeromonas* bacteria and phage. Mosquitoes were then harvested as larvae, pupae, or adult for DNA extraction and PCR analysis. Here we report the preliminary findings from the phage time course in both lab-reared and wild mosquitoes.

Bacterial secreted BefA protein disrupts cellular membranes and stimulates pancreatic Œ≤-cell expansion

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The microbiome's interaction with cellular membranes is increasingly emerging as an important factor in organismal patterning and development at the cellular and tissue level. Previous work has demonstrated that a bacterial protein, Beta Cell Expansion Factor A (BefA), secreted by *Aeromonas veronii* is both necessary and sufficient for the proliferation and expansion of pancreatic Œ≤-cells in the developing zebrafish (*Danio rerio*). Although BefA's impact on Œ≤-cell proliferation has been characterized, the mechanism through which BefA elicits Œ≤-cell proliferation remains elusive. BefA contains a predicted membrane-interacting SYLF domain that is sufficient for Œ≤-cell proliferation. Membrane permeabilization assays utilizing synthetic membranes and bacterial cells have demonstrated BefA can permeabilize membranes, and mutagenesis of the SYLF domain has revealed residues that modulate BefA's membrane permeabilizing activity. Preliminary data suggests BefA may assert its pro-proliferative effect by disrupting Œ≤-cell membranes, causing depolarization and an influx of extracellular Ca^{2+}. Treatment of developing zebrafish larvae with FK506 (Tacrolimus), an inhibitor of the calcium-dependent pro-proliferative phosphatase calcineurin, is sufficient to block BefA-induced expansion of Œ≤-cells during zebrafish development. These data suggest a mechanism through which BefA's membrane permeabilizing activity promotes Œ≤-cell proliferation in the developing zebrafish.

Clostridiales supplementation alters the microbial profile and induces social behavior deficits in mice in a sex-specific manner following prenatal immune insult

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Autism spectrum disorder (ASD) is a heritable and complex neurodevelopmental disorder with both genetic and environmental risk factors, with a male:female diagnosis ratio of 3:1. ASD can be modeled in rodents using Maternal Immune Activation (MIA), in which pregnant mice undergo an immune challenge that leads to ASD-relevant phenotypes in their offspring. Previous work has shown that the microbiota of the gastrointestinal (GI) tract can drive the development of abnormal behavior in male MIA mice. In this study, we tested whether modulating the microbial composition of the MIA mice could induce behavioral deficits and if these deficits were sex-specific. To modulate the microbial community, we supplemented MIA offspring with oral *Clostridium celatum,* a gut commensal bacterium found to be enriched in autistic children. We then compared behavioral phenotypes and the microbial structure of the mice that received the bacteria to those which did not. We found that exposure to Clostridium resulted in sex-specific social impairment, affecting only male MIA mice. 16S amplicon analysis of the duodenum and colon microbiota showed that the MIA model as well as *C. celatum* addition altered the gut microbiome in a sex-specific manner, and that specific taxa were associated with social behavior outcomes. These data reveal that sex-specific resilience to disruptions of the gut microbiome may play a role in the efficacy of the MIA model of ASD.

Amphibian pathogen surveillance through a course-based undergraduate research experience (CURE)

Jennifer Perez, Dr. Jenifer Walke, and Dr. Bo Idsardi, Eastern Washington University, Department of Biology

There are calls for more inclusive, hands-on research experiences to be accessible to all undergraduate students. This has led to the development of course-based undergraduate research experiences (CUREs) which can provide an opportunity for a larger and more diverse group of students to engage in authentic research. CUREs can make a positive improvement in student comprehension, confidence, and awareness within their learning. The North American Bsal Task Force Surveillance and Monitoring Working Group developed a CURE called the Student Network for Amphibian Pathogen Surveillance (SNAPS) in 2020-2021 across three institutions. SNAPS incorporates amphibian disease surveillance into undergraduate courses where students learn about amphibian pathogens through the lens of diverse academic disciplines. Students actively contribute to disease surveillance efforts by sampling for two fungal pathogens among local amphibians. Before and after participation in SNAPS, students completed surveys that measured their self-reported knowledge of amphibian disease, interest in the environment, and self-efficacy towards conservation. In the preliminary analysis we found that SNAPS significantly increased students' self-reported knowledge on amphibian disease (Kruskal-Wallis test, Chi-square = 7.9374, p-value < 0.02). For 2022, SNAPS expanded to 29 participating institutions across North America which can allow for the opportunity to further evaluate the impact of SNAPS CUREs on students' learning as well as continue amphibian pathogen surveillance.

Effects of a Neonicotinoid Insecticide on the Growth of Honey Bee Gut Microbes

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The gut microbiome plays an essential role in the overall health of organisms, including important pollinators such as bees. However, the presence and abundance of these microbes may be altered by environmental factors, such as exposure to pesticides, potentially resulting in altered host health. The goals of this project were to understand 1) how widespread pesticide residues are in honey bees in eastern Washington, and 2) whether the honey bee gut microbiome is impacted by pesticide exposure. Accordingly, we tested bees from 24 hives at five sites across eastern Washington using HPLC-MS for the presence and concentration of six commonly used agricultural pesticides; Carbaryl, Chlorpyrifos, Coumaphos, Cypermethrin, Imidacloprid, and tau-Fluvalinate. Five of the six tested pesticides were found in the following percentages across all hives sampled: Imidacloprid (41.7%), Cypermethrin (41.7%), tau-Fluvalinate (12.5%), Chlorpyrifos (4.2%), and Coumaphos (4.2%). The prevalence of hives with detectable pesticides, and to some extent the concentration of pesticide residues in the bees, varied across the different sites. We also found that bees from hives with imidacloprid had significantly different gut microbiomes than bees without the pesticide, based on next-generation 16S rRNA gene amplicon sequencing. Next, we tested the effects of one of these abundant pesticides, the neonicotinoid insecticide imidacloprid, on the growth of honey bee gut microbes in laboratory assays. We sampled bees from two different locations and two time points in eastern Washington, sterilely dissected the mid and hind gut regions, and isolated bacteria on four media types. We identified the isolates by sequencing the 16S rRNA gene, and tested them against different concentrations of imidacloprid in 96-well plate assays. We found that the majority of isolates tested exhibited reduced growth compared to controls, even in low concentrations of imidacloprid. However, several isolates were robust to altered growth in the presence of the pesticide. Our combined field and laboratory results about the host-microbe-pesticides interactions can have important implications for bee, and thus ecosystem, health.

The impact of caspase-8 and STING interactions on extrinsic apoptosis during *Coxiella burnetii* infection

Chelsea Osbron (School of Molecular Biosciences, Washington State University) and Alan Goodman (School of Molecular Biosciences and Paul G. Allen School of Global Health, College of Veterinary Medicine, Washington State University)

Coxiella burnetii is an obligate intracellular bacterial pathogen and the causative agent of the global zoonotic disease Q Fever. A greater understanding of how *C. burnetii* interacts with host immune signaling pathways is needed to develop new, targeted therapeutics. Previous research has shown that *C. burnetii* inhibits host cell apoptosis, but bacterial interactions with extrinsic apoptotic signaling, such as with caspase-8, remain largely unknown. Intriguingly, insect homologs of caspase-8 have been shown to interact with the type I IFN regulator STING to promote immune signaling, but whether this interaction or its significance to immunity is conserved in mammals has not been examined. In this research, we investigate interactions between human caspase-8 and STING, as well as how the activity of these proteins impacts *C. burnetii* infection. We hypothesize that STING and caspase-8, both individually and through their interactions, have roles in regulating apoptotic and interferon signaling that are important during *C. burnetii* infection. To evaluate caspase-8 interactions with STING, we examined the co-localization and physical interactions of STING and caspase-8 in human cells using immunofluorescence microscopy and co-immunoprecipitation techniques. Further, we assessed the impact of STING signaling on caspase-8-mediated apoptosis by western blot and annexin V/PI staining. Finally, we interrogated how this cell death signaling is influenced by *C. burnetii.* The results of this study will aid in uncovering the relationships between STING, caspase-8, and *C. burnetii* infection, thereby providing necessary information for effectively targeting these pathways to enhance the immune response against *C. burnetii*.

Determining the role of obgE during Elementary Body germination in the human pathogen *Chlamydia trachomatis*

Colleen C. Monahan, Hong Yang, Anders Omsland, Paul G. Allen School of Global Health, Washington State University

Chlamydia trachomatis (Ct) is an obligate intracellular bacterial pathogen with distinct serovars. Serovars A-C cause blinding ocular infections while serovars D-L cause sexually transmitted infections. During the life cycle, Ct transitions between the non-replicative, infectious Elementary Body (EB) and the non-infectious, replicative Reticulate Body (RB). The molecular mechanisms that regulate these developmental transitions are unknown. ObgE is a GTPase that is essential for efficient chromosome partitioning and initiation of DNA replication in E. coli. In Ct, obgE is a midcycle gene with transcript first detected at 8hpi and maximal expression observed 16-24hpi. Given the proposed function of obgE in E. coli cell cycle progression and its expression profile in Ct, it is hypothesized that ectopic expression of obgE in Ct will promote EB germination. Ectopic expression of obgE was achieved with Theophylline (0hpi) upon infection of HeLa cells with bacteria carrying an inducible gene construct. Ectopic expression of obgE resulted in a 52% decrease of genome equivalents (GE) at 25hpi and a 72% decrease in infectious EBs at 30hpi. Changes in the production of infectious EBs was measured by infectivity assays while changes in GE was determined by qPCR as a measure of overall replication. These phenotypic effects were dependent on the concentration of Theophylline suggesting the effects of ectopic expression on the production of infectious EBs and GE are directly correlated with obgE expression. The production of infectious EBs and GE will be quantified at 6,12,18, and 25hpi to determine if ectopic expression of obgE specifically affects EB germination. The role of obgE in Ct chromosome partitioning will be explored through qPCR analysis.

Labile Antimicrobial Mechanisms of Manuka Honey Differentially Impact Pathogenic Bacteria

Rayna Carlson and Andrea Castillo, PhD

Manuka honey (MH) has been documented to possess powerful anti-microbial properties through multiple mechanisms. These mechanisms include a low pH, high osmolarity, iron chelation, and its unique manuka factor (UMF), methylglyoxal (MGO). Although MGO is purported to be a major pillar in the honey's antimicrobial properties, its activity is variable against different bacterial species. We hypothesize that MH's other antimicrobial mechanisms may also exhibit variable activity against different bacterial species. Preliminary experiments to determine the Manuka honey minimum inhibitory concentrations (MIC) for bacterial species, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus,* suggested that some Manuka honey antimicrobial mechanisms lose effectiveness over time, but only against some species. To investigate this further, I am conducting MIC experiments weekly with newly prepared MH and aging MH. I hypothesize that the aging MH MICs will increase for bacterial species that are affected by the antimicrobial mechanism that is degraded in the aged MH. In future studies, we will endeavor to compare the chemical composition of the newly prepared and aged MH. This will indicate which MH antimicrobial mechanisms are most effective against the three major pathogenic bacterial species, *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

A possible role for *Citrobacter rodentium* effector Tir in reducing intestinal epithelial cell extrusion

Marin V. Miner, Isabella Rauch

One of the first host responses to intestinal bacterial infections is the process of cell extrusion whereby an infected intestinal epithelial cell is forced into the lumen. Upon infection an innate immune sensor termed NAIP-NLRC4 inflammasome triggers pyroptotic cell death and a cascade of actin rearrangement that causes the cell to be extruded, preventing further infection. Given the critical role that the inflammasome and subsequent extrusion process play in infection it is unsurprising that bacteria employ effectors to interfere with inflammasome function. It has yet to be determined, however, if bacterial secreted effectors, such as *Citrobacter rodentium* Tir, that aim to derail actin structural integrity have an impact on the extrusion process initiated by the inflammasome during infection. We have collected promising data connecting a dysfunctionality of Tir to a reduced extrusion phenotype. Infection of intestinal epithelial cell monolayers with wild type *C. rodentium* greatly increases the percentage of pyroptotic cells in the monolayers when compared to the Tir mutant. These monolayers are likely accumulating dead cells over time that are struggling to extrude. This is indicative of a role for Tir in slowing the process of extrusion through its actin manipulation capabilities. Elucidating the impact of bacterial effectors on host actin rearrangement in the context of inflammasome activation is a crucial and missing component to characterizing how bacteria subvert host defense.

Bacterial Cell Wall Components in Healthy Murine Brain Fluctuate with Rest and Activity Cycles

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Bacteria, their components, and their products have important roles in host physiology. The interactions that underlie these roles result from coordination of molecular events and signaling between microbes and the host and are often rhythmic in nature. Rhythmicity is reflected in daily oscillations of microbiome composition, metabolic activity patterns of both the host and the microbiome, and the release of microbial products into the host circulation. These processes are susceptible to perturbation from altered host behaviors including sleep loss. Bacterial cell wall components, peptidoglycan (PG) and muramyl peptides (MPs), isolated from mammalian brains and urine following sleep deprivation (SD), promote non-rapid eye movement sleep. These PG/MPs likely originate from the host microbiome and are present in neonatal murine brain, where their concentration increases in the first weeks of life alongside colonization of the gut. PG/MP amounts and dynamics in healthy, adult murine brain have recently been reported, yet a full description linked to rest and activity cycles is lacking. Wildtype mice acclimated to standard lab conditions were sacrificed at Zeitgeber time (ZT) 3, corresponding to peak rest; ZT6, corresponding to the middle of the rest period; ZT12, corresponding to the light to dark transition; and ZT15, corresponding to peak activity (n= 8). At times corresponding to peak rest and activity, additional groups of mice were sacrificed following 3h sleep deprivation (n= 8). Hypothalamic (HT), somatosensory cortex (Sctx), brain stem (BS), and olfactory bulb (OB) areas were dissected, homogenized in phosphate buffered saline, and centrifuged. PG contents in resultant supernatants were determined using an ELISA (MyBioSource), sample PG interpolated from the standard curve, and values expressed as ng PG per mg tissue wet weight (ng/mg). In all brain areas, PG levels varied with time of day, with lowest PG concentration occurring at ZT12 when mouse sleep duration is lowest. BS PG values were significantly higher than PG values for all other brain areas at all times of day. At ZT3, mean PG values from control mice were: 3.6 in HT, 3.7 in Sctx, 4.8 in OB, and 8.6 ng/mg in BS. After 3h SD ending at ZT3, corresponding values were: 3.0, 4.8 (statistically significant increase, $p<0.05$), 3.9, and 7.5 ng/mg. Further, after SD ending at ZT3, PG values in Sctx were higher than corresponding HT values in all eight individual mice ($p<0.001$). At ZT15, PG control values were: 4.6 in HT, 4.6 in Sctx, 4.5 in OB, and 8.9 ng/mg in BS. After SD ending at ZT15, PG levels were not significantly changed in any brain area assayed. However, compared to PG values after SD ending at ZT3, values at ZT15 were significantly higher for HT and BS ($p<0.0005$ and $p<0.005$, respectively). Results indicate PG fluctuates with rest and activity in healthy, adult murine brain, has unique regulation by brain area, and is altered following sleep loss, suggesting brain PG is involved in host physiology and behaviors such as sleep. Thus, the dynamic nature of bacterial products in mammalian brain are consistent with PG involvement in sleep-wake regulation and various associated cognitive states. The observed changes are suggested to be the product of millions of years of co-evolutionary symbioses between microbes and their hosts i.e., representing the mammalian holobiont.

Disrupting SARS-CoV-2 infection with alpaca-derived antibody fragments

Jules B. Weinstein[1], Tim A. Bates[1], Michelle Garcia[1], Savannah K. McBride[1], Hans C. Leier[2], Eric Barklis[1], Fikadu G. Tafesse[1]

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The ongoing SARS-CoV-2 pandemic highlights a need for basic research of coronavirus replication to drive novel therapeutic development. A promising technology to address this is antiviral alpaca-derived antibody fragments. These single-domain antibodies, known as VHHs or nanobodies, exhibit binding capabilities comparable to full-sized antibodies composed of heavy and light chain domains. VHHs also are highly stable and retain their function even in reducing intracellular environments. To exploit these qualities, we raised VHHs capable of blocking viral entry and replication by immunizing an alpaca with purified recombinant spike (S) glycoprotein and nucleocapsid (N). We then used phage display to pan for high-binding VHHs against different portions of S and N. From our S binding candidates, we isolated an RBD specific VHH that neutralizes both S-pseudotyped lentivirus and live SARS-CoV-2 at nanomolar concentrations. This VHH, saRBD-1, also neutralizes multiple SARS-CoV-2 strains with little loss of efficacy, including the previously circulating Delta variant. From our N binding candidates, we have isolated VHHs with affinity and specificity towards the globular N and C terminal domains. One family of these candidates reduces viral progeny when stably expressed in cell culture lines through a mechanism not yet fully understood. Next, I hope to discern the mechanism of the inhibitory N-specific VHHs by identifying their target epitopes and studying their ability to block crucial N functions including RNA binding, multimerization, and phase separation. Further development of these nanobodies will help us move towards pan-coronavirus nanobody therapeutics.

Merocytophagy contributes to dissemination of *Francisella tularensis* and is enhanced by SYK signaling in macrophages

Kelly Deobald, Shaun Steele, and Tom Kawula. Washington State University Paul G. Allen School for Global Health

Merocytophagy is a phagocytic process characterized by the transfer of cytosolic content between cells and the formation of a double-membraned vesical within recipient cells. Studies conducted in our laboratory have demonstrated that proteins and intracellular pathogens can be transferred between cells by this contact-dependent mechanism. In particular, *Francisella tularensis*, a highly infectious pathogen that spreads from cell to cell by hijacking the phagocytic pathway in macrophages, has been shown to replicate within cells that receive cargo via merocytophagy. While these observations may point to an alternative route of *F. tularensis* dissemination in vivo, it is also possible that this mechanism of bacterial spread could amplify immune responses. Ongoing studies aim to characterize molecular and cellular players in merocytophagy to gain a better understanding of this phenomenon and to develop strategies to assess merocytophagy in vivo in the future. In cell-to-cell contact studies, inhibition of spleen tyrosine kinase (SYK) expression and activity showed decreased cargo transfer by merocytophagy. Pharmacologic inhibition of SYK was also associated with decreased surface expression of several integrins, giving evidence that cell-to-cell contact is stabilized by integrins during merocytophagic transfer in macrophages.

Dissecting the unfolded protein response gene regulatory network in *Ixodes scapularis* during infection

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In 2019, over 50,000 tickborne disease cases were reported to the CDC. Of these cases, 68% were caused by the Lyme disease spirochete, *Borrelia burgdorferi*, and 15% due to *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. Both pathogens are transmitted by the North American deer tick, *Ixodes scapularis*. The majority of research on arthropod immunity has been modeled in *Drosophila*, however, arthropod immunity is divergent across species. How *I. scapularis* immunity influences acquisition, maintenance, and transmission in these pathogens is incompletely understood. The unfolded protein response (UPR), a cellular stress response, is comprised of three transmembrane receptors, IRE1Œ±, PERK, and ATF6, and has recently been linked to innate immunity in ticks. This led to our current question; what is the UPR gene regulatory network that responds to infection and how does it influence pathogen dynamics in ticks? Using a UPR reporter plasmid assay, we determined that ATF6 and NRF2, a transcription factor downstream of PERK, were activated in response to both *B. burgdorferi* and *A. phagocytophilum* infection. To predict ATF6 and NRF2 regulated genes, we designed a computational transcription factor binding site prediction model of the *I. scapularis* genome. We identified 263 genes that were predicted for NRF2 and 22 genes for ATF6. Orthologs were identified through NCBI BLAST and used in combination with Enrichr pathway analysis to identify putative biochemical and cellular pathways responding to bacteria. Our results indicate that stimulator of interferon genes (STING) and ER-associated degradation (ERAD) may be involved in this response. Overall, we have generated a novel approach for predicting gene regulatory networks through a combination of surrogate expression systems and computational framework that can be applied to other transcription factors in non-model organisms.

Euo a Repressor of Late Gene Expression in *Chlamydia trachomatis*

Cody Appa, Nicole Grieshaber, Scott Grieshaber. University of Idaho

Chlamydia trachomatis is the most prevalent bacterial sexually transmitted infection globally with over 1.5 million cases in the United States alone in 2020. Untreated chlamydial infections lead to ectopic pregnancy, sterility, and birth defects in women. It also can lead to trachoma, an infection of the mucosal tissue surrounding the eye. This infection is the leading cause of preventable bacterial blindness in the world and is especially hard hitting in regions without adequate access to antibiotic treatments. All *Chlamydiae* species rely on a complex multicellular developmental cycle involving transitioning between non-infectious replicative bodies, the reticulate bodies (RBs) and infectious, non-replicative bodies the elementary bodies (EBs). To transition between these two cell types a non-replicative, non-infectious cell form known as the intermediate body (IB) has long been thought to be a transitory body baring little significance. Expression of the protein Euo has long been thought to repress development in the RB amplification phase of development. Through ectopic expression of plasmid vectors, automated live cell microscopy, confocal microscopy, IREP assays, and IFU (inclusion forming unit) assays. We have determined that Euo arrests the cycle in the IB phase and not the RB phase of development.

The Type IV secretion effector BspA interferes with MARCH6-dependent ER-associated degradation to promote *Brucella* intracellular growth

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Brucella abortus, the causative agent of brucellosis, is a Gram-negative facultative intracellular pathogen that relies on Type IV secretion (T4SS) effector-mediated modulation of host cell functions to establish a replicative niche, the Brucella-containing vacuole (BCV). Upon infection, *Brucella* exploits the host endocytic, secretory and autophagic compartments to facilitate its intracellular persistence, proliferation, and dissemination. A recent report has suggested a role for the host endoplasmic reticulum (ER) quality control system in *Brucella* intracellular cycle, as a newly characterized effector, BspL, was found to control bacterial egress at the late stages of infection by enhancing the ER-associated degradation pathway (ERAD). However, this effector was not required for intracellular growth. Here we show that another T4SS effector, BspA, also engages the ERAD machinery yet to promote *Brucella* intracellular proliferation. We demonstrate that BspA is required for *Brucella* replication in bone marrow-derived macrophages (BMMs) and interacts with the host protein MARCH6, a membrane-associated E3 ubiquitin ligase involved in host ER proteostasis. While siRNA-mediated silencing of MARCH6 in BMMs does not significantly affect the intracellular replication of wild-type *Brucella*, it does restore the replication of the bspA deletion mutant, which suggests that BspA interferes with MARCH6 function. Furthermore, pharmacological inhibition of ERAD with the EDEM I inhibitor Kifunensine and siRNA depletion of another ERAD component, UbxD8, exert the same effect on the bspA deletion mutant. Altogether, these findings suggest that BspA is modulating MARCH6 function to inhibit the host ERAD pathway and promote *Brucella* intracellular growth. Consistently, ectopic expression of BspA in BMMs stabilizes established ERAD- and MARCH6-specific substrates, indicating ERAD inhibition. Finally, ectopically expressed BspA displaces UbxD8 from the MARCH6 ubiquitin ligase complex in HeLa cells, suggesting that BspA interferes with MARCH6-UbxD8 interaction in order to inhibit ERAD. Altogether, our data reveal that targeting ERAD components by Type IV effectors emerges as a multifaceted theme in *Brucella* intracellular pathogenesis.

Disrupting SARS-CoV-2 infection with alpaca-derived antibody fragments

Jules B. Weinstein[1], Tim A. Bates[1], Michelle Garcia[1], Savannah K. McBride[1], Hans C. Leier[2], Eric Barklis[1], Fikadu G. Tafesse[1]

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Dimmer or Switch: Illumination of quorum-sensing gene activation in *Pseudomonas aeruginosa*

Bryce Pettit Estell, Parker Neal Smith, Martin Schuster, Department of Microbiology, Oregon State University, Corvallis, OR

Pseudomonas aeruginosa is an opportunistic pathogen that utilizes quorum sensing (QS) via diffusible signal molecules to activate the expression of many genes in a cell density-dependent manner. It is not clear whether QS gene activation is either a threshold response (switch-like) or a gradual response (dimmer-like) to increasing cell density. Our work investigates this fundamental behavior, using both batch and continuous culture formats with a minimal growth medium. QS induction was measured via the use of a reporter plasmid containing the promoter of the lasI gene, encoding the signal synthase, fused to green fluorescent protein (GFP). Preliminary batch culture experiments with varying concentrations of succinate as the limiting carbon source showed a step-wise, density-dependent increase of lasI-gfp, with an apparent threshold between 0.25 and 0.5% of succinate. Analogous experiments in continuous-culture chemostats are under way to quantify lasI-gfp expression in response to cell density. In chemostats, bacteria grow under constant, steady-state conditions, allowing us to assess the QS response type and its stability more clearly. Our work will provide insights into the emergent properties of a cell-cell signaling pathway in bacteria, with implications for pathogenesis and synthetic biology.

Novel viruses of the family *Partitiviridae* discovered in *Saccharomyces cerevisiae*

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A large-scale screen to determine the diversity of double-stranded RNA (dsRNA) viruses in the model yeast *Saccharomyces cerevisiae* identified novel viruses from the family *Partitiviridae*. Most S. cerevisiae partitiviruses (ScPVs) were found associated with strains of yeasts isolated from coffee and cacao beans. The existence of partitiviruses was confirmed by sequencing the viral dsRNAs and purifying and visualizing isometric, non-enveloped viral particles. ScPVs have a typical bipartite genome encoding an RNA-dependent RNA polymerase (RdRP) gene and a coat protein (CP) gene. Phylogenetic analysis of ScPVs identified three species of ScPV, which are most closely related to viruses of the genus *Cryspovirus* from the mammalian pathogenic protozoan *Cryptosporidium parvum*. Molecular modeling of the ScPV RdRP revealed a conserved tertiary structure and catalytic site organization compared to the RdRPs of the Picornaviridae. The ScPV CP is the smallest identified in the *Partitiviridae* and has structural homology to those of other partitiviruses. ScPV CP appears to lack a protrusion domain that is a conspicuous feature of other partitivirus particles. It is possible to transfer ScPVs to laboratory strains of *S. cerevisiae*, which provides future opportunities to study partitivirus-host interactions using the powerful genetic tools available in this model organism.

Manuka Honeys' Impacts on Bacterial Persister and VBNC Formation in Key Cutaneous Pathogens

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Long-term antibiotic use has allowed microbes to develop resistance and cause chronic infectious diseases that were previously treatable. Resistance is a phenomenon in which bacteria survive treatment with a concentration of antibiotics that was once lethal. Bacteria resist antibiotic treatment by genetic change or initiating states of dormancy called persisters or Viable but Non-Culturable (VBNCs) cells. These subpopulations of antibiotic resistant persisters and VBNCs increase with exposure to stresses, including antibiotic treatment, and are a major cause of reoccurring infections that result in significant morbidity and mortality. Manuka honey (MH), a well-known alternative broad-spectrum antimicrobial, is a promising treatment for cutaneous infections. Although genetic resistance to MH has yet to be detected in bacteria, it remains unknown on how MH impacts persister and VBNC formation. In this study, we quantified persister and VBNC cells induced by MH treatment in the clinically relevant bacterial species, Staphylococcus aureus and Pseudomonas aeruginosa. At 1/2 MIC concentrations, MH did not cause a significant increase in percent persisters in either species when compared to untreated samples or those treated with conventional antibiotics. MH did significantly reduce the percent VBNCs formed in S. aureus when compared to those treated with conventional antibiotics, however, not in comparison to the untreated group.

Investigation of mechanisms used by the *Brucella* type IV secretion effector BspF to modulate vesicular transport at the recycling endosome

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Intracellular bacterial pathogens modulate their host cell environment to promote their survival, proliferation, and dissemination. Species of the genus *Brucella* are the causative agents of brucellosis, one of the most commonly encountered zoonotic diseases worldwide. To proliferate within mammalian cells, Brucella generates a replicative Brucella-containing vacuole (rBCV) and expresses a Type IV Secretion System (T4SS) that functions as a molecular syringe to deliver effector proteins into the host cell during infection. The rBCV membrane is generated from host endoplasmic reticulum (ER) membranes in a T4SS-dependent manner, indicating that Brucella modulates host vesicular trafficking via delivery of T4SS effector proteins. The T4SS effector protein, BspF, promotes Brucella replication via modulation of plasma membrane to Golgi traffic at the recycling endosome by inhibiting an Arf6-/Rab8a-dependent GTPase cascade resulting in trans-Golgi network (TGN)-derived vesicle recruitment to the rBCV. Here, we investigated the mechanism by which BspF modulates host retrograde transport to promote replication. BspF contains a Gcn5-N-acetyltransferase (GNAT) domain and both self-acetylates and precipitates with acetylated-lysine containing proteins. Mutations to conserved residues within BspF's GNAT domain suppress BspF-dependent blockage of retrograde transport and increase binding to ACAP1 which in turn affects Arf6 activation. In addition, BspF interacts with two Brucella effector proteins, BspH and BspI. BspF, BspH, and BspI colocalize on Rab5-positive early endosomes and stabilize the tubular recycling endosome compartment, suggesting that a BspF/BspH/BspI effector complex remodels early endosomes to contribute to BspF-dependent disruption of vesicular transport. These findings suggest BspF has acetyltransferase activity, providing a potential mechanism by which BspF may alter the Arf6-/Rab8a-GTPase cascade to promote Brucella replication, and identify BspF as part of a novel effector protein complex that may contribute to BspF-dependent modulation of plasma membrane to Golgi network vesicular transport.

The Role of the Gut Microbiome in Intestinal Colonization with Extended Spectrum Œ≤-Lactamase-Producing *Escherichia coli* in Healthy Children

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Antimicrobial resistance is one of the major public health concerns. Globally in 2019, an estimated 4.95 million deaths were associated with antibiotic-resistant bacterial infections. The most troubling types of antibiotic resistant organisms consist of extended-spectrum Œ≤-lactamase (ESBL)-producing *E. coli* (ESBL-Ec), considered by the CDC to pose significant health risks. Intestinal carriage of ESBL-Ec has been rising at alarming rates in low-income countries, and carriage of these organisms is associated with an increased risk of drug-resistant infections, treatment complications, and mortality. Our previous study found that ESBL-Ec colonizes 74% of healthy infants aged 1-12 months in Bangladesh. Increasing this concern, colonization with ESBL-Ec enables antimicrobial resistance genes (ARGs) to be exchanged with other bacterial species in the gut via horizontal gene transfer. In this study, we aimed to understand the mechanism of the persistence of ESBL-Ec in the gut by analyzing the stool samples from healthy children in Bangladesh with and without ESBL-Ec carriage. We hypothesize that the composition of an individual's gut microbiota has a vital role in ESBL-Ec colonization among healthy children, which is mediated by the selection and expansion of metabolically dominant resistant clones. Our preliminary metagenomic analysis of 23 stool samples, 11 positives for a higher number of ESBL-Ec (>103 CFU/g stool), revealed that ESBL-Ec positive stool samples have a relatively less diverse bacterial population compared to ESBL-Ec negative samples. We identified the differentially abundant organisms in ESBL-Ec positive samples that might have roles in high carriage of ESBL-Ec. Further analysis will investigate the differentially abundant bacterial populations in these samples along with the identification of ARGs and metabolic genes which may shape the gut microbial composition and effect ESBL-Ec carriage.

Differential Restriction of Viral Lifecycles by the Type VI CRISPR System

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Bacteria have evolved diverse immune systems to interfere against viral predation, but phages can also provide fitness advantages to their prokaryotic hosts. In the case of temperate phages, bacteria can benefit from infection when antibiotic resistance genes, toxins, and phage-defense systems are transferred from phage to host. Some immune systems may restrict lytic predation while allowing lysogeny by targeting hallmarks of lytic replication e.g., sensing of portal / capsid proteins. Some immune systems, however, seem incapable of differential lifecycle restriction as they detect phage nucleic acid i.e., CRISPR-Cas and restriction modification (RM). While most CRISPR systems sense viral DNA, others sense viral mRNA transcripts. By sensing RNA, these CRISPR systems respond to specific transcriptional programs rather than the phage itself. Thus, RNA-sensing CRISPR systems can be uniquely tolerant of temperate phage integration. The type VI CRISPR system employs the RNA targeting, RNA cleaving nuclease Cas13 to restrict phages. Upon activation, Cas13 cleaves both phage and host RNA, resulting in a reversible cell dormancy that is incompatible with phage replication. While bacteria can benefit from both temperate phages and effective immune systems, the interactions of the two remain poorly understood. Here, using a marked phage and an array of Cas13-targeting constructs, we demonstrate that the type VI CRISPR system can restrict lytic replication of temperate phage while allowing lysogeny.

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Cooperation is ubiquitous in nature, and yet challenging to explain evolutionarily through a Darwinian ″survival of the fittest″ lens. This is because cooperating requires the cooperator to take on an additional cost, reducing their fitness. Many mechanisms to sustain cooperation have been identified, such as kin selection and reciprocal altruism, however, models for these methods are often phenomenological in nature and are therefore missing important biological processes. The chemostat is a continuous bacterial culturing device that also provides a way to mathematically model the growth and interactions of bacteria mechanistically. A chemostat nicely reflects larger ecological systems in that it has an inflow of nutrients of and an outflow of the mixed solution containing the bacteria and nutrient (which models a naturally occurring turnover of nutrients and death, emigration, and predation of the species). This relationship between the chemostat and larger ecological systems allows us to utilize it to test some of these fundamental evolutionary questions about cooperation. Here, we have taken a mechanistic modeling and empirical approach to explore the evolution of cooperation in Pseudomonas aeruginosa, which produces an extracellular metabolic enzyme as a public good. Both the empirical and mathematical modeling work showed that the cooperating enzyme-producing bacteria can sustain their population when grown independently, however, when a cheating strain is introduced that does not produce the enzyme but benefits from its presence, a Tragedy of the Commons occurs and the population collapses. Our work experimentally validates the frequently predicted Tragedy of the Commons in a microbial population, quantifies and parametrizes a social behavior, and underscores the importance of integrating empirical and theoretical approaches to understanding a given biological system.

A phage endolysin produced by recombinant Escherichia coli exhibits bacteriostatic activity against Cutibacterium acnes

Kathryn M Collins and Luis Matos

Endolysins are highly conserved enzymes utilized by bacteriophages to disrupt the bacterial host cell wall and release the phage progeny. Endolysins have shown high bacteriostatic activity in vitro and have shown to be safe for topical application and oral ingestion. Therefore, endolysins could be a viable anti-bacterial agent against *Cutibacterium acnes* proliferation in acne vulgaris. We propose that the endolysin from the C. acnes P100.1 bacteriophage, which belongs to the amidase family of enzymes, will have bacteriostatic activity against C. acnes. The P100.1 endolysin gene was codon optimized for Escherichia coli, and the DNA was synthesized. The gene was cloned into the pET11a expression vector and expressed in the BL21 (DE3) E. coli strain. The lysate from induced and uninduced (negative control) cultures were filtered and analyzed for bacteriostatic activity by measuring its effect on bacterial growth (turbidity) of C. acnes over time. The C. acnes cultures grown with the lysate from induced E. coli (+ENDL) exhibited a significant reduction in turbidity ($p<0.05$) when compared to C. acnes grown in the presence of uninduced lysate (-ENDL). These preliminary results suggest that ENDL has potential as a bacteriostatic agent against C. acnes proliferation. In future studies, we will characterize the purified ENDL stability in storage and its activity at various temperatures, concentrations, and over different time periods. Additionally, we will determine the enzymes' host specificity.

Novel Brevinin-2 Peptide Design, Prediction, and Synthesis

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With more than 100 known members, the anuran Brevinin-2 peptide family exhibits a broad range of antimicrobial activity against Gram-positive and -negative bacteria, including antibiotic-resistant strains. Despite some variability, these peptides are largely cationic, amphipathic, and Œ±-helical with a conserved disulfide Rana-box motif (C-K-X4-C) at the C-terminus. Although electrostatic interactions and the hydrophobic effect are thought to guide the membranolytic activity of most antimicrobial peptides, the precise mechanism of selectivity for the Brevinin-2 family remains unclear. Indeed, several factors inhibit the development of these peptides as therapeutic drugs: a high experimental hemolytic activity, a poorly conserved primary sequence, an incomplete understanding of the factors influencing the peptide-membrane interaction, and a dependence on local environmental conditions. To investigate the factors guiding the Brevinin-2 membrane interaction, we designed a novel set of four representative Brevinin-2 consensus sequences from the positional residue frequency of 80 natural sequences. We predict that these consensus sequences are cationic, amphipathic, and Œ±-helical in a membrane environment using available computational tools. We will obtain high-purity synthetic peptides that maintain the conserved Rana-box motif by employing standard Fmoc solid phase peptide synthesis, fast protein liquid chromatography, and MALDI-TOF mass spectrometry. We will determine critical endpoints as a measure of specificity by quantifying the peptides' activity against a panel of antibiotic-resistant bacteria, toxicity against erythrocytes, and killing kinetics under different environmental conditions. By concurrently investigating the peptides' structural criteria and binding mechanism, we will posit the conditions necessary to elicit a specific endpoint such that the next generation of peptides can be designed, synthesized, and evaluated. Ultimately, we aim to provide a recursive framework for future Brevinin-2 peptide design capable of predicting peptide specificity and thus enhance the development of these peptides as therapeutic drugs.

A Partnership Between Tribal Colleges and Montana State University to Create STEM CUREs

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Many students face a difficult transition when transferring from smaller 2-year colleges to larger 4-year institutions. In order to support the success of students, who transfer from tribal colleges to Montana State University, we first created strong relationships with instructors and administrators at Montana's tribal colleges. Subsequently, collaboratively with faculty from Montana's 7 tribal colleges, we developed course-based research experiences (CUREs) targeted at tribal college students in STEM who wish to transfer. This CURE offers an introduction to research that will help students transfer and inspire participation in undergraduate research. The CURE uses zebrafish as model to study developmental biology in the contexts of both human and environmental health. We have conducted this course at MSU for 8 years (2015- 2022) and have brought the course to 6 tribal colleges: Aaniiih Nakoda College, Chief Dull Knife College, Fort Peck Community College, Blackfeet Community College, Stone Child College, and Little Big Horn College. We have also taught at Gallatin College and Great Falls College, which are 2-year institutions in the Montana system. Offering the course at different campuses allows us to serve students in their local communities, while the courses at MSU give them the opportunity to visit the 4-year campus they might attend in the future. We observed the biggest positive impact on the students' self-assessment of their ability to think like a scientist and work like a scientist and their overall research skills. We measured significant improvement in laboratory skills and skills associated with presenting their research. A future goal of this program is to facilitate creation of additional CUREs hosted by tribal college faculty. Additional goals are to bring the original CURE to tribal colleges outside the State of Montana and to invite out-of-state tribal college students to participate in the CURE at MSU. This program is funded by the NSF.

The Molecular Mechanism of Cellular Attachment for an Archaeal Virus

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Sulfolobus turreted icosahedral virus (STIV) is a model archaeal virus and member of the PRD1-adenovirus lineage. Although STIV employs pyramidal lysis structures to exit the host, knowledge of the viral entry process is lacking. We therefore initiated studies on STIV attachment and entry. Negative stain and cryo-electron micrographs showed virion attachment to pili-like structures emanating from the Sulfolobus host. Cryo-electron tomography and sub-tomogram averaging revealed pili recognition by the STIV C381 turret protein. Specifically, the triple jelly roll structure of C381 determined by X-ray crystallography shows that pilus recognition is mediated by conserved surface residues in the second and third domains. In addition, the STIV petal protein (C557), when present, occludes the pili binding site, suggesting that it functions as a maturation protein. Combined, these results demonstrate a role for the namesake STIV turrets in initial cellular attachment and provide the first molecular model for viral attachment in the archaeal domain of life.

Evolving Phages to Treat American Foulbrood Disease in Honey Bees

Keera Paull (University of Idaho, undergrad), Emma Spencer (University of Idaho, lab tech), James T. Van Leuven (University of Idaho, research professor)

In 1915, bacteriophages (phages) were discovered and characterized as infecting and killing bacteria. Phages are now known to be very specific to which bacterial species and strains they can infect. This makes them a potential way to treat pathogenic bacterial infections' process called phage therapy. My research applies phage therapy techniques to a bacterial pathogen in honey bees called American Foulbrood Disease (AFB). Hives infected with *Paenibacillus* larvae, the causative species of AFB, are greatly weakened, often dying within the season. Infected hives are often burned to prevent the spread of this bacterial pathogen. However, my research with phage therapy offers a less destructive treatment to AFB. I currently work with eight P. larvae strains and eight AFB-specific phages. Phages differ in how well they can infect and lyse different AFB strains and, conversely, some P. larvae strains are resistant to many phages. An effective phage therapy treatment contains a cocktail of phages that, in combination, can kill a broad range of P. larvae strains. To develop such a cocktail, I have evolved phages together on multiple P. larvae strains using the Appelmans protocol. This method of evolving phage by passaging promotes valuable phage recombination each round of growth and inclusion of novel phage at low concentrations. The ability of P. larvae strains to develop resistance to specific phages creates an obstacle to successful phage therapy. To combat this, I am using P. larvae strains that have evolved phage resistance in my current Appelmans trial. My goals are twofold: to produce a phage cocktail that will be successful against most P. larvae strains, including resistant ones, and give insight to how the Appelmans protocol can best be used to evolve phage. With this research, I hope to establish phage therapy as an efficient and effective treatment to AFB.

Host Range Expansion of Phage Against *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a bacteria that commonly infects wounds and the lungs of people with cystic fibrosis. While generally treated using antibiotics, P. aeruginosa readily evolves multi-drug resistance, making it a difficult infection to clear. An alternative uses bacteriophages to specifically target and eliminate the infectious bacteria. This is called phage therapy. Strain diversity of pathogenic bacteria presents a challenge to phage therapy treatment because most natural phages are specific to only a narrow range of host strains. My research focuses on this challenge by evolving P. aeruginosa specific phages to have broader host ranges. To accomplish this, I used the Appelmans protocol. This method for phage host range expansion promotes recombination between phages through passaging and selects for phages that are successful at infecting multiple hosts. I started with a "cocktail" of three *P. aeruginosa*-specific phages with different host ranges, and five unique P. aeruginosa strains, two of which were resistant to all three phages at the start. After one round of selection, the phage cocktail expanded its host range to the two resistant strains. After completing nine rounds, all *P. aeruginosa* strains were killed at lower phage cocktail concentrations than at the beginning of the experiment. Host range analysis of individual phage isolates from the endpoint cocktail revealed that some phages retained their ancestral host range while others expanded the ability to infect a new host. Three phage isolates gained the ability to infect resistant P. aeruginosa strains but lost the ability to infect strains they previously could infect. This demonstrates that there are evolutionary tradeoffs to an expanded host range. Fully understanding tradeoffs like this could be a breakthrough in the development of broad host range phage for phage therapy applications in the future.

The effect of rugose small colony variants on *Pseudomonas aeruginosa* biofilm dispersion

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One of the most common sources of hospital-acquired infections and the leading cause of chronic infections in cystic fibrosis patients is the opportunistic pathogen *Pseudomonas aeruginosa*. Pathogens such as P. aeruginosa exist in structured, bacterial communities encased in a self-produced matrix known as biofilms. These biofilms provide protection to the bacteria, which is largely why *P. aeruginosa* is so difficult to treat. There are several stages to biofilm development with the final stage being dispersion. During dispersion, bacteria leave the biofilm. Dispersion is a growing area of interest for researchers as bacteria are more susceptible to antibiotic treatment and immune cell clearance than biofilm cells. P. aeruginosa develops a unique phenotype called the rugose small colony variant (RSCV). This phenotype is characterized by an overproduction of the polysaccharides that make up the matrix. Studies reveal that this phenotype arises in cystic fibrosis patients. Little information is known about RSCVs and dispersion. My research focuses on finding out whether RSCVs disperse in response to stopped flow. To investigate this question, biofilms were grown over the course of five days using a tube reactor system. This apparatus supplies bacteria with a constant source of nutrients and flushes out their waste products to create an ideal environment for biofilms to grow. To induce dispersion, the flow of nutrients to the biofilm is stopped. RSCV forming strains were grown in the reactor to compare their dispersion responses to control strains. Samples were collected from the reactor, serial diluted, and the colony forming units (CFUs) were counted. We found that biofilms formed from RSCVs release less bacteria when dispersion was induced. The data suggests that RSCVs do not disperse. Future work may be directed towards understanding what aspects about RSCVs cause them to release less bacteria even when signaled to disperse.

Inflammatory discordance between the small molecule C3aR inhibitor SB 290157 and genetic ablation of C3aR in activated primary bone marrow macrophages

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Complement receptor C3aR is activated by its cognate ligand C3a and subsequently modulates innate immune cell inflammatory state, inflammasome activation, and bacterial infection outcome. C3aR is a putative inhibitory G protein-coupled receptor (GPCR) that is highly expressed on monocytes and macrophages, important innate immune effector cells that help initiate and maintain inflammation during bacterial infection. Our previous research identified a novel role for C3aR in modulating the production of inflammatory cytokines by macrophages downstream of TLR4 activation via LPS challenge. Specifically, we found C3aR1-/- knock-out mice exhibited decreased expression of Il-6 in the blood in response to systemic LPS, compared to WT mice. Further, we found in primary human macrophages treated with SB 290157, a small molecule inhibitor of C3aR (C3aRi) showed decreased IL-6 and TNF production when challenged with LPS. We concluded that C3aR activation is required for wild-type levels of inflammatory cytokine production downstream of LPS challenge in macrophages. Currently, it is unknown how C3aR activation is modulating TLR4-dependent cytokine production in macrophages. Here we aim to understand the mechanistic role of C3aR within macrophages by studying both C3aR1-/- BMDMs and WT BMDMs treated with C3aRi or Vehicle (Veh) alone. In agreement with our previous findings, we find that WT BMDMs treated with C3aRi display significantly decreased relative expression and fold-change expression of Il-6 and IfnŒ≤ and IL-6 secretion after LPS treatment, compared to Veh-treated BMDMs. In contrast, C3aR1-/- BMDMs challenged with LPS show significantly increased relative expression and a significant increase in IL-6 secretion, compared to WT BMDMs, suggesting that the inhibitor of C3aR and genetic ablation are inducing discordant inflammatory outcomes downstream of LPS. However, C3aR1-/- BMDMs still showed decreased fold-change expression of Il-6, IfnŒ≤, and tnf after LPS challenge, compared to WT BMDMs. Further, C3aR has been shown to mediate metabolic homeostasis in T cells; however, it is unknown whether C3aR ablation or inhibition in BMDMs affects basal metabolism or LPS-induced metabolic changes. Here we show mitochondrial respiration is differentially regulated in C3aR1-/- BMDMs compared to C3aRi-treated BMDMs. Basal and LPS-induced oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) differ significantly between C3aR1-/- BMDMs and WT BMDMs treated with C3aRi, suggesting genetic ablation of C3aR and inhibition of C3aR perturbs metabolic homeostasis that leads to dissimilar phenotypes in BMDMs. Thus, our data further cautions against the use of C3aRi as the sole tool to delineate C3aR biology and function in BMDMs, and highlights the continuing need to develop true C3aR antagonists for therapeutic use.

Killer Yeast as a Biological Control Against Spoilage Caused by Diastatic Strains of *Saccharomyces cerevisiae*

Zhenhao Zhong, Ximena Garcia, Dr. Paul A. Rowley

Diastatic yeast contamination is a problem that many craft-brewing companies struggle with as it can prolong fermentation after packaging, increasing gravity, off flavors, and the explosion of cans and bottles. Diastatic yeasts are a strain of *Saccharomyces cerevisiae* that contain the STA1 gene that allows these strains to hydrolyze residual carbohydrate polymers of dextrin and starch. Killer yeasts have the unique ability to release protein toxins that can inhibit the growth of competing yeast. In this study, we tested 8 canonical killer yeast against 38 diastatic strains and discovered that the killer toxin K1 was effective at inhibiting 89.7% and K2 inhibited 55% of these spoilage yeasts. Four killer toxin-resistant strains of diastatic yeasts were found to secrete killer toxins that were identified as K2 by reverse transcriptase PCR. The production of K2 by diastatic yeasts would possibly explain the reason why these strains were resistant to K2. These four resistant strains were tested against a collection of 200 unknown killer yeast and were susceptible to ten or more of the novel killer yeast. Based on their spectrum of activity it appears these unknown killer toxins spectrum of activity are similar to K2 toxin, this indicates that these novel killer yeast strains may produce a variant K2 that is effective against strains that are resistant to the canonical K2. Fermentation trials have shown that K1 killer yeasts are also effective in inhibiting the growth diastatic yeast in a simulated contamination event, without affecting the gravity of the beer. Therefore, killer yeasts appear to be an effective intervention to prevent the spoilage of craft beers by diastatic yeasts. Continued collaboration with Rhinegeist breweries will enable the further commercialization of this novel biological control.