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Abstract Book

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UNDERGRADUATE STUDENT ORAL PRESENTATIONS

1. Reducing Chromosomal Antibiotic Resistance in *Escherichia coli*

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Midwestern State University

Antibiotic resistance is an expanding clinical challenge. Bacteria often gain resistance via horizontal transfer of plasmids with resistance genes. Plasmid-located resistance is lost when the bacteria are cured of plasmids. However, clinical studies demonstrated that chromosomally located resistance elements can also be lost. Lacey, et al. concluded that a strain of *Staphylococcus aureus* lost chromosomal resistance genes after treating patients with cloxacillin, an antibiotic to which the strain was susceptible. We hypothesize a similar loss of resistance could occur in Gram-negative bacteria such as *Escherichia coli*. We further hypothesize that the targeting mechanism of the drug influences reversion to susceptibility. Other students the lab isolated an *E. coli* strain (QH020816-1.1) from seagulls that is erythromycin and tetracycline resistant. When cured of its plasmids using Acridine Orange, the strain lost its resistance to tetracycline. The plasmid-cured strain was pressured with 0.5 × minimum inhibitory concentration of tetracycline, as determined by an E-test, and subcultures were obtained every 24 hours for 10 days. Erythromycin resistance in the tetracycline-treated samples was assayed using the Kirby-Bauer disc test. We observed a linear increase in the zone of inhibition over days 1-5, which plateaued at ~20mm days 6-10. PCR and gel electrophoresis analysis of the erythromycin resistance gene in the strain before and after treatment revealed an insertion in the gene. Current efforts include characterization of this insertion. To test our hypothesis regarding targeting mechanism, we are extending our experiments using imipenem instead of tetracycline to pressure the *E. coli*. We hope to identify mechanism(s) of reversion that could lead to strategies of antibiotic use that will increase the lifespan of drugs.

2. Rapid evolution of CRISPR-associated Cas proteins Dorcie Gillette[1], Hyuk Cho[2], and M. Choudhary[1]

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a naturally occurring genetic defense system in bacteria and archaea; it is comprised of a series of DNA repeat sequences with spacers derived from previous exposures to plasmid or phage. This system has revolutionized our capacity for gene or genome editing of prokaryotes and eukaryotes. There are three major types of CRISPR systems, Type I, Type II, and Type III; each system type possesses an associated signature protein, Cas3, Cas9, and Cas10, respectively. Since the CRISPR loci originated from past independent exposures of the foreign genetic elements, it is likely that their associated signature proteins have rapidly evolved. In addition, their domain structures might have experienced different selective pressures, and therefore they have differentially diverged. We employed genomic, phylogenetic, and structure-function

constraint analyses to determine the evolutionary relationships of Cas3, Cas9, and Cas10 proteins. Results reveal that all three Cas proteins are most highly represented in the phyla Bacteroidetes, Firmicutes, and Proteobacteria. Additionally, Cas proteins are prevalent in both pathogenic and nonpathogenic species among some of these phyla. Phylogenetic analysis reveals that each of these Cas proteins originated monophyletically. Genomic analysis of the homologous proteins reveals that these proteins shared ~30-50% amino acid identity; therefore these proteins within and across Cas protein families have rapidly evolved. Additionally, structure-function constraint analysis shows that the whole protein experiences moderate levels of selective pressure. Further analysis of Cas9 shows that several protein domains (RecI, Bridge Helix, HNH, and RuvC) are highly conserved; these domains must have experienced purifying selection. However, two domains (Rec II and PAM interacting domains) must have experienced positive selection and therefore exhibit rapid divergence.

3. Inhibition of *Staphylococcus aureus* by Thai, Brazilian and Panamanian Plant Extracts

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Antibiotic resistance is increasing with more than 2 million people in the US becoming infected by resistant bacteria resulting in more than 23,000 deaths annually. At the current rate, if there is no intervention, there will be more than 10 million deaths due to antimicrobial resistance globally by 2050 and this will dwarf the deaths currently caused by cancer. This is a serious threat to global health because modern medicine is becoming less effective as antibiotic resistance increases. Factors propagating this threat are over-prescribing, misuse, and agricultural use of antibiotics. One bacterium that frequently develops resistance is *Staphylococcus aureus,* which is commonly found in the nose and on the skin of 30% of Americans. It causes no harm until the skin is punctured or there is some other wound which can become serious and possibly fatal. The purpose of this study was to examine plant extracts for possible antibacterial properties in order to offer alternatives or co-therapies for current antibiotics. Over 500 plant extracts received from the National Cancer Institute have been assayed. In this project, plant extracts from Thailand, England, Uganda, Brazil, and Panama were assayed for antibacterial attributes by re-suspending them in ethanol or methanol, impregnating paper disks with this solution, and placing them on an agar plate inoculated with *S. aureus.* The plates were incubated at 37 degrees Celsius for about 24 hours, then examined for zones of inhibition. Four plant extracts showed zones of inhibition ranging from 8 millimeters to 30 millimeters. The other extracts, *Leandra barbinervis, Terminalia Amazonia,* ethanol extract of *Shorea roxburghii,* and methanol extract of *Shorea roxburghii,* showed antibacterial activity towards *S. aureus* ranging from 8 millimeters to 12 millimeters. The methanol extract of *Melochia umbellata* showed the greatest antibacterial activity towards *S. aureus* of 30 millimeters. To further analyze the extracts, a minimum inhibitory concentration (MIC) is needed to better understand the antibacterial activity which could potentially lead to more effective clinical treatments.

4. (REV)ised: a New Search for Reticuloendotheliosis Virus

Camille Trautman[1], Brittany Stewart[1], Heidi Spann[1], Melissa Hopkins[2], Josh Katuri[1], T. Wayne Schwertner[2], Jeff Breeden[2], and Dustin Edwards[1]

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Reticuloendotheliosis virus (REV) is an immunosuppressive avian retrovirus. REV can infect B-cells, a component of blood, and has been identified in wild galliform birds, such as turkeys. The virus also infects endangered Attwater's prairie chickens (APC) and has been an obstacle in conservation efforts. Infected individuals may exhibit symptoms such as anemia and neoplasia. There is evidence that blood-feeding insects may act as mechanical vectors. The mode of transmission and prevalence in nature is little known. We hypothesize that a population of wild turkeys in Texas are infected with REV and are a reservoir for transmission by insects to other closely related birds, such as APCs. By understanding the sources of new infections, additional measures could be implemented to protect at-risk populations. Our aims include determining the prevalence rate of REV infection in wild turkeys in Texas, isolating and propagating virus from recently infected APCs, and determining whether there is genomic variation of the virus. Blood samples from 331 wild Rio Grande turkeys from 18 counties were collected between January 2016 and April 2017. DNA was extracted and viral DNA amplified by polymerase chain reaction using REV-specific primers. REV was detected in six counties, primarily in central Texas, at prevalence rates of 5% – 10%. Further, we tested 88 imported Eastern wild turkeys from three states and detected REV in one individual from West Virginia. Blood from 31 infected APCs was collected between December 2015 and July 2017, and B-cells were isolated from one sample by Ficoll gradient. Isolated cells were co-cultured with chicken DF-1 cells to propagate the virus. Virus titers were determined by immunofluorescence assays at 6.8×10^5 50% tissue culture infectious dose, demonstrating successful isolation and propagation of REV. Initial next generation sequencing of the virus resulted in 77% coverage of the whole genome. Sequence alignment analysis showed a highly conserved genome, however a short region was variable as compared to sequences from 2001. This variable region may identify sources of new infections. Future experiments will determine whether REV can be detected in quail, whether mosquitoes can harbor the virus, and develop novel diagnostic methods for field-collected blood samples.

5. Identification of Antibiotic Resistant *Ochrobactrum intermedium* and the Utilization of Bacteriophage in Cell Lysis

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Ochrobactrum intermedium, a gram-negative, rod shaped, and non-lactose fermenting opportunistic pathogen, has long been known to infect animals and immunocompromised humans, especially those that are hospitalized and/or have recently undergone surgical procedures. In the hopes of discerning potential antibiotic resistance and bacteriophage susceptibility as an alternative to current infection treatment, a number of assays were

performed on an *O. intermedium* isolate obtained from soil located near the Animal Rehabilitation Keep (ARK) in Port Aransas, Texas, including 16S rRNA sequencing to determine the species, Kirby-Bauer antibiotic resistance assays, predatory phage isolation and purification, phage DNA isolation, restriction digests of phage genetic material, and lysogeny assays. Moreover, transmission electron microcopy (TEM) was performed in order to visualize the isolated phage that use *O. intermedium* as a host for replication. As a result of these experiments, various degrees of resistance were observed to six of the eight antibiotics used, and the isolated bacteriophage was shown to produce lysogens that incorporate viral genetic material into their own genome. Based on genomic sequence data, this bacteriophage contains three hundred and eighty genes, with sixteen tRNA genes. A majority of the genes present as reverse genes. Continued research will allow for the annotation of the aforementioned phage genome and the potential development of a bacteriophage solution to help combat *O. intermedium* infections in both humans and animals – particularly native aquatic species – as an alternative to traditional antibiotic treatments.

6. Testing Anti-Microbial Activity of Medicinal Plants

Joy Collins and Dr. Robert Corbett

Lamar University

Medicinal plants are used in rural countries all over their world as an alternative to drugs because vaccines aren't always accessible. Shamans and medicine men make concoctions of plants from their environment to heal wounds and treat illnesses. Many medical professionals and researchers have tested the effects of plants and created new drugs based off their findings. The rise in the occurrence of multidrug resistant bacterial infections and the lack or newly developed antibiotics to fight these infections has caused great concern in the medical field. In an effort to find new treatments, many are turning to ancient remedies – medicinal plants. Antimicrobial activities of crude ethanol extracts of known medicinal plants and other plants were tested against Escherichia coli (Gram negative) and Staphylococcus epidermidis (Gram positive) as a preliminary study to identify possible alternatives to current antimicrobial treatments used in treating bacterial infections. Over a dozen plants were tested throughout 4 months. Alcohol extracts of witch hazel, dried ginger, fresh rosemary, and dried forsythia were the most effective in preventing growth of bacteria. As we continue the research in medicinal plants, the goal is to determine what phytochemicals work best and what simple procedure could be done to treat and prevent further infections.

GRADUATE STUDENT ORAL PRESENTATIONS

7. Protein Binding Partners of the CpsA Virulence Factor in Group B Streptococcus

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Streptococcus agalactiae, Group B Streptococcus (GBS), is an important human pathogen,

causing life-threatening sepsis and meningitis in newborns. The virulence of GBS in part can be attributed to its ability to produce a polysaccharide capsule that helps with immune cell evasion. CpsA has been recognized as an integral part of capsule synthesis. We hypothesize that protein-protein interactions with CpsA play a role in virulence and may be required for CpsA function. Elucidating CpsA protein-protein interactions may provide a better understanding of CpsA function in the capsule synthesis pathway. Using a Bacterial Adenylate Cyclase Two-Hybrid (BACTH) (Euromedex) system we have evaluated CpsA interactions using two different methods. Using screening techniques on LB/X-gal and MacConkey/maltose media we have demonstrated CpsA interactions with previously shown partner CpsC protein, providing a positive control for this system. We have also quantified the interactions by using β- galactosidase assays. Using the BACTH system has allowed us to investigate whether protein interactions are occurring at the N-terminus or the C-terminus of the proteins. The N-terminus of CpsA is being brought into close enough proximity to the N-terminus of CpsC to show a positive interaction. Interactions between CpsA and predicted partners CpsY and CpsE have shown that interactions are not occurring via the amino terminal end, interactions via the C-terminal end are still being evaluated. Furthermore, with i*n vivo* MBP-Nanotrap immunoprecipitation assays (ChromoTek) we have identified additional proteins that have interactions with the CpsA protein that were not previously predicted partners in *S. agalactiae*. One of the pull down proteins, FtsZ, which is a cell division protein, will be evaluated in the BACTH system to identify whether this is a direct interaction with CpsA or an indirect interaction through the formation of a larger protein complex. Understanding protein-protein interactions of CpsA will help in designing new antimicrobial therapies to target capsule synthesis.

8. Environment has Minor Effect on Microbiome Restoration after Rifampicin Disruption

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Disruption by antibiotics causes instability in the native microbiota, which typically increases host susceptibility to opportunistic infections (e.g. *C. diff*) and can result in correlated diseases, such as antibiotic-associated diarrhea. Our knowledge remains limited in understanding the complex interplay between host, microbiota, and environment. Previous work in our lab has shown that exposure to a broad-spectrum antibiotic, rifampicin, results in a brief bacterial bloom dominated by one organism, followed by establishment of an altered community composition during recovery in artificial pond water (APW), using mosquitofish as a model organism. My study asks if recovery in the original aquarium water, as compared to APW, will result in return of the original pre-treatment community composition. This explores how environment may affect the microbiome following disturbance. We found that short-term recovery (12 hrs post- treatment) in APW had the largest inter-individual variability within Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR community fingerprint profiles and 16S rRNA gene profile analysis, whereas the community biochemical activities returned more similar to pre-treatment, unlike aquarium water. High bacterial counts on treated fish skin after 60 hrs in APW were not associated with increased mucus levels. Despite stochastic fluctuations, after a week microbial communities recovered in different conditions shared higher ERIC profile similarity than either one when compared to pre-treatment. Also, regardless of water bacterial counts reflecting skin numbers, these communities were

structurally distinct. Findings from this study did not support environment as a major influence on the microbiome recovered after antibiotic. Future studies will explore probiotic interventions in microbial community recovery.

9. Development and use of a *G. mellonella* infection model to discover novel virulence mutants in *B. anthracis*

Jacob Malmquist

Texas Christian University

Understanding bacterial virulence is important because it provides insight into the molecular basis behind bacterial infections. With the decreased efficacy of antibiotics due to the development of drug resistance, this knowledge could be used to identify specific targets for new pharmacological targets thereby strengthening our arsenal against these pathogens. Currently, our main mechanism by which to evaluate *in vivo* virulence is the mouse model (*Mus musculus*). While this model is effective, there are substantial ethical and resource constraints associated with vertebrate use. In order to provide alternative *in vivo* testing models, this study investigated the invertebrate wax worm larvae, *G. mellonella*, as an *in vivo* infection model for *B. anthracis*. To validate the ability of *G. mellonella* to discern attenuated bacterial strains, previously identified virulence mutants were constructed and assessed. This model proved capable of distinguishing between virulent and avirulent strains. Next, we tested whether *G. mellonella* could identify novel virulence mutants. A small collection of transposon mutants was screened for deficits in reactive oxygen species (ROS) survival and iron acquisition using *in vitro* screens. This yielded 10 attenuated mutants. These mutants were then assessed in *G. mellonella* and 2 were found to have an *in vivo* phenotype. These results demonstrate the potential effectiveness of *G. mellonella* as a future infection model and could increase the efficiency in the identification of novel bacterial virulence mutants.

10. Detection of a Fas-L like protein in *Candida albicans*

John Ford and James Masuoka

Midwestern State University

Candida albicans is a dimorphic fungus occupying a position as a significant member of the human microbiome. Through colonization of mucosal surfaces *C. albicans* has become an important nosocomial pathogen and source of chronic infection for immunocompromised individuals. The microenvironment of human mucosal surfaces has imbued *C. albicans* with a litany of survival strategies that blur the line between mutualism and parasitism. In these areas of high cell turnover, *C. albicans* and other commensal microbes are regularly exposed to the cellular mechanisms controlling programmed cell death (apoptosis). Some primary research suggested that *C. albicans* is capable of manipulating apoptosis within the host by a protein similar to Fas-L. The present research provides experimental evidence of the presence of a Fas-L-like protein in the *C. albicans* cell wall. This putative protein was detected experimentally by adherence to Fas coated polystyrene and indirect immunofluorescence. Additionally, the basic local alignment search tool (BLAST) program on the NCBI and Candida Genome Project

websites were used to identify protein sequences similar to Fas-L and other Tumor Necrosis Factor proteins. Initial searches revealed several *C. albicans* proteins with sequence similarity to Tumor Necrosis Factor proteins, with more detailed analysis of these sequences to follow. Taken together, we believe these findings suggest the presence of a Fas-L like protein in the *C. albicans* cell wall. This could further characterize an existing disease model as well as add to our understanding of this pathogen/host interaction.

EUGENE AND MILLICENT GOLDSCHMIDT GRADUATE STUDENT AWARD

Diverse pathogenicity mechanisms promote *Candida albicans* survival upon macrophage phagocytosis

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Interactions between *Candida albicans* and mammalian phagocytes is a key determinant of disease initiation and progression whose investigation has proven to be a valuable experimental tool to uncover key virulence traits. To gain further insights into the fungal response to phagocytosis, we used RNA-sequencing to characterize the transcriptome of *C. albicans* after phagocytosis by murine bone marrow-derived macrophages. These data confirmed earlier observations of a significant reorganization of carbon metabolism, downregulation of translation and cell cycle progression, and induction of responses to oxidative and nitrosative stresses and DNA damage repair. Beyond these changes, though, nearly half of all upregulated transcripts are uncharacterized. Included in this list we found a surprising number of newly annotated genes that encode proteins of less than 100 amino acids. Such small proteins are often important as secreted mediators of host-pathogen interactions, and therefore we have begun a genetic analysis of these upregulated small ORFs (*SMA1* through *SMA13*). One of these, Sma12, is a 71aa, serine/threonine rich protein that was newly annotated in Assembly 22 based on conservation in other *Candida spp. SMA12* has been shown in previously published transcriptional profiling experiments to be upregulated in response to serum and the FungalRV algorithm predicts it to be an adhesin. While Sma12 does not have a signal sequence, it is indeed a functional microadhesin necessary for robust adhesion to polystyrene surfaces and mammalian cells. Heterologous expression of *SMA12* in *Saccharomyces cerevisiae* increases adhesion to polystyrene, suggesting the mechanism by which it promotes adhesion is conserved. Importantly, the *sma12*Δ mutant is deficient in survival upon co-incubation with macrophages, indicating that this small protein is critical for interactions with phagocytes. Current studies aim to understand the role of this microadhesin in mammalian infection models and to determine the subcellular localization using immunohistochemistry and an epitope-tagged variant of Sma12.

Additionally, initial characterizations of a second small putative adhesin, Sma5, are currently in progress. Two additional small ORFs, *SMA6* and *SMA13* have antioxidant properties and are also required for survival in macrophages, demonstrating that these newly identified genes are significant mediators of host-pathogen interactions.

UNDERGRADUATE STUDENT POSTERS

PATHOGENIC MICROBIOLOGY
UGP1

Characterization of Volatile Organic Compounds in Chronic Wound Pathogens

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Chronic, non-healing wounds are an ever-increasing problem in the U.S. affecting over 5.7 million people, with treatment costs accumulating to upwards of $20 billion annually. The persistence of these wounds and their related costs is contributed to by microbes that colonize the wound, many of which are highly resistant to antibiotics, creating treatment challenges. Treatment options rely upon culture methods to identify the organisms present in the wound. However, some microbes are difficult to culture or may not be present at quantifiable levels, posing additional challenges to effective treatment. Individual species of microbes have specific molecular makeups, including specific volatile organic compounds (VOC's) which are also thought to be unique to each organism. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are two of the most common chronic wound pathogens and are highly multidrug resistant. Utilizing solid-phase micro-extraction (SPME) and gas-chromatography mass-spectrometry (GC-MS), we used an *in vivo* model to compare VOC's present in the chronic wound environment to those present *in vitro*. While the number of relevant compounds were reduced *in vivo*, these compounds were still comparable to the *in vitro* baseline. Interestingly, in the dual species infection, the dominant compounds belonged to *P. aeruginosa* with lower levels of *S. aureus*. While relationships between organisms in a chronic wound vary from symbiotic to highly competitive, these data indicate that even at low levels, pathogen VOC profiles vary between species, both qualitatively and semi-quantitatively. This method could lead to identification of bacterial species present in wound infections without the need for cultures.

UGP2

Synergistic Interactions Between S. aureus And B. fragilis in NSTIs Are Likely Mediated By A Heat Resistant Small Molecule

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Necrotizing soft tissue infections (NSTIs) are often polymicrobial in nature, rather than monomicrobial. When data from patients with polymicrobial NSTIs is analyzed, two microorganisms are often isolated; *Staphylococcus aureus (Sa)*, a gram-positive, facultative anaerobe, and *Bacteroides fragillis (Bf),* a gram negative, obligate anaerobe. We have preliminary evidence in mice showing that NSTIs are often larger in size when both *Sa* and *Bf* are present, compared to the mono-infections of either organism. This suggests a synergistic interaction between *Sa* and *Bf*. We have also shown that *Sa* cell free spent media (supernatant) alone can stimulate *Bf* growth *in vitro*, suggesting that a possible contributor to this synergy is a *Sa* secreted substance increasing *Bf* growth. In this study, we aimed to identify the secreted *Sa* factor(s) that stimulates *Bf* growth *in vitro*. In order to characterize the physical properties of this factor, we treated *Sa* supernatant with two treatments; a heat treatment to denature any proteins in the supernatant, and an activated charcoal treatment that would bind any small molecules present in the supernatant. We tested whether these treated supernatants would enhance *Bf* growth when compared to *Bf* grown in untreated *Sa* supernatant and nutrient broth alone. We found that heat-treated supernatant still stimulated *Bf* growth, while charcoal treated supernatant did not. Based on our data, we conclude that the source of the synergistic interaction between *Sa* and *Bf* is at least partially mediated by a small molecule.

UGP3

Effect of Growth Conditions on the Cell Surface Hydrophobicity of *Candida albicans*

Melissa Rose and James Masuoka

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Candida albicans is an important commensal fungus inhabiting the gastrointestinal and genitourinary tracts of healthy humans. In immunocompromised individuals, this dimorphic fungus can opportunistically cause diseases such as vaginal and oral candidiasis (“yeast infections” and thrush, respectively). Individuals immunocompromised by chronic infections, such as those with HIV infections, or by drugs given for transplantation or cancer treatment, are at particular risk. The prevalence of candidiasis, especially mucosal infections, reflects the colonization rate in the population. Adherence is the first step in colonizing the host, and cell surface hydrophobicity (CSH) is an important component of adhesion. Hydrophobic cells have been shown to adhere better to mouse tissues than hydrophilic cells. Hydrophobic cells are more resistant than hydrophilic cells to phagocytic killing. Both characteristics greatly contribute to the ability of *C. albicans* to cause disease. The growth conditions found within the human host greatly influence the ability of microbes to effectively colonize and subsequently cause disease. Environmental conditions such as growth temperature were previously shown to affect cell surface hydrophobicity. Cells that were grown to stationary phase at room temperature were almost entirely hydrophobic, while cells grown at 37°C are almost entirely hydrophilic. We hypothesized that other growth conditions would also influence hydrophobicity particularly the composition of growth media and pH of growth environment. The nutrient composition of growth media and pH were tested as these were previously shown to influence other fungal cellular process. The pH affects the rate of germination and expression of genes

essential for survival, and growth media composition influences the length of the lag phase. The effect of growth media composition on CSH was tested using four different media. Our results indicate that relative CSH is affected by media composition and that this effect is strain dependent. The effect of pH on CSH was tested at pH 4.5, which corresponds to the vaginal canal, and pH 7.2, which corresponds to blood and the oral cavity. Our results suggest that pH does not influence CSH. By understanding how cell surface hydrophobicity changes with these parameters we will gain a better understanding of how *Candida albicans* successfully colonizes multiple niches within the human host.

UGP4

Quantitative Analysis of Bacteria Overgrowth after Skin Microbiome Disruption

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Microbiomes play a major role in health of the host. Disruption of microbiomes can lead to negative consequences for the host, including increased susceptibility to infection, diarrhea, and other consequences. A fish skin model of mucosal microbiomes is used because it contains vertebrate immunity yet is simple and easy to experimentally manipulate and sample. This model showed a bacterial overgrowth effect following disruption by a physical rinse, antibiotics, or an antiseptic, as measured by counting colonies on plates. In order to confirm the plating results via a culture-independent method, we Gram stained skin mucus samples directly before and after a rinsing disruption. Mucus was retrieved off the surface of the skin of the fish and applied to a slide with Gram positive cocci (*Micrococcus luteus*) and Gram negative rod (*Escherichia coli*) controls. To quantify results, we viewed ten fields of each sample and counted the approximate number of bacteria per field. The gram stain results confirmed an overgrowth of bacteria on day two of recovery.

UGP5

Development of CRISPR-Cas Nickase tool for genetic manipulation in *Clostridium difficile*

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Clostridium difficile is an opportunistic pathogen that causes the disease known as *C. difficile* associated diarrhea (CDAD). CDAD causes $12.6 billion dollars in healthcare related costs every year. Many clinical studies have been done to show the impact of CDAD. Despite this, very little is known about the molecular biology and genetics, in reference to *C. difficile*'s pathogenicity. A lack of effective tools for gene editing in *C. difficile* is the main reason for this gap in knowledge. Clustered Regularly Interspaced Short Palindromic Repeats-and associated proteins (CRISPR-Cas) is a now well-founded system for genome editing in all organisms. This system can be modified to only make single-stranded DNA breaks. Clostridia, as a family, have been shown to not survive double-stranded DNA breaks. Here, I propose the

implementation of a CRISPR-Cas Nickase system to increase the efficacy of CRISPR-Cas genome editing in *C. difficile*. Homologous recombination is induced via single-stranded DNA damage and can be used to induce mutations via Homology Arms (HAs) provided on a plasmid. Previous studies have used the nickase based method in conjunction with HAs on the plasmid for homologous recombination for removal of genes in other Clostridium species.

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UGP6

Analysis of Murine Cytomegalovirus Interactions with Normal Flora Bacteria to Promote Infectivity

Thu Doan and Dr. Laura K. Hanson

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Cytomegalovirus (CMV) is a double-stranded DNA herpesvirus that causes mild flu-like symptoms in individuals with a normal immune system. However, it can cause severe disease in immunocompromised patients. Furthermore, CMV is a major cause of birth defects. Infants who develop congenital CMV infection experience seizures, intellectual disability, muscle weakness, and/or hearing loss. According to the National CMV Foundation, 1 in every 150 babies are infected with CMV per year, making it one of the most common congenital viral infection in the United States. In previous studies, researchers found enteric viruses binding to intestinal bacteria, which could enhance infectivity and association with host cells. As of currently, there are no publications on whether viruses that enter through other routes may similarly exploit normal flora. Inspired by this, we are investigating if CMV, which is normally transmitted through saliva, urine, vaginal secretions, or semen, can use bacteria to promote infectivity. Using a variety of methods, we are testing for potential binding of CMV with normal flora bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, and the impact on survival and infectivity of CMV. Initially, we wanted to figure out a method to fully kill off the bacteria so they will not replicate in our mammalian cell culture. We tried heat and UV exposure to kill off the bacteria. However, with both of these methods, we found evidence of bacterial growth indicating not all bacteria were eliminated. Next, we tried inhibiting the bacteria using antibiotics. After testing ampicillin on murine CMV in NIH 3T3 fibroblast cells, we observed a decreased amount of plaques compare to our control. This indicates ampicillin has an effect on viral infectivity. Currently, we are testing for an appropriate amount of ampicillin that will kill off our bacteria with minimal impact on the virus before proceeding to our experiments on whether the bacteria can promote infectivity.

UGP7

Cryopreservation of Fecal Microbial Communities with Dimethyl Sulfoxide

Krystal Ann Mundell

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Cryopreservation is a standard laboratory technique for archiving pure cultures of microbial cells but few studies have attempted to preserve microbial communities. Cryopreservation of microbial communities would enable archiving of microbiome libraries for microbial discovery programs and other biotechnological applications. Since gut microbes appear important as a source of microbes that produce bioactive compounds of therapeutic value, in fecal-transplant therapy and as potential probiotics, we determined the suitability of DMSO as a crypresevation agent for feces collected from dogs. We compared samples from four dogs before (fresh) and after treatment with DMSO and storage at -80 °C (cryopreserved). Serial dilutions from fresh and cryopreserved samples were plated on Trypic Soy Agar (TSA) to support a range of aerobic heterotrophs and on MacConkey (MCA) and ChromoSelect agar (CSA) to select for fecal bacteria. We compared the number of colony forming units (CFUs) and the species of bacteria, as determined by MALDI-TOF, recovered. Fresh samples yielded about 5X more CFUs than cryopreserved samples. Cryopreservation decreased readily culturable bacteria from 2.6 to 0.5 x 10^8 CFU g^{-1} (wet weight) on TSA, 5.5 to 0.7 x 10^7 CFU g^{-1} on MCA, and 2.4 to 0.2 x 10^6 CFU g^{-1} on CSA. *Escherichia coli* was the most abundant microbe for libraries generated from both fresh and cryopreserved fecal samples and was relatively abundant in the library generated from the cryopreserved samples. *Enterobacter aerogenes, Enterobacter asburiae*, and *Entercoccus faecium* were relatively abundant in libraries generated from fresh samples. These results suggests that cryopreservation of fecal samples with DMSO changes the structure of fecal microbial community and selects against the *Enterobacter* and *Enterococcus* species.

UGP8

Using bioinformatics tools to decipher regulatory gene networks in *Leishmania major* infected dendritic cells

Emily Ensley

University of Mary Hardin-Baylor

Bioinformatics approaches can be used to decipher complex gene regulatory networks in biological systems. Here, bioinformatics tools were used to identify transcription factors that play a role in the response against *Leishmania major* infection in human dendritic cells. Cutaneous leishmaniasis is a vector borne disease resulting in ulcerative lesions near the sight of infection. It is found primarily in the Tropics, Subtropics, and Southern Europe. Previous studies highlighted the role of major transcription factors, such as NFkB and C/EBP, that drove the responses of dendritic cells against *L. major*. However, these studies did not focus on lesser known transcription factors that may also contribute to the complex responses against *L. major*. In this study, unanalyzed gene expression data was obtained from a public data repository and used to examine changes in gene expression at different time points during *L. major* infection of dendritic cells. The obtained gene expression data was normalized and differentially expressed genes were identified using the TAC software. The data was clustered and queried for transcription factors that might play a role in the dendritic cell responses against *L. major*. The genes that shared expression patterns (i.e. appeared in the same cluster) with the identified transcription factors were considered co-expressed and could also be possible targets of these transcription factors. We are currently searching for existing targets and new candidate targets for these transcription factors in the co-expressed groups of

genes aiming to identify groups of co-regulated genes.

UGP9

Mutational Effects on Complex Formation of a Viral Virulence Protein

Karen Meacham, Lisa Bolin, Laura Hanson

Texas Woman's University

Cytomegalovirus (CMV) is a common infectious cause of birth defects, including hearing loss and mental retardation, as well as complications in transplant patients. Murine CMV (MCMV) can be used as an analogue to study human CMV because of similar genetic structure and disease presentation and the availability of well-established mouse cell lines. Three MCMV proteins, M139, M140, and M141, have been previously identified as required to cause serious disease in the host. These proteins form a complex that is larger than would be expected with only one copy of each protein, but the exact makeup is unknown. The M140 protein binds M141 to protect it from proteasomal degradation, but whether this is due to multiple copies of M140 or recruitment of M139 is unknown. Previous experiments have shown that mutant forms of M140 will bind M141 but not protect it. We are using both full-length and truncation mutant M140 plasmids to determine whether self interactions occur and how this affects the ability to recruit M139 and protect M141 from degradation. As M140 is most important in macrophages, we are comparing both macrophages and fibroblasts to see whether functions are general or specific.

UGP10

Coevolution of host-pathogen interactions in a *Pseudomonas aeruginosa* chronic infection model of *Drosophila melanogaster*

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Pseudomonas aeruginosa is an antibiotic resistant, opportunistic pathogen that causes both acute and chronic infections in a variety of hosts. Notably, chronic *P. aeruginosa* infections are the leading cause of morbidity and mortality in cystic fibrosis patients. The innate immune response in *Drosophila melanogaster* makes it a suitable host for examining the chronic infection of *P. aeruginosa*, and allows for examination of host-pathogen interaction over multiple generations. The Red Queen Hypothesis predicts that under coevolution, rare genetic variants will have a selective advantage and will rise in frequency until common. It posits that new mutations that arise in pathogen populations will rise in frequency until common when the host population becomes so diseased that it undergoes a population crash. Subsequently, new mutations in host populations may arise to resist the virulent pathogen. In this study, a novel *P. aeruginosa* oral feeding infection model in *D. melanogaster* was utilized to examine evolutionary dynamics during host-pathogen coevolution. Three evolutionary treatment groups

were used: coevolution, host evolution, and pathogen evolution. Each of these three treatment groups exhibited the fluctuating dynamics predicted by the Red Queen hypothesis through generations of decreasing and increasing host mortality.

UGP11

Evaluation of Sequences in a Viral Pathogenesis Factor Involved in Complex Formation

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Human cytomegalovirus (HCMV) is the leading infectious cause of birth defects as well as a cause of complications in organ transplants. To research pathogenesis, the mouse model MCMV is used. There is a large amount of sequence similarities between human and mouse CMV, and proteins with sequence similarities perform similar functions, however each virus is specific to one species as the host. Three genes in the MCMV genome (genes M139, M140, and M141, homologues of US22, US23, and US24 of HCMV) are important to the virus' ability to cause severe disease in the host. The M139 gene has a long and a short product, pM139 long and pM139 short. The long binds to pM140 and pM141, while it is not clear whether or not pM139 short binds. In infected cells, pM140 and pM141 are co-precipitated with anti-pM139 antisera, however only pM139 long is detected upon precipitation with anti-pM140 antisera. A caveat to this is that the co-precipitation of pM139 with anti-pM140 antiserum is very inefficient. As pm139 short is less abundant than pm139 long, it could be below the limit of detection. Previously, a plasmid was made which expresses only pM139 long. The M139 long plasmid was digested and ligated so that only pM139 short would be expressed. The M139 plasmids will be co-transfected with plasmids expressing epitope-tagged pM140 and pM141. Immunoprecipitations will be performed to study the binding ability of pM139 short. It is expected that the short pM139 will not bind with the pM140 and pM141. If pM139 short does not bind, smaller deletions will be made to identify the sequences required for binding. If pM139 does bind, this would indicate that pM139 short is capable of binding with pM140 and pM141, and the previous failure to detect this binding with anti-pM140 in infected cells may reflect relative amounts pM139 short and pM139 long in the complex.

ANTIMICROBIAL MICROBIOLOGY

UGP12

Evaluating Anti-Viral Properties and Cell Toxicity of Photochemically Activated Silver Nanoparticles

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Sexually transmitted infections (STIs) have become an increasing problem in recent years. Our

goal is to discover whether silver nanoparticles (AgNPs) could be used as an effective anti-viral against viral STIs. The AgNPs we use are from the Omary lab at the University of North Texas and have varied surface properties. We have been testing PAA (-) and chitosan (+) functionalized AgNPs. We use the herpes virus, mouse cytomegalovirus (MCMV), to determine the anti-viral properties of these AgNPs. Our preliminary tests have been in cell culture, this would allow transition into the animal model. Our cell cultures consist predominantly of IC-21 macrophages. We use these cells because they are phagocytic and are more likely to react negatively and die than other cells because macrophages take in the toxic debris. When there is toxicity we are also testing the AgNPs with NIH3T3 fibroblasts to determine if the toxicity is similar for non-phagocytic. In toxicity tests, we have found the (-) AgNPs kill the mouse cells at concentrations as low as 0.01mM and at a concentration of 0.1 mM the cells are dead within 6 hours, but the (+) AgNPs have no apparent toxicity as high as 0.25 mM. The 3T3's did not die as rapidly suggesting that the toxicity was present but delayed. Due to their low toxicity, we are testing the (+) AgNPs by mixing them with the cells and virus at varying times. We have begun these tests by simultaneously adding the virus and (+) AgNPs to the cells with no pre-mixing. We analyzed our data for viral protein expression using a western blot and found that there appears to be no inhibition of the virus. We are continuing these tests by using varying pre-mixing incubation times. We know that the toxicity of the (-) AgNPs is due to the AgNPs themselves, as 0.1 mM PAA has no apparent toxicity. To test the anti-viral activity of the toxic (-) AgNPs we are mixing the virus and AgNPs for set amounts of times, centrifuging out the AgNPs, and testing the supernatant for viral infectivity. We can do similar experiments with the (+) AgNPs to determine if the virus binds to the AgNPs, even if there is no inhibition of infection. If we find direct inhibition, the AgNPs could be incorporated in products such as vaginal creams or foams. Since an important mode of transmission of CMV is urine, AgNPs that bind CMV could be used as a second layer of protection in diapers to decontaminate the urine so it is less likely for care workers or parents to contract CMV.

UGP13

Identification and Characterization of Two Soil Bacteria Isolates Displaying Antimicrobial Activity

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The risks of acquiring a bacterial infection with resistance to antibiotics are on the rise worldwide. In order to address this issue, the Small World Initiative™, an innovative program for the discovery of new antibiotics, has partnered with universities to identify microorganisms from the soil that display antimicrobial properties against bacterial pathogens. The rationale for the program is the more students searching, the better chances of finding microbes with antibiotic properties. As part of this program, two antibiotic-producing bacteria isolates were identified from soil collected on the northeast side of San Antonio, Texas. For this project, bacteria was isolated by first performing a serial dilution and plating the dilutions on nutrient agar to determine the number of colony forming units (CFUs) per gram of soil. Thirty diverse colonies were selected and tested for antimicrobial activity against ESKAPE relatives, bacteria that represent common human pathogens. From the colonies tested, two isolates displayed

antibiotic properties against *Erwinia carotovora* and *Bacillus subtilis*. The two antibiotic-producing isolates, numbers 20 and 23, have distinct colony morphology. Number 20 is white with an irregular shape and undulate edges, and are moist and creamy in texture. Isolate number 23 is also moist and creamy texture, but more of a brown/yellow pigment and shaped circular with entire edges. Both isolates showed antimicrobial activity, determined by measuring the zones of inhibition, towards ESKAPE relatives. Isolates displaying antimicrobial activity were further characterized by 16s rRNA sequencing and secondary assays, including Gram staining and biochemical tests. The information gathered from these soil isolates has been documented in the Small World Initiative™ worldwide database. In conclusion, this research is clinically relevant as there is an urgent need to identify new antibiotics that are effective against the ESKAPE pathogens.

UGP14

Anti-Biofilm Activity of Chloroxine against *Candida albicans*

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Candida albicans, while a common inhabitant of the human microbiota, represents an increasing health threat to immune and medically compromised individuals. As an opportunistic pathogen, *C. albicans* is capable of causing disease ranging from superficial to life-threatening systemic candidiasis. Furthermore, *C. albicans* ability to form biofilms, complex microbial communities, heightens this issue of resistance as biofilms are intrinsically less susceptible to these commonly used antimicrobials as well as host immune responses. Considering the role of biofilm formation in *C. albicans* infections, it represents a valuable target for the development of anti-virulence treatment strategies. In effort to address the urgent need of developing new treatment strategies targeting the resistant *C. albicans* biofilms, we screened 707 small molecule from the NIH Clinical Collection compound library to discover novel inhibitors of *C. albicans* biofilms using a 96-well microtiter plate model of biofilm formation. Of the compounds screened, 12 compounds were deemed successful for inhibition of *C. albicans* biofilm formation, and only 2 compounds inhibited preformed biofilms. Chloroxine, a synthetic antibacterial drug, was identified from the library of compounds to have the greatest effects against biofilm formation with an SMIC80 between 0.5-1 µg/ml. Other compounds identified to inhibit *C. albicans* biofilm formation by more than 75 percent included hexachlorophene, cervastatin, MK 886, and tocainide. Our current studies are aimed towards screening the compounds for potential additive or synergistic effects with clinically used antifungals, amphotericin B and fluconazole. In conclusion, the compounds identified in this study represent potentially novel antifungal agents, which are urgently needed, to treat *C. albicans* biofilm infections.

UGP15

Identification and Antibacterial Activity of Cellulose-Degrading Symbiotic Bacteria from Grass-feeding Termites

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Midwestern State University

Most organisms are thought to contain microbial symbionts that assist in everyday life functions such as providing additional and essential nutrients, and assistance in digestion. In return, the host provides a constant environment and stable food supply. This mutualistic relationship can be observed in a termite, where the host insect provides stable living conditions and the microbes provide the ability to digest cellulose; a metabolic activity a termite and many other organisms cannot accomplish themselves. In the familiar wood-eating termites, cellulose digestion is performed by protozoa. However, in grass-feeding termites such as *Gnathamitermes perplexus,* bacteria appear to perform this function. A graduate student in the lab, isolated cellulose-degrading bacteria from the termite gut. Many of these isolates show morphological similarity to *Streptomyces* species, some of which are known to produce antimicrobial agents. We hypothesized that these bacterial symbionts produce antimicrobial compounds to protect the host in addition to degrading cellulose. The isolates were identified using traditional biochemical and morphological techniques, and by sequence analysis of the 16S ribosomal DNA. Antimicrobial activity of the termite isolates is tested using two bioassay methods, with *Streptomyces kanamyceticus,* a known antibiotic producer, as a control. The results of this study will help us better understand the symbiotic relationship between these bacteria and the termite host. In addition, it may lead to the discovery of new antimicrobial compounds.

UGP16

Antimicrobial Capabilities of Globally Sourced Plant Extracts

Isaiah Lange

Saint Edwards University

Drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA), have affected populations around the globe and brought forth the necessity for the development of new alternative antibiotics. The use of standard antibiotic treatments has become increasingly ineffective due to antibiotic resistance, which has been attributed to: overprescribing by physicians, excessive agricultural use, and patient misuse. If this growing epidemic is not addressed by 2050, antibiotic resistant bacterial infections will become the major leading cause of death. In previous studies, indigenous plants used in traditional medicine have been investigated for their secondary compounds and have been effective against the bacterium *Staphylococcus aureus*. In this study, as part of an ongoing study in which over 300 extracts were examined, plant extracts from the National Cancer Institute were investigated using the Kirby Bauer Disk Diffusion Assay. Out of the extracts tested, 6 exhibited antimicrobial properties against *S. aureus* and resulted in inhibition zones ranging from 9 to 13 mm. Further research could be done to investigate the minimum inhibitory concentration (MIC) of the promising extracts. The antimicrobial components of these extracts could be developed into possible treatments for bacterial infections in conjunction with or instead of antibiotics.

UGP17

Identification of Plant Extracts with Antimicrobial Activity to *Staphylococcus aureus*

Blanca Garcia

St. Edward's University

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been classified as a serious health threat by the Centers for Disease Control and Prevention (CDC). In the United States annually, 2 million people become infected with antibiotic resistant bacteria causing more than 23,000 deaths. Antibiotic resistance can be largely attributed to misuse and overuse of antibiotics leading to selection for antibiotic resistant strains and this results in an increased cost of care and higher risk of death. As a result, finding alternatives to antibiotics is crucial in the effort to control infectious diseases. Alternative therapies can be developed from plants that have antimicrobial substances. The goal of this study was to identify plant extracts with antimicrobial properties to *S. aureus*. In this study, 352 plant extracts from various countries obtained from the National Cancer Institute were assayed for antimicrobial properties against *S. aureus*. The Kirby Bauer Disk Diffusion method showed that four plant extracts, two from Gabon and two from Vietnam showed inhibition ranging from 7 mm to 10mm. Two of the four extracts assayed, *Terminalia nigrovenulosa* and *Instia bijuga*, have been previously used in traditional medicine to treat some infectious diseases while use of the other plant extracts, *Paraphyadnathe flagelliflora* and *Calpocalyx dinklagei*, has not been reported. Next, a minimum inhibitory concentration assay (MIC) will be used to analyze the four extracts in order to better quantify their antimicrobial properties. The MIC has been accomplished using known antibiotics in preparation for testing the extracts in this assay. In the future, these extracts may be developed into therapies to be used in addition to or as an alternative to antibiotics. The significance of this research is that these therapies could result in more effective treatment of infections currently resistant to known treatments.

UGP18

Evaluation of the Antimicrobial Properties of the Native Texas Plant Euphorbia bicolor (Euphorbiaceae)

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The main objective of this work was to evaluate *Euphorbia bicolor* extracts for antimicrobial activity. Secondary objectives include observing extract activity in different conditions such as exposure to different temperatures and homogenization in different solvents. This could aid in identifying the phytochemicals of E. bicolor with antibacterial properties. Broth microdilution assays using *Escherichia coli* aided in determining the minimum inhibitory concentrations (MIC). Extracts were prepared as both alcohol and aqueous solutions and solvents were used as controls. As much as 30% bacterial inhibition has been observed using aqueous extracts. When using alcohol extracts, 25% bacterial inhibition was observed, not significant from the 20% bacterial inhibition by alcohol controls - suggesting that inhibitory phytochemicals are

found in the aqueous extracts. In conclusion, *E. bicolor* extracts display inhibitory properties in experimental conditions. Future studies will focus on finding the inhibition mechanisms of *E. bicolor* towards *E. coli* and other microbial species.

UGP19

Analyzing Plant Extracts for Bacterial Inhibition of *Staphylococcus aureus*

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The need for antimicrobial agents has increased as antibiotic resistance has become one of the major threats to global health. Antibacterial agents have become less effective with time and repeated use. This is a source of major concern for hospitals since there is a higher risk of antimicrobial resistance and the patients represent an especially vulnerable population due to the underlying conditions that brought them here. The World Health Organization (WHO), estimates that by 2050 there will be an estimated 317,000 deaths per year in the United States alone from antimicrobial resistant diseases. For many years, traditional healers have used plants to treat infectious diseases. Plants have an abundant assortment of secondary metabolites that have been found to have antimicrobial properties such as *Syzygium aromaticum* also known clove. To test plants for antimicrobial qualities, our research group chose *Staphylococcus aureus* because it is the leading cause of skin and soft tissue infections. This year, the WHO announced that *S. aureus* is among the most commonly reported antibiotic resistant bacteria and the Office of Disease Prevention and Health Promotion reported that MRSA is still a common healthcare associated infection. To identify plants with antimicrobial activity our research group used the Kirby-Bauer Disk Diffusion Method to test a variety of 50 ug of a variety of plant extracts from Madagascar that were resuspended in ethanol. Out of 188 extracts tested, 8 demonstrated inhibition. The zones of inhibition ranged from 7mm to 12mm. Further testing with these extracts would include performing a minimum inhibitory concentration assays and safety tests to see if they would be effective to use as treatments.

UGP20

Antimicrobial properties of novel zinc oxide nanoparticles

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The rise of antimicrobial resistance has caused an increase in patient morbidity and mortality and has deepened the need for new antimicrobials to combat what the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) has defined as a global health threat. Zinc Oxide is commercially available as non-distinct, mixed nanoparticle

morphology and frequently used as an astringent protectant in bandages, pastes, and creams. Here, a novel synthesis method was used to generate polar terminated, flat surfaced “plate” morphologies and non-polar terminated, long hexagonal surfaced “rod” morphologies. In this study, the effectiveness of the novel zinc oxide nanoparticle morphologies as an antimicrobial was tested *on Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Commercially available zinc oxide inhibited growth in *E. coli* and *S. aureus*, and had no effect on *P. aeruginosa*. In all three species the antimicrobial properties of zinc oxide was enhanced by the plate and rod morphologies. Overall, the plate morphology was observed to be the most effective, suggesting oxygen radical production occurring at the polar faces of the zinc oxide as a possible mechanism.

BASIC AND ENVIRONMENTAL MICROBIOLOGY

UGP21

Construction of Myxococcus xanthus EPS-deficient strains for motility analysis

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Myxococcus xanthus is a Gram-negative bacterium that lives in the soil and feeds on other microorganisms. *M. xanthus* moves in swarms to hunt its prey, a process that is regulated by a complex system of extracellular signals. These bacteria utilize two distinct methods of movement: the movement of a single bacterium, which is known as adventurous motility (A-Motility), and movement within the swarm, which is known as social motility (S-Motility). The mechanisms of these systems are not completely understood, but it is known that S-motility requires the production of Type IV pili and exopolysaccharide (EPS). To understand the role of EPS in motility, we have created mutant strains of *M. xanthus* that are deficient in EPS production. The *epsZ* gene encodes for a putative bacterial sugar transferase necessary for the production of EPS. To generate *ΔepsZ* strains, we electroporated a construct that creates an in-frame deletion within *epsZ* into DK1622 (WT) and into DK1218 (A-S+). These mutant cells are unable to produce EPS. Our next steps will be to conduct motility assays and characterize any phenotypic differences comparing them to their parental strains.

UGP22

Chemical signaling in the phycosphere: Growth response of Chromists to bacterial signals and Phytohormones

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Plants and algae contain multiple chemical and physical mechanisms to overcome biotic and abiotic stressors in their environment, such as changes in salinity, temperature, and in the constituencies of their microbiota. Algae adapt to environmental and population dynamics through the production of and response to allelochemical signals, called semiochemicals, which are disseminated into the immediate surroundings of the algal cell, its phycosphere, by the algae and by other microorganisms. A number of studies have investigated signaling dynamics in robust green microalgae and diatoms, but few reports have examined growth and pigmentation responses in species from the kingdom of Chromista. In these experiments, a group of colorful Chromists have been grown in a multi-well plate format to study the differences in growth rate and pigmentation observed in the presence of select phytohormones and bacterial signals. Cell viability and pigmentation in microalgal cultures were evaluated using flow cytometry. Signal degradation in live cultures and by crude microalgal extracts were examined in signaling assays and by C18HPLC. The Eustigmatophytes, *Eustigmatos vischeri* and *Nannochloropsis oculata*, showed decreased growth in the presence of some bacterial signals, but degraded other signals as crude extracts and in live culture. The golden algae, *Tisochrysis lutea*, demonstrated small but detectable growth increases in the presence of each of the phytohormones tested. Crude extracts from each of the marine Chromists, *Phaeodactylum tricornutum* and *Rhodomonas salina*, showed limited degradation of bacterial signals. Slight differences in the growth rate of xenic microalgal cultures versus the axenic cultures were also observed.

UGP23

Entomopathogenic Fungi: an Alternative to Pyrethroids and other Chemical Pesticides

Clarissa Mae de Leon

St. Edward's University

Due to the new resistance of Genetically Modified Organisms the use of Chemical pesticides has increased significantly, thus adversely affecting the way we eat and produce our crops. The use of chemical pesticides in the United States has increased by approximately 50% in the past 6 years. Chemical pesticides may degrade in sunlight (e.g. Imidacloprid) however, once in the soil they persist for months and have unintended effects on non-target organisms. Aquatic environments are similarly impacted due to increased pesticide levels in runoff. Use of entomopathogenic fungi, natural pesticides, can reduce the quantity of synthetic pesticides in use. As fungi can also take up unusual molecules as an energy source, they can reduce the level of pesticide and pesticide metabolites already in the environment. We examined the impact of entomopathogenic fungus, using the soil-dwelling nematode *Caenorhabditis elegans*, a detrivore, as a model non-target organism. Following a 24hr exposure to *Beauveria bassiana (*10 mg/L*),* we assessed *C. elegans* for chemotaxis, motility, and fecundity, which are needed for individual and species success, every other day. The data indicates a 25% decrease in mortality, with no physical nor neurological deficits, compared to controls. In contrast, a typical target organism *Reticulitermes flavipes*, a subterranean termite, under the same conditions showed a 50% increase in mortality. This study suggests that *Beauveria bassiana* can be safely used in agricultural settings as it impacted targets, without detriment to

non-target organisms.

UGP24

Utilizing CRISPR technology to construct recA gene deletion in Rhodobacter sphaeroides

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The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system and its associated signature Cas protein evolved in bacteria and archaea as their genetic defense systems against plasmid and phage. The CRISPR locus consists of repeat sequences separated by spacers derived from previous exposure to foreign genetic elements. When the same foreign genetic elements reenter the bacterial cell, the spacer is transcribed into RNA, which then forms a complex with the Cas9 protein. The complex then binds to the invading genetic element and produces a double strand break, which is repaired then through either non-homologous end joining (NHEJ) or homology directed repair (HDR) mechanism. This endonuclease activity of the Cas9 protein to selectively target and cleave DNA has revolutionized our capacity for high fidelity gene or genome editing of prokaryotes and eukaryotes. Our laboratory has examined the evolutionary relationships and structure-function constraints of the CRISPR associated signature proteins (Cas3, Cas9, and Cas10). Results reveal the monophyletic origins of these three Cas proteins; these proteins share 30-50% amino acid identity suggesting their high divergences. Further analyses reveal that several functional protein domains have differentially diverged indicating different levels of selective pressures. Application of the CRISPR technology in *R. sphaeroides* presents a novel opportunity for gene deletions and gene editing. We are currently constructing a recombinant plasmid, which contains essential elements of CRISPR system including the guide RNA gene for targeting the *recA* gene deletion in *R. sphaeroides*. The goal of this project is to construct an *in-vivo recA* deletion in *R. sphaeroides*, and examine the role of the *recA* gene in the ultraviolet light mediated DNA damage-repair system.

UGP25

Genomic and physiological characterization of *Sphingobium yanoikuyae* JS1018

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A variety of plants have an ability of producing allelopathic chemicals to fight off pathogenic fungi. One example is the legume peanut plant *Arachis hypogaea* L. which is capable of producing a variety of stilbene-derived compounds such as resveratrol and pterostilbene as antifungal phytoalexins. Bacteria which can degrade these allelopathic chemicals can inhibit

plants from fighting potential microbial disease. Little is known about these bacteria that can degrade phytoalexins. To understand the microbial role in plant allelopathy, the stilbenoid degrading bacteria were isolated from the rhizosphere soil of peanut plant by using resveratrol or pterostilbene as a solo carbon source. Our initial experiment has showed that *Sphingobium yanoikuyae* JS1018 is able to efficiently degrade resveratrol and pterostilbene. We sequenced the genome of this species in order to identify the functioning gene which encodes the enzyme cleaving the stilbenoids. We further examined the physiological culture conditions of *S. yanoikuyae*. The pH ranges for growth were from 5 to 8. The temperatures tested for the growth of *Sphingobium yanoikuyae* JS1018 were between the range of 4°C and 45°C, with the optimum growth temperatures ranging from 22 to 33°C. The analysis of the genes and the pathway for stilbenoid degradation are underway.

UGP26

Growth enrichment of *Rhodobacter sphaeroides* with different carbon sources

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Rhodobacter sphaeroides is a purple non-sulfur α-*Proteobacteria* with the ability to grow under a wide variety of environmental conditions. It grows aerobically (20% O2), semi-aerobically (2% O2), and photosynthetically. It also grows anaerobically but with much slower growth rate. However, anaerobically grown *R. sphaeroides'* cells serve as potential biocatalyst for oxygen removal from a specific environment. Our hypothesis is that additional carbon sources could provide increased bacterial growth under both aerobic and anaerobic growth conditions. Bacterial cells were grown under aerobic and anaerobic growth conditions in Sisrom-minimal medium (succinate carbon source) separately with additional carbon sources (pyruvate, acetate, butyrate, citrate, arabinose, fructose, maltose, or sorbitol) at 50mM concentration. The growth characteristics and growth kinetics were analyzed by measuring optical density and colony forming units at every 24-hour interval up to 120 hours. Results reveal that bacterial growth under aerobic condition was significantly increased in media supplemented with pyruvate and butyrate. This suggests that additional carbon source could increase the growth potential of *R. sphaeroides* under anaerobic-dark condition. We are currently growing bacterial culture under anaerobic-dark growth condition with pyruvate or butyrate supplement in presence of DMSO as a terminal electron acceptor

UGP27

Controlling the mutagenic activity of the error-prone DNA polymerase RumA'2B: contributions of host and integrative conjugative element 391 factors

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Southwestern University

Triggered by the SOS response, the activation of the error-prone polymerase RumA'$_2$B

functions to replicate past DNA lesions following severe DNA damage. Located on the highly mobile conjugative transposon ICE391, the mutagenic nature of this polymerase can accelerate the spread of antibiotic resistance among gram-negative and gram-positive bacteria. The mechanisms activating these low fidelity polymerases in the SOS response are widely accepted; however, the factors regulating the mutagenesis of RumA'$_2$B within ICE391 remain undiscovered. To identify these regulatory mechanisms, fragments of ICE391 were cloned into appropriate plasmid backgrounds. Following cloning, *Escherichia coli* was transformed with the resulting plasmids, and mutagenesis assays were performed to evaluate regulators on ICE391 affecting the rate of RumA'$_2$B-mediated mutagenesis. Regions reducing and augmenting the mutagenic activity of this error-prone polymerase have been identified and characterized. Future work will continue mutagenesis assays and deletion analysis to elucidate the regulatory mechanisms within ICE391.

UGP28

Comparison of Microbiome of Two Varieties of Maize by Culture-Dependent Methods

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The plant microbiome appears important in the host plant's growth and health but the controls of this microbiome remain poorly described. To determine how the microbiome changes with plant variety, we compared libraries of readily-culturable bacteria isolated from an agronomically important hybrid variety and a heritage variety of maize (*Zea mays*). Sets of plants were sampled at different stages of development and from two locations of plant. A total of 197 bacteria were isolated on either full or quarter-strength tryptic soy agar (TSA) from the rhizoplane and endosphere of these varieties and analyzed by matrix assisted laser desorption – time of flight (MALDI-TOF) mass spectrometry. Several species of bacteria were exclusively isolated from either the rhizoplane or endosphere. *Enterobacter aerogenes* dominated the library generated from the rhizoplane. *Bacillus asahii* dominated the library generated from the endosphere, particularly from the heritage variety. Together these species accounted for the majority of isolates. Minor members of the community included *B. cereus*. Some of these relatively rare species were also found exclusively in libraries generated from either the rhizoplane or endosphere. The strength of TSA did not appear important. Together, these results suggest that maize variety may be less important than location within the rhizosphere in controlling microbiome and this translates into the species of bacteria isolated.

UGP29

Bacteria Obtained From Organic Verses Conventionally Raised Beef May Have Differences in Species Profiles

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Antibiotic resistance in bacteria is a widespread problem in many environments and industries. The use of prophylactic antibiotics alters the species of bacteria present in the poultry and swine industry, and it has been shown to promote antibiotic resistance among these bacteria (Reyes-Herrera 2005). It is unknown if prophylactic antibiotics impacts beef in the same way. Gram negative bacteria from organic and conventionally raised beef were tested for resistance to four common antibiotics and also tested for lactose fermentation capabilities. There was no significant difference in antibiotic resistance profiles between bacteria found on either meat sample. However, bacteria from beef that had been previously exposed to antibiotics contained both lactose and non-lactose fermenting bacteria compared to the bacteria from meat that had no previous exposure to antibiotics, which had no lactose fermenting bacteria.

UGP30

Density-dependent Colony Expansion of Socially Motile *Myxococcus xanthus* Cells is Driven by Exopolysaccharides

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Myxococcus xanthus, a model organism for studies of multicellular behavior in bacteria, moves exclusively on solid surfaces using two distinct but coordinated motility mechanisms. One of these, social (S) motility, is powered by the extension and retraction of polar-localized type IV pili and requires the presence of exopolysaccharides (EPS) produced by neighboring cells. A unique characteristic of *M. xanthus* S-motility is cell density-dependent colony expansion, in that the initial expansion rate increases with cell density. To understand the dynamics of S motility-driven colony expansion, we developed a mathematical model describing the effects of cell density, EPS deposition and nutrient exposure on the expansion rate with reaction-diffusion equations. This model explains the density-dependence of the colony expansion by predicting the presence of a lag phase – a transient period of very slow expansion with a duration dependent on the initial cell density. We propose that at a low initial density, more time is required for the cells to accumulate enough EPS to activate S-motility resulting in a longer lag period. We validated these model predictions by studying long-term (up to 96 hours) colony expansion of a socially motile only strain (DK1218) of *M. xanthus* at different initial cell densities on 0.5% agar nutrient plates. Next, to determine the threshold concentration at which EPS triggers cell movement, we examined the effects of purified EPS on colony expansion of *M. xanthus*. Specifically, we spotted *M. xanthus* DK1218 cells (3×10^5 cells/3 µl) directly adjacent to different concentrations of dried EPS and incubated the 0.5% agar nutrient plates at 32˚C for 96 h. Our analysis showed that the presence of purified EPS increases *M. xanthus* colony expansion with increasing EPS concentration. The decreased colony expansion at lower concentrations of EPS can be interpreted as a reduced net cell movement in the growing colony. This decrease in net movement can be achieved either through a reduction in single cell speed or through an increase in the reversal frequency. Notably, a recent experiment by Zhou and Nan (Mol. Microbiol. 103:729–743, 2017) showed that cells deficient in EPS production have higher reversal frequency than wild-type cells. Upon further analysis of their experimental data prompted by our model, we determined that these mutants also exhibit

reduced speed. Taking into consideration the effect of EPS on single cell speed and reversals the model explains the observed reduction in the colony expansion rate between EPS-deficient and wild-type cells. All of these observations indicate that our computational model provides insights into the mechanism of *M. xanthus* S-motility and will be useful in our continued experimental analysis of the effects of polysaccharides on S motility.

UGP31

Analysis of crude oil degradation by a halotolerant *Vibrio* isolate

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Bioremediation uses microorganisms to restore a polluted environment to its previous quality after an ecologically unfriendly event occurs. Hydraulic fracturing is used by the oil industry to break loose subterranean pockets of oil by shooting chemicals into the ground at high pressures. This can cause uncontrolled spills to occur and often harms the ecosystem around the well, and additionally creates a high salt environment polluted with crude oil. We seek to restore these ecosystems using microorganisms to degrade the oil present and allow for natural regrowth and population of the land's flora and fauna. Bacteria from Truscott Brine lake may possess the qualities required to survive in a high salt environment and degrade crude oil. Preliminary testing of the *Vibrio tubiashii* isolate from Truscott lake shows promising results on its ability to degrade oil as determined by gas chromatography. We determined that *Vibrio tubiashii* reduced crude oil by 55.93% in the presence of 15% NaCl, while *Pseudomonas aeruginosa*, which has known crude oil degradation properties, at its peak only reduced crude oil by 21.33% at 3% NaCl. Infrared spectroscopy performed on the crude oil after incubating with the *Vibrio tubiashii* isolate showed an increase in transmittance. This is further evidence of crude oil in the sample being degraded. These results indicate that our *Vibrio tubiashii* isolate could be a good candidate for bioremediation.

BACTERIOPHAGE MICROBIOLOGY

UGP32

Isolation and Genomic Annotation of Novel Bacteriophage 'Blufalcon'

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Without the ability to replicate on its own, metabolize, and respire, viruses have fallen short of being classified as living entities. However, this inability to self-replicate has not stopped viruses from becoming the most abundant life form within the biosphere. Viruses have

achieved their vast numbers through infection and subsequent replication within their host: bacteria, plants, or mammals. When viruses infect the aforementioned bacteria, they are known as bacteriophage or phage. The uses of these primal bacteriophages continue to expand as the discovery of new bacteriophage occurs on a regular basis. In this study, the phage named ‘Bluefalcon’ was discovered and showed a temperate life cycle. Further purification of ‘Bluefalcon’ included spot test and a series of streak tests. A transmission electron microscopy image was obtained via uranyl acetate staining of the high titer phage lysate. Genomic DNA of ‘Bluefalcon’ was extracted and purified, and restriction enzyme digests was performed. Lastly, the ‘Bluefalcon’ genome was sequenced via the ion-torrent method and determined to have a genome length of 51,052 bps with 60.9% GC content. The bioinformatics programs ‘DNA Master’ and ‘Phamerator’ were used to annotate the genome. Phage ‘Bluefalcon’ has 81 genes and classified under the A5 subcluster. This process is the first step in the isolation of a phage that has the potential to eradicate life-threatening diseases caused by antibiotic resistant bacteria.

UGP33

Isolation and Characterization of Novel Bacteriophage ‘Rabbs’ and Annotation of Gene 32 Tyrosine Integrase

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Bacteriophages, or phages, are important to study because they are used to develop gene therapy, cloning technology, and treatment for antibiotics resistance. There are two life cycles that phages can utilize; lytic that kills their hosts and temperate that integrates their DNA within their hosts DNA. Tyrosine integrase is fundamental to the temperate life cycle and it is used in labs for cloning and recombination techniques. A group of novel bacteriophage was isolated during the fall of 2017; one of them, ‘Rabbs’, was characterized by spot test, Transmission Electron Microscope, restriction digest, and the complete genome was then sequenced. Afterwards, the annotation of the ‘Rabbs’ genome was completed using bioinformatics tools like DNA Master, Phamerator, and HHpred. ‘Rabbs’ was observed as having a predominately lytic life style with a capsid size of 85 nm in diameter and a genome length of 42,430 base pairs containing 64 genes. It is classified as a G1 phage. Within the ‘Rabbs’ genome, gene 31 encodes for tyrosine integrase, an enzyme that is integral for the life cycle shift from lytic to temperate with longer incubation.

UGP34

Genomic Annotation of phage ‘Rabbs’ and study of tail protein coding genes

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Bacteriophage (phage) are one of the most abundant, diverse, and seemingly ever present in

every location across the world. There are more than 10^{31} bacteriophage that exist throughout all factions of life including soil, oceans, and microbiomes. The battle between phage and bacteria has been ongoing in an increasing arms race that precedes even that of bacteria and fungi. Phage have developed an arsenal of molecular tools that are used to cause cell lysis within bacterial cells. This includes their ability to integrate DNA through recombination into their bacterial host, which can be orchestrated by first attaching to the bacterial host through its tail fibers. Research on bacteriophage has yielded tools that are useful to scientific fields such as genetics for the aforementioned recombination technology and additionally, gene therapy. Students at Del Mar College, located in Corpus Christi, Texas in 2017, discovered the bacteriophage 'Rabbs'. 'Rabbs' was classified as belonging to the G1 subcluster of mycobacteriophage, 'Rabbs' has 42,116 base pairs within its genome, and 65 coding regions. DNA Master and Phamerator were utilized in order to examine the genomic conservation, and protein homology of this new phage. HHpred was used in identifying pair-wise comparisons of putative genes to potential matches in various databases. Based on the results of annotation, it's been indicated that genes 15 and 16 are both involved in the tail construction of the phage, being tail chaperone and tape measure proteins respectively. Notably, is Gene 15 also contains a "slippery sequence", which is unique for virus genes. Together, these genes dictate the length and structure of the phage tail; the vital interconnect between the capsid and its host.

UGP35

The Annotation of Streptomyces phage Haizum

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Bacteriophages are increasingly becoming an area of interest in scientific study. In our lab, we studied the genome of Streptomyces phage Haizum which was isolated on the host bacterium *Streptomyces xanthochromogenes*. Haizum is in the Cluster BD2 and was originally isolated from a mixed lysate with another Cluster BD2 phage, Thestral. Haizum was sequenced at the Pittsburgh Bacteriophage Institute using the shotgun sequencing method on the Illumina platform. The genome is 50,660 bp in length with an 11 bp 3' overhang and a GC content of 66.8%. After performing the procedures in the lab, we were able to annotate the total of 81 potential protein-encoding genes from our phage using PECAAN, Glimmer, Genemark, Phamerator, and Starterator. Based on the similarities from previously annotated phages we were able to find the gene functions and identify novel characteristics. Our research throughout the year has provided us with insight and knowledge about the genome of our phage, Haizum.

UGP36

Analysis of Genome Structure and Gene Content of *Streptomyces xanthochromogenes* Phage Thestral

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Streptomyces xanthochromogenes phage Thestral was isolated from soil in Denton, Texas and is a member of Cluster BD, subcluster BD2. There are 19 members of the BD2 subcluster, all of which infect only the *Streptomyces* genus. The members of this cluster have an average GC content of 66.96% and an average genome length of 50,064 base pairs. Thestral was discovered by Megan Webb from a soil sample from the campus of UNT. Thestral was sequenced September 7, 2017 at the Pittsburgh Bacteriophage Institute. The GC content is 67.7%, and the total genome length is 52,628 base pairs. Annotation of this phage is being conducted during the spring semester at the UNT, and gene functions were assigned using a combination of bioinformatic algorithms and human review. Common functions found in Thestral were helix-turn-helix, DNA binding domain, minor tail protein, and portal protein. One tRNA was identified in the genome. Overall Thestral also had a large number of unidentified functions for its genes with a staggering number of 72, showing the novelty of the phage as a whole.

UGP37

Isolation and Annotation of the Bacteriophage EGole

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Bacteriophages are the most abundant organisms on Earth, with approximately 10^{31} individuals existing. The SEA-PHAGES program at the University of North Texas is aimed at isolating and analyzing bacteriophages to better understand these unique and abundant organisms. This presentation details the isolation and annotation of Streptomyces phage EGole, found in soil from Fort Worth, Texas and isolated on the bacterial host *Streptomyces griseus*. The materials and methods used for this research followed the protocols in the SEA-PHAGES Phage Discovery Guide and SEA-PHAGES Bioinformatics Guide. The bacteriophage was isolated through the method of direct isolation. The plaques created were clear, between two and three millimeters in diameter, and comet shaped. With an electron microscopy picture, we saw that EGole has a Siphoviridae structure. When tested for its host range, EGole tested positive with *Streptomyces griseus* along with *Streptomyces azureus*. The isolation of EGole included the processes of separating individual phages, several phage titers, and an analysis of the phages electrophoresis results. EGole was sequenced at the Pittsburgh Bacteriophage Institute. This genome contains 135,653 base pairs, with a terminal repeat of 11,341bps. The GC content is 49.9%. We identified approximately 240 potential genes of which around 40 are tRNA genes. Most of the protein-encoding genes have unidentified functions. This phage is classified into Cluster BE1 of the actinobacteriophages. .EGole is a novel phage that presents a new and interesting subject to study.

UGP38

Experiment to Demonstrate the Programmed Translational Frameshift in Tail Assembly Chaperone Genes of Streptomyces phage BryanRecycles

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The programmed translational frameshift in genes encoding tail assembly chaperones of various bacteriophages has previously been characterized. This phenomenon often occurs as a result of a “slippery sequence,” or a repetitive stretch of nucleotides which causes the ribosome to “stutter” and slip into an alternate reading frame. In the case of BryanRecycles, a phage belonging to Cluster BD1 of the actinobacteriophages, genes 17 and 18 (which encode tail assembly chaperones) appear to contain a -1 frameshift based on bioinformatics analysis. The purpose of this study is to experimentally demonstrate the frameshift in BryanRecycles using traditional cloning techniques. First, PCR was performed to amplify the target gene, followed by gel electrophoresis and extraction. Primers were designed without sequences that would be digested by the restriction endonuclease, SmaI. The product was again amplified using a second set of primers, which resulted in the addition of a His-tag to the N-terminal end of the expected protein product. The PCR product was inserted into the pUC18 plasmid vector. This was done by linearizing the plasmid using SmaI and inserting the phage DNA using T4 DNA ligase. The vector was introduced into chemically-competent DH5α *E. coli* by transformation. Future work involves blue-white screening to identify recombinant *E. coli*, followed by protein expression, isolation, and identification techniques. These include induction, SDS-PAGE, and Western blotting. Using these methods, we hope to show experimentally that the frameshift occurs and to identify the exact frameshift location.

UGP39

The Isolation and Annotation of Streptomyces phage LazerLemon

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The SEA-PHAGES program at the University of North Texas offers students the opportunity to conduct research and discover and analyze new types of bacteriophages using multiple sequences of procedures. By undergoing a series of enrichments, titers, extractions, and gene annotations, we were able to fully study our phage which we isolated from *Streptomyces griseus*, and classify it within the actinobacteriophages. Isolation over this phage first began on August 27th, 2017 at 2200 hours in Denton, Texas. We obtained our sample three inches below the surface where soil conditions at the time were moderately dry. Then, we began our characterization of this bacteriophage through a series of enrichment protocols. By documenting each process and comparing our results to those of our peers and other SEA-PHAGE students, we were able to better understand our annotated bacteriophage, Streptomyces phage LazerLemon. LazerLemon was sequenced at the University of Pittsburgh where we determined that it had a genome sequence length of 54798 and circularly-permuted genome ends. We then proceeded to annotate the genome using the PECAAN platform; including databases such as PhagesDB BLAST, HHPred, NCBI BLAST, and the Conserved Domain Database. From the DNA sequence, we were able to determine that *LazerLemon* is a

member of Cluster BH of the actinobacteriophages; one of six members of this cluster. Our analysis identified 83 putative protein-encoding genes. We will continue analyzing the genetic features of this phage using the skills and tools obtained from last semester and this current semester in the SEA-PHAGES program.

UGP40

Isolation and Characterization of Streptomyces phage Salete

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Streptomyces phage Salete was isolated from a soil sample using *Streptomyces griseus* as the host. This research was conducted as part of the University of North Texas SEA-PHAGES program. *S. griseus* is characterized as being Gram-positive and having a high GC content. Upon sequencing and annotation, Salete was determined to be a Cluster BG actinobacteriophage containing 70 predicted genes over the span of 57,243 bp, 21 of which were able to be assigned known functions. The genome ends are characterized as being circularly permuted. In addition, the GC content was found to be 69.2%. Salete is a *Siphoviridae* bacteriophage and was determined to be temperate. Bioinformatic comparison of Salete with other BG cluster phages revealed a strong similarity to phages BayC, BabyGotBac, and Maih. Post-annotation analysis of Salete's genome led to the discovery of 10 potential membrane proteins, 2 potential promoters, and 2 potential terminators. There is no evidence to support the presence of tRNAs. It was discovered during annotation that specific sections of Salete's genome act as functional modules, such as genes in the first section pertaining to capsid development. This discovery, in addition to all other discovered characteristics of BG phage Salete, may lead to a further understanding of actinobacteriophage genome structure and function.

UGP41

The Characterization of Streptomyces phage Comrade

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A bacteriophage is a virus that infects a bacterium and reproduces inside its cell. The phage will attach to the host bacterium and empty its nucleic acid core into the bacteria's cell. *Streptomyces* is a bacterium that as a whole includes over 500 species that can be found in the soil and water. Some species of *Streptomyces* have been found to produce medically-useful antibiotics. The isolation host used for this study was *Streptomyces griseus*. *Streptomyces griseus* is a bacterium that lives in the soil and is responsible for the smell we most associate with dirt. *Streptomyces* phage Comrade was isolated through the SEA-

PHAGES program in 2017. The isolation temperature was 35 degrees Celsius which is important because the typical incubation temperature of the host is 30 degrees Celsius. Comrade was isolated from an enriched culture. Comrade is a member of Cluster BK1 of the actinobacteriophages. Cluster BK1 currently has only 5 members, none of which have been completely annotated at this time. The BK1 cluster has an average size of 128,659 base pairs and an average GC content of 47.7%. Comrade has 129015 bp with a GC content of 47.1%. The preliminary annotation of Comrade includes 232 protein-encoding genes. Out of these 232 genes, 73 have found to have identifiable functions. Some of the functions include DNA methylase, RuvC resolvase, and WhiB family transcription factor. There are also 37 tRNA genes in our initial review. The bacteriophage Comrade's annotation will shed light on the newly discovered BK1 cluster, revealing how these bacteriophages function and interact with the world around them.

UGP42

Genomic comparison between two closely-related Cluster BD1 bacteriophages, Nabi and Rana, that infect *Streptomyces griseus*.

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Bacteriophages are viruses that infect and use a host bacterial cell in order to replicate and produce more of itself. They can be found nearly everywhere bacteria grows. The genus *Streptomyces* is one of the most diverse of the Actinobacteria, and members of this genus are ubiquitous in soil. Streptomyces phage Rana and Streptomyces phage Nabi were isolated from soil collected along a trail in Spurger, Texas using the host strain *Streptomyces griseus*. After isolation, the phages were purified and their DNA was extracted. They were then sequenced and annotated using various bioinformatics tools. Based on genomic comparisons, both Rana and Nabi were found to be in the same subcluster (BD1) of the actinobacteriophages, each with 76 ORFs and GC content of 65.8%. Out of 76 ORFs, 21 ORFs did not completely matchup between the two phages. They differ in length by 147 base pairs with Rana at 50,980bp and Nabi at 51,127bp. With the difference in base pairs, there is a difference in protein structures and functions. Nabi had more different ORF functions compared to Rana. Putative functions included tail spike protein, transcriptional regulator, helix-turn-helix DNA binding domain, and flagellar assembly protein FliH. Through bioinformatic analysis, Rana showed to have 40 ORFs with known functions while Nabi has 42 ORFs with known functions. By comparing these two very similar phages, we can expand our knowledge in the differences of their genetic makeup which leads them to be two different bacteriophages.

UGP43

Potential DNA Primase Frameshift in Streptomyces phage BryanRecycles

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Bacteriophages can contain programmed translational frameshifts (PTF's) in their genomes. These are programmed shifts in the translational reading frame that cause the ribosome to move by one or two nucleotides, resulting in a new codon that may code for a new protein. Prior studies on Tail Assembly Chaperones (TAC) were able to confirm the existence of a frameshift at that site. The TAC exhibits one of the most established examples of a frameshift in a phage. Our research focuses on the DNA primase gene in BryanRecycles, a Cluster BD1 phage isolated at the University of North Texas in 2016 by Bryan Burton. The Phamerator map shows 2 consecutive overlapping DNA primase genes, which is highly characteristic of a PTF. We are attempting to experimentally validate the existence or absence of the DNA primase PTF, working in tandem with another group of UNT students that are focusing on the same task with BryanRecycles' TAC gene. Through primer design and PCR, we will isolate the desired section of the genome. The product will be run through a gel, extracted, and run through a double digestion. pGlo vector DNA will be digested with corresponding restriction enzymes and then purified. Finally, the plasmid will be transformed into *Escherichia coli* DH5α and used to produce the gene product sizes. Moving forward, we will re-annotate the relevant portion of the genome to reaffirm our results bioinformatically. If our approach is successful, the methods we used can be applied to potential PTFs in other BD1 phages. Standardization of testing methods will save time and resources in the future.

UGP44

The Scientific Discovery of Streptomyces phage Austintatious

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What started out as mere plaques on an agar plate could be the key to understanding the secretive world of the most prominent parasites on the planet. The scientific field is always advancing and seeking to find new solutions to new problems. Therefore, research is essential for societal advancement. At the SEA-PHAGES program at the University of North Texas, we conducted research on the most abundant and mysterious parasite on Earth, bacteriophages. The host used in our study, *Streptomyces griseus*, is well known in the microbiology world as being the source of the "magic bullet" antibiotic, streptomycin. This bacterium is commonly found in soil as it helps to decompose organic material, is Gram-positive and has a high G+C content of about 72%. Studying its viruses could help us better understand this important bacterium. This could also lead to discovering how viruses could be defeated as we analyze their genes and functions. The bacteriophage that we annotated is Austintatious, a Cluster BC3 actinobacteriophage that was isolated and sequenced in 2015. Isolated from a soil sample in Denton, Texas, Austintatious is on the smaller end of the genome size spectrum, with 36,213 base pairs. There is a total of 56 genes that have been annotated and the G+C content is relatively high at 72.6%. Although it has been known for bacteriophages to carry tRNAs and tmRNAs, this particular one did not have any. The guide for isolation and annotation can be both found on PhagesDB, SEA-*PHAGES Discovery Guide,* and *SEA-PHAGES Bioinformatics Guide.*

UGP45

Bacteriophages and the Current Research on Streptomyces phage Gibson

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Bacteriophages are viruses that infect a host bacterium and use the host to replicate their genome and proteins. There are many bacteriophages to be discovered in the world, in fact, they are the most abundant entity on Earth. Because of this, they are also highly dynamic and forced to evolve quickly with the changing environment. In this particular lab, we used the bacterium *Streptomyces griseus* as our host. This experiment followed the SEA-PHAGES Discovery Guide procedures with the exception of a few adjustments to the enzyme restriction digestion procedure. Soil was taken from near the melon patch in a family garden where the soil was sandy loam. After processing the sample further, we discovered that the plaque morphology was circular, clear, roughly 1-1.5mm wide. The overall result is an isolated, purified, amplified, and sequenced bacteriophage named Gibson. Looking at the EM pictures, the phage appears to have siphoviridae morphology, with its hexagonal capsid head and medium length, thick tail. After being sent for sequencing at the University of Pittsburgh, Gibson was found to have a genome size of 69,439 bp and 107 protein-encoding genes. We did not find any tRNA. So far, we have found 28 identifiable gene functions in this phage such as hydrolase and HNH endonuclease. In conclusion, the experiment has yielded a successfully isolated and annotated bacteriophage that can be used to further our knowledge and advance modern science.

UGP46

Isolation and Genomic Analysis of the Wofford Bacteriophage

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Streptomyces griseus is a soil bacterium known widely for its use in producing antibiotics. Bacteriophages are viruses that infect bacteria. Wofford is a lytic bacteriophage of the siphoviridae morphotype isolated from soil using *S. griseus* as a host. Wofford was isolated using direct plating methods and its DNA was collected and sequenced. Wofford belongs to Cluster BE2 of the actinobacteriophages and its genome contains 133,007 bp, including a large direct terminal repeat of 11,214 bp. Wofford's genome has a guanine-cytosine content of 47.7%. Wofford's DNA was annotated using the Phage Evidence Collection and Analysis Network program (PECAAN). Its genes were analyzed for function association using the National Center for Biotechnology Information Base Pair Local Alignment Search Tool (NCBI BLAST), and HHPred. While annotating the gene sequence, we found many interesting

functions, such as HNH endonuclease and glycosyltransferase. These findings open the door to further study of Wofford and other *Streptomyces* phages.

UGP47

The Discovery of the Streptomyces phage LukeCage and the Further Analysis of its Qualitative and Genomic Properties

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Bacteriophages are highly abundant, but largely unstudied, viruses that infect and lyse bacteria. Phages are the most abundant organisms in the biosphere, yet are still largely unexplored. The bacteriophage population is extremely diverse and fast growing, making them a good area to research in order to understand their evolution. Also, the majority of phage genes are largely unknown in function, as only about 30% of genes in sequenced phages have confirmed function. This leaves much to still be learned about these organisms. During this lab, a bacteriophage named LukeCage was discovered and isolated on the host bacterium *Streptomyces griseus*. This phage was amplified and its genetic material extracted and sequenced for further genome annotation. This annotation used a bioinformatics program, the Phage Evidence Collection And Annotation Network, that collects the comparative and other evidence tools. LukeCage's biological characteristics are yet to be fully explored but there are many characteristics that make this phage interesting to explore. It has homology to multiple other phages in Cluster BE of the actinobacteriophages, but it would be one of the first to be fully annotated in the BE2 subcluster. With its relatively large genome, believed to contain 263 genes, aggressive nature indicated by the high volume of clear plaque shapes it forms, and genetic homology to other phages, LukeCage is a promising avenue to uncover the unknowns in the function and evolution of bacteriophage genomics.

UGP48

Annotation of Bacteriophage Olicious from *Streptomyces griseus*

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Viruses are particularly small and unable to be seen with the naked eye. There are diverse types of viruses that will infect different types of cells which are called the host cell. When a virus infects the host cell they are replicating and will go through their life cycle. Viruses carry their genetic information needed to replicate and synthesis the protein needed for reproduction. In this phage, we will be using the bacteria host of *S. griseus* which has already changed the medical field with its antibiotics. Our phage was sequenced at the University of Pittsburgh and with these steps Olicious was found to be in the Cluster BF with a genome size

46,139 bp and a direct terminal repeat length of 274bp. We were also able to find out the GC content to be 59.7% and the morphotype to be Podoviridae (short non-contractile tail). Bioinformatics includes a variety of techniques like sequencing structural alignment, prediction of protein structure and function, gene finding and expression data clustering. After DNA sequencing Olicious was then annotated using the database PECAAN which includes PhagesDB, HHPRED and NCBI. Through these databases we were able to identify the number of protein encoding genes potentially to be 65, tRNA genes potentially to be 22 ,and numerous functions within Olicious like metallophosphoesterase and helix-turn-helix DNA binding domain.

UGP49

Screening of Translational Frameshifting in Streptomyces phage BryanRecycles' Tail Assembly Chaperone Genes

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Programmed translational frameshifts are naturally occurring phenomena that allow the production of two peptide products for one mRNA transcript. In *Streptomyces* phages, a translational frameshift has been well documented in the tail assembly chaperone (TAC) genes, which was originally discovered and annotated in *E. coli* phage λ. The strongest identified regulatory factor of translational frameshifts is the "slippery sequence" which typically follows a 5'-XXXYYYX-3' structure. The TAC gene's slippery sequence is well documented as 5'-GGGAAAG-3' and thus is used to easily find the location of translational frameshifts in the TAC genes of unannotated phages. However, while the TAC gene's translational frame shift is well documented, frameshifts in other phage genes is less understood. In phage BryanRecycles, we have found a candidate for a potential translational frameshift in its DNA primase gene. The DNA primase gene has similar characteristics to the TAC gene's components that are evident of the translational frameshift. In its currently annotated state, the DNA primase gene has a large gap downstream of the gene which has significant coding potential in a -1 frame starting before the stop codon of the gene. Despite this evidence, a slippery sequence similar to TAC's could not be identified in the DNA primase gene. To prove the presence of translational frameshifts in BryanRecycles, our group is cloning the TAC genes into plasmid pGLO resulting in a protein fusion of the GFP gene with the product of the TAC gene. If the translational frameshift exists, then we will be able to observe two differently sized GFP tagged protein products that share the same N-terminal domain and have unique C-terminal domains. This experimental method is being used in parallel with a companion group who is attempting to do the same with the DNA primase genes. Given the well-characterized nature of the TAC frameshift in other phages, we expect to show the presence of this frameshift in BryanRecycles and show the viability of this method for use with other potential frameshifts in phage genes.

UGP50

Isolation of the DNA Primase Gene from Streptomyces phage BryanRecycles for Examination of a Potential Slippery Sequence.

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Production of the tail assembly chaperone protein (TACP) gene in bacteriophages is known to result in two products: a short, abundant tail assembly chaperone protein, and a longer version produced at a low rate. The gene contains a slippery sequence in which the guanine and adenine frequency is higher than normal upstream of the stop codon, allowing the ribosome to shift back into a different reading frame to bypass the stop codon of the short protein to produce the longer version. This is well documented for the TACP but has not been shown for the DNA primase (DNAPr) gene. The goal of this experiment is to examine if the DNAPr gene of a Cluster BD1 bacteriophage, Streptomyces phage BryanRecycles, contains a functional frameshift in the DNA primase genes. To do this, we are inserting the DNA primase genes into a plasmid to be expressed in *E. coli* DH5-alpha. To isolate and amplify the gene of interest, PCR using selective primers was used. The genes were first amplified using primers specifically designed to remove the start codon of the first DNA primase gene and the resulting PCR product was amplified once again with primers to reintroduce the start codon and a His-6-tag. This two-step PCR procedure was used to increase the likelihood of the His-6-tag primer annealing to the template DNA. Incorporation of HIS-6 tagged DNAPr gene into pUC18 plasmid vector is then done by blunt-end ligation. The gene product will be tagged using a Hexa-His-tag during PCR in order to selectively isolate the translated primase protein using immobilized nickel, cobalt or copper columns. The purified protein will be analyzed using SDS-PAGE utilizing the molecular weight as identification reference. The approximate weight of the short sequence is estimated to be 16 kDa and the long product is estimated to be 35 kDa. For further identification the purified protein from the bands on the SDS-PAGE, bands will be extracted and sequenced. Once completed this experiment will provide experimental data to support or refute the hypothesis that the DNAPr gene found in cluster BD1 bacteriophages results in two proteins of differing size, short and long sequence, due to a slippery sequence causing the ribosome to switch reading frame and bypass the stop codon of the short sequence.

UGP51

Discovery of Cluster A1 and O Bacteriophages, Arlo and Ryadel

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We isolated 16 bacteriophages that infect *M. smegmatis* mc^2155 from soil samples in north Texas. DNA was extracted and whole genomes of mycobacterophages Arlo and Ryadel were sequenced at the Pittsburgh Bactriophage Institute. Arlo is a temperate cluster A1

bacteriophage with medium plaque size and Ryadel is a lytic cluster O bacteriophage. Transmission electrom microscopy identified both Arlo and Ryadel to have siphoviridae morphology, however Ryadel has a prolate capsid which is characteristic of Cluster O bacteriophages.Arlo genome is 52,069 base pairs in size and contains a 10 base overlap of CGGATGGTAA, while Ryadel genome is 72,658 base pairs in size with a 4 base overlap of GTGT. Analysis of potential viral open reading frames indicate that Arlo could encode for a Cas4 protein, a component of CRISPR genome editing systems. These phages could provide further insights into bacterial immune functions and function of elongated capsid structures.

UGP52

Isolation of Novel 'Addmin' and Study of Immunity Suppressor Gene by Genomic Annotation of 'Rabbs'

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Tuberculosis is one of the oldest contagious illnesses across the globe, significantly, it would claim nearly 2 million lives in 2015 and it stands out as the leading killer of those infected with HIV. Standard tuberculosis treatments are usually centered on the use of antibiotics. As bacteria continue to adapt to the tuberculosis antibiotic treatments, the increase of multi-drug-resistant and extensively drug-resistant strains have been found. The isolation and study of novel non-pathogenic bacteriophages, specifically phages that infect *Mycobacterium* bacteria, could assist in developing phage therapy and aid in fighting against antibiotic resistant pathogenic bacteria. During the fall semester of 2017, a novel bacteria phage named 'Addmin' was cultivated from the Coastal Bend Area and analyzed via the spot test, transmission electron microscope, and restriction digest. During spring semester of 2018, the genomic DNA of bacteriophage 'Rabbs' was sequenced and annotated with DNA Master, HHPRED, and Phamerator. Gene 35 of 'Rabbs' demonstrates a high coding potential as an immunity suppressor gene. It is well known that bacteria host use immunity suppressors to respond to the phage attacks. The presentation of similar gene in bacteriophage could be a product of DNA recombination between the two genomes. Study of these genes will shed light on better understanding on the defense mechanisms of both phage and its host.

GRADUATE STUDENT POSTERS

GP1. Correlation between *Streptomyces griseus* Phages' Genome Length and their Burst Time and Burst Size

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Many previous studies have shown that bacteriophage burst time is manipulated by the endolysin-holin system. However, we have found no reports regarding a relationship between burst time and phage genome size. We hypothesized that smaller phage genomes would burst

the host cells faster than larger ones, and vice versa. In this study, 19 phages isolated on *Streptomyces griseus* were selected and grouped based on their genome sizes, ranging between 40-133 kb. The phage burst time was determined by one-step phage growth curve with MOI of 0.1. The preliminary results showed that the approximate constant period for Raleigh (40 kb), BryanRecycles (50 kb), Wentworth (68 kb), Comrade (129 kb), and Blueeyedbeauty (130 kb) ended at 5, 3, 3.5, 4, and 2.5 h, respectively. These results would indicate that phage genome size is not correlated with burst time. Further investigations are ongoing to confirm the validity of this small size sample's result, and to explain the occurrences observed.

GP2. Regulation of the PCA1 transcript by the Nonsense Mediated mRNA Decay (NMD) Pathway

Kaitlin Murtha

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The Nonsense-Mediated mRNA Decay (NMD) pathway is a highly-conserved mRNA surveillance pathway with a dual function. First, the pathway degrades mRNAs with Premature Termination Codons (PTCs). Secondly, it acts to control gene expression by degrading mRNA transcripts which do not contain PTCs. These targeted transcripts are called "natural mRNAs". Natural mRNAs are targeted by the NMD pathway by certain internal *cis* elements, for reasons largely unknown. Natural mRNAs have been found to belong to subsets of functionally related genes in both yeast and mammals. *PCA1* is one of these natural mRNAs that has been found to be a target of NMD. The *PCA1* gene is an alias of *CAD2*, a plasma membrane P_{1B}-type ATPase that transports cadmium. It also has a role in copper homeostasis. Peccarelli et al., 2016 showed that *PCA1* mRNA accumulates to higher levels in NMD mutants. We have determined which features of the *PCA1* transcript mark it a target of NMD. Interestingly, half-life experiments show that *PCA1* is differentially regulated by NMD in rich media versus low copper media. We aim to understand the mechanisms by which *PCA1* is regulated by NMD under different environmental conditions.

GP3. Role of phospholipid in *Mycoplasma pneumoniae* Community Acquired Respiratory Distress Syndrome toxin-mediated binding, intracellular transport and cytotoxic effects

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Background: The mollicute *Mycoplasma pneumoniae* is a frequent cause of human bacterial community acquired pneumonia in children and adults. In addition, *M. pneumoniae* infections can result in a wide array of extrapulmonary manifestations that can range in severity from mild to life-threatening. *M. pneumoniae* can colonize the human respiratory tract and exploit the host cell machinery using its virulence determinants, such as the adhesion proteins and Community Acquired Respiratory Distress Syndrome (CARDS) toxin. CARDS toxin is a unique bacterial protein that exhibits both ADP-ribosylating and vacuolating activities on

sensitive target cells and is largely responsible for recapitulating the inflammatory and histopathological effects seen in the airway cells of *M. pneumoniae* infected individuals. Nonetheless, in order to exert its activity on intracellular targets, CARDS toxin must first interact with its target cells' surface receptors, surfactant protein-A (SP-A) and Annexin A2 (AnxA2). However, our prior studies demonstrated that even in the absence of these proteinaceous receptors, CARDS toxin can still bind to host target cells and exert its activity, suggesting that CARDS toxin could use alternative receptors. This observation then prompted the search for a non-proteinaceous receptor. Materials and Methods: Using full length and truncated CARDS toxin derivatives, enzyme linked immunosorbent assay, immunoblotting, small interfering RNA (siRNA), live-cell imaging, and immunofluorescent & phase contrast microscopy, we investigated the possibility that membrane phospholipids might act as alternative receptors. Results: Earlier, we reported the binding of CARDS toxin to SP-A and Anx- A2, and here we show how CARDS toxin uses an alternative receptor to execute its pathogenic properties. In the present study, we report how CARDS toxin binds to a non-proteinaceous phospholipid in a concentration-dependent and saturable manner, which reinforces the specificity of the interaction and the potentiality of the phospholipid to act as a functional receptor for CARDS toxin. Using truncations of CARDS toxin, we demonstrated that the carboxy terminus of CARDS toxin mediated binding to phospholipid. Immunofluorescence analysis and live-cell imaging of CARDS toxin-treated cells demonstrated that CARDS toxin exploits phospholipid for binding and intracellular trafficking. Further, reduction of surface-associated phospholipid significantly decreased CARDS toxin binding, internalization and CARDS toxin- induced vacuole formation in human alveolar epithelial (A549) cells as well as in other human cell lines tested. Conclusion: CARDS toxin recognizes phospholipid as a functional receptor leading to CARDS toxin-mediated changes in mammalian cells. This type of characterization of CARDS toxin interactions with host cells will provide insights into how the toxin exploits host phospholipids for *M. pneumoniae* pathogenicity, which should facilitate future therapeutic developments and improve public health at large.

GP4. Functional analysis of M139, a pathogenesis factor of mouse cytomegalovirus

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Cytomegalovirus (CMV) is the major infectious cause of congenital complications and also a common cause of morbidity and mortality in bone marrow and organ transplant patients. Since, human cytomegalovirus (HCMV) can only infect humans, mouse cytomegalovirus (MCMV) is studied in the mouse model for understanding the function of viral pathogenesis factors. CMV genes can be classified as: immediate early, early and late. Expression of immediate early genes doesn't require viral transcription factors. Early genes are expressed before viral DNA replication. Late genes are expressed after viral DNA replication and many of these gene products are responsible for virus assembly and formation of mature virus particle. One gene family in cytomegalovirus is the US22 gene family, members of which play important roles in pathogenesis. This gene family includes M139, M140 and M141 in MCMV which are homologous to US22, US23 and US24 respectively in HCMV. Previous work has shown that the protein products of M139, M140 and M141 form a stable complex and the deletion of any one of these genes leads to replication impairment of MCMV in macrophages. The M140 gene product, pM140, is required for stable viral gene encapisdation in macrophages, but the role of

pM139 (protein product of M139) in virus replication is still unknown. Dr. Clive Sweet's lab showed that a mutant virus with a C-terminal truncation of 79 amino acids in pM139 replicates normally at 37°C but the replication is impaired at 40°C in fibroblast cells. The main objective of this study is to determine which step in the viral replication cycle is affected by M139 truncation towards the long term goal of clarifying the role of pM139 in infection. We found that there is no difference in the stability of Wildtype and Sweet's mutant virus particles at 37°C and 40°C indicating that the temperature sensitive defect in Sweet's mutant is intracellular. Truncation of M139 didn't alter the level of E1 (early protein) or major capsid protein (late protein) suggesting that the defect is in the assembly or release of virus which is under investigation. However, the M140 deletion mutant wasn't temperature sensitive indicating that this is separable from the previously identified functions in macrophages.

GP5. Microbial Hg Methylation Characterized by Illumina Sequencing in Caddo Lake, TX

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Caddo Lake in northeastern Texas is one cypress-Spanish moss dominated lake ecosystem. Contamination of mercury (Hg) especially methylmercury (MeHg), which is a neurotoxicant in major fish species and reptiles, has been reported in this lake decades ago. Due to the feature of bioaccumulation and biomagnification, Hg contamination in the lake fishes causes health concerns on the wildlife and local people. However, the source and synthesis of MeHg in this lake, primarily from microbial Hg methylation mechanisms, has been little studied. We investigated the lake for the past two years, by taking sediment and plant samples in several areas of the lake wetland habitats which showed high MeHg levels in fish from previous studies. We employed a culture-independent molecular approach to identify the Hg-methylating microbial community present in sediment as well as the sporangia of the invasive species Giant salvania (*Salvania adanta*). Total Hg, MeHg, sulfate and other biogeochemical factors were analyzed. For the first year total Hg concentrations in lake sediment (123.2 – 147.7 ng dw g^{-1}) were significantly higher than those in Spanish moss (*Tillandsia usneoides*) tissues (27.1-39.8 ng dw g^{-1}). However, MeHg levels in Spanish mosses (1.2-1.4 ng dw g^{-1}) were obviously higher than those in the sediment. Giant Salvania is an invasive species with the ability to decrease sunlight and oxygen concentrations to the detriment of fish and other aquatic animals. This increase in anoxic conditions is likely to increase the conditions for Hg methylation. We extracted genomic DNA from all the samples, and conducted the detection of functioning genes including the Hg methylation genes (*hgcAB*), methyl-coenzyme M reductase genes (*mcrA*) as well as 16S rRNA gene. The 16S rRNA genes were sent for high throughput next generation sequencing on Illumina MiSeq. In lake sediment samples, a total of 6402 OTUs were discovered, dominated with *Crenarcheales* (9.7%), *Bacteroidales* (5.2%), *Sinobcteracea* (4.5%). Our results indicated that the lake sediment samples contained potential mercury methylators, which included *Syntrophobacteraceae* (1.4%), *Geobacter* spp. (1.1%), SRB *Desulfovibrio-Desulfobulbus-Desulfobacter* (0.6%), and methanogenic archaea (0.6%). It seems that microbial MeHg production in this wetland habitat could be influenced by

a complex syntropy between methanogens and SRB.

GP6. Cell Surface Hydrophobicity of *Candida albicans* Upon Release of Cell Wall Protein by Dithiothreitol Treatment

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Midwestern State University

Candidiasis, disease caused by *Candida albicans* and related fungal species, is widely spread and constitutes a serious healthcare issue, particularly in the increasing number of immunocompromised patients. Disease is preceded by colonization, which is dependent on adhesion of *C. albicans* cells to host tissue surfaces. Cell surface hydrophobicity (CSH) facilitates microbial colonization by increasing the ability of the fungal cells to adhere. It also increases yeast cell virulence by increasing fungal resistance to phagocytosis. Our current model of CSH states that hydrophobic proteins are embedded within the cell wall. Changes in surface fibrillar protein structure mask or expose these hydrophobic proteins. Glycosylation of the fibrillar proteins, particularly the presence of b-1,2-oligomannosides, correlates with these changes. Electron micrographs of cells treated with dithiothreitol (DTT) show surface fibril protein arrangements similar to those seen in hydrophobic cells. Further, DTT-treated cells became hydrophobic. Although the exact mechanism of DTT action is still unclear, we hypothesize that it is causing the release of the fibrillary glycoproteins, leading to exposure of the hydrophobic proteins. Following treatment with DTT, *C. albicans* CSH was determined by a hydrophobic microsphere assay. Proteins released by DTT treatment were separated by SDS-PAGE and stained for both total protein and for glycoprotein. Localization of the b-1,2-oligomannosides was determined using galectin-3, a lectin that specifically binds to these carbohydrate groups. The results of these studies may help identify candidate fibril proteins and will provide insights into how changes in cell wall protein composition can influence interactions between *C. albicans* and the human host.

GP7. Identifying the relationship between bacterial dysbiosis, inflammation, and antibiotic use in chronic inflammatory bowel disease

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A complex relationship exists between gastrointestinal commensal microorganisms and their hosts. While it is not entirely understood how commensal microbiota influence the host immune system, it is evident that the two are largely dependent on one another, each one affected by the actions of the other. Disharmony of the healthy GI tract can result in chronic inflammatory bowel diseases, such as Crohn's disease (CD) or ulcerative colitis (UC). In the healthy GI tract, the lower intestine is largely hypoxic, thus it is expected to be largely dominated by anaerobes. However, inflammation in the large bowel results in dysbiosis of the microflora such that obligate anaerobes decrease in number while the presence of facultative anaerobes increases. As previous literature demonstrates, this could be due to the fact that inflammation in the host generates reactive nitrogen and reactive oxygen species, molecules

that facultative anaerobes can use as final electron acceptors in anaerobic respiration. As has also been documented, use of antibiotics can result in persistent alterations in the gut microbiome composition that mimic the alterations seen in the inflamed gut. The *Gambusia affinis* fish model is good for investigating IBD because it has the necessary components of a vertebrate immune system yet is less expensive than mice or rats. Dextran sulfate sodium (DSS) is used to induce clinical-grade colitis in rodent models. Our preliminary data suggests that DSS can likewise generate intestinal inflammation in a dose-dependent manner in fish. We predict that inflammation induced by DSS will affect the gut microbiome of the *Gambusia affinis* similarly to treatment with rifampicin, a broad-spectrum antibiotic. In this way, we hope to demonstrate the potential overlap between inflammation and antibiotic usage, especially effects on microbiome community composition.

GP8. Effects of Microalgal Extracts on Stenotrophomonas maltophilia Biofilms

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Biofilms are extracellular matrices produced by bacteria for adhesion and are a major factor in cell-to-cell gene transfer, promoting the development of antimicrobial drug resistance (AMDR). AMDR is becoming a more prevalent and life-threatening feature of human infections, especially in hospital settings. Extracts of both marine and freshwater microalgae have been shown to have antimicrobial effects, including some activity against bacterial biofilms. This study compares the antimicrobial activity of two types of lipid extracts from select species of microalgae of the kingdom, *Chromista.* Assays for bacterial biofilm viability and bacterial growth have been performed according to established methods to assess the effect of microalgal extracts on film and planktonic growth in *Stenotrophomonas maltophilia*. Antibiotic sensitivity of *S. maltophilia* in established biofilms and planktonic culture was also assessed in the presence of microalgal extracts as a means of examining whether algal extracts increase sensitivity of *S. maltophilia* cultures to common antibiotics. Preliminary results demonstrate significant decreases in the viability of *S. maltophilia* biofilms treated with extracts from *Botryococcus braunii, Nannochloropsis oculata, Eustigmatos vischeri*, and *Clorochromonas danica* and *Rhodomonas Lens*. Furthermore, a slightly enhanced sensitivity of *S. maltophilia biofilms* to both Azeotram and Cefatriaxone in the presence of microalgal extracts from *Tisochrysis Galbana* and *Mesostigma viride* was observed*.* The extracts did not exhibit a significant impact on drug sensitivity in planktonic cultures of *S. maltophilia*, as demonstrated by the minimum inhibitory concentrations (MICs) of select antibiotics with and without extracts present.

GP9. Assessing the impact of stormwater runoff on the bacterial community structure and the prevalence of fecal indicator bacteria in an urbanized bay

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Enteric or gut-associated bacteria can accidentally be introduced to aquatic environments through wastewater (e.g., wastewater treatment plant outfalls and leaking sanitary sewer systems) and stormwater (e.g., urban, agricultural, and industrial runoff). Consequently, these

potentially harmful microbes can disrupt the natural balance of marine ecosystems, threatening both marine and human health. In particular, the coastal bays of Texas, where barrier islands limit exchange with the Gulf of Mexico and increase residence time, are sinks for stormwater runoff. Moreover, the loading of runoff in coastal Texas is predicted to worsen with population growth, urbanization, and climate change. Despite the growing concern of increased stormwater runoff, its impact on coastal marine ecosystems is largely unknown. To address this issue, we are conducting a multifaceted study that asks four important questions: 1) Do storm events increase the concentration of fecal indicator bacteria (FIB)? 2) What are the most probable sources of the FIB? 3) How does the loading of stormwater runoff affect the overall bacterial community composition? and 4) Is antibiotic resistance more prevalent among FIB following storm events? Our findings indicate that the levels of enterococci increase significantly ($p < 0.001$) following storm events. Additionally, the overall community structure is significantly less diverse ($p < 0.005$) after rainfall, indicating that storm events act as environmental disturbances in marine environments. The results of this study address open questions about the fate of FIB in an urbanized bay and the impact of stormwater runoff in coastal ecosystems.

GP10. Microbiota biodiversity and anatomical comparison of the intestinal tracts amongst the Order *Rodentia* occupying similar environmental niches

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The microbes that live within animal intestines participate in a multitude of important functions such as digestion, pathogen exclusion, and the generation of vitamins/amino acid precursors. Current work has provided numerous insights into this dynamic population, but often relies on comparisons within a single species, or between numerous distantly related species. While intestinal microbe populations are generally conserved between animals of the same species, the population is variable and sensitive to the host's diet, geographic location, and immune system. Along with their microbial populations, an organism's morphometries, such as overall length and weight, intestinal length, or cecum size, provides insight to the species specialized diet and a suggestive link to the expected microbiota population. Here, we will assess intestinal morphology and microbial populations of four species from the order *Rodentia* found in the same ecological niche. We hypothesize that the microbe populations will be unique each species given the variability unique natural histories and diet preferences of each species, despite occupying similar environmental ecological niches. Here we report our preliminary efforts to collect target animals and begin to quantify their morphometric differences and microbiologic populations using the V4 and V5 sub regions of the 16S rRNA.

GP11. Sterol regulatory element binding protein 2 (SREBP2) activation is a common pathway between HCMV and MCMV

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Cytomegalovirus (CMV) is a herpesvirus, with strict host specificity, having a high prevalence among the human population ranging between 70 to 100%. It is one of the largest enveloped viruses, with a linear double stranded DNA genome with a size of 230 kb. There is clinical correlation between CMV infection and atherosclerosis in both humans and the mouse (MCMV) model system. Studying similarities and differences in host responses may help to identify some important factors in pathogenesis. We found that, like HCMV, MCMV infection induces SREBP2 (involved in cholesterol synthesis) and ATP-citrate lyase downstream in the same pathway. However, unlike HCMV the fatty acid synthesis pathway involving SREBP1 was not induced by MCMV. These results support the presence of a common conserved feature between HCMV and MCMV for one pathway but not the other and, if lipogenesis is important for pathology, implicates SREBP2 over SREBP1. Since CMV is an enveloped virus, increased lipid synthesis in infected cells may provide more envelope components and promote production and spread in infected tissue. Ways to control or reduce lipogenesis might be used as tools for virus inhibition. Blueberry extract has previously shown an inhibitory effect on lipogenesis both *in vitro* and *in vivo*. We hypothesized that using blueberry extract may inhibit or reduce viral progression and/or lipogenesis induced by MCMV. To test this hypothesis blueberry extract was applied to NIH-3T3 fibroblast that were either mock infected or infected with MCMV. Cells were harvested at various time points and tested for SREBP1 and 2 as lipogenesis regulation factors. Blueberry extract failed to inhibit either virus production or the induction of SREBP2. These results do not support our hypotheses on the effect of blueberry on CMV infection. However, the results indicate that blueberry extract may have reduced efficacy on regulating lipogenesis with CMV sero-positive individuals.

GP12. Photo-chemically activated AgNPs with varying antibacterial activity

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There is growing interest in utilizing the unique antimicrobial properties of silver nanoparticles (AgNPs) to address medicinal therapy, antisepsis or disinfection. The antimicrobial activities of AgNPs may be affected by many factors like synthesis method of AgNPs - physical, chemical and biological, shape, size, electrical charge, and modification to their surface. Antimicrobial properties of AgNPs have been well documented but their mechanism of action remains still unclear. The present study was aimed to investigate the antibacterial activities of two novel AgNPs and address the mechanism behind any antibacterial activity. Here, we showed that photo-chemically synthesized, negatively charged AgNPs made in poly(acrylic acid) (PAA), are bactericidal. However, photo-chemically synthesized, positively charged AgNPs made in chitosan, have no evidence of any antibacterial activity. $AgNO_3$ was used as a positive control. The functional AgNPs have antimicrobial activity against Gram-negative bacteria like *Escherichia coli* and *Serratia marcescens* which are well characterized human pathogens. The negatively charged AgNPs were found to be stable for at least 10 months with the same efficacy of antibacterial activity. The preliminary results under the conditions we used, indicated that some components in media are important to have antibacterial activity and we are working on which factors are important. Understanding what leads to inactivation of AgNPs

could be important for their disposal to limit environmental impact. A better understanding of mechanisms of action underlying the antibacterial effects of AgNPs could contribute to design of new silver containing nanomaterials to use in clinical and therapeutic interventions.

GP13. A TAXONOMIC AND FUNCTIONAL ANALYSIS OF BACTERIAL DIVERSITY IN AGRICULTURAL SOIL TESTING FOR ORGANOPHOSPHATE BIODEGRADATION

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The organophosphate (OP) insecticide chlorpyrifos and herbicide glyphosate are most among the most widely used pesticides in the United States. Chlorpyrifos, a potent neurotoxin and glyphosate, recently classified as a probable carcinogen by the World Health Organization, therefore represent xenobiotics that can significantly affect both environmental and human health through contaminated soil. The purpose of this research is to evaluate the capacity and extent bacterial populations found within agricultural soil collected across the Houston metropolitan area able to degrade these pollutants. Whole genomic DNA was isolated from collected soil samples and sent to Genewiz (Plainsfield, NJ) for metagenomics sequencing, library construction and species abundance analysis to compare changes in bacterial population and presence of bacterial OP degradation biomarkers. Sequence annotation was performed through the Metagenomic Rapid Annotation using Subsystem Technology (MG-RAST) server. NCBI BLAST tools and the Kegg protein database were then used to identify putative degradation biomarkers. Sequencing of collected soil was found to harbor multiple microorganisms putatively capable of degrading chlorpyrifos and glyphosate with species representing the genera *Pseudomonas*, *Rhizobium*, *Stenotrophomonas*, and *Klebsiella* predominating across all sites. Chlorpyrifos degradation biomarkers belonging to a family of diverse OPHC2-like metallo-β-lactamase (MBL) enzymes that catalyze OPs at low catalytic rates were observed in all samples. Glyphosate oxidase (GO) biomarkers were also found in high abundance in all samples collected. A single hit for a putative bacterial phosphodiesterase (PDE) enzymes that may target chlorpyrifos was found only in a single sample collected from ranch soil. Mobilized biomarkers such as bacterial phosphotriesterase (PTE) were not observed in any of the samples.Taken together, the results demonstrate that intrinsic OP biomarkers are well-dispersed throughout agricultural soil, but catalytic activity is likely to be low. The addition of a well-characterized bacterial consortium may therefore significantly improve microbial degradation of both chlorpyrifos and glyphosate in soil and have significant bioremediation applications. In the future, the degradation of chlorpyrifos or glyphosate-polluted soil samples spiked with an OP-degrading consortium will be compared with non-augmented soil over a period of 30 days to assess how the consortium impacts native OP degradation in soil.

GP14. Microbiome Overgrowth Following Disruption is Microbial, not Host Driven

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The microbiome refers to the bacterial communities that intimately interact and live on or in a host. These bacterial communities form complex interactions with the host, environment, and with foreign bacteria, and play an important role in the developing of the host's immune system. Also, it has been shown how disruption of this stable microbiome can lead to many health problems and diseases. In our lab, *Gambusia affinis*, the Western Mosquitofish, is used to model vertebrate mucosal microbiomes. A physical rinse, a rapid non-selective mechanical removal of bacteria, was used to deplete and disrupt the skin microbiome of *G. affinis.* During recovery after the rinsing protocol, a large increase (over 3 log) in culturable bacterial colony occurs, peaking at two days after rinsing and returning to pre-treatment levels around day twelve. We hypothesized that fish were overexpressing mucus after rinsing, driving the overgrowth effect. However, skin mucin quantification did not show a correlation with bacterial CFUs during recovery. This result suggests the bacterial overgrowth effect is a function of the microbiome community and not host driven.