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Abstract Book

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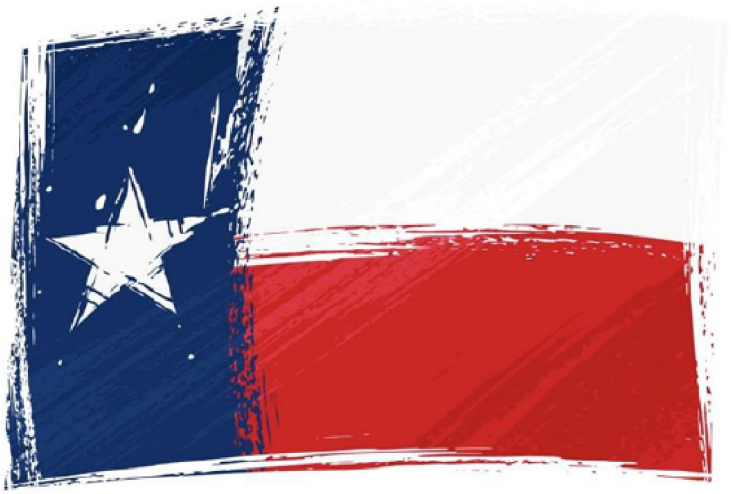

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Undergrad Oral Presentation

UO1 – **Case Study: relapsing urinary tract infection and subclinical bacteriuria due to Corynebacterium amycolatum in a cat**

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Introduction
Over a year, numerous clinical isolates of *Corynebacterium amycolatum* were recovered from a male cat with a history of chronic kidney disease, perineal urethrostomy, subcutaneous ureteral bypass (SUB), urolithiasis, and recurring urinary tract infection (UTI). Despite brief clearance of UTI after each treatment with β-lactams and SUB flush, *C. amycolatum* was repeatedly isolated from the patient's urine. *C. amycolatum* is rarely recorded as a cause of infection in animals and has not been reported in feline UTI to date.
Methods
The 5 isolates were subjected to MALDI-TOF mass spectrometry and RapID CB Plus system. In addition to sequencing the *16S rRNA* and *rpoB* genes, the lipophilia of the isolates was evaluated by using Brain Heart Infusion (BHI) media with (and without) 1% Tween 80. The isolates were also tested for antimicrobial susceptibility by broth microdilution.
Results
All isolates were identified by MALDI-TOF mass spectrometry as *C. amycolatum*. In contrast, the RapID CB Plus system identified the isolates as *Corynebacterium jeikeium* with $\geq$99.9% probability. Partial *16S rRNA* sequences showed 100% nucleotide (nt) similarity to numerous *Corynebacterium* spp. A hypervariable region of the *rpoB* gene had $\geq$ 98% nt identity with *C. amycolatum* and *C. jeikeium*, with all others being $\leq$ 91%. The identification of *C. amycolatum* was ultimately confirmed by using BHI agar, whose supplementation with Tween 80 did not enlarge bacterial colonies of the isolates. Over the several months of treatment, *C. amycolatum* developed increasing resistance to β-lactams. However, the most recent isolate was surprisingly susceptible, which could be suggestive of an internal (e.g., biofilm) or external source of reinfection.
Conclusions
To the best of our knowledge, this is the first report of *C. amycolatum* associated with feline UTI. In contrast to the mass spectrometry, the genotypic testing and commercially available biochemical system were insufficient to speciate *C. amycolatum*. If MALDI-TOF mass spectrometry is unavailable, it is recommended that lipophilia be assessed to differentiate *C. amycolatum* from *C. jeikeium*.

UO2 – **Plasmid Mediated Antibiotic-Resistant Environmental *Enterobacteriaceae***

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Antibiotic resistance is a significant concern across the globe. Specifically, members of *Enterobacteriaceae* constitute a significant cause of multi-drug resistance nosocomial infections. Antibiotic resistance in this family is drastically increasing, and many of the antibiotics used to treat these infections are no longer effective. In this study, 19 environmental isolates were isolated from water across Texas using eosin methylene blue and xylose lysine deoxycholate media. The 16s rRNA gene from each isolate was sequenced to determine the genus and predict the isolates' molecular phylogeny. Based on the 16s sequencing, 47% of the isolates are members of *Enterobacteriaceae*, 21% *Pseudomonadaceae*, 16% *Moraxellaceae*, 5% *Comamonadaceae*, and *Neisseriaceae*, and the remaining are likely members of *Flavobacteriaceae*. Antibiotic susceptibility testing was conducted on each isolate to determine the resistance or susceptibility against a panel of common antibiotics. Our data shows that several of the environmental isolates were resistant to commonly prescribed antibiotics. *Aeromonas*, *Enterobacter*, *E. coli*, and *Acinetobacter* were found to be resistant to trimethoprim, rifampin, ampicillin, and amoxicillin/clavulanic acid, respectively. The opportunistic pathogens, *Pseudomonas* and *Chromobacterium*, were both resistant to ampicillin, amoxicillin/clavulanic acid, and rifampin. These data indicate that *Enterobacteriaceae* isolated from the environment are exhibiting resistance to multiple antibiotics used to treat these infections. Current and future work includes the molecular identification of plasmid and strain genotypes, which play a role in horizontal gene transfer events leading to the spread of antibiotic resistance. These findings demonstrate multidrug-resistance occurring in environmental isolates with potential implications of horizontal dissemination.

UO3 – **Investigating the host-pathogen mechanism between Madagascar Hissing Cockroaches and the parasite *Blabericola migrator***

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Gregarines are a vast, diverse but understudied group of parasitic protists that commonly infect the intestinal epithelia of different species of invertebrates. Gregarines are closely related to the human pathogen *Cryptosporidium spp.*, which causes cryptosporidiosis, a serious and sometimes fatal diarrheal illness. Gregarines and *Cryptosporidium* are also similar in their epicellular lifestyle and specialized life cycle, suggesting that Gregarines may serve as a useful model to study these pathogens. In this study, a laboratory colony of Madagascar Hissing Cockroaches *(Gromphadorhina portentosa)* was used to propagate and isolate the intestinal stages of the Gregarine *Blabericola migrator*. The goal of this study was to develop microscopic tools to investigate host-pathogen interactions between *G. portentosa* and *B. migrator*. Parasites were fixed either isolated from the intestine or cryosectioned *in situ* and stained with a variety of commercially available reagents recognizing evolutionarily conserved structures. One of these, *Vicia villosa* Lectin (VVL), has been shown to stain cell structures in other apicomplexan parasites. We observed staining of characteristic Gregarine surface structures called epicytic folds as well as a septum-like structure with VVL and anti-Myosin antibodies in *B. migrator*.Anti-$mathstuff\alpha$-Tubulin staining *B. migrator* also localized to the epicytic folds. The DNA dye, Hoechst, stained an unknown punctate structure that was transferred from the satellite parasite to the primite parasite. Future research will be done using anti-histone H3 antibody and Na+/ K+ ATPase $mathstuff\alpha 1$ antibody to better locate the movement of DNA between the parasites.

Graduate Oral Presentation

GO1 – **Uncovering the role of penicillin-binding protein 1A in cell division and multidrug resistance in *Acinetobacter baumannii***

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The increasing prevalence of antibiotic treatment failure due to multi-drug resistant infections highlights the need to understand underlying resistance mechanisms. Of particular concern is resistance to last-line antimicrobials, such as colistin (polymyxin E). Colistin targets the ubiquitous lipooligosaccharide (LOS) outer membrane anchor, lipid A, which was considered essential for viability in diderm bacteria. However, several LOS- *Acinetobacter baumannii* clinical isolates were recovered after colistin selection, suggesting a conserved resistance mechanism. Previously, we identified that inactivation of the conserved cell wall synthase, penicillin-binding-protein 1A (PBP1A), is required for colistin selection of LOS- *A. baumannii*. The transglycosylase (TGase) enzyme activity of PBP1A is key for *A. baumannii* survival without LOS, whereas the transpeptidase (TPase) activity is not. However, the impact of PBP1A loss was not characterized. Current knowledge of PBP1A is based on studies in E. coli, which suggests the primary function is cellular elongation. In contrast, our data demonstrate that PBP1A inactivation produces nondividing multi-septate cells, pointing to an alternative role in *A. baumannii* cell division. Fluorescent cell wall precursors incorporated in the peptidoglycan cell wall were used to identify septation defects leading to cell chaining. Using a fluorescent protein fusion, we found that PBP1A was enriched at the midcell divisome complex and its localization was dependent on TGase activity. To further understand the implications of PBP1A on antimicrobial resistance, clinical isolates with mutations in PBP1A were tested against cell-wall targeting β-lactam antibiotics. Our analysis suggested that PBP1A is a major cell wall synthase required for proper cell division in *A. baumannii*. Additionally, inactivation of PBP1A primes for both colistin and β-lactam resistance through enzymatic loss of function. Our studies support a model where the loss of PBP1A function conditions *A. baumannii* to rapidly develop multidrug or extensively drug-resistant infections.

GO2 – **The roles of *dksA*-like genes in *Paracoccus denitrificans***

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Paracoccus denitrificans, a facultative anaerobe, uses nitrogen oxyanions and oxides as respiratory electron acceptors under anaerobic conditions. Nitrate (NO_3^-) is reduced sequentially to dinitrogen (N_2), via nitrite, nitric oxide (NO) and nitrous oxide (N_2O) in the pathway known as denitrification. The intermediate NO is both toxic and a critical signaling molecule that mediates the transition from aerobic to anaerobic respiration. NO is scavenged by reduction to N_2O, or by oxidation to nitrate by the flavohemoglobin, Hmp. Our unpublished work demonstrates that Hmp plays a crucial role in mediating the switch from aerobic to anaerobic respiration. In *Escherichia coli*, the ppGpp and RNA polymerase binding protein DksA is required for transcription of *hmp* and for NO detoxification. Thus, we are interested to investigate the potential role of DksA in regulating NO metabolism in *P. denitrificans*. Our literature review and analysis of the *P. denitrificans* genome revealed two *dksA*-like genes having increased expression during anaerobic growth. We constructed strains with deletions of the *dksA*-like genes (*Pden_0916* and *Pden_0547*), and a double deletion mutant. Strains deleted for *Pden_0547* exhibit an aerobic growth defect in media containing butyrate as the carbon and energy source and nitrate. Optimal growth on reduced substrates such as butyrate requires the disposal of excess reducing equivalents, for example through nitrate reduction by the periplasmic nitrate reductase NAP. Unlike the wild-type strain, *Pden_0547* mutants fail to up-regulate NAP expression in butyrate-grown cells. Thus, one DksA-like protein is implicated as a regulator of expression of the *nap* operon. NO stimulates biofilm production in *P. denitrificans*, either directly or indirectly. We observed a 4-fold reduction in biofilm production in strains deleted for *Pden_0916*. Assays of a reporter fusion to the *hmp* promoter show that *Pden_0916* is a negative regulator of *hmp* expression. We suggest that increased *hmp* expression in the *Pden_0916* mutant reduces NO accumulation in static cultures leading to reduced biofilm formation. Our study reveals distinct phenotypes associated with mutations in two *dksA*-like genes suggesting roles for DksA in the regulation of nitrate and NO metabolism.

GO3 – **Expression of cold shock transcriptional regulator CaspA and the putative virulence-associated protein VacB/RNaseR restores growth at low temperature in pandemic O3:K6 serotype Vibrio parahaemolyticus**

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Vibrio parahaemolyticus is the leading cause of shellfish consumption associated gastritis worldwide, partly as a result of clonal expansion of the highly pathogenic O3:K6 serotype. Genomic analysis of environmental *V. parahaemolyticus* 03:K6 strains isolated from the Pacific Northwest (PNW) led to the discovery of a novel, predominant environmental ecotype missing an entire genetic locus spanning from *VP1884* through *VP1890*. This locus was found previously to be transcriptionally upregulated upon bacterial culture at cold temperatures. Among the missing genes is *VP1889* annotated to encode the cold shock transcriptional regulator *CspA*. We deleted the *VP1884-VP1890* locus from clinical O3:K6 laboratory strain BAA-239 and complemented *cspA* and its neighboring ORF *VP1888* in the chromosome. We hypothesized that the absence of clinical O3:K6 serotype *V. parahaemolyticus* as the primary serovar causing vibriosis in the PNW in spite of broad distribution of environmental O3:K6 isolates is caused by their inability to withstand temperatures associated with shellfish harvest. Allelic exchange with the *lacZ* reporter gene from *V. cholera* and the *tetR* selection marker from pBR332 was employed to generate a Δ*VP1884-VP1890* O3:K6 strain to test the hypothesis that *cspA* and *VP1888* affect bacterial growth kinetics at cold temperatures. The Δ*VP1884-*Δ*VP1890::lacZ/tetR* strain displayed no growth defect at 30°C but did at 10°C. The growth defect of the Δ*VP1884-*δ*VP1890::lacZ/tetR* strain was partially complemented by *cspA* alone and; interestingly, over complemented by expression of both *cspA* and *VP1888* at 10°C. These results suggest that the *VP1884-VP1890* genetic locus codes, at least, two genes essential for growth at temperatures within the range employed to store shellfish harvested from the ocean. This may be contributing to the unusual pattern of *V. parahaemolyticus* O4:K12 serotype dominance as the principal cause of vibriosis in the PNW, whereas O3:K6 is the predominant clinical serovar in the rest of the world.

Poster – Session 1

Basic and Environmental

U1 – **Assembly and characterization of metagenome assembled genomes to uncover the hidden subsurface microbial life of Blackwood Sinkhole, Bahamas**

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Prokaryotes make up the majority of the biomass in sediment, where they cycle organic carbon and regulate the transformation of organic matter. Microbes inhabiting the sediment display a great diversity, but the role of each species remain cryptic. Here, we used metagenomics to better understand the potential roles and functions of the microbial communities inhabiting sediment from an anoxic coastal basin. We analyzed six metagenomes from separate stratigraphic layers of a 90 cm core (dated to ∼1,500 years old) from Blackwood Sinkhole, Bahamas. Each layer was found to have distinct microbial community structures with sulfur reducers and methanogens present in the upper layers, while sulfur oxidizers and hydrocarbon degraders were found at the lower depths (72.5–76.9 cm and 79.5–86.9 cm). We assembled 166 high quality metagenome assembled genomes (MAGs), with the majority belonging to the phyla Proteobacteria (38%), Actinobacteria (15%), and Chloroflexi (9%). We also generated a nutrient profile for nitrate, nitrite, silica, and phosphate. Our results will provide connections between specific species and their role in the global geochemical cycles.

U2 – **Determining Effective and Comfortable Face Coverings to Decrease SARS-CoV-2 Transmission**

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During the COVID-19 pandemic, non-pharmaceutical interventions were introduced to decrease its spread. Since SARS-CoV-2 is airborne, face coverings were recommended. We wanted to identify face coverings that are both comfortable and effective. During summer 2020, we initially tested the effectiveness of seven face coverings using aerosolized Glo Germ with each covering placed on a wig stand. UV light was used on the uncovered wig stand to assess how much of the substance was transmitted. Of the seven materials, the scarf, fishing scarf, and bandana failed to block the Glo Germ, while the surgical mask, T-shirt mask and hand sewn cotton and cotton-chiffon hybrid masks showed no Glo Germ penetration. Next, we tested for the comfort of the masks since an effective mask that is difficult to wear may not be used consistently. Nine students wore the four masks that had been effective in the previous test and then performed 8 activities for 1 minute each. They then completed an online mask comfort survey. Results indicated that the surgical mask, sewn cotton mask, and sewn cotton-chiffon mask were all acceptable for long-term use and comfort but the T-shirt mask scored poorly. In fall 2020, the 3 masks that blocked Glo Germ and were found comfortable were tested against aerosolized *Serratia marcescens*. To accomplish this, 8ml of a 0.5 McFarland Standard was nebulized through each mask into a filter holder collected bacteria that were able to penetrate the mask. The filter was vortexed in peptone water and serially diluted and plated on BHI plates and incubated. Colony forming units were measured and the number of bacteria determined and compared with a no mask control. The percent effectiveness was calculated, and statistical analysis were performed. A one-way ANOVA test was used to compare each mask to the others and to the no mask control. Usage of any mask compared with the no mask control included a p-value of 0.001 which indicates statistical significance while the p-values comparing the masks with each other did not show significance (0.1089). These data indicate that wearing a mask is effective in transmission of *S. marcescens* but that using this method of testing was not sensitive enough to reveal differences in the effectiveness of each mask.

Pathogenic Micro

U3 – **Clinical Considerations and Treatment Efficacy for Biofilm-Associated Chronic Wound Infections**

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Biofilms comprise a community of microorganisms encased within a self-produced extracellular matrix which acts as a mechanical barrier to both the immune system and antimicrobials, often leading to the development of a chronic wound. Chronic wound infections boast high morbidity and mortality rates and are responsible for profound medical costs in the U.S annually. The presence of bacterial biofilms, coupled with the absence of information regarding the best course of treatment, deems these wounds difficult to eradicate. Biofilm-associated wounds are usually polymicrobial, whereas most acute infections present as monomicrobial; biofilm-associated bacteria are up to 1000X less susceptible to antimicrobial treatment compared to planktonic bacteria. Current standard-of-care methodologies in clinical laboratories are often not representative of the chronic wound environment and therefore may lead to improper reporting of microbial diversity and antimicrobial susceptibility to clinicians and hospital units. Due to the inability to perform sufficient diagnostic testing, many patients are receiving inadequate therapies - leading to amputation, prolonged hospital stays, and possible loss of life. There exists a disconnect between basic science, clinical laboratorians, and clinical care providers in the diagnosis and treatment of biofilm-associated infections. While a wide breadth of knowledge exists about the basic science of biofilm-associated chronic wound infections, there is a notable paucity of studies regarding evidence-based clinical interventions and best-practices. This review encompasses a summary of the available literature on the efficacy of treatment options displaying clinical evidence, as well as recommendations for the clinical management of such infections. Translation of biofilm knowledge into laboratory diagnostics and clinical care remains paramount in improving patient outcomes and quality of life as well as reducing hospital costs and prolonged stays.

U4 – **Investigating *Pseudomonas aeruginosa* cyan fluorescence utilizing the MolecuLight *i:X* bacterial imaging device**

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Chronic wound infections are a cause of patient morbidity and mortality and are a significant concern in the clinical setting. Chronic wounds are defined as those that persist six weeks or longer and are often refractory to medical intervention. Chronic wound infections are known to be polymicrobial and harbor multiple species of bacteria, with one of the most notorious species being the pathogen, *Pseudomonas aeruginosa*. MolecuLight *i:X* is a handheld bacterial imaging device designed to visually detect auto-fluorescent characteristics of most clinically relevant pathogens in real-time. A red fluorescence signature is detected with the *i:X*device from the bacterial exoproduct porphyrins. Additionally, *P. aeruginosa* exhibits a unique, cyan fluorescence signature; clinically, when a chronic would exhibits cyan fluorescence with the *i:X* device, there is over a 90% positive predictive value that the wound will harbor *P. aeruginosa*. It is thought that the cyan fluorescence is due to virulence factor exoproduct production, and previous work in our laboratory has demonstrated that *P. aeruginosa* cyan fluorescence can be induced in response to stressful bacterial environments (such as immune system challenge or nutrient deprivation). Investigation of the mechanisms responsible for the observed cyan fluorescence is necessary to better understand the clinical detection of *P. aeruginosa* in chronic wounds using the MolecuLight *i:X* device.

Phage and Antimicrobial Resistance

U5 – **Isolation and Annotation of Cluster EG Bacteriophage Fizzles**

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Bacteriophages are a potential novel treatment for bacterial infections. Bacteriophage Fizzles was directly isolated from host *Microbacterium foliorum* NRRL-24224 SEA incubated with a soil sample from dry soil in an ant nest in a subdivision in Stephenville, Texas. Following two rounds of serial dilution and plaque assay, Fizzles formed small, lytic plaques with turbidity throughout the plaque. Negative-staining transmission electron microscopy showed Fizzles has *Siphoviridae* morphology with an approximate tail length of 150 nm and capsid diameter of 50 nm. Phage DNA was extracted with a modified zinc chloride precipitation method and then sequenced to 2719-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina Next Generation Sequencing to determine a double-stranded DNA genome of 62,078 base-pairs that contains direct terminal repeats of 181 base-pairs. Whole-genome sequence analysis using a suite of bioinformatic software revealed Fizzles has 68.2% G+C content and 103 protein-coding genes transcribed rightwards (48.1% of genome) and leftwards (51.9% of genome), which encodes for structural proteins, a histidine triad nucleotide binding protein, hydrolase, MazG-like nucleotide pyrophosphohydrolase, HNH endonuclease, RuvC-like resolvase, DNA primase/helicase, RecA-like DNA recombinase, and nucleotide pyrophosphohydrolase. Fizzles is a cluster EG bacteriophage and most closely related to Microbacterium phages Squash and Nike.

U6 – **Modulation of Bacterial Host Phenotypes by Mycobacteriophage Pixie Gene Products**

*Matt Bristerpostma[1], Denise Andreotti-Phillips[1], Yabram Basurto[1], Miguel Carrillo[1], Christopher Freeland[1], Shady Kuster[1], Chamey Suchors[1], Madalyn Patton[1], Josue Zuniga[1], Selina Alvarado[1], Faith Cox[1], Harold Rathburn[1], Dustin Edwards[1]

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Bacteriophage genes are being studied for their potential clinical use in phage therapy for antibiotic resistant infections. With the support of the Howard Hughes Medical Institute, as part of the Science Education Alliance Gene-function Exploration by a Network of Emerging Scientists (SEA-GENES), we have amplified 45 out of 100 genes of interest from Mycobacteriophage Pixie and cloned 32 genes for study in cytotoxicity and superinfection assays. Genes were amplified from Pixie high titer lysate by PCR amplification, and the products purified and ligated into a pExTra plasmid by isothermal assembly. Plasmids were cloned into 5-alpha F'Iq *Escherichia coli*, and the extracted DNA was electroporated into *Mycobacterium smegmatis* mc2155. Cytotoxicity assays were conducted by plating serial dilutions of pExTra-containing *M. smegmatis* on inducer plates containing anhydrotetracycline (aTc). Cytotoxicity was determined based on plasmid-containing *M. smegmatis* growth versus controls. Superinfection assays were conducted using aTc induction of *M. smegmatis* plasmid-containing lawns inoculated with serially diluted bacteriophages D29, Larva, and Pixie and compared to control *M. smegmatis* to determine the efficiency of plating. Our study revealed 4 potentially cytotoxic genes (a putative major capsid protein, uncharacterized gene downstream of the lysin A/B proteins, putative tyrosine integrase, and uncharacterized gene adjacent to the immunity repressor) and screened 18 genes for superimmunity. This information broadens the understanding of bacteriophage-bacterial interactions and can allow us to apply it to clinical use of bacteriophages.

U7 – **Bacteriophage Tank18, Isolated from a Microbacterium foliorum Culture**

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Bacteriophages are viruses that replicate within the domain bacteria. Tarleton State University, as part of the Howard Hughes Medical Institute SEA-PHAGES Program, has worked to collect liquid and soil samples to isolate and characterize novel bacteriophages for potential use in phage therapy for antibiotic resistant bacterial infections. Bacteriophage Tank18 was discovered in a sandy environment, at approximately one centimeter depth, near a horse barn in Stephenville, Texas, and was directly isolated and incubated with the host *Microbacterium foliorum* NRRL-24224. Following four rounds of serial dilution and plaque assays 2mm lytic plaques were formed. Negative-staining transmission electron microscopy showed that Tank18 had a *Siphoviridae* morphology with a tail length of 115 nm and a capsid diameter of 45nm. Restriction enzyme digest identified enzymes HaeIII, NspI, and SalI as able to digest bacteriophage Tank18 DNA. The phage Tank18 was archived at the Pittsburgh Bacteriophage Institute and Tarleton State University.

U8 – **Testing plant extracts from Asia and South America for antimicrobial properties**

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Antibiotic resistance (AR) is a major threat to public health, causing more than 35,000 deaths and 2.9 million infections in the US annually. These infections are more difficult to treat and result in repeated physician visits and increased hospital stays. With increasing AR many common antibiotics are no longer effective against infections. Medicinal plants have been used in traditional medicine in many countries due to their accessibility and low cost. In this project I, tested plant extracts from Asia and South America that were obtained from the National Cancer Institute to see if any had antimicrobial properties. I used a Kirby Bauer disk diffusion method to test 120 plant extracts against *Staphylococcus aureus*. It was hypothesized that most samples would not exhibit zones of inhibition. I resuspended 50 ug of each lyophilized extract in 200 proof ethanol and impregnated a disk with each extract. Ethanol alone was used as a control. The disks were placed on Mueller Hinton agar inoculated with *S. aureus* and examined for zones of inhibition detected 24 hours later. I used 25 ul of ethanol to resuspend the samples and found that impregnating the disk with half of the sample then drying the disk for 15 minutes and then adding the rest of the sample did not produce background inhibition. Eleven samples from Asia and 4 samples from South America exhibited zones of inhibition ranging from 9mm to 15mm, indicating that they have antimicrobial properties. Future research will focus on separating the extracts into their components and determining the identity of the antimicrobial substance which may then be developed into a therapeutic agent.

Graduate

G1 – **Regional and sex-dependent differences in the microbiome of Ixodes ricinus ticks**

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Introduction: Understanding the microbial ecology of disease vectors may be useful for development of novel strategies aimed at preventing transmission of vector-borne pathogens. Although *Ixodes ricinus* is one of the most medically important tick species, the microbiome of *I. ricinus* ticks has been dissected for only limited parts of the globe. To date, the microbiome of *I. ricinus* from Eastern Europe has not been defined. The objective of this study was to compare microbiomes of *I. ricinus* ticks within (males vs. females) and between collection sites that represented three administrative regions of Ukraine, Dnipropetrovs'k (D), Kharkiv (K), and Poltava (P).
Methods: A total of 89 individual microbiomes of questing *I. ricinus* adults were analyzed by targeting the V6 region of 16S rRNA gene through the Illumina 4000 Hiseq sequencing.
Results: The alpha diversity analyses demonstrated that, regardless of tick sex, patterns of bacterial diversity in ticks from regions K and P were similar, whereas the microbiome of region D ticks was quite distinct. A number of inter-regional differences were detected by most beta diversity metrics for both males and females. The inter-regional variations were also supported by the unweighted UniFrac results with three region-dependent clusters of female ticks and one distinct cluster of region D males. Lastly, numerous region- and sex-specific differences were also identified in the relative abundance of various bacterial taxa.
Conclusions: Collectively, the present findings demonstrate that the microbiome of *I. ricinus* ticks can exhibit a high degree of variation between tick sexes and geographical regions.

G2 – **Chronic Wound Polymicrobial Communities and the Impact to Antimicrobial Susceptibility**

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Recent advances in sequencing technologies have demonstrated that many chronic infections are polymicrobial in nature. In polymicrobial communities, multiple species interact and can synergize activities, leading to decreased antibiotic efficacy and worse patient outcomes. Chronic wounds are known to be polymicrobial biofilm-associated infections, and are highly refractory to clinical treatment. While there is an extensive body of literature demonstrating changes to antimicrobial efficacy in response to the biofilm environment, there is a notable paucity of studies examining the effects of polymicrobial synergism. This project investigates the shifts to antimicrobial efficacy of four clinically relevant wound pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterococcus faecalis*) when grown in a polymicrobial community. When comparing antimicrobial susceptibility in the monomicrobial verses polymicrobial condition, shifts in antimicrobial efficacy were observed. This demonstrates that current clinical methods for determining antibiotic susceptibility may not fully represent the clinical environment. Acknowledging the role of the polymicrobial community in infectious processes and their impact on antimicrobial susceptibility is crucial in order to more effectively treat patients and manage chronic infections.

G3 – **Development of a SYBR Green-Based RT-qPCR for the Detection and Quantification of Lone Star Virus**

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Lone Star Virus (LSV) is a newly characterized tick-borne phlebovirus in the *Phenuiviridae* family isolated from the lone star tick, *Ambylomma americanum*. LSV was first identified in 1967 but remained unstudied for over 40 years until its genome was sequenced in 2013. LSV was determined to be a negative single-stranded, tri-segmented RNA virus. Genome sequencing data indicated LSV is related to members of the Bhanja group of phenuiviruses, and Bhanja group viruses are associated with febrile illness with central nervous system involvement in clinical and laboratory settings. LSV is phylogenetically closely related to two newly emerged viruses that present with serious illness, Heartland Virus and Severe Fever with Thrombocytopenia Syndrome Virus. LSV is a potential pathogen as evidenced by infection and cytopathic effect in human (HeLa) and nonhuman primate (Vero) cell lines. LSV does not currently have a diagnostic assay available, and the prevalence or location of the virus is unknown in the environment. RT-qPCR assays are beneficial for the prompt diagnosis of disease, monitoring of viral load, and biosurveillance. A SYBR green-based RT-qPCR assay is advantageous over probe-based methods due to its low cost, time efficiency, and simplistic design. The development of a SYBR green-based RT-qPCR assay for LSV will provide a rapid, sensitive, and specific detection method for diagnostic and research purposes as well as determine the level of risk this virus poses in medical and veterinary settings.

G4 – **Characterization of the Microbiome of the Barton Springs Salamander**

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Globally, over 70% of amphibians are suffering a decline, with the urbanization of ecosystems being a threat to amphibians. The Edwards Aquifer provides water for the city of Austin, Texas, as well as as a habitat to the federally endangered *Eurycea sosorum*. The Austin Nature and Science center is a permanent facility where salamanders are held for a captive rearing program with the aim of reintroduction of salamanders into Barton Springs. We hypothesize that the microbiome of host captive salamanders differs from that of wild salamanders and could be contributing to negative effects on host fitness. The skin microbiota of amphibians is a vital organ that regulates different physiological processes and has been shown to impact overall host fitness by providing protection against fungal pathogens. Microbial community composition between captive and wild populations of amphibians varies dramatically in species richness and abundance. In partnership with the City of Austin, we collected skin microbiota, fecal, substrate, and water samples from salamander habitats to characterize the differences in microbial communities between captive and wild salamanders. Bacterial and fungal DNA was extracted from the samples and bacterial 16S rRNA and the ITS region of fungal rRNA was amplified using Earth Microbiome Project primers. Amplicons were cleaned using AMPure brands to enrich target and remove non-target DNA prior to index PCR with barcoding primers. Index products were pooled to equimolar concentrations and purified using Pippin Prep. Amplicons were sequenced at Texas A&M Genomics and Bioinformatics Services. Sequence analysis was conducted using QIIME and USEARCH. Operational taxonomic units (OTUs) will be assigned using Greengenes database and USEARCH then aligned with PyNAST. The OTU table was clustered at 97% similarity, filtered to remove rare OTUs, and normalized. Maximum likelihood phylogenetic trees will be constructed using FastTree. Unweighted UniFrac distance metrics will be utilized to compare differences in beta diversity due to temporal, geographical, and wild versus captive microbiomes.

G6 – **Metagenome assembled genomes of Galveston Bay show resiliency following Hurricane Harvey**

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On August 24, 2017, Hurricane Harvey made landfall in south Texas and subsequently traveled north to Houston and stalled for five days, where it set the record for the highest rainfall amounts ever recorded in the United States. The resulting storm water runoff created a massive pulse event carrying terrestrial, freshwater, and anthropogenic nutrients, chemicals, and microbes into Galveston Bay. Over a five week period following Hurricane Harvey, metagenomic samples at four locations were taken along a transect from the San Jacinto River to the Gulf of Mexico. The metagenomic samples were used to generate metagenome assembled genomes (MAGs) to better understand how the pulse event affected particular taxonomic groups as well as the genomic potential of those groups. In total 1,395 MAGs were created from a co-assembly of the samples of which 103 were high quality and 349 were medium quality MAGs that were used for further analysis of taxonomy and genomic potential. Comparison of the taxonomy and abundance of the MAGs show no overall trends; however, phyla such as Actinobacteria and Verrucomicrobia were more prevalent in the earlier samples. At the whole metagenome level, there were slight shifts in the genomic potential of certain metabolic pathways such as photosynthesis and nitrogen and sulfur metabolism, but these changes were not apparent within the MAGs. The lack of a clear pattern in the genomic potential of the MAGs indicate that while whole ecosystem changes were occurring there was no major change in the function of the microbial groups that could be identified. While the increasing weather events remain a large challenge, these results would suggest that the microbial populations in coastal ecosystems are resilient to pulse disturbances both at the taxonomic and functional level.

G7 – **The Effect of Debaryomyces hansenii on Clostridium difficile sporulation**

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Clostridium difficile is a gram-positive, spore-forming, opportunistic pathogen. This obligate anaerobe is naturally present in low abundance in the gastrointestinal (GI) microbiome. At low levels the bacterium does not pose a threat to the health of the host, but when a stressor is introduced to the environment the bacteria can become an opportunistic pathogen and cause an infection. A common stressor associated with and *C. difficile* infection (CDI) is antibiotics; certain antibiotics used to treat other bacterial infections can alter the composition of the microflora. To survive the presence of the antibiotics *C. difficile* will form spores which not only aid in the colonization of the bacterium in the GI tract but are also the main cause for hospitalization and community acquired infections. Probiotics help to restore the balance of the microflora and can decrease the chance of having a relapse infection. There are some probiotics, termed next generation probiotics (NGPs) that produce secondary metabolites which have broad-spectrum antimicrobial properties. Here, we present preliminary data using a potential NGP, *Debaryomyces hansenii*, showing its effect on the sporulation frequency of *C. difficile*.

Poster – Session 2

Basic and Environmental

U9 – **Validation of the MolecuLight i:X bacterial imaging device for use in the food safety industry**

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The MolecuLight *i*:X is a hand-held bacterial imaging device that detects the autofluorescent properties of most clinically relevant species of bacteria. It is FDA-approved to detect bacteria within chronic wound infections in real-time and has been shown to aid in the point-of-care treatment and diagnostics. Specifically, many species of bacteria in the presence of endogenous aminolevulinic acid (ALA) will produce the exoproduct porphyrin which exhibits autofluorescent properties detectable with the i:X device. Currently, there is interest in expanding the use of the MolecuLight *i*:X device to detect microorganisms relevant to food safety for use in commercial industry. Preliminary data has demonstrated that the i:X device can detect numerous food pathogens, including *Listeria monocytogenes, Salmonella enterica*, and *E. coli* O157:H7. This work will describe further efforts to validate the use of the i:X device in food safety, including expanding testing of relevant food pathogens and contaminants detectable with the device, detection of bacteria on a variety of industrial surfaces, and induction of bacterial fluorescence on non-living food contact surfaces.

U10 – **Evaluating Face Masks for Comfort and Effectiveness to Protect the Public**

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During the COVID-19 pandemic, interrupting the spread of SARS-CoV-2 protects people. With no vaccine initially, non-pharmaceutical measures were sought to decrease the reproduction rate of the virus. Since face coverings have been found to reduce virus transmission, we examined which face coverings and fabrics could be worn comfortably and are most efficient in blocking droplets and aerosols. In the summer of 2020, we used GloGerm to determine which common 7 face covering styles (winter scarf, fishing scarf, bandana, surgical mask, no-sew t-shift mask, cloth mask (100% cotton), and cloth hybrid (100% cotton/chiffon)) were most effective against simulated respiratory droplets using a mannequin head. After treatment, the coverings were removed and any droplets that penetrated the coverings were detected via UV light. We found that all coverings, except the fishing scarf, protected the mannequin against the aerosol. To test for comfort, nine students wore several masks types and completed a survey. These included a disposable surgical mask, a no-sew t-shirt mask, and double layer sewn masks with 100% cotton or cotton and chiffon layers. The surgical mask, sewn cotton mask, and sewn cotton-chiffon mask were all acceptable for long-term use and comfort. During fall, aerosolized *Serratia marcescens* was used to further determine the effectiveness of the masks. To accomplish this, 8mL of a McFarland standard of *S. marcescens* was aerosolized using a nebulizer and the bacteria penetrating the masks were trapped using a filter using a vacuum. The filter was then placed into 9mL of peptone water and was serially diluted in peptone water then 100ul plated onto BHI agar and incubated for 36 hours at 37°C. Colonies were counted, and colony forming units were calculated. The percent effectiveness was determined by comparison with a no mask control. Preliminary results showed that the CFU penetrating of each of the three masks was below the level of detection (fewer than 30 colonies on each plate). The future goal is to refine this procedure to quantify the results and analyze these using statistical analysis more precisely.

Pathogenic Micro

U11 – **Development of a Triplex qPCR assay for Avian Retroviruses**

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Reticuloendotheliosis virus (REV) is an immunosuppressive avian retrovirus that infects the B cells of Galliformes, Passeriformes, and Anseriformes. Modern REV testing utilizes a duplex quantitative polymerase chain reaction technique (qPCR) developed by the Texas A&M Veterinary Medical Diagnostic Laboratory. We modified this technique to a triplex TaqMan qPCR assay to test simultaneously for REV and an emerging avian retrovirus, lymphoproliferative disease virus (LPDV), which has not yet been detected in Texas but has been found in neighboring states. We amplified the pan-avian GAPDH gene as a DNA extraction control and the REV and LPDV Env genes to test for viral infection. Primers and hydrolysis probes were designed using IDT PrimerQuest and ThermoFisher Scientific Multiple Primer Analyzer software to determine targeted conserved regions of the DNA and to minimize cross-reactivity using Cy5, FAM, and HEX fluorophores with double non-fluorescent quenchers to reduce false positives. Target duplexed DNA sequences were synthesized to serve as positive amplification controls and serially diluted to known concentrations to form a standard curve for proviral DNA quantification. Reaction conditions and primer-probe concentrations were optimized and reactions performed in triplicate with a 384-well plate. The validated triplex assay will be used to test for REV and LPDV proviral DNA in blood samples collected between 2018-2020 in Texas in collaboration with the Texas Parks and Wildlife Department.

U12 – **A Method of Biofilm Diagnostics: Exploring Autofluorescent Properties of a Biofilm Extracellular Matrix**

*Rachel C. Diaz[1], Laura M. Jones[3], Monique Y. Rennie[3], Allie Clinton Smith[2]

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Chronic wounds are polymicrobial biofilm-associated infections that fail to progress through the normal stages of healing and remain open for six weeks or longer. Biofilm-associated bacteria are able to secrete an extracellular polymeric substances (EPS), which acts as a mechanical barrier to both the immune system and antimicrobial treatment, and biofilm-associated infections have been shown to be more difficult to treat and can lead to adverse patient outcomes. The MolecuLight *i:X* handheld real-time bacterial imaging device has been developed that detects autofluorescent properties of bacteria via the exoproduct porphyrin to aid in chronic wound diagnostics. The device has been previously demonstrated to detect bacteria both planktonically (free-living) and within biofilm, and there is an interest of developing a mechanism to determine if there is a unique fluorescence signature associated with biofilm EPS that can be detected with the *i:X* device. To investigate this, a polymicrobial mixture of chronic wound pathogens *S. aureus, E. coli, E. cloacae,* and *P. mirabilis* were evaluated as both planktonic bacterial suspensions and biofilms to identify potential changes in patterns of autofluorescence. In a full emission spectral scan, a panel of excitation and emission wavelengths from different bacterial growth environments were evaluated to determine if there was a specific fluorescence peak associated with bacterial EPS that could be indicative of biofilm. This could potentially inform a specific setting within the MolecuLight *i:X* device for detection of a biofilm within chronic wound infections, allowing clinicians to diagnose not only bacterial infections but also the presence of a biofilm in real-time. The MolecuLight *i:X* imaging device has the potential to alter how biofilm-associated chronic wounds are diagnosed and treated in a clinical setting, improving patient care.

U13 – **Genetic implications of viral encephalitis: Up regulation of DRD2 gene potentially linked with psychological symptoms of the virus**

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Viral encephalitis a direct invasion of the brain by viral pathogens that causes inflammation of the tissues due to the infection or an autoimmune response. The inflammation causes the brain to swell, which can lead to headaches, a stiff neck, sensitivity to light, and even cognitive symptoms such as confusion, disorientation, psychosis, hallucinations, and memory loss. One out of every 10 people die from encephalitis. There is still a lack of understanding as to why patients acquire certain symptoms with viral encephalitis and what leads to such rapid mental deterioration. The aim of this bioinformatics study was to identify a common set of differentially expressed genes in multiple data sets by examining control human DNA and human DNA that has been infected with viral encephalitis and determine what changes in gene presentation are contributing to the symptoms of encephalitis, especially the neurological changes and inflammation. Three raw gene expression data sets of viral encephalitis were obtained from a public data repository and analyzed with the TAC software. Gene expressions were exported to Microsoft Excel and organized from largest linear fold change to the smallest. Numerical rank and linear fold change were considered in the selection of significant up-regulated genes. From the top 100 upregulated genes in each of the three data sets, 21 were found to be commonly up regulated. Out of the 21 genes, the most significant up regulated gene in relation to the psychological symptoms of encephalitis was the DRD2 gene. This gene is a subtype of a dopamine receptor, and previous studies have found up regulations of this gene to be a causative factor of the symptoms in schizophrenia cases. Since the psychological symptoms of schizophrenia and viral encephalitis are very similar (hallucinations, impaired concentration, confusion and disorientation), there is the potential that the up regulation of this gene in viral encephalitis is also causing the psychological symptoms that these patients experience. To further prove this hypothesis, MRI's of patients with viral encephalitis show inflammation of the brain specifically in the thalamic regions of the brain, which happens to be where dopamine production occurs as well. All of these results provide information for a testable hypothesis suggesting that the up regulation of the DRD2 gene contributes to the psychological symptoms in viral encephalitis.

Phage and Antimicrobial Resistance

U14 – **Isolation and Annotation of Cluster EE Bacteriophage PurpleGoat**

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Antibiotic-resistant bacteria continue to be a global concern, and alternative treatments, such as bacteriophage therapy, are of increased interest. Sequencing of novel bacteriophages further elucidates what is known about their evolution and application. Bacteriophage PurpleGoat was extracted from an ant pile in Stephenville, Texas, in 2020 and isolated using host bacterium *Microbacterium foliorum* NRRL-24224. Following two rounds of serial dilution, PurpleGoat formed slightly turbid circular plaques 3mm in diameter. PurpleGoat was then amplified and purified from webbed plates for a high volume lysate. Transmission electron microscopy identified PurpleGoat to have Siphoviridal morphology with a 145nm tail and 45nm capsid. PurpleGoat is a Cluster EE bacteriophage, 17,542 bp in length with 68.7% G+C content and 9 bp overhang of 5'-CCCGCCCCA-3'. PurpleGoat has 26 predicted protein-encoding genes, including structural proteins, DNA binding proteins, an endolysin, and endonuclease. PurpleGoat was determined to be 100% identical to bacteriophage Scamander, previously isolated in a vegetable garden over 1,600 km away in Rockville, South Carolina, in 2017. Bacteriophages Rowley, also found in Stephenville in 2018, and Bernadetta, found in Pensacola, Florida, in 2020, are also identical. This particular phage might be associated with commercial soil, but the discovery of Bernadetta may suggest otherwise, as it was found in a water meter box.

U15 – **Prevalence of antibiotic resistance in bacteria isolated from the Comal Springs riffle beetle, Heterelmis comalensis**

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The widespread use of antibiotic has led to increase of antibiotic resistant genes in pathogenic, commensal and environmental bacteria. Most animals are inhabited by diverse communities of microbes, also known as the microbiome; and the gut microbiome of animals is believed to be an important reservoir of antibiotic resistance genes. Little is known about the prevalence and distribution of antibiotic resistance genes in the microbiome of wild animals. The aim of this study is to evaluate the prevalence and distribution of antibiotic resistance genes of bacteria isolated from the Comal Springs Riffle Beetle, *Heterelmis comalenis*. We used CARD-RGI (https://card.mcmaster.ca/analyze/rgi) to characterize the repertoire of antibiotic resistance genes in the genome of 60 bacterial strains isolated from *H. comalensis*. We found that all except four strains isolated from the riffle beetle presents at least one form of antibiotic resistance gene against one or more drug classes. We are currently performing antibiotic resistance assays to assess the susceptibility of the 60 bacterial strains to 9 different antibiotics. This study can provide further information on whether the bacteria from the microbiome of wild animals, such as the aquatic beetle *H. comalenis* can serve as a reservoir of antibiotic resistance in the environment.

U16 – **Isolation and Annotation of Cluster EB Bacteriophage IndyLu**

*Ashley Suris[1], Raylon Huckaby[1], Jaime Merrill[1], Selina Alvarado[1], Tommy Butler[1], Carlos Canales[1], Matthew Castro[1], Julia Gaston[1], Marlee Goppert[1], Jesse Laposky[1], Jessica Lee[1], Elizabeth Mullins[1], Victoria Saadeh[1], Damla Ustundag[1], Josue Zuniga[1], Faith Cox[1], Dustin Edwards[1]

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With the growing concern surrounding antibiotic resistance, there is increased interest in bacteriophage-based therapies as an alternative treatment strategy. Bacteriophage IndyLu was directly isolated from a soil sample taken from a dry area near a horse barn in Stephenville, Texas, and incubated in host *Microbacterium foliorum* NRRL-24224 SEA. Following two rounds of serial dilutions and plaque assays with a soft agar overlay, IndyLu formed small, defined lytic plaques less than 1cm in diameter. Negative-staining transmission electron microscopy revealed *Siphoviridae* morphology with an approximate tail length of 140 nm and capsid diameter of 60 nm. Phage DNA was extracted with a modified zinc chloride precipitation method and sequenced to 1156-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina Next Generation Sequencing. A double-stranded DNA genome of 41,958 base-pairs with a 10 base 3' sticky overhang (ACTCCCGACA) was determined, making IndyLu the sixth largest member of cluster EB, with an average G+C content of 66.2% for the cluster, and most closely related to Microbacterium phages Didgeridoo (96% coverage) and Lahqtemish (95%). Whole-genome sequence analysis using PECAAN, PhagesDB, NCBI BLASTn and BLASTp, HHPRED, and TmHmm revealed 72 protein-coding genes transcribed rightwards (94.5% of genome) and leftwards (5.6% of genome). Putative genes include structural proteins, a HNH endonuclease, Holliday junction resolvase, and Cas4 family exonuclease have already been identified.

Graduate

G8 – **Infection of Human Endothelial Cells with Colorado Tick Fever Virus Stimulates Cyclooxygenase 2 Expression and Vascular Dysfunction**

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Colorado tick fever (CTF) is a potentially life-threating illness caused by Colorado tick fever virus, a double stranded RNA virus in the Reoviridae family. CTF generally self-limiting manifestation of myalgia, fever, headache, leukopenia, and a petechial rash. However, severe CTF symptoms include meningitis, hemorrhagic fevers, and meningoencephalitis. The mechanism of CTFV mediated pathology is currently unknown. Furthermore, there are no detailed studies that define the molecular mechanisms underlying CTFV associated endothelial damage. Cyclooxgenase-2 (COX-2) is a known mediator of inflammation and associated with various pathophysiological conditions. We demonstrate in this study that CTFV infected HMEC-1 show induction of COX-2, but no apparent effects on COX-1 isoforms using transcriptomics analysis and qPCR. Angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2) are known biomarkers produced during endothelial and vascular dysfunction in many infections. The ratio of ANG-2/ANG-1 is a biomarker of endothelial activation and vascular damage that could be used to identify severe infections. Additionally, Tie-2 is an endothelial reception involved in inflammation and vascular leakage that is associated with CTFV infected HMEC-1s. The analysis of our transcriptomics data shows the increase of pro-inflammatory cytokines, interleukin-8 (IL-8), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1), that are recognized markers of vascular inflammation. Our data suggests that CTFV induces pathological characteristics of vascular activation and dysfunction as measured through enhanced COX-2 expression, skewed ANG-2/ANG-1 ratio, and increased levels of pro-inflammatory cytokines. We uncover specific biomarkers for CTFV-induced vascular dysfunction and highlight future therapeutic research for this neglected tick-borne disease.

G9 – **Targeted High-Throughput Cultivation of Methylotrophic Bacteria from Oso Bay Sediment**

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We have developed a method for high-throughput isolation and cultivation of bacteria from marine sediment using fluorescent activated cell sorting. Isolation of individual cells has aided in the ability to bypass traditional enrichment techniques that often select for fast growing generalists. Sorting individual cells has decreased time required to obtain pure isolates, which can take months depending on the isolate. Increased culturing efforts coupled with whole genome sequencing has also aided in expanding known metabolic capabilities within genera. This project expanded upon these methods to use selective media to isolate methylotrophic bacteria from wetland sediment and characterize them using whole genome sequencing. Sediment was collected from Oso Bay, Corpus Christi, TX using a push core. Sediment from push cores were either 1) enriched using methanol and methane combinations, or 2) an unamended slurry was made with PBS. Cells were separated from the sediment via gentle vortex and filtered to remove large particulates. Single cells within filtrates were fluorescently labelled using BacLight LIVE/DEAD differential stain and sorted into media containing methanol as a sole carbon source with a BD FACSJazz cell sorter. Growth was monitored via measurements of optical density using a VarioSkan Lux plate reader, taxonomically screened using 16S rRNA gene sequencing, and three isolates were selected for whole genome sequencing using Illumina MiSeq sequencing platform. Two isolates were preliminarily identified within the genus *Methylophaga*, a facultative methylotroph. Phylogenetic tree construction is being done to accurately place isolates within the genus. Genome analysis is currently underway to determine carbon metabolic pathways including denitrification and sulfur oxidation, which has been noted in other *Methylophaga* species. Expansion of isolates for this less well defined genera generally increases in known diversity of metabolic capabilities for said genus. *Methylophaga* is similar in this way; over the past 20 years, additions to *Methylophaga* have demonstrated the ability to use nitrate as sole electron acceptor under denitrification conditions as well as oxidation of a variety of sulfur compounds. These discoveries, along with our cultivation efforts, will aid in understanding methylotrophs further as well as expanding their ecological importance in various nutrient cycles.

G11 – **Increased production of aromatic amino acid precursor-derived carboxylic acids by methanotroph Methylomicrobium alcaliphilum in response to carbon dioxide.**

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Methanotrophic bacteria (methanotrophs) play an essential role in the biogeochemical cycling of methane (CH4) due to their unique capacity to use this gas as a carbon and energy source. These bacteria are also promising agents for the bioconversion of natural gas and anaerobic digestion-derived biogas to high-value products. We previously demonstrated that exposure of the methanotroph Methylomicrobium alcaliphilum 20ZR to mock diluted biogas (30% CH4/20% CO2) inhibits bacterial growth but induces the production of the polylactic acid (PLA) bioplastic precursor lactic acid. To gain further insight into this phenotype, we evaluated the metabolic response of M. alcaliphilum 20ZR to biogas using untargeted metabolomics. Our results show that M. alcaliphilum 20ZR not only increased lactic acid production in response to biogas, but also significantly induced (∼100-fold) synthesis of aromatic amino acid biosynthetic pathway-derived metabolites, including indolelactate, phenyllactate and 3-(4-hydroxyphenyl)lactate. Transcription of genes encoding pyruvate reductase or prephenate dehydrogenase was significantly decreased during cultivation with biogas compared to CH4 alone, which may be responsible for an increase in available hydroxypyruvate and phenylpyruvate substrates available for enzymatic conversion by lactate dehydrogenase (LDH) under these conditions. Consistent with our initial increased lactic acid observations, we measured a 2.5-fold increase in LDH activity in whole cell lysates from bacteria cultivated with mock biogas. Collectively, our data support a model wherein CO2 elicits a physiological response by M. alcaliphilum 20ZR that balances metabolic enzyme activities to support increased pyruvate moity-containing metabolite conversion to their respective lactate derivatives by lactate dehydrogenase. The knowledge gained in this study will guide future metabolic engineering efforts to increase methanotroph production of versatile PLA synthons from CH4.

G12 – **Role of a NosP-like protein in the regulation of formaldehyde metabolism in Paracoccus denitrificans**

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Growth of *Paracoccus denitrificans* on methanol or methylamine generates formaldehyde as a toxic intermediate. Formaldehyde is oxidized to formate by a glutathione-dependent formaldehyde-activating enzyme (GFA), a glutathione-dependent formaldehyde dehydrogenase (GD-FALDH), and an S-formyl glutathione hydrolase (FGH). Expression of the genes encoding these is regulated by a two-component regulatory system, FlhSR. The *flhS* and *flhR* genes flank a gene of unknown function previously designated *orf2*. The product of orf2 is similar to NosP, a heme-containing nitric oxide (NO) binding protein which, in other organisms, regulates the activity of a histidine kinase or a diguanylate cyclase. GD-FALDH also has S-nitrosoglutathione (GSNO) reductase activity. Since GSNO is toxic and may accumulate in cells exposed to NO, there is a potential physiological rationale for increased expression of the formaldehyde oxidation machinery in response to NO. We predict that FlhS and Orf2 are interaction partners and that Orf2 is the recognition element in a signal transduction pathway that regulates formaldehyde metabolism. We are interested to test this hypothesis and to determine whether Orf2 functions as a sensor of formaldehyde and/or nitric oxide. We created in-frame deletions in genes of Orf2, FlhR and FlhS, and generated lacZ reporter fusions to the promoter of the operon encoding the GD-FALDH, GFA and FGH and the promoter of the *moxF* gene (encoding the methanol dehydrogenase), also regulated by FlhSR. Previous work has shown that *flhS* and *flhR* mutants are unable to grow on solid media with either methanol or methylamine as the sole source of carbon and energy. We have repeated this observation, but also found that these mutants can grow in liquid media, albeit at a greatly reduced rate. The *orf2* mutant has a similar phenotype to the *flhS* and *flhR* mutants, consistent with the suggestion that Orf2 serves as the signal recognition protein to activate FlhSR. Accordingly, the activities of FlhSR regulated promoters are greatly reduced or eliminated in an *orf2* mutant. Based on our current data, there is no indication that Orf2 mediates a response to NO. Thus, we are led to the prediction that Orf2 acts with FlhSR to up-regulate transcription in response to formaldehyde. It appears that NosP family members have diverged to mediate responses to different small molecule signals.

G13 – **Synergistic Effects of Biofilm Dispersion and Antimicrobial Therapies**

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Biofilms are sessile bacteria communities adhered to a substrate. Biofilms are noted to provide many advantages to bacterial cells, including increased resistance to antimicrobial compounds. Following dispersal from biofilms, antimicrobial susceptibility returns. The present study investigated the efficacy of combination treatment of a dispersant and antibiotic on monoculture biofilms of *Pseudomonas aeruginosa*PAO1, *Escherichia coli*F11, preformed on silicone disks. Antibiotics tested included tobramycin (effective against *P. aeruginosa*PAO1) and nitrofurantoin (effective against *E. coli*F11). Variables considered included biofilm maturation state, growth conditions, concentration of dispersal agent, and treatment time. The combination therapy showed most promising results with *Pseudomonas aeruginosa*and these results varied with growth conditions.

G14 – **Large scale rain events alter marine viral communities in composition and function in coastal bays**

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With an estimated $\sim 10^8$ viruses ml^{-1} in productive coastal waters, viruses are the dominant biological entity within the ocean. Viruses play many important roles in the ecosystem, including top-down control, nutrient recycling, aiding in host metabolisms and increasing fitness of the host in unfavorable conditions. In freshwater environments, it has been shown there is an impact of stormwater runoff on viral community composition due to increased rainfall and bacterial abundance. However, the extent of pulse disturbances on marine environments has not been explored. Hurricane Harvey was a large-scale pulse disturbance that brought record breaking rainfall, $1.4 - 1.7 \times 10^{10} m^3$, to the greater Houston area in August of 2017 and flushed into Galveston Bay. Utilizing Hurricane Harvey as a pulse disturbance and large rain event, we aimed to understand the impact large scale rain events have on coastal bay marine ecosystems. The objective of this study is to demonstrate the role of viruses in ecosystem recovery by showing how the viral community adapts and changes with host abundances following Hurricane Harvey. This objective was accomplished through sampling four stations along a transect in Galveston Bay once a week for five weeks. Following sampling, metagenomic methods were used for the hosts and viruses and viral production experiments were utilized to characterize viral activity. Common marine viruses such as *Podoviridae* and *Myoviridae* were removed from the ecosystem and there was an increase in auxiliary metabolic genes associated with nitrogen, sulfur and methane metabolisms. While the prokaryotic community almost recovered, the viral community remained different than the pre-Hurricane Harvey community with the families of viruses present differing and increases in auxiliary metabolic genes associated with the disturbed system. With climates changing, it is predicted that hurricane and rainfall intensities are going to increase over the years; therefore, it is important to understand how pulse disturbances like large rain event impact the marine microbial community, how the ecosystem recovers, and the impact of the changes on the global ecosystem.