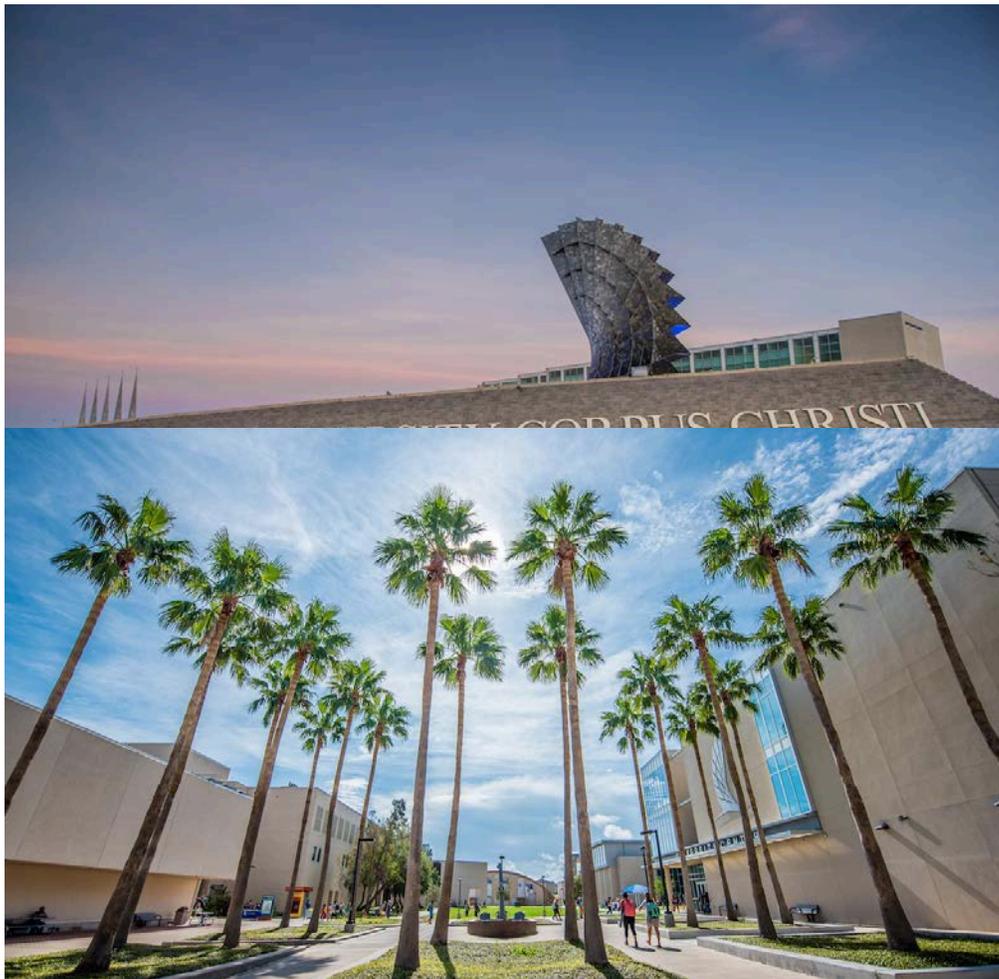




**Abstract Book
2018 Fall Meeting**

November 8 - 10, 2018

**Hosted by Texas A&M University-Corpus Christi (TAMU-CC)
6300 Ocean Drive, Corpus Christi, Texas**



Thursday, November 8th

7:00-8:00pm

Keynote address by Dr. Jason Gill (Assistant Professor, Bacteriophage Biology and Microbiology, Texas A&M University) in the University Center's Ballroom 147C.

Phage therapy for treating MDR bacterial infections: A 100-year development cycle

Jason Gill

Department of Animal Science, Center for Phage Technology, Texas A&M University

Infections caused by extreme-drug resistant bacteria may be the greatest emerging threat to immunocompromised and critically ill patients worldwide. The current antibiotic resistance crisis has revitalized interest in bacteriophages, which are bacterial viruses, as an antibacterial strategy. This practice, termed “phage therapy”, was used extensively in the pre-antibiotic era but fell from favor with the introduction of chemical antibiotics in the early 1940's. Phages are ubiquitous and highly successful natural predators of bacteria, and resistance to phages is generally unlinked to antibiotic resistance. They are also highly specific for their bacterial targets, which reduces collateral damage to beneficial microflora. In the modern era, phage therapy has been studied extensively in preclinical model systems but its translation from bench to bedside has been slow due to multiple factors. These include phage specificity, which poses challenges for the development of broadly effective treatments, the development of bacterial resistance to individual phages, and the compatibility of these naturally-occurring biologicals with traditional drug development pathways. This talk will focus on recent advances in this field and efforts to develop phages against multidrug-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii*, two Gram-negative opportunistic pathogens of concern in immunocompromised patients. In contrast to the traditional therapeutic development pathway, our model has been to develop well-characterized libraries of therapeutic phages which can be rationally selected and rapidly deployed on a personalized basis.

Friday, November 9th

8:30-9:30am

ASM Distinguished Lecturer Dr. Cheryl Nickerson (Professor, Center for Immunotherapy, Vaccines and Virotherapy, The Biodesign Institute, Arizona State University) in the University Center's Ballroom 147C.

Outpacing Infectious Disease - Mimicking the Host-Pathogen Microenvironment

Cheryl Nickerson

Center for Immunotherapy, Vaccines and Virotherapy, The Biodesign Institute, Arizona State University

As the world's leading killer of children and young adults, infectious disease presents a formidable threat to global security. Bold approaches are urgently needed to combat new and re-emerging pathogens, many of which are multi-drug resistant. Toward this goal, my team's research takes a multidisciplinary approach that blends microbiology, tissue engineering and physics to mimic the dynamic interactions between the host, its microenvironment, and the pathogen that lead to infection and disease. We focus on bacterial pathogen and human host cellular and molecular responses to physical forces (*e.g.*, fluid shear and gravity) that are physiologically relevant to those encountered *in vivo* during the natural course of infection. While these forces are relevant to those experienced during the normal lifecycles of bacterial and human cells, they have been widely overlooked as environmental stressors with potential to dictate the outcome of infection. We have developed several innovative model pathogenesis systems to study these processes, including i) 3-D organotypic tissue culture models as predictive platforms to study host-pathogen and -commensal interactions, and ii) approaches that characterize pathogen responses to physiological fluid shear forces encountered in the infected host, as well as in the microgravity environment of spaceflight. This presentation will cover our current research findings and future perspectives on the use of these pathogenesis platforms to provide novel insight into the mechanobiology of infectious disease and enable the convergence of basic research discoveries into biotechnological and clinical applications.

9:30-11:30

Session A: Graduate Student Research I General Microbiology, Ballroom 147A

Session Chair: Robert McLean, PhD

Institution: Texas State University

1. Invisibles having visible impact- Effects of Arbuscular Mycorrhizal Fungi (AMF) on growth and herbivore defense in Sudan Grass

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Beneficial plant-microbe interactions in the rhizosphere have been found to enhance plant growth and development. Arbuscular mycorrhizal fungi (AMF), a major group among these microbes, improve plant fitness through the establishment of mycorrhizal symbioses. Despite being successfully established in various natural and domesticated study-systems, relatively little is known on whether organic farming techniques such as cover cropping can be used to harvest the benefits of AMF, and whether AMF has cascading effects on plant defense traits. To test this, we planted Sudan grass (*Sorghum drummondii*), a dry land tolerant species as a summer cover crop, either inoculated with AMF or left as control. We hypothesized that AMF will alter plant defense pathways in Sudan grass influencing the attractiveness of the species to beneficial and damaging herbivores, besides other potential benefits for plant growth and development. Our results suggest that while AMF inoculated plants had significantly better growth and establishment (fitness traits), they also experienced lower initial incidence and damage by the herbivore fall armyworm (*Spodoptera frugiperda*). In addition, our insect community trapping experiment revealed that AMF inoculated *S. drummondii* attracted more beneficial insects (predators and parasitoids) and less number of damaging herbivores. In total, our data suggest that AMF treated *S. drummondii*, can positively influence both growth and defense traits and has the potential to be an excellent cover crop.

2. 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), as a neurotoxicant in major fish species and reptiles, has been reported in this lake decades ago. Hg contamination by trophic transfer 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, have been little studied. We investigated the Hg-methylating microbial community in the lake for the past two years, by taking sediment and plant samples in lake wetland habitats which showed high MeHg levels in fish from previous studies. 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. By using the new primer sets of Hg methylation genes (*hgcA*), we have detected *hgcA* genes in almost all sediment samples, in aquatic invasive species and in terrestrial Spanish mosses hanging on Cypress trees in Caddo Lake. The finding that Spanish mosses contained *hgcA* genes are quite novel since the epiphyte Spanish moss (*Tillandsia usneoides*) is generally aerobic year-around. The mystery mechanisms for mosses containing *hgcA* genes in the lake are under further investigation. The 16S rRNA genes were sequenced by Illumina MiSeq. In lake sediment samples, a total of 6402 OTUs were discovered, dominated with Crenarchaeales (9.7%), Bacteroidales (5.2%), Sinobacteraceae (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 syntrophy between methanogens, Syntrophs, and SRB

3. Life on a nurdle: the microbial response to plastic and bioplastic in the Laguna Madre, TX

Lee J. Pinnell* and Jeffrey W. Turner

Texas A&M University-Corpus Christi

Plastic is abundant in marine environments, accounting for up to 95% of all debris in coastal areas, yet little is known about the microbial assemblages or individuals that colonize this substrate. Moreover, the contribution of microbe-plastic interactions to biogeochemical processes is virtually unknown. This study reports the shotgun metagenomic sequencing of biofilms associated with plastic and bioplastic [polyethylene terephthalate (PET) and polyhydroxyalkanoate (PHA), respectively] microcosms staged at the water-sediment interface of the Upper Laguna Madre, TX. Community composition analysis revealed that sulfatereducing bacteria (SRB) dominated bioplastic biofilms whereas plastic biofilms were indistinguishable in comparison to a ceramic biofilm control. Analysis of bioplastic enzyme pools revealed the enrichment of depolymerases, esterases, adenylyl sulfate reductases (*aprAB*), and dissimilatory sulfite reductases (*dsrAB*). Phylogenetic analysis of a highly enriched polyhydroxybutyrate (PHB) depolymerase indicated that it was genetically diverse, suggesting the presence of a mixed microbial assemblage. Biodegradation rates of each plastic type were calculated by comparing

the pre- and post-exposure mass and scanning electron microscopy (SEM) was used to visualize both the microorganisms forming biofilms, and any signs of degradation. Results show that after 424 days' exposure PHA samples decreased by over 1500mg, representing a drop of approximately 51% from the pre-exposure mass. In contrast, the mass of PET did not change. Visual analysis with SEM demonstrates that while biofilms form on both plastic types, there is a disparity in biodegradation. Overall findings indicate that the introduction of plastic did not alter the microbial community. By contrast, the introduction of bioplastic promoted a rapid and significant shift in microbial diversity and enzyme pools.

4. Novel Asgard archaea capable of short-chain hydrocarbon cycling

Kiley W. Seitz¹, Nina Dombrowski¹, Laura Eme², Anja Spang², Jonathan Lombard², Jessica Sieber³, Andreas P. Teske⁴, Thijs J.G. Ettema², and Brett J. Baker^{1*}

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The recently discovered Asgard superphylum is believed to have given rise to the eukaryotic cell and encodes for eukaryotic signature proteins (ESPs) that have enhanced our understanding of the origin of cellular complexity. These archaea are widespread in sediments, yet despite their evolutionary importance their ecological roles are largely unresolved. In this study, *de novo* assembly and binning of high-throughput metagenomic sequences of samples collected from hydrothermal vent sediments from Guaymas Basin (Gulf of California, Mexico) yielded seventeen new Asgard genomes. Two of those genomes fell into a previously undescribed phylum that is phylogenetically related to Lokiarchaeota. We propose that they are named "Helarchaeota" after Hel, Goddess of the Underworld and Loki's daughter. Helarchaeota genomes encode for a similar repertoire of ESPs to those described in Lokiarchaeota, however, they possess distinct metabolic features that set them apart from other Asgard genomes. Most importantly, Helarchaeota appear to be capable of hydrocarbon oxidation as their genomes encode for proteins similar to those involved in short-chain alkane utilization using methyl-coenzyme M reductases (MCR). Activated alkanes get further metabolized via the butyryl-CoA oxidation and Wood-Ljungdahl pathways. Phylogenetic placement of the *mcrAB* gene supports butane as potential substrate, however, MCR could utilize other short-chain alkanes as Guaymas Basin sediments are rich in both methane and ethane. While the *mcr* genes are absent from other Asgard, butyryl-CoA oxidation and Wood-Ljungdahl pathways are prevalent throughout the superphylum. Acquisition of more Helarchaeota and Asgard genomes are needed to determine whether the MCR is a unique feature of this phylum or if short-chain hydrocarbon oxidation may have played a role in early eukaryotic evolution. Nevertheless, this discovery makes Helarchaeota the first member of the Asgard archaea involved in anaerobic alkane degradation.

5. MOLECULAR CHARACTERIZATION OF FUNGI IN THE OLIGOTROPHIC MARINE SUBSURFACE

Morgan Sobol¹, Tatsuhiko Hoshino², Fumio Inagaki², Martha Ariza³, Brandi Kiel Reese¹

Texas A&M University – Corpus Christi, Department of Life Sciences
Japan Agency for Marine-Earth Science and Technology – Kochi Core Center
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The marine subsurface was previously thought to be devoid of eukaryotic life, but recent advances in molecular technology have unlocked the presence and activity of fungi in this isolated environment. In continental systems, fungi are important members of the global carbon cycle because of their ability to transform recalcitrant carbon into labile carbon. In order to understand the adaptations that have led to the fungal ecological and nutrient cycling roles within marine subsurface sediments, genomes of these fungi need to be analyzed. Here, we present the draft genomes of two fungi (isolates SPG-F1 from site U1371E and SPG-F15 from U1368D) isolated from subsurface sediment collected within the South Pacific Gyre through the Integrated Ocean Drilling Program Expedition 329. Total sedimentary organic carbon was generally less than 0.02 wt%, with recalcitrant carbon comprising the majority. Isolates SPG-F1 and SPG-F15 were previously identified as *Penicillium* via morphological analysis and 18S rRNA gene sequencing. The genomes of both isolates were sequenced using the high-throughput sequencing platform Illumina HiSeq. The resulting genome sizes were 32 megabase pairs (Mbp) for SPG-F1 and 36 Mbp for SPG-F15, which was comparable to continental *Penicillium* species. The transposable element content for each genome was 3% for SPG-F1 and 10% for SPG-F15. A total of 11,581 genes were predicted in the SPG-F1 genome and 11,243 genes in SPG-F15. Approximately 58% of the annotated genes for both isolates were assigned to gene ontology for biological, cellular, and molecular functions. Isolate SPG-F1 had 653 genes assigned to carbohydrate-active enzymes, whereas, a total of 626 were assigned to SPG-F15. Of these genes, SPG-F1 had 86 and SPG-F15 had 91 specifically involved in potential recalcitrant carbon degradation. Degrading inorganic carbon or recalcitrant carbon is crucial to sustaining life in the deep subsurface. This sheds light on the need to target more fungal genomes to determine the extent fungi plays in the subsurface carbon cycle.

6. Nodule formation of typical and atypical *Frankia* isolates from *Casuarina* sp. on their potential host plant, *Casuarina equisetifolia*

Spandana Vemulapally, Trina Guerra, Dittmar Hahn

Texas State University, Department of Biology, 601 University Drive, San Marcos, TX 78666, USA

Members of the nitrogen-fixing actinobacterial genus *Frankia* form root nodules with specific host plant species. Isolates of *Frankia* are typically obtained from root nodules and are generally infective on the same plant species. Several *Frankia* strains originally isolated from *Casuarina* species, however, have been found to be non-infective on *Casuarina* species, but instead infective on *Elaeagnus* species. Since *Frankia* infective on *Elaeagnus* sp. represent a different host infection group, the goal of this study was to investigate the potential role of typical infective isolates from *Casuarina* species on the potential establishment of these atypical, non-infective

Frankia strains in root nodule formation on *Casuarina equisetifolia*. Soil microcosms were established with plants of *C. equisetifolia* and inoculated with the typical Frankia strain CcI3 or the atypical strain R43, or combinations of both at different densities. Plant growth performance of *C. equisetifolia* assessed by plant height measurements 3 months after inoculation was significantly better in the presence of strain CcI3 compared to that of strain R43 alone, with no additional effect of coinoculation of strain CcI3 with either 104, 105, or 106 cells (g of soil)⁻¹ of strain R43. Similar results were obtained for root nodule formation where R43 alone did not produce any nodules while numbers of lobes produced in the presence of strain CcI3 alone or in combination with different cell numbers of strain R43 were not significantly different from each other. qPCR analyses on separated periderm and cortex samples from selected nodule lobes revealed the presence of strain CcI3 in cortex samples in all treatments, while strain R43 was not detected in any cortex samples but in 40% of the periderm samples from lobes of treatments with highest inoculation values. In situ hybridization detected cells of strain R43 on the outside of the nodules, i.e. on the periderm only. These results demonstrate that Frankia strain R43 is not co-infecting root nodules formed by the typical Frankia strain CcI3 on *C. equisetifolia*. With its absence from the cortex, but pronounced abundance specifically on the periderm of some, though not all nodules, the atypical isolate R43 exhibits properties of a surface contaminant on nodules of *C. equisetifolia*.

Session B: Undergrad Student Research

Ballroom 147B

Session Chair: Daisy Zhang, PhD

Institution: Del Mar College

1. Genomic analyses reveal mechanisms of extreme osmoregulation in hypersaline Vibrionaceae

Sandra M. Amend, Sarah A. Tominack, Hailey R. Wallgren, Lee J. Pinnell, Jeffrey W. Turner

Texas A&M University-Corpus Christi, Corpus Christi, Texas

The Vibrionaceae family is cosmopolitan in distribution and is very diverse, containing pathogenic and non-pathogenic species. A majority of the organisms in this family inhabit fresh to brackish aquatic environments, with some residing in soil and sediment habitats and few preferring hypersaline conditions. *Salinivibrio costicola* is one of the first described species with tolerance to hypersaline conditions, and since numerous others have been identified. Through genomic investigations, it has been discovered that these hypersaline adapted vibrios possess active transport mechanisms that aid in osmoregulation. Here, culture methods were used to isolate presumptive *Vibrio* species from the hypersaline Los Olmos Creek (salinity = 86-103), an ephemeral creek connected to the Laguna Salada in Baffin Bay, Texas. Genomic DNA was sent for whole-genome sequencing and was then assembled, annotated, and analyzed by genome-scale phylogenetics and ANI comparisons. Preliminary investigations suggest that one of the environmental isolates is strongly related to *Salinivibrio* strain KP-1, while the other is most closely related to *Vibrio alginolyticus*. It is not surprising that a potential *Salinivibrio* was

isolated from Los Olmos Creek, but the isolation of *V. alginolyticus* from a hypersaline environment was novel. Further work will include ortholog analysis to describe similarities between these and other members of the Vibrionaceae, and to query the genomes for potential unique osmoregulation mechanisms. Research on osmoregulation and other mechanisms that allow for survival in extreme and variable conditions is timely given the expected impacts of climate change on aquatic environments.

2. Utilization of Polymerase Chain Reaction (PCR) to detect the presence of *vcgC* in *Vibrio vulnificus* isolates from the Coastal Bend region of Texas

LarReshia Brumfield, Alyssa Garcia, Tolulope Okuyemi, Tyler Vance, and Dr. Gregory Buck

Department of Life Sciences, TAMU-CC

Vibrio vulnificus is a Gram-negative bacterium found in estuarine and marine waters and shellfish. *Vibrio vulnificus* is known to cause diseases such as gastroenteritis, septicemia, and necrotizing fasciitis. *Vibrio vulnificus* possesses a virulence correlated gene locus (*vcg*) that currently has no known function. The locus has two alleles: *vcgC*, the clinical type, or *vcgE*, the environmental type. PCR was used to identify whether isolates possessed the *vcgC* allele, using primers to anneal a region producing an amplicon of 428bp. Nineteen *V. vulnificus* isolates that were originally collected in August 2006 to July 2007 from multiple sites in the Coastal Bend region, were regrown from cryogenic preservation at -80°C in Brain-Heart infusion broth with 2% NaCl, subcultured in LB broth with 2% NaCl and nucleic acid extracted by a crude lysate method, followed by endpoint PCR. Thirteen isolates possessed the *vcgC* allele as seen in duplicate. Three isolates showed an amplicon in only one reaction and will be retested. The remaining isolates did not show amplicons. The data show that PCR can be used to identify the presence of *vcgC* in isolates of *Vibrio vulnificus*. Future research will use PCR to identify the presence of *vcgC* and *vcgE*, in additional isolates.

3. The Interdependence between Iron Acquisition and Biofilm Formation in *Pseudomonas aeruginosa*

Donghoon Kang, Natalia V. Kirienko

Department of Biosciences, Rice University, Houston, TX

Multi-drug resistant *Pseudomonas aeruginosa* is an increasing threat to immunocompromised patients, particularly those with cystic fibrosis. *P. aeruginosa* infections are difficult to treat due to the bacterium's ability to form biofilms that limit the penetration of antibiotics or host immune cells. Though the mechanism remains unclear, iron starvation disrupts biofilm formation. In vivo, hosts secrete proteins such as lactoferrin that restrict extracellular iron availability, mitigating pathogen biofilm formation. We can recapitulate this in vitro by the addition of synthetic iron chelators or iron antagonists such as gallium. However, biofilm formation can also inversely affect iron acquisition by regulating the production of the siderophore pyoverdine. We

conducted a high-throughput genetic screen to identify genes necessary for pyoverdine production. Amongst 5810 *P. aeruginosa* PA14 transposon mutants, 5% exhibited severely attenuated pyoverdine levels without growth defects. Mutations in flagellin, type VI pili, and exopolysaccharide synthesis were enriched, suggesting that biofilm formation is necessary for pyoverdine production. Deletion mutants validated the results of the screen. These mutants also exhibited significantly attenuated pathogenicity against *Caenorhabditis elegans* compared to wild-type *P. aeruginosa*. Furthermore, we demonstrated that the secondary messenger c-di-GMP, a master regulator of virulence factors, modulated pyoverdine production in a biofilm-dependent manner. Moreover, the addition of a known biofilm inhibitor, 5,6-dimethylbenzimidazole, attenuated both biofilm formation and pyoverdine production (without affecting bacterial growth), ultimately rescuing *C. elegans* hosts during pathogenesis. These findings suggest that biofilm inhibitors, in addition to increasing pathogen susceptibility to antimicrobials, have potential as novel anti-virulents that can be used in combination with chelators to restrict pathogen iron uptake.

4. Utilization of Codon Bias and Bioinformatics to Predict Host Range of Streptomyces Bacteriophages

Claudia McCown, Sonya Layton, and Lee E. Hughes

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Through the SEA-PHAGES program, 173 *Streptomyces* bacteriophages and counting have been isolated, characterized, sequenced, and logged into the database known as PhagesDB. *Streptomyces* bacteriophages have been isolated from a variety of approximately 27 different *Streptomyces* host strains. *Streptomyces* bacteriophages are categorized into 14 clusters based on genomic similarities. There have been few studies analyzing the viral infectivity patterns of *Streptomyces* bacteriophages; therefore analyzing *Streptomyces* phage host range is essential to better understand these phages. Since few *Streptomyces* bacteriophage clusters encode their own tRNA genes, many are thought to strictly utilize the replication mechanism of the host that they infect. If a bacteriophage's genome is not compatible with the host's replication mechanism, it is suggested that infection is not possible. Because of the nature of bacteriophage replication, codon bias is suggested to be a strong indication of bacteriophage infectivity. Using several bioinformatics tools, we created a phylogenetic dendrogram, which we used as a prediction tool of *Streptomyces* bacteriophage infectivity. By comparing the codon bias of *Streptomyces* strains to the codon bias of *Streptomyces* bacteriophages, we proposed that we could predict the likelihood of infection of a bacterium by specific bacteriophages. We hypothesized a range of novel information about the host range of phage clusters. This dendrogram was unable to identify a *Streptomyces* strain that may be highly associated with a certain cluster of phages, but the clustering on our dendrogram tends to reveal patterns of infection through groups of phages. We took a closer look at phages with highly similar codon bias trends and noted their differences in host range. Our host range testing provides that generally phages with similar codon bias have conserved host range, but do tend to vary occasionally. Our results showed variance in host range as well as replication efficiency with phages of similar codon bias, thus suggesting that

phage infection is in fact multifaceted. These results leave the opportunity to take a closer look at *Streptomyces* phage infection and other factors that may contribute to infectivity

5. Increased *Salmonella enterica* serovar Typhimurium-host cell invasion in vitro through induction of SPI1 Type Three Secretion System

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Salmonella Typhimurium is a facultative intracellular pathogen that causes gastroenteritis in humans and a systemic typhoid-like disease in susceptible mice. Expression of the *Salmonella* Pathogenicity Island 1 (SPI1)-encoded Type Three Secretion System 1 (T3SS1) is crucial for invasion of non-phagocytic cells, such as intestinal epithelial cells. This process is regulated by environmental cues and must be induced prior to invasion of host cells. The goal of this project was to develop a protocol to optimize growth parameters of *S. Typhimurium* in lysogeny broth (LB) to maximally induce SPI1 expression and subsequent invasiveness. We monitored SPI1 induction using a transcriptional *prgH* fusion to a destabilized variant of GFP, through whole population and single cell analysis. Invasiveness was monitored by measuring internalization into HeLa cells using a gentamicin protection assay. Our results indicate that culture vessel and culture surface-to-volume ratio are important for optimal SPI1 induction. Sub-cultures grown in 5 ml LB-Miller in 14-ml tubes with aeration resulted in SPI1 reporter expression in nearly 76% of the population, compared with the 31% seen when using previously described growth conditions with a larger surface-to-volume ratio. Bacteria grown under the new conditions resulted in increased invasion efficiency of HeLa cells by 2.64% of the input inoculum. We conclude that at least two-fold higher levels of SPI1 induction occur with *Salmonella* Typhimurium when grown aerobically with a low surface-to-volume ratio.

6. Exopolysaccharides Drive Density-dependent Colony Expansion of Socially Motile *Myxococcus xanthus* Cells

Kimberley Kisson¹, Pintu Patra², M. Gabriela Bowden¹, Oleg Igoshin² and Heidi B. Kaplan³

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Social (S) motility is type IV pili-mediated flagella-independent group movement on a solid surface, which is best studied in the gram-negative soil bacterium *Myxococcus xanthus*. It is related to twitching motility in the gram-negative pathogen *Pseudomonas aeruginosa*. The cells move due to extension, adhesion, and retraction of the polar-localized pili. A unique characteristic of *M. xanthus* S-motility is cell density-dependent colony expansion. Our previous quantitative

analysis and mathematical modelling data supports the hypothesis that this expansion is dependent on the accumulation of exopolysaccharide (EPS), a sugar-based polymer material, excreted by *M. xanthus* cells onto the agar surface: low-density colonies delay expansion until a threshold concentration of EPS accumulates, whereas, high-density colonies expand directly upon plating as they quickly produce EPS above the threshold value. We validated the model's predictions by studying long-term (up to 96 hours) colony expansion of a S-motile only strain (DK1218) of *M. xanthus* at different initial cell densities on 0.5% agar nutrient plates. Our data strongly suggest that EPS is critical for S-motility colony expansion. To test this further, we generated an EPS biosynthesis mutant (*epsZ*) in wild-type and S-motile only backgrounds. First, we examined colony expansion in the of *epsZ* mutants and observed that both strains moved much less than their EPS-producing parents. It appeared that the *epsZ* S-motility only strain expanded only due to cell growth. Then, we examined the effects of purified EPS on colony expansion of the *epsZ* S-motility only strain and its parent by spotting cells of these strains (3×10^5 cells/3 μ l) adjacent to or overlapping increasing concentrations of purified EPS on 0.5% nutrient agar plates at 32°C for 48 h. Our analysis showed that the presence of purified EPS increased *M. xanthus* colony expansion of all strains tested with increasing EPS concentration. These results support the conclusion that EPS is absolutely required for *M. xanthus* S-motility colony expansion. We are currently examining the effects of EPS on single cell motility.

7. Identification of Regulatory Structures Located Within Mycobacteriophage Genomes

John Ramirez, Jamie L. Vulgamore, M. Clayton Speed, Daiyuan Zhang, and J. Robert Hatherill

Department of Natural Sciences, Del Mar College, Corpus Christi, TX

Bacteriophages are viruses that survive by infecting and then replicating using a bacterial host's genetic machinery, leading to the destruction of the host cell. Bacteriophages and their hosts are locked in an evolutionary struggle that has led to the development of new mechanisms of defense and infection. Bacteria have regulatory structures known as a riboswitch, an ancient regulation mechanism composed of a single piece of RNA that alters its self-annealed structure in the presence or absence of cellular metabolites. The cellular metabolite interacts with the portion of the RNA structure forming a hairpin-loop and making the Shine-Delgarno sequence unavailable to ribosomes. We used an enrichment method to isolate phage that infect the host *Mycobacterium smegmatis*. Phage were purified and classification was assisted by TEM imaging. The quality and quantity of DNA harvested from 'Chupacabra' were measured through restriction enzyme digest. After genomic sequencing, bioinformatic analyses of the isolated phage genome were used to classify the phage and annotates its genome. Putative riboswitches were located in our isolated phage using the Denison Riboswitch Detector (DRD). In addition, FASTA files available from GenBank for four bacteria and 94 bacteriophage were analyzed using the DRD. During this project, the novel bacteriophage 'Chupacabra' was isolated. This phage belongs to the cluster A and subcluster A10 of bacteriophages. In culture, 'Chupacabra' exhibits a temperate life cycle and forms plaques approximately 3 mm in diameter. Its capsid and tail were 60nm in diameter and 140nm long, respectively, and its genome was 50,286 base pairs in length. Annotation of the 'Chupacabra' genome revealed genes that were atypical when compared to related lytic phages. Among the four bacterial

species included in our study, we identified 322 total putative riboswitches. We also located 110 putative riboswitches across the bacteriophage genomes. Lysogenic and lytic phages appear to favor different types of riboswitches. These findings suggest that riboswitches may have functioned as metabolite sensors in primitive organisms and actinobacteriophage and modern cells still retain some of the ancient regulatory control systems. We postulate that riboswitches are able to regulate gene expression and are, therefore, able to control the transition from a lysogenic to a lytic lifestyle.

Session C: Graduate Student Research II Medical Biology, Ballroom 147E
Session Chair: Heidi B. Kaplan, Ph.D.

1. Identification of surface epitopes associated with protection against highly immune-evasive VlsE-expressing Lyme disease spirochetes

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The tick-borne pathogen, *Borrelia burgdorferi*, is responsible for approximately 300,000 Lyme disease (LD) cases per year in the United States. Recent increases in the number of LD cases in addition to the spread of the tick vector and a lack of vaccine highlights an urgent need for designing and developing an efficacious LD vaccine. Identification of protective epitopes that could be used to develop a second-generation vaccine is therefore imperative. Despite the antigenicity of several lipoproteins and integral outer membrane proteins (OMPs) on *B. burgdorferi* surface, the spirochetes successfully evade host antibodies primarily due to the VlsE-mediated antigenic variation. VlsE is thought to sterically block antibody access to protective epitopes of *B. burgdorferi*. However, it is highly unlikely that VlsE shields the entire surface epitome. Thus, identification of subdominant epitope targets that induce protection, when made dominant, is necessary to generate a good effective vaccine. Towards the identification, we repeatedly immunized immunocompetent mice with live-attenuated VlsE-deleted *B. burgdorferi* and then challenged the animals with VlsE-expressing (host-adapted) wild type. Passive immunization and western blot data suggested that the protection of the 50% of repeatedly-immunized animals against the highly immune-evasive *B. burgdorferi* was antibody-mediated. Comparison of serum antibody repertoires identified in protected and non-protected animals permitted the identification of several putative epitopes significantly associated with the protection. Most of the linear putative epitopes were conserved between the main pathogenic *Borrelia* genospecies and found within known subdominant regions of OMPs. Currently, immunization studies testing whether the protection-associated epitopes are protective under way.

2. Pseudomonas aeruginosa changes its carbon metabolism transcriptome in response to blood from trauma patients, which influences its virulence in-vitro

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Trauma accounts for 41 million emergency department visits across the United States yearly. Trauma patients are more susceptible to sepsis, which is the leading cause of death in days after the initial injury. Sepsis is associated with different bacterial pathogens including *Pseudomonas aeruginosa*. Despite numerous studies, the pathogenesis of *P. aeruginosa* infection during trauma-induced sepsis has not been described. In this study, we examined the effect of trauma-induced changes in blood on the expression of *P. aeruginosa* genes. This was accomplished using our newly developed model system that enables us to grow *P. aeruginosa* directly in blood. *P. aeruginosa* strain UCBPP-PA14 (PA14) was grown in blood samples from trauma patients (TPs) and healthy volunteers (HVs). Transcriptomic analysis of PA14 that was grown in blood from both TPs and HVs was done using RNA-seq technology. We used the Rockhopper 2 system for downstream analysis of the RNA-seq data. Additional statistical analysis was performed using orthogonal partial least square discriminant analysis (OPLS-DA) as a novel approach to filter the significantly expressed genes. Genes whose expression was significantly increased were related to carbon metabolism including malonate utilization, maltose and mannitol uptake. We confirmed the increase in the mannitol and maltose concentrations in the serum of trauma patients using metabolomic analysis. Using the murine model of thermal injury, we found that the mutation of *mdcA* did not reduce the mortality rate compared to PA14, however, the bacterial burden recovered from the spleen and liver was significantly lower than that of PA14. Then, we hypothesized that the malonate utilization contributes to *P. aeruginosa* virulence since malonate is important in fatty acid metabolism and energy production. We compared the effect of growth of PA14 in M9, a minimal medium containing either glycerol (GM9) or malonate (MM9) as a sole carbon source, on the expression/production of virulence factors. In contrast, the growth in MM9 significantly reduced pyoverdine production, the QS autoinducers (C12 and C4), as well as the QS-controlled virulence factor, LasA, and LasB.

3. Characterization of novel MBL-inhibitors

Misha I. Kazi, Joseph, M. Boll

Metallo- β -lactamases (MBLs) demonstrate uncharacteristically broad substrate specificity that enables inactivation of virtually all bicyclic β -lactam antibiotics, including last-line carbapenem antibiotics. Specifically, New Delhi MBL (NDM-1) recently emerged and quickly spread throughout many nosocomial Gram-negative populations, which include some of the most difficult to treat antibiotic-resistant pathogens. Despite the importance and prevalence of NDM-1 in

healthcare settings, no inhibitors have been approved for clinical use. Here, we use an innovative discovery platform to sample chemical space within peptide motifs that inhibit NDM-1-mediated carbapenem resistance in *E. coli*. We screened >1,000,000 random peptide sequences using high-throughput sequencing and discovered ~1700 unique sequences that inhibit NDM-1 catalytic activity in an *E. coli* cell-based system. Furthermore, 37 inhibitor sequences sensitized NDM-1-encoded *E. coli* to a broad range of β -lactam antibiotics. Using genetic and biochemical validation, we demonstrate that the top three candidate sequences enhance carbapenem-dependent killing via direct binding to the NDM-1 MBL. Our results support biologically relevant interactions between the inhibitor chemistries discovered in our screen and NDM-1 in *E. coli*, suggesting that amino acid motifs can potentially target and inhibit clinically relevant MBLs to restore the efficacy of our available β -lactam antibiotic repertoire.

4. A bacteriophage Mu releasin protein unleashes the endolysin to accomplish host lysis

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In Gram-negative bacterial hosts, phage escape requires overcoming the three topological barriers between assembled phage particles in the cytoplasm and the extracellular milieu. Classically, a holin in the inner membrane enables an endolysin to access and degrade the peptidoglycan, followed by spanin-mediated outer membrane disruption. In bacteriophage Mu, we used bioinformatic and genetic approaches to identify the proteins that provide the three canonical lysis functions and discovered an additional lysis protein required for endolysin release. In Mu, overlapping inner and outer membrane spanin genes, called 23 and 23a, are adjacent to the endolysin gene 22, also called *lys*. The endolysin protein, Lys, has a SAR (signal-anchorrelease) domain at its N-terminus. Genes 19 and 20 encode integral membrane proteins with four and one transmembrane domains, respectively. Phenotypic analysis indicates that gp19 is a pinholin and gp20 functions as the anti-pinholin. After identifying the known lysis protein types in the Mu lysis cassette, we were surprised to find that nearby gene 25 also exhibited a knockout phenotype indistinguishable from that of *lys*. Here, we demonstrate that co-expression of *lys* and 25 is necessary and sufficient for host lysis. Typically, membrane depolarization by the pinholin results in discharge of the weak endolysin anchor from the membrane, liberating endolysin to degrade peptidoglycan in the periplasm. However, the short N-terminal cytoplasmic segment of Lys contains three positively charged residues that could prevent the protein from being pulled through the membrane. Mutations neutralizing those positive charges relieve dependence on gp25 for lysis. We hypothesize that the acidic cytoplasmic domain of gp25 creates a microenvironment to deprotonate the basic residues and allow release of Lys. This is the first evidence for active regulation of SAR endolysin activity at the level of membrane localization, leading to the designation of gp25 as the first releasin.

5. Evolution of trimethoprim resistance in *Escherichia coli* is driven by epistasis between dihydrofolate reductase mutations with distinct resistance mechanisms

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Fitness landscapes of antibiotic targeted enzymes have been repetitively mapped to understand the genetic interactions between mutations leading to high levels of antibiotic resistance. Although ruggedness of these landscapes tells us about high order epistasis between mutations at the cellular level, understanding these effects at the biochemical and molecular level remains elusive. Here, we carried out an extensive experimental and computational study to quantitatively understand the evolutionary dynamics of *Escherichia coli* dihydrofolate reductase (DHFR) enzyme in the presence of trimethoprim induced selection. Previously, mutations in ten residues spanning the active site of DHFR were shown to cause trimethoprim resistance at the cellular level. In this project, we biochemically and structurally characterized these residues and revealed distinct resistance mechanisms at the molecular level. We then characterized the biochemical parameters (k_{cat} , K_m , and K_i) of a mutant library carrying all possible combinations of six resistance-conferring mutations and we calculated the epistatic interactions between these mutations. Our results suggest that there is a higher order epistatic network for catalytic power (k_{cat} , and K_m) of DHFR but epistatic interactions regarding trimethoprim affinity (K_i) is less prevalent. In conclusion, our data presents a showcase for how biochemical parameters can give rise to complex fitness landscapes and offers new ways of developing mutant-specific inhibitors.

6. The high-risk human papillomavirus subtype-18 E6 oncoprotein induces the *Tp53*-induced glycolysis and apoptosis regulator (TIGAR), an antioxidant effector that is essential for in vivo tumorigenesis

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The molecular mechanisms by which oncogenic viruses deregulate cellular growth and proliferative pathways to cause human cancers are not completely understood. The high-risk subtype human papillomaviruses (HPVs) transform epithelial tissues associated with the development of squamous-cell cervical carcinomas, as well as head-and-neck cancers which often

have high mortality rates and poor clinical outcomes. The E6 oncoprotein of high-risk HPVs cooperates with c-Myc and induces the degradation of the p53 tumor suppressor through interactions with the E6AP ubiquitin pathway, leading to aggressive cellular proliferation. The HPV E6 oncoprotein further inhibits p53-dependent apoptosis by destabilizing the TIP60 acetyltransferase and preventing the acetylation of p53 on lysine residue K120 which differentially regulates the expression of pro-apoptotic versus cell-survival/metabolic effector genes. Intriguingly, the HPV18 E6 oncoprotein doesn't degrade p53 as efficiently as other high-risk HPVs, in part, due to interference by the short E6 isoforms, E6*I and E6*II. Our preliminary studies have demonstrated that the HPV18 E6 protein, but not HPV16 E6, induces the *Tp53-induced glycolysis and apoptosis regulator* (TIGAR) –a mitochondrial 2,6-bis-fructose-phosphatase that suppresses the accumulation of damaging reactive oxygen species (ROS) induced by c-Myc. Moreover, using a HeLa xenograft model of HPV18-induced tumorigenesis in NIH III nude mice, we found that both TIGAR and c-Myc are expressed at high levels in the tumor (i.e., HPV18 E6- and huKi67-positive) tissues of engrafted animals. The siRNA-knockdown of TIGAR expression using a lentiviral-siRNA-*tigar* vector inhibited HPV18-induced tumor development, growth, and angiogenesis in vivo –as evidenced by the reduced infiltration of endothelial progenitors into the tumor stroma. These findings suggest that the HPV18 E6 oncoprotein cooperates with c-Myc by inducing p53-dependent pro-survival signals, including TIGAR, which suppresses oncogene-induced damaging ROS and promotes tumor growth through the robust chemoattractive recruitment of endothelial stem cells during viral carcinogenesis

12:30-2:30pm

Poster Session. Poster presenters will be stand by their poster for a 1-hour shift: group A 12:30pm to 1:30pm and group B 1:30pm to 2:30pm. Posters will be on display in the University Center's Ballroom 147A.

GP1: Genome-scale phylogenetic analysis of 43 environmental *Vibrio vulnificus* from the Texas Coastal Bend region of the northern Gulf of Mexico

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Vibrio vulnificus is a halophilic, Gram-negative, and opportunistic pathogen with a single polar flagellum. This bacterium is capable of producing necrotizing fasciitis if it enters the human body through broken skin or may cause sepsis if ingested, usually through the consumption of raw oysters. Genomes of clinical *V. vulnificus* strains have been well-documented, however, environmental strains have been poorly characterized. The focus of this investigation was to characterize 43 newly sequenced environmental strains (this study) in the context of all publicly available environmental strains and select canonical clinical reference strains. A total of 43

isolates, cryogenically-preserved in a prior study, were chosen for this analysis. These isolates were collected from seven locations in the northern Gulf of Mexico from August 2006 to July 2007, with salinity at these locations ranging from 0.62 to 52.7 ppt. Isolates were confirmed as *V. vulnificus* by species-specific *vvhA* PCR and MALDI-TOF mass spectrometry. All 43 environmental strains were sequenced using the Illumina MiSeq technology using paired-end 251 bp chemistry. Overlapping sequence reads were merged using FLASH, low quality bases and adapters were removed using Trim_Galore, and draft genomes were assembled de novo using SPAdes. Contigs smaller than 500 bp were removed and the draft genomes were submitted to NCBI GenBank. A comparative genomic analysis was completed using get_homologues to infer phylogenetic relatedness and genome content. Current analyses are focused on the characterization of strain diversity and novel genome content. Future studies will benefit from the availability of 43 newly sequenced genomes and this first comprehensive analysis of environmental strains

GP2: Characterizing an aquatic bacterial isolate with preferential growth in modeled microgravity

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The microgravity environment on the International Space Station (ISS) can be modeled in the laboratory. While several organisms have been investigated in both spaceflight and modeled microgravity (using a low shear modeled microgravity (LSMMG) simulator), there have been no studies on whether bacteria preferentially grow in LSMMG. Using an enrichment protocol, we isolated a bacterium from the San Marcos River that prefers growth in a LSMMG environment. Using 16S sequencing, we found that this organism likely belongs to a new genus. We have characterized this organism using a variety of biochemical tests and growth conditions. We found that this species utilizes D-gluconic acid, aztreonam, prefers a neutral pH, and can withstand osmotic stress. Based on growth patterns, we know that this organism grows poorly in high fluid shear conditions and is motile under certain conditions. Further research will consist of full genome sequencing using Illumina MiSeq and Nanopore sequencers. In addition to spaceflight, low shear conditions may exist in other regions such as the spaces in between the microvilli in the intestines, deep terrestrial sub-surface, and regions of water bodies that lack turbulent flow. Identifying organisms that experience this condition and characterization of their genomes will aid in the identification of mechanisms that promote growth in LSMMG, with potential implications in spaceflight and other low shear environments.

GP3: Insights into the Multiple Roles of Programmed Cell Death Markers at Different Growth Stages and Diel Cycle in Toxic Dinoflagellate *Karenia brevis*

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Historically, marine phytoplankton were presumed to replicate indefinitely by binary fission. However, drastic changes in environmental conditions have been shown to induce Programmed Cell Death (PCD) in algal cells, and may greatly affect development of harmful algal blooms (HABs). The Gulf of Mexico (GoM), a vast region with extensive shellfish resources, is subject to frequent toxic blooms of the dinoflagellate *Karenia brevis*. Although the formation processes of *K. brevis* blooms have been extensively investigated, the mechanisms of bloom decline and termination are not well known. Here we used established PCD markers, such as reactive oxygen species (ROS) and caspase-like activity, to define stress-related death processes at different growth stages in *K. brevis* under oxidative stress and elucidate the variation of these markers over the diel cycle. ROS and caspase-like activity were observed to precede the death process of *K. brevis*. Similar stress responses were found at different growth stages, even though vulnerability to oxidative stress increased as the culture aged. In untreated cells, however, prevalence of ROS and caspase-like activity fluctuated strongly, but did not correlate with cell death, which may reflect variable ROS scavenging process or preparation for quiescence. In diel experiments, results suggested that light cycles played an important role in determining cell cycle pattern in *K. brevis*, while neither light conditions nor cell cycles were correlated with ROS diel variations. Our research revealed detailed cell death responses on *K. brevis* at different growth stages which may provide useful insights into HABs decline mechanisms and the development of bloom-declined indicators. Meanwhile, the presence of PCD markers in untreated populations may also highlight their house-keeping functions in cell survival and aging process.

GP4: Assessment of microbial communities involved in mercury methylation in a tropical freshwater ecosystem: Mapping of microbiome data with *hgcA*-*hgcB* gene cluster

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Sam Houston State University

Mercury can be a serious hazard to human health, especially in aquatic systems surrounded by gold mining areas. Mercury (Hg) is introduced to the environment from a wide variety of human activities; in alluvial gold mining, for example, Hg is used to amalgamate gold ore from alluvial deposits. When different ionic forms of Hg are circulated into freshwater environments, they can get methylated by microorganisms to produce MeHg. The relationships between microbial community composition and environmental factors, with respect to mercury methylation potential in the environment, could be investigated using functional genes. For example, the identification of the two gene-cluster *hgcA* and *hgcB* in Hg-methylating microbes has resulted in the identification of many microbial species involved in this process, such as iron-reducing bacteria (IRB), sulfur-reducing bacteria (SRB), and methanogens. In this study, we used homologous genes of *hgcA* and *hgcB* to identify the genetic bases for microbial Hg-methylation in the Mazaruni River in Guyana, South America, a river that has been increasingly impacted by alluvial gold mining activities, leading to concerns about the potential MeHg contamination in this aquatic ecosystem. Water and river sediments samples were collected from mined and non-mined sites and were analyzed for total Hg and MeHg using ICP-Mass Spectrometry. Microbial

community characterization of sediment samples was performed by analyzing 16s rRNA gene sequencing. Results revealed a significantly higher concentration of both total Hg and MeHg mined sites when compared to non-mined sites. Differences in microbial community structure were also observed between mined and non-mined sites, with a higher abundance of SRB, IRB, and methanogens present in mined sites. A majority of genera belonging to these three groups possess *hgcA* and *hgcB* genes in their genomes. Future studies will involve the gene expression analysis of *hgcA* and *hgcB* by employing reverse transcription polymerase chain reaction (RT-PCR) on microbial communities collected from mined and non-mined sites.

GP5: Genetics and physiology of the soybean symbiont *Bradyrhizobium japonicum* under acidic conditions.

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Symbiotic association between soybean and its symbiont bacterium *Bradyrhizobium japonicum* is of economic and ecological importance because this process can enhance soybean yield and minimize adverse effects of synthetic nitrogen fertilizers. Inoculation of soybeans with *B. japonicum* facilitates biological nitrogen fixation in soybean root nodules, thereby providing a nitrogen source for plant growth. However, soil acidity in the rhizosphere is one of the major constraints that hinder the performance of the *B. japonicum* inoculant in the field. Therefore, understanding of genetics and physiology of the bacterium in response to acidic conditions is a key step to develop a better inoculant. In this study, survivability tests were conducted by exposing the bacterium to various acidic conditions ranging from pH 3.0 to pH 6.8 (the neutral condition). Then, pH 4 was chosen to further study acid-shock responses, because it was the threshold pH for bacterial growth. RNA extracted from cells exposed to pH 4 for 4 h was used for whole-genome expression profiling of *B. japonicum*. Functional group assignment of differentially expressed genes revealed that 582 genes were up-regulated while 619 genes were down-regulated at 2 fold cut-off. Among the up-regulated genes, *blr7593* encoding multidrug resistance efflux pump showed the highest fold induction (37.9-fold). Interestingly, other efflux pump and transporter-related genes (i.e., *bll6622* and *blr1091*) were also highly up-regulated. In addition, genes (i.e., *bll0729* and *bsl4595*) involved in heat shock and cold shock responses were strongly expressed. This result indicates that *B. japonicum* could govern a similar mechanism to cope with heat, cold, and acid shock. It would be worthwhile to select the highly expressed genes for site-specific mutagenesis and, therefore identify their function and physiological roles in the *B. japonicum*-soybean symbiosis under acid-stress conditions.

GP6: CHLORPYRIFOS BIODEGRADATION CAPACITY OF BACTERIAL CONSORTIA IN AGRICULTURAL SOIL

Nelufa Yesmin Islam¹* & Dr. Rupa Iyer²

1. Graduate student, Department of Engineering Technology-Biotechnology Track, College of Technology, University of Houston (*Contact Author).
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Abstract

Chlorpyrifos (CP) is the most widely used organophosphate insecticide and has drawn major health concern due to its persistence and toxicity to both human and the environment. This research project focused on the potential of bacterial consortiums to completely degrade CP and its toxic byproducts in contaminated agricultural soil. The capacity of different agricultural bacteria to grow and utilize CP in minimal media was assessed by Tecan UV-Vis spectrophotometer and gas chromatography mass spectrophotometry (GC-MS) analysis respectively. The effect of CP degradation in polluted soil samples spiked with 3 bacterial species, *Pseudomonas putida* CBF10-2, *Ochrobactrum anthropi* FRAF13, and *Rhizobium radiobacter* GHKF11, individually or in consortium was compared with CP degradation in non-augmented soil samples over a period of 7 days. The observation of positive growth from each individual bacterial strain in minimal media supplemented with suggests each strain is capable of metabolizing CP as a carbon source. GCMS analysis revealed 4 metabolites, 2 hydroxy-3,5,6-trichloropyridine, phosphorothioic acid, fumaric acid and ethanol during the 7-days inoculation period. CP inoculated soil samples spiked with bacterial consortia consisting of 3 strains has higher degradation rate (78.55%) than nonaugmented soil samples (38.63%) at 2nd day of incubation. Among various sources, Fairchild ranch soil and Pecan Grove crop field soil showed overall greater degradation capacity than Jersey Village garden soil possibly due to likelihood of greater CP pesticide application at these sites. Overall degradation kinetics for augmented and non-augmented soil samples was $0.79d^{-1}$ and $0.19d^{-1}$ and half-life 1.03 and 5.45 days respectively. The outcome of this study suggests a microbial remediation approach is an effective alternative method for degradation of CP in agricultural soil. Future goals of this project include the development of effective microbial consortia capable of efficient biodegradation of other organophosphate insecticides, herbicides and nerve agents

GP7: Genomic expansion of Deltaproteobacteria biodiversity from marine sediments

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Deltaproteobacteria are a class of ubiquitous bacteria that play a substantial role in biogeochemical nutrient cycling in marine sediments. However, our understanding about their role in the environment is biased towards cultured exemplars. Thus, there is a gap in knowledge about the metabolic capabilities of many Deltaproteobacteria. To address this, we reconstructed 81 highquality genomes using metagenomic approaches (via de novo assembly and tetranucleotide and coverage-based binning). These genomes were recovered from coastal (Mesquite Bay, Texas) and deep-sea (Guaymas Basin, Gulf of California) sediments and

compared to Deltaproteobacteria genomes available in public databases. Phylogenomic analyses revealed nine new, uncultured lineages that significantly expand the biodiversity of this class. Metabolic reconstructions indicated that these uncultured groups are versatile and encode for key genes involved in dissimilatory nitrate reduction to ammonium (DNRA), dissimilatory sulfite reduction, and CO₂ fixation via the reductive acetyl-CoA pathway (Wood Ljungdahl). Some of the uncultured groups encode for a relatively large variety of hydrogenases, including groups 1, 3, and 4, suggesting that hydrogen cycling is crucial to their physiology. Overall, this study expands the biodiversity of Deltaproteobacteria, providing new insights into the ecological roles of this ubiquitous group in marine sediments.

GP8: Targeted cultivation of basaltic crustal fluids from the western flank of the MidAtlantic Ridge

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The marine subsurface is an isolated poorly understood biome, due in large part to limited energy and low biomass. Previous efforts to study microorganisms from the subsurface have previously focused on culture-independent methods such as genomics. Substantial progress has been made in culturing the unculturable through the use of basal media that mimics the natural environment, rather than attempt to enrich the whole community through generalized carbon and nutrition needs. This study aims to isolate and cultivate microorganisms through a newly developed method of high throughput culturing (HTC) using fluorescence activated cell sorting (FACS). Samples for this study were collected from the western flank of the Mid-Atlantic Ridge onboard the R/V Atlantis in October 2017. Circulation Obviation Retrofit Kits (CORK) were previously installed at this site to access the basaltic crustal fluids below the sediment during Integrated Ocean Drilling Program (IODP) Expedition 336. We collected fluid from the basaltic crust at U1382A and U1383C CORKs, bottom seawater from 4500 meters, and shallow sediment from 0-2 cm below sediment surface. Live cells from the samples were sorted using a BD FACSJAZZ into 96-well plates containing marine broth and Czapek broth and incubated at 26°C. Sterile media used onboard and at the Reese Lab were also sorted as quality control to verify lack of contamination. Each plate was analyzed using a plate reader to measure absorbance as a proxy for growth and growth curves were plotted for individual wells. Isolates that exhibited the most growth were selected for Sanger sequencing of the 16S rRNA gene. Future steps include characterization of the unique isolates by studying their ecophysiology including salinity, pH, and temperature tolerances. Additionally, whole genome sequencing will allow further elucidation of what contributions these unique isolates make to Mid-Atlantic Ridge basalt and sediment

GP9: Metagenomic analysis of microbial communities from the Arctic soil in Svalbard, Norway

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About one fourth of the earth's terrestrial ecosystem remains under extremely cold conditions ($\leq 0^{\circ}\text{C}$). Global warming and environmental changes have caused the increase in the active layer thickness of glaciers, which passes through seasonal freeze-thaw cycle. The objectives of this research are to analyze the distribution of microbial communities and biogeochemical cycle due to water runoff in mountain topography of Svalbard. Permafrost soil samples were collected from 10 research sites at different elevations from Midtre Lovénbreen in Svalbard. Physicochemical properties of soil samples were evaluated, and metagenome sequencing was performed in Ion Torrent Personal Genome Machine platform. A total of 6,804,794 quality filtered reads (680,479 reads on average) were assembled for 10 samples individually using MEGAHIT and annotated in DOE Joint Genome Institute. Principal component analysis (PCA) was conducted to examine correlations among variables foreland, middle and seashore samples, where first two principle components explained 80.9% of total variability. Higher percentage (3.41%) of Eukaryotes were found in the middle zone, where Streptophyta were increasing down the hill. Relative abundance of Proteobacteria and Acidobacteria was found to be higher in seashore followed by foreland and middle zones of the mountain. This metagenome analysis reveals changes in microbial community composition and functional responses to the water runoff in high altitude due to glacier retreat.

GP10: Molecular Analysis of Haemogregarinidae in Freshwater Turtles

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Turtle conservation frequently requires turtles to be removed from at-risk areas, and to be bred in captivity. These turtles are often stressed, and consequently are more susceptible to diseases and parasites. In freshwater turtles, parasites are specifically represented by members of the Haemogregarinidae. Two genera, *Haemogregarina* and *Hepatozoon*, are the most common parasites found in different turtle species. We determined the prevalence of *Haemogregarina* and *Hepatozoon* parasites in different freshwater turtles and related the infecting parasite species to the species of turtle. Samples had been collected from eight different locations, and were classified as Wild, Captive, or Wild Caught Captive Raised. Analyses included 326 blood samples from six turtle families, Bataguridae, Chelidae, Emydinae, Kinosternidae, Pelomedusidae, and Trionychidae. SybrGreen-based *qPCR* detected Haemogregarinidae in 88 of these samples. Preliminary data of comparative sequence analyses of a subset of these Haemogregarinidae identified 25 *Haemogregarina* infections and 11 *Hepatozoon* infections. Positive samples were found in all eight locations, with no significant correlations between geographic location yet. *Haemogregarina* infections seem to be specific for the turtle families Kinosternidae and Emydinae, while *Hepatozoon* infections were found only in the turtle family Bataguridae.

GP11: The effects of gold mining and mercury contamination on microbiome composition in a tropical freshwater ecosystem

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Mercury (Hg) contamination of freshwater ecosystems due to anthropogenic activities, such as gold mining, poses a serious problem for environmental sustainability, global human health, and food and economic security in many developing and underdeveloped countries. Previous studies demonstrated a high concentration of Hg and methyl mercury (MeHg) in soil sediments collected from gold-mined sites, and the concentration of MeHg increases through food chain, highest at the upper trophic level. The formation, biomagnification, and bioaccumulation of MeHg are facilitated through metabolic processes in iron- and sulfur reducing bacteria, green algae, and plants. This study focusses on the effects of mercury contamination, due to gold mining operations, on microbiome composition in a tropical river in South America. The Mazaruni River in Guyana has been highly impacted by gold mining operations, which use Hg for gold amalgamation. Mined and non-mined sites were surveyed and data on the physical and chemical water composition, and habitat descriptors were recorded. Likewise, water and soil sediment samples were collected from corresponding sites for elemental and microbiome analysis. Results revealed that specific physical and chemical water characteristics (temperature, turbidity, pH, and electrical conductivity) are significantly different between sites, suggesting that mining activities alter these parameters, which can potentially be useful for monitoring Hg contamination. Elemental analysis performed on sediment samples reveal concentrations of gold (Au), arsenic (As), and sulfur (S) were significantly higher at mined sites than the non-mined sites. Results further demonstrated that gold-mined sites had significantly higher concentrations of Hg and MeHg than non-mined sites. Microbiome analysis revealed that mining activities significantly affect overall microbial community composition and diversity. These sites harbor abundance of iron- and sulfur reducing bacteria, which are known to mediate mercury methylation. Our findings will increase the understanding of mercury methylation and the metabolic role of microorganisms in aquatic ecosystems, which in turn will provide tools for mercury biomonitoring and bioremediation.

GP 12: Variation in bacterial communities followed by DOC released from *Heterosigma akawhio* and the impact of this variation on the growth of three phytoplankton species, *Prorocentrum minimum*, *Chattonella marina*, and *Skeletonema costatum*

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In the marine environment, phytoplankton blooms result in increased dissolved organic carbon (DOC) concentration which is major carbon source for the bacterial community. Due to

differences in DOC consumption amongst bacterial species, this increased DOC can lead to variation in bacterial community structure (BCS), which can in turn affect phytoplankton growth and species composition. However, the effect of this variation in BCS on phytoplankton community is largely unknown. In this study, we investigated variation in BCS after addition of DOC released from *Heterosigma akashiwo*, and examined the effect of the resulting bacterial community on the growth of three phytoplankton taxa: the dinoflagellate *Prorocentrum minimum*, the diatom *Skeletonema costatum*, and the raphidophyte *Chattonella marina*. The initial bacterial community was isolated from a field sample (Masan Bay, South Korea) during an *H. akashiwo* bloom and incubated for three days after addition of DOC from *H. akashiwo* culture. Then, the resulting bacterial community was inoculated into the three target algal species. The growth of *P. minimum* ($76\pm 15\%$) and *C. marina* ($232\pm 153\%$) in bacterial treatments was clearly enhanced at day 14. However, the growth of *S. costatum* was suppressed ($-28\pm 1.5\%$) at day 14. Cluster analysis based on BCS similarity showed that the BCS after DOC treatment was relatively close to BCSs in *P. minimum* and *C. marina*, compared to BCS in *S. costatum*. Together with these findings, the effect of *H. akashiwo*-elicited DOC on bacterial community may affect differentially depending on phytoplankton taxa.

GP13: Effects of a drought-tolerant Bradyrhizobium on soybean yield and nodulation

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The soil bacterium *Bradyrhizobium japonicum* is an agriculturally important microbe in regards to its symbiotic association with the soybean plant, *Glycine max*. Soybean, one of the most valuable crops in the U.S., has been utilized for human and livestock consumption. As the demand for this crop continues to rise, breeders and farmers have been developing various ways to maximize their yield. One such method involves inoculating soybeans with *B. japonicum*, which forms nodules on the roots of the soybean plant, where the microbe converts atmospheric nitrogen (N_2) into ammonia (NH_3). Optimizing this process has the potential to greatly reduce or even eliminate the use of chemical nitrogen fertilizers which have had negative impacts on the surrounding environment. However, drought is the major constraint for not only survival of inoculants, but also the maintenance of their symbiotic efficacy in soybean fields. Our lab has developed a molecular marker system to select for drought resistance, which resulted in identifying a Texas-native rhizobial strain (i.e., TX-VA) with novel drought tolerance. The objective of this research is to compare soybean yield and root nodulation between our TX-VA strain and a non-inoculated control under irrigated and non-irrigated conditions. The field trial was performed at the USDA crops research site in Stoneville, Mississippi. By analyzing nodulation and final soybean yield, we hypothesize that TX-VA will have a positive impact on soybean growth and production even under the non-irrigated condition. This research is an expansion of previous trials where we strive to provide local farmers in Texas with inoculants that ultimately increase the soybean yield at a lower cost than using chemical nitrogen fertilizers.

GP14: Metabolic relationships of uncultured bacteria associated with the microalgae *Gambierdiscus*

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Background: Communities of bacteria inhabit algae cell surfaces and produce a variety of compounds, such as vitamins and secondary metabolites, that can impact the fitness of the host. These bacteria and their host interactions have been studied via culturing, single-gene diversity, and metagenomic read surveys. However, these methods are limited by culturing biases and fragmented genetic characterizations and often overlook the community context of these interactions. A higher-resolution genomic framework is needed to resolve the physiological interactions within these complex algal-bacterial communities. **Results:** Here, we infer the metabolic capabilities of uncultured bacterial genomes (obtained using metagenomic assembly and binning) associated with the marine microalgae *Gambierdiscus carolinianus* and *G. caribaeus*. Phylogenetic analyses of ribosomal proteins and 16S rRNA genes revealed that these bacteria belong to the commonly algae-associated families Rhodobacteraceae, Flavobacteriaceae, and Phycisphaeraceae. The Phycisphaeraceae genomes represent the first algae-associated representatives within the uncultured “SM1A02” Planctomycetes group. Metabolic reconstructions indicate all these bacteria are motile, facultative aerobes, and that some are capable of metabolizing organosulfur compounds and catabolizing phytoplanktonic carbon sources, such as dimethylsulfoniopropionate. Through metabolic handoffs these communities are capable of biosynthesizing compounds beneficial to both the algal host and other bacteria including iron chelators, B and K vitamins, methionine, lycopene, and squalene. **Conclusions:** Extensive pathways for the biosynthesis of vitamins, iron chelators, and polyketides in these uncultured bacteria may benefit other bacteria in the community as well as *Gambierdiscus*. These findings provide a greater depth of understanding regarding the complex physiological interactions between algae and their associated bacterial consortia.

GP 15: Planning a Microbiological Experiment for the International Space Station

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The International Space Station (ISS) is a built environment that has been continuously inhabited since November 2000. Living with the resident crew are the microorganisms carried to the ISS as flora in and on the astronauts. Microorganisms have established a biofilm in the water filtration unit of the Environmental Control and Life Support System (ECLSS) that provides drinking

water for the resident crew. To investigate the architecture and disinfection of this biofilm, we are sending an experiment to the ISS using a Biocell plate designed by Bioserve (University of Colorado, Boulder). Because of the cost of sending material to space, experiments on ISS must be completed in one run, which is complicated by the unique environment of the ISS. Of special consideration is the low gravity environment, termed microgravity. Thus, the Biocell must be investigated for feasibility to the experiment. We have tested the ability to support biofilm growth, the oxygen gradient induced by the design of the Biocell, and mixing solutions in the Biocell in a microgravity environment, where fluid shear forces are not active. The chosen growth media, artificial urine, must also be analyzed for longterm stability. Of particular consideration are the stability of urea, which quickly degrades to cyanite in aqueous solutions, pH stability over long-term storage, such as during storage, scrub time, and flight to the ISS, the ability to support bacterial growth in microgravity, and the stability of chromosomally-expressed fluorescent markers in the presence of PFA over longterm storage for the return flight. Preliminary investigations have resulted in redesigns to the Biocell plate, the artificial urine media intended for use in the space flight experiment, and the post flight analysis protocol. Determining the optimal disinfection protocols to treat biofilms in microgravity will increase the safety of astronauts on future spaceflight missions.

GP 16: Brockarchaeota, a new widespread hot spring archaeal phylum

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Our understanding of microbial diversity has been biased by those taxa that are readily cultured and those targeted by rRNA-based surveys. Innovations in metagenomic approaches have provided genetic information for several uncultured lineages on the tree of life. However, compared to bacteria, the number of described phyla within the archaea is limited. Here we describe a new uncultured phylum of archaea, Brockarchaeota, which has been overlooked in rRNA-based diversity surveys. The first eight genomes of this phylum were obtained from hydrothermal-associated sediments and hot springs (up to 70°C). Brockarchaeota are a member of the TACK superphylum and surveys of other metagenomic datasets revealed they are globally distributed in geothermal springs. Despite belonging to the same phylum, the hot spring-associated Brockarchaeota are more metabolically versatile than the deep-sea lineages. They are capable of respiratory reduction of a variety of geothermal derived substrates including; hydrogen, sulfur, thiosulfate, selenate, nitrite, and arsenate. They are also able to couple the reduction of these

electron acceptors to the oxidation methanol and methylamines. This enhances our understanding of biodiversity and physiological capabilities of archaea in hot anoxic environments.

GP 17: Sorting and Cultivation of Single Cells from Sediment

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It has been estimated that less than 1% of the world's microbes can be successfully cultured. The limited success of culturing and isolating individual microbes is a consequence of the difficulties that would be involved in analyzing them within their natural environment, which is complex and contains many unknown variables. This method provides a new way to skip the traditional enrichment step in which a majority of the rare biosphere is lost due to not providing the needed nutrients at the onset. Identification of microbial communities based on 16S rRNA gene target analysis relies on public databases such as National Center for Biotechnology Information (NCBI), which contains a high percentage of uncultured representatives. Increasing the number of cultured representatives within these databases not only strengthens the identification of unknown organisms, but also aids in our understanding of their functionality. This project will utilize single cell sorting to culture cells from coastal and deep subsurface sediment. Approximately 5 g of sediment was suspended in a 1X phosphate-buffered saline (PBS) solution, gently vortexed to separate cells from sediment matrix, then centrifuged on a low speed. The supernatant containing suspended cells was sorted into 96-well plates at 1, 5, and 10 cells per well using a BD JazzFACS. Each well was filled with either marine broth (e.g., peptone, yeast extract, sodium chloride, and nutrients common to seawater) or Luria-Bertani (LB) broth (e.g., peptides and casein peptones, vitamins). Growth was monitored daily via optical density on a plate reader for 10 days and absorbance was recorded. Visible changes within the wells such as clarity and coloration were also observed. Cells sorted into LB broth, on average, had greater growth rates whereas the cells grown in marine broth showed lower overall growth. In the future, this method will be applied to deep subsurface biosphere sediments, aiming to cultivate and characterize this microbial community.

GP 18: Genomic analysis of Hep-1B-8, a novel *Vibrio* sp. isolated from aquacultured Pacific white shrimp (*Litopenaeus vannamei*) during a bacterial disease outbreak

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The Earth's population has become increasingly reliant on aquaculture as a major high-protein food source. Demand due to a growing global population has facilitated innovation within this

field, allowing systems to increase production with fewer resources. In the case of shrimp farming, the use of zero exchange, biofloc-dominated, recirculating raceways is now a common practice as the systems minimize water discharge and supplemented feed. However, the high biosolid content and continuous antibiotic addition within these closed systems promotes the growth of both beneficial and non-beneficial bacteria. During a bacterial disease outbreak in Pacific white shrimp (*Litopenaeus vannamei*), occurring during the grow-out phase in a biofloc dominated system, 13 Gram-negative bacteria were isolated and cultured. A genome-scale phylogenetic analysis revealed that strain Hep-1B-8 was a novel *Vibrio* species within the Harveyi clade. Hep-1b-8 is most closely related to *V. brasiliensis* LMG 20456 but the two share only 83.46% average nucleotide identity (ANI). A query of subsystem features using RAST's SEED Viewer revealed Hep-1B-8 harbors a large number of genes associated with resistance to copper, fluoroquinolones and tetracycline as well as multiple drug efflux pumps. In addition, Hep-1B-8 also contains multiple proteins secretion systems (i.e. T3SS and T6SS), further implicating the isolate in virulence and pathogenesis. Findings suggest that intensive aquaculture systems may unknowingly select for the evolution and maintenance of antibiotic resistance and virulence. A full phenotypic and bioinformatic analysis is focused on the systematics of this novel species.

GP 19: During Bacteremia, *Pseudomonas aeruginosa* Adapts by Altering the Expression of Numerous Virulence Genes

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Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that causes serious infections in immunocompromised hosts including severely burned patients. After multiplying within the burn wound, *P. aeruginosa* translocate into the bloodstream causing sepsis and septic shock. While previous studies analyzed the influence of infection sites on *P. aeruginosa* virulence, little is known regarding the effect of blood during systemic infection. We hypothesize that human blood significantly alters the expression of *P. aeruginosa* genes. To address this, we used RNA-seq analysis to compare the global expression of the *P. aeruginosa* strain PAO1 that was grown to an early logarithmic phase in either a laboratory medium (Luria Bertani broth, LB) or whole blood from healthy volunteers. Compared with LB broth, the growth of PAO1 in whole blood significantly ($q < 0.05$) altered the expression of 769 genes. Among the genes whose expression was significantly reduced are the quorum sensing (QS) genes, including the phenazine operons that encode the virulence factor pyocyanin. These results showed on average a 218-fold significant decrease in the expression of the phenazine biosynthesis genes in the presence of blood. We confirmed these results using qRT-PCR. To determine if the effect resides

within the serum fraction, we grew PAO1 to a late stationary phase in LB broth or LB containing 10% commercially-available pooled adult human serum. Samples were collected every 2 h and the level of expression of selected genes was determined using qRT-PCR. At early log phase, serum significantly repressed the expression of different QS genes, including the phenazine operons. However, at late logarithmic and early stationary phases, serum significantly enhanced the expression of these genes. These results suggest that during sepsis, and depending on the stage of growth, serum differentially influences the expression of different *P. aeruginosa* virulence and virulence-related genes.

GP 20: ROLE OF TEMPERATURE DEPENDENT REGULATION ON *Pseudomonas aeruginosa* BIOFILM FORMATION

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Biofilms are surface-associated groups of microorganisms that adhere to surfaces and interact with each other using an extracellular polymeric substance matrix. Microorganisms have developed complex mechanisms to sense and react to their constantly changing environment under these conditions. One key regulatory cue for them is temperature. Studies have shown that different factors, such as temperature, can cause behavioral and morphological changes in the microbial communities. *Pseudomonas aeruginosa* is a common nosocomial gram-negative bacterium, that can cause various serious diseases in infected humans. The severity of the infections is compounded by *P. aeruginosa*'s ability to form robust biofilms in all the various niches it occupies. Biofilm-associated infections are particularly recalcitrant to clearance by both antimicrobial therapy and immune function. We hypothesize that the fluctuations in temperature in the different niches that *P. aeruginosa* occupies drive the formation of biofilms specifically adapted to survival within that niche. Using MALDI IMS, we have demonstrated that biofilms grown under these different temperature conditions exhibits dramatically different protein expression profiles, which supports the contention that these biofilms are uniquely adapted to different niches. The objective of this project is to elucidate the genes involved in the temperature regulation of biofilm formation of *P. aeruginosa*. For this purpose, a biofilm screen was run on a commercially-available transposon mutant library containing over 5,000 unique mutants of *P. aeruginosa* at four different temperatures (room temperature, 30°C, 37°C, and 40°C) to identify genes required for temperature-dependent biofilm formation. This temperature range was chosen to simulate conditions relevant to both medical and industrial settings. These strains were replicated out into 96-well plates and incubated for two days at the specified temperatures. Finally, total biomass and biofilm growth were monitored using specific absorbance readings combined with staining analysis. The biofilm assay becomes more variable as temperature increases, but we were still able to identify potential

primary screen hits that exhibit reproducible biofilm phenotypes. We identified mutants with temperature dependent as well as few with temperature-independent phenotypes. The potential hits were then categorized into groups based on their function and carried forward towards the secondary screen. Effectively this project will reveal the genetic mechanisms utilized by *P. aeruginosa* to establish biofilm growth at temperatures relevant to medical, industrial, and natural environments and will provide a wealth of information regarding the adaptive potential of *Pseudomonas aeruginosa* towards the colonization of various niches including the human.

GP21: The Path to PYRAMMID (PRedict Metagenome-Medication Interaction)

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The completion of the Human Genome Project led to the recognition that many human phenotypes, including variation in disease susceptibility and drug response, cannot be merely explained by human genetic variations. Thus, the Human Microbiome Project and other initiatives were focused on investigating the structure and composition of the human-associated microbiomes and their contribution to such human phenotypic variations. The human microbiome is a complex community of microorganisms that has evolved with us over thousands of years and it has been involved in the host central processes such as drug metabolism. The human microbiome with its highly diverse metagenome encodes a broad spectrum of functional enzymes that expand the repertoire of metabolic reactions occurring within the host. For example, gut microbiomes are known to have the ability to alter the bioactivity, bioavailability, and toxicity of different medications. Despite the significance of these modifications, relatively little is known about the specific genes and enzymes that affect medication metabolism. Understanding the role of the microbiome and metagenome in medication response, aka PharmacoMicrobiomics, may enable the future development of metagenome-targeting approaches that optimize drug efficacy. To achieve this goal, we are using Magic-BLAST tool to investigate the potential metabolizing functions of different genes in the human metagenome. We are collecting publicly available Sequence Read Archive (SRA) of the human metagenomes from 5 different body sites including gut, vaginal, skin, oral and nasal. Then, we are aligning their sequences to the genes that are known to play a role in the metabolism of different medications. These genes were collected from the DrugBank database that has a list of 1311 drugs, besides 336 human enzymes, which are involved in the metabolism of those drugs. We used Conserved Domain Architecture Retrieval Tool (CDART) to find similar bacterial proteins to that of humans that are involved in drug metabolism based on their domain architecture. Our results suggest that different body metagenome/microbiome serves a different role in drug metabolism. Future work that compares the metagenome of healthy and diseased humans will be proven beneficial in predicting the metabolic capacity of human metagenomes in dysbiosis. Thus, achieving our goal and predict potential metagenome medication interactions and present future therapeutic option that acknowledges the role of the human-associated metagenome.

GP 22 Pioneer Species Affect Community Composition and Function During Recovery from Physical Disruption of the Mucosal Microbiome of *Gambusia affinis*.

A Gomez, Chelcy E Brumlow, Lindsey A Burcham, Madison B Cowdrey, Todd P Primm.

Department of Biological Sciences, Sam Houston State University. Microbiome communities have major effects on the health of humans, and when disrupted, can have negative impacts, including opportunistic infections and inflammatory conditions. When disrupted by antibiotics, microbiome communities in humans and research animals become temporarily dominated by one or a few species, presumably serving as pioneer species during secondary succession. The mechanisms behind and results of this domination are still unclear. We use the fish *Gambusia affinis* to model mucosal microbiomes and have a system to add a selected bacterial strain after disruption to be the dominant organism. The fish skin microbiome was depleted and disrupted by a physical rinse, and then *Escherichia coli* strain K-12 was added as a pioneer species. K-12 dominated after eight hours of exposure and became a rare species after two and ten days of recovery. The bacterium *Chryseobacterium indoltheticum* was the dominant organism after two days, whether K-12 was present or not. However, the K-12 exposed fish did have a different final community composition on day ten and also altered biochemical profile. These changes suggest that the pioneer species influences the final community composition and function. Further understanding of this process may allow us to use probiotics in a predictable fashion to restore disrupted microbiomes.

GP 23: Roles of TIGAR in HTLV-1-induced tumorigenesis using an in vivo model of T-cell lymphoma

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The human T-cell leukemia virus type-1 (HTLV-1) infects and transforms CD4⁺ T-cells and causes adult T-cell leukemia/lymphoma (ATLL) – an aggressive lymphoproliferative malignancy that is often fatal. The HTLV-1 genome encodes several nonstructural/regulatory products (Tax, Rex, HBZ, p8^I, p12^I, p13^{II}, p30^{II}) within a highly conserved 3' nucleotide sequence, known as pX. Although the roles of the major transactivator protein Tax and the regulator of mRNA-splicing, Rex, have been extensively studied, little is yet known about how the other pX factors contribute to viral pathogenesis. We recently demonstrated that the p30^{II} protein interacts with the TIP60 acetyltransferase and cooperates with the viral oncoproteins, Tax and HBZ, by inducing the expression of p53-regulated pro-survival genes (Hutchison *et al* 2018, *Virology* 520:39-58; Romeo *et al* 2018, *Virology* 518:103-115). Further, we have shown that p30^{II} inhibits oncogene-induced cytotoxicity by inducing the *Tp53-induced glycolysis and apoptosis regulator* (TIGAR) – a mitochondrial antioxidant effector that suppresses the accumulation of damaging reactive oxygen species (ROS). Using an infectious proviral clone of HTLV-1, ACH.wildtype or an ACH p30^{II}-

defective mutant, we demonstrated that p30^{II} prevents mitochondrial damage and autophagy induced by Tax and HBZ. The TIGAR protein is highly expressed in primary HTLV-1+ ATLL clinical samples and HTLV-1+ lymphoma T-cell-lines, as compared to cultured human PBMCs. Moreover, we established a highly penetrant in vivo model of HTLV-1-induced T-cell lymphoma by engrafting NOD/scid mice with HTLV-1+ SLB1 or Met-1 lymphoma T-cells. The tumor tissues from these animals contained high levels of c-Myc, p53, and TIGAR, as well as the pro-angiogenic markers HIF-1 α and VEGF. siRNA-knockdown of TIGAR using a lentiviral-siRNA-*tigar* vector inhibited the infiltration of HTLV-1+ tumor cells into secondary tissues (spleen, liver, or pancreas) and resulted in reduced angiogenesis in vivo. These findings suggest that the dysregulation of TIGAR promotes HTLV-1-induced lymphomagenesis and metastatic infiltration by HTLV-1+ tumor cells which may contribute to ATLL disease progression.

GP 24: Molecular detection of fecal indicator bacteria and human-associated bacteroidales in a Texas River impacted by Hurricane Harvey

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Hurricane Harvey has caused unprecedented devastation to huge parts of southeastern Texas, particularly damaging the wastewater infrastructure resulting in release of sewage contamination into environmental waters. In response to Hurricane Harvey, a National Science Foundation RAPID project was launched to perform a comprehensive assessment of the microbial water quality in a Texas River impacted by the hurricane floodwaters. For this project, water samples were collected along the Guadalupe River during September–December 2017. In this presentation, we will share the results of the sampling, including the presence of fecal indicator bacteria, human-associated fecal genetic markers and pathogens, measured using qPCR and ddPCR assays. In general, results of this initial microbiological contaminant assessment will serve as baseline information for follow-on studies to monitor existing and emerging public health risks to residents of Texas and potential long-term environmental impacts on the water resources in the impacted regions.

GP 25: Effects of sub-lethal cobalt cation concentration on *Chromobacterium violaceum* quorum signaling in the absence and presence of oxygen

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Biofilms are communities of bacteria that can adhere to a surface or to each other by producing an extracellular matrix. Biofilms are more resistant to antibiotics than unattached (planktonic) bacteria, a process that is mediated through cell density-dependent gene expression (quorum

signaling – QS). QS and QS inhibition (QSI) has been primarily studied under aerobic conditions. However, during a recent investigation of QSI in the facultative anaerobic, pigmented bacterium, *Chromobacterium violaceum*, we observed differences in QSI and motility during aerobic and anaerobic growth. A previous study indicated that *C. violaceum* QS is non-competitively inhibited by sublethal concentrations of Cd²⁺. In this study, we are investigating QS inhibition (QSI) of *C. violaceum* using sublethal concentrations of Co²⁺ (as cobalt chloride). Preliminary studies indicate that *C. violaceum* motility is inhibited anaerobically but not aerobically by Co²⁺. We will also be investigating biofilm formation, chitinase production, AHL signal production, and flagellar production using a series of phenotypic assays as well as TaqMan qPCR. Evidence to date suggests that QSI affects transcript levels of quorum-regulated genes differently under aerobic and anaerobic growth. This represents the first report of altered quorum inhibition as a function of anaerobic growth in this organism

GP26: Investigation of a cryptic aminoglycoside resistance gene in *S. Typhimurium*

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Wild-type *Salmonella* are not typically resistant to aminoglycosides despite the presence of a cryptic aminoglycoside resistance gene termed *aac(6)-Iy*. Most cases of aminoglycoside resistance in *Salmonella* occur due to horizontal transfer, although expression of this cryptic gene has been described in *Salmonella enterica* subspecies *enterica* serovar *Enteritidis*. In *S. Enteritidis*, the *aac(6')-Iy* gene is found downstream of a group of putative metabolic genes termed the *sgc* cluster. It is unknown whether *aac(6)-Iy* has a functional role aside from antimicrobial resistance. The chromosomal position of the *sgc* cluster and *aac(6')-Iy* was investigated using NCBI Nucleotide BLAST and PATRIC. Mutants of the *sgc* genes individually and in combination with the *sgc* genes were made in *S. Typhimurium*. Growth and survival of these mutants *in vitro* and in cultured macrophages was compared to wild-type. The *aac(6')-Iy* gene is found in all species and most *Salmonella* serovars except *Salmonella arizonae* and remains in the same genomic environment adjacent to the *sgc* cluster. The mutants did not exhibit an *in vitro* growth defect. The mutants lacking both the *sgc* genes and *aac(6')-Iy* exhibited a survival defect in macrophages.

GP 27: Characterization of *agr* Groups of North American *Staphylococcus pseudintermedius* Canine Clinical Isolates

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Staphylococcus pseudintermedius is an important commensal and potential pathogen in dogs and, increasingly, in humans. It is the leading cause of canine pyoderma as well as a major cause of post-surgical infections, bacteremia, and toxic shock. Increasing multiple drug resistance has complicated treatments. Staphylococcal virulence is regulated, in part, by the quorum sensing accessory gene regulatory system (*agr*). The signal molecule for *agr* is the autoinducing peptide (AIP), which has four known alleles: I, II, III, and IV. In *S. aureus*, different *agr* alleles are significantly associated with infection type, virulence factor carriage, and phylogenetic relationships. In this study, we examine such associations in *S. pseudintermedius*. We whole genome sequenced and performed biofilm assays on 160 isolates from four specific disease groups (healthy, pyoderma, urinary tract, and surgical infection). The *agr* type, biofilm characteristics, toxin gene carriage, antimicrobial resistance gene carriage, and sequence type were identified in all isolates. Six main methicillin resistant sequence types were identified for the region (ST64, ST68, ST71, ST84, ST150, and ST155) and *agr* type II isolates were found to be significantly less common in diseased dogs, less likely to carry drug resistance, and less likely to carry several toxin genes than the other *agr* types.

GP 28: *Monodelphis domestica* as a Model to Study Zika Virus Pathogenesis.

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In 2016, Zika virus (ZIKV) transmission was found in 20 different countries or territories. Common clinical manifestation of the virus include fever, rash, conjunctivitis and, in some cases, severe neurological defects including Guillen Barre syndrome and fetal microcephaly. Currently, there is no vaccine or specific treatment for ZIKV. Several model organisms have been used to study viral pathogenesis such as transgenic mice and nonhuman primates. Yet, each of these model organisms have limitations such as issues manifesting pathogenesis, failure to manifest infection, maintenance cost, small sample size of animals and long developmental time. In the present study, we examined a new potential model organism for ZIKV pathogenesis research, *Monodelphis domestica*. In order to determine whether this model organism would be a viable option for future ZIKV research, an intracerebral study was conducted to determine if the animals were susceptible to ZIKV. To further characterize the potential of this model organism, a follow-up study to examine viral dissemination using three routes of administration was

performed. Opossums were infected with Zika virus, and tissues were collected at 26 weeks of age (comparable to a 13-year-old child) and examined through the use of antibody staining and *in situ* hybridizations to detect viral replication. In the intracranial experiment, the brains of the opossum pups showed significant signs of cellular death, and some of the infected pups showed a spongiform-like morphology. Interestingly, one of the pups infected with ZIKV grew to less than half the size of its littermates and demonstrated a global growth restriction that has been shown in other animal models, termed congenital Zika syndrome (CZS). In the route study, examination of the infected organ tissues showed widespread dissemination of the virus. Upon evaluating the weights of the infected organs compared to those from the control animals, it was found the ovaries, vagina, and uterine weights were significantly different than organs extracted from uninfected animals. This new model organism could help in the development of future ZIKV vaccines and treatments and help increase our understanding of the pathogenesis of the virus *in vivo*, by serving as a ‘bridge’ model between the transgenic mouse and nonhuman primates.

GP 29: Microbiological survey of *Procyon lotor* intestines from South Texas

Molly McClurg

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Procyon lotor, the North American Raccoon, poses a unique health risk to humans. Their predilection to washing their food in water before digesting and easily adapting to a diverse array of environments target them as being a key species in the identification of pathogens that can be potentially harmful to humans. Identifying a pathogen and how it is transmitted is crucial to the control of infectious outbreaks. Studies have shown raccoons to be an asymptomatic carrier of many zoonotic pathogens, such as *Salmonella* species. As raccoons are frequently found living in close proximity with humans, we sought to determine the types of microbes they might be carrying, and what type of risk this might pose to human health. To better understand the bacteria in these animals, we enriched and selectively cultured gastrointestinal tract samples for *Salmonella*, *Escherichia*, *Enterococcus* species as well as cellulose-degrading bacteria. Culture samples from the gastrointestinal tract were taken from each species and grown in LB broth. Selective tests were cultured using Tetrathionate broths and agar plates of Eosin Methylene Blue, Cellulose, Columbia CNA blood agar, Bile Esculin, and XLT4 agars.

GP 30: Expansion and taxonomic update of *Vibrio rotiferianus*

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Vibrio is a genus of ubiquitous aquatic Gram-negative bacteria. Several species of this genus are zoonotic pathogens that cause disease in humans and many species of marine animals. In aquaculture, *Vibrio* disease outbreaks are often mitigated by preemptive use of antibiotics. However, this practice likely leads to increased antibiotic resistance in potentially pathogenic

bacteria. Here, we compared the abundance of antibiotic resistant genes (ARGs) between four aquaculture-sourced *Vibrio rotiferianus* isolates and an environmental isolate from the Bohai Sea, China. The four aquaculture-sourced *V. rotiferianus* were isolated from the hepatopancreas of vibriosis infected Pacific white shrimp from the Texas A&M AgriLife Research and Extension Center (Corpus Christi, TX, USA). The draft genomes were sequenced in this study. Raw sequence reads were trimmed of adapters and low-quality bases, filtered for length (500 bp cutoff), assembled de novo, and submitted to RAST for genome annotation. The four draft genomes were compared to the Bohai Sea isolate to identify differences in ARG content. The type strain of the species was then compared to all publicly available *Vibrio* species in an effort to expand the number of strains assigned to this species. All *V. rotiferianus* genomes were also compared in an all-versus-all pairwise comparison with the goal of generating a phylogenetic tree that updates the taxonomic status of the species.

GP 31: Identifying the relationship between bacterial dysbiosis, inflammation, and antibiotic use in a model organism

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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. 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. Further, use of antibiotics could result in persistent alterations in the gut microbiome composition that mimic the alterations seen in the inflamed gut, as antibiotic use in humans sometimes improves irritable bowel conditions and sometimes worsens them. We used the fish *Gambusia affinis* as a model for gut inflammation because it has the necessary components of a vertebrate immune system yet is less expensive than rodents. Dextran sulfate sodium (DSS) was used to 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. Preliminary data shows that both treatments strongly alter the composition of the gut microbiome.

GP 32 Computational identification of regulatory small RNAs in *Rhodobacter sphaeroides*

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Bacterial gene-expression is regulated by multiple factors, including small, noncoding RNAs (sRNAs). Small RNAs bind to their complementary target sequences at gene-promoter sites or bind to mRNA transcripts to repress transcription or translation. It has been previously shown that when sRNAs bind to mRNA targets, sRNA-mRNA hybrids are sequestered from reaching to the ribosomes and induce degradation of mRNA targets. These regulatory RNAs have been discovered in a number of bacterial species and are generally 40-500 nucleotides in length, with an average length around 50-200 nucleotides. The objective of this study is to identify putative sRNAs in *Rhodobacter sphaeroides*, a metabolically diverse microorganism. A putative sRNA search was performed through RNAspace, a noncoding sRNA annotation platform, as it integrates various algorithms such as INFERNAL, BLAST, and RNAz coupled with CG-sequence aggregation. Additional putative sRNAs were identified using a Support Vector Machine (SVM) classifier trained with known sRNAs from other organisms whose GC-content is similar to that of *R. sphaeroides*. Results indicated that the amount of putative sRNAs identified through RNAspace exceeded the number of predictions made by the SVM model. RNAspace resulted in a higher sensitivity for trans-acting sRNAs, while the SVM model showed a higher specificity. Future research will involve the sequencing of small RNAs from *R. sphaeroides* grown under different conditions in order to validate identified putative sRNAs. These bona fide sRNAs will be used to identify their corresponding target genes and further the study of sRNA regulation mechanisms.

GP 33: Structural and biochemical studies of MurAA, an enolpyruvate transferase that contributes to daptomycin resistance in *Enterococcus faecium*

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Overuse of antibiotics has contributed to the evolution of multi-drug resistance and as a result, bacterial infections have re-emerged as a critical crisis in human health. Among these, enterococci infections pose a serious threat. Enterococci cause 66,000 infections in the United States each year and resistance to the frontline antibiotic vancomycin leaves limited treatment options for patients. The CDC estimates that vancomycin-resistant enterococci (VRE) are responsible for about 1,300 deaths annually in the U.S. Daptomycin (DAP) is an antibiotic of last resort for many patients and is used frequently to treat VRE infections. Less than 2% enterococci

isolates are DAP nonsusceptible, indicating the high efficiency of DAP and thus, highlighting the importance of maintaining this efficacy of this drug in the absence of other viable treatment options. A clear understanding of the biochemical basis of resistance can provide targets and pathways for the development of new therapeutic approaches and antibiotics. Mutations in the gene *murAA* have been identified in DAP resistant *Enterococcus faecium* in previous studies from our lab. MurAA is an enolpyruvate transferase, catalyzing the transfer of enolpyruvate from phosphoenolpyruvate (PEP) to UDP-N-acetylglucosamine (UNAG), which is the first step of peptidoglycan synthesis. Since mammalian cells do not have a cell wall, MurAA is considered an excellent target for drug discovery. Fosfomycin (FFQ) is a naturally occurring inhibitor that binds to the active site of MurAA. Our preliminary results have shown that a MurAA A149E mutation identified in DAP resistant strains retains nearly wild type steady state activity but potentially reduced stability. Understanding the structure of MurAA can provide valuable insights into the function of this enzyme and its interactions with the substrate and the inhibitor, thereby providing structural basis for the design of novel antibiotics or co-drugs that can be administered with DAP to delay the development of resistance.

Undergraduate Poster Abstract:

UP1: Metagenomic analyses of viral communities carried by mosquitoes in the Greater Houston area Alhaarbaf,

A., Irfan, A., Hasan, A., Silva, S., Brittain, D., Tran, V., Larios-Sanz, M., and Rosell, R.

Mosquitoes are vectors for viral pathogens including West Nile and Chikungunya, which are found in the Houston area. Viral diversity is an emerging area of exploration due to limited universal assays and insufficient sample analyses. Metagenomics allows for the characterization of insect/microbial/viral communities in a sample. Our approach is to use metagenomic techniques to broadly survey the viral communities associated with mosquitoes present in Houston. We collected mosquitoes during the peak season (Spring and Summer) and separated them by sex and genera using morphological markers. These samples were divided into homologous groups of 5 individuals and nucleic acid extraction was performed on these samples. DNA and RNA were extracted in order to account for both DNA and RNA viruses. DNA samples were used to genotype the mosquitos using insect cytochrome oxidase 1 (COI) primers and PCR. The remaining DNA and the RNA samples were used to generate DNA/cDNA libraries which were sequenced using nextgen (454) sequencing. We developed a data workflow that allowed us to batch-analyze our sequence library with the NCBI database, helping us identify any known sequences. Current analyses of sequenced data shows the presence of a number of viruses, including both single-stranded and double-stranded DNA as well as single-stranded RNA viruses. Some of our hits include Epstein-Barr, West Nile, and the Nam Dinh Houston Virus. This work will aid in the characterization of the diversity of viral populations in mosquitos in Houston, important information that is useful when considering epidemiology and prevention strategies.

UP2: Quantifying Bacterial Interactions *in vitro* within the Mucosal Microbiome of *Gambusia affinis*

Lindsey A Burcham, Madison B Cowdrey, Jeffrey M Belanger, Javier A Gomez, Todd P Primm

Microbiomes are complex microbial communities that affect host health in multiple ways. While the composition of microbiome communities has been determined under many different circumstances, how the individual bacterial species interact is still largely unknown. This information is important in gaining further information on the functions of the microbiome and potentially aid in the development of more effective probiotics. Our fish model organism, *Gambusia affinis*, is a tractable system for microbiome study. Nineteen bacterial strains were isolated from the fish skin and biochemically characterized and identified. Three experimental methods were used to identify 27 positive and nine negative strain-strain interactions. We developed a liquid co-culture method to quantify interactions between two strains in more detail. Future work will use this approach to validate suspected interactions from experimental 16S gene sequencing data.

UP3: Biochemical Oxygen Demand in Local River Systems and Sediments Post-Hurricane Harvey

Ryan Cabico, Darcia Gonzalez, Alexander Solis, Christopher Patrick, Brandi Kiel Reese

Texas A&M University at Corpus Christi,

Texas River health and composition are important factors in river ecology, and monitoring both is an effective way to track ecological disturbance and recovery over time. Anthropogenic waste, natural disasters, and natural fluctuations in seasonal precipitation can cause shifts in microbial community composition measured by biochemical oxygen demand (BOD), the amount of dissolved oxygen needed by heterotrophic organisms to respire organic material over a given time. We measured the effect of macro-consumer exclusion on sediment BOD in two streams (SFC & GC) in Summer 2018. SFC has a benthic substrate rich in silt and fine particles whereas GC has a benthic substrate high in sand and coarse gravel. Sediment oxygen demand (SOD), a specific form of BOD, was measured in treatment and control plots before macro-consumer exclusion cages were installed and at time points 1 month and 2 months after installation. We observed relative increases in BOD in exclusion cages of both streams, however, we observed very different temporal trajectories of BOD among streams. There was a total positive change between treatments of $\Delta 0.241 \pm 0.03 \text{ mg L}^{-1} \text{ day}^{-1}$ in SFC and $\Delta 0.108 \pm 0.06 \text{ mg L}^{-1} \text{ day}^{-1}$ in GC from June to August. From June to August SOD declined in both the control and experimental treatments in SFC, but declines were lower in the exclusion treatments ($-\Delta 17.698 \pm 2.24 \text{ mg L}^{-1} \text{ day}^{-1}$) than the control treatments ($-\Delta 22.776 \pm 1.93 \text{ mg L}^{-1} \text{ day}^{-1}$). From July to August, SOD increased in both treatments in GC, but increases were greater in the exclusion treatments ($\Delta 0.226 \pm 0.03 \text{ mg L}^{-1} \text{ day}^{-1}$) than the control treatments ($\Delta 0.128 \pm 0.20 \text{ mg L}^{-1} \text{ day}^{-1}$). The results document how sediment composition interacts with macroconsumers to impact BOD. In both cases, consumer exclusion increased BOD relative to the control, potentially via an increase in organic matter deposition within the cages, providing a substrate for microbial growth. However these effects varied among streams, potentially due to differences in substrate and hydrologic regime.

UP4: Identification of Antibiotic Resistant *Morganella morganii* and its Bacteriophage

Rodney Cantu, Lorie Leyva, Tara L. Clancy, J. Robert Hatherill, and Daisy Zhang

Del Mar College

For eighty years, antibiotics have been discovered and used to treat bacteria infection and increased significant antibiotic resistant strains along the way. An antibiotic bacterium was isolated using soil sample collected from south Texas farmland. Using colony PCR, 16s rRNA gene was amplified and later sequenced. Bioinformatics analysis identified this bacteria as *Morganella morganii*, a micro flora bacteria that lives in the gut of mammals and reptiles and has been proved to be pathogenic in some studies. The soil sample collected from the same location was enriched using *Morganella morganii* and then used for phage assay. A novel bacteriophage was discovered and to be lysogenic for its host bacteria. The study shines a light on possibility to use phage to treat infections caused by *Morganella morganii* strains.

UP5: Arbuscular Mycorrhizal Fungi (AMF) influences insect community dynamics in an organic cropping system

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Arbuscular Mycorrhizal Fungi (AMF) has been shown to enhance plant growth and development in various ecosystems. Insect community dynamics, including herbivores and beneficial insects, also play a major role in both natural and agricultural ecosystems. These interactions at multiple trophic levels, directly and indirectly, influence plant growth, development, defense against herbivores, and consequently, their fitness. However, whether AMF can influence these interactions is less understood, especially in organic systems. Using Sudan grass (*Sorghum drummondii*) as our host, we examined the effects of AMF using AMF inoculated and untreated control Sudan grass in their ability to attract beneficial insects and repel damaging herbivores in an organic farm in Mission, South Texas. We employed a combination of three different trapping methods that collectively trap both flying and crawling insects, replicated in time and space. Our preliminary analyses suggest that AMF inoculated *S. drummondii* attracts more beneficial insects and less damaging herbivores when compared to their control counterparts. These mainly include beneficial predators and parasitoids in Hymenoptera, and herbivores in Coleoptera and Diptera. Since most of these interactions are mediated by plant volatiles, we are currently extracting and quantifying plant volatiles from these treatments to explain the mechanisms underlying the on-field results, and to determine if AMF inoculated *S. drummondii* could be a viable cover crop option for organic farming.

UP 6: PCR amplification of *mcrA* gene from organic rich terrestrial riverine subsurface sediments. Relative quantification and phyla confirmation of cultivated *Euryarchaeotau* and *Verstraetearchaeota* methanogens from organic rich sediments in a Texas riverine system

Clay Clarkson and Dr. Brandi Kiel Reese

Texas A&M University – Corpus Christi

Methane has not only played a significant part in the evolution of Earth's climate, but also contributes to current greenhouse conditions. With the current state of atmospheric methane shifting towards biogenic signatures there is a call to understand the underlying sources. Methanogens dwelling in habitats impacted by anthropogenic activity are potential sources of increased methane fluxes, and recently, the essential gene encoding for methanogenesis (*mcrA*) has been identified outside the *Euryarchaeotau* phylum. This study sets out to understand the phylogenetic diversity of methanogenic and *mcrA* communities in subsurface sediments from an organic rich riverine system using cultivation-dependent molecular techniques. Enriched cultures supplemented with a methanogenic media that satisfies multiple methanogenic pathways was used to amplify culturable communities. Methanogenic genes were then amplified via polymerase chain reactions (PCR) utilizing phyla specific *mcrA* gene primer sets targeting the following phyla; *Bathyarchaeota*, *Euryarchaeotau*, and *Verstraetearchaeota*. This study confirms the presence and cultivation of *Euryarchaeotau* and *Verstraetearchaeota* methanogens enriched from freshwater riverine subsurface sediments.

UP 7: The Effect of Mineral Oil Overlays on the Diversity of Bacteria in Bioaerosols Collected with an Anderson Impactor

Dillon Cline, Shelly Tran, Kristina Hagerman, Olivia Advincula and Michael G. LaMontagne

The Anderson impactor is widely used to collect bio-aerosols in environmental research and to evaluate indoor air quality. This device collects particles by impaction on agar media. The flow of air over the agar can desiccate the media during collection. This desiccation increases particle bounce and stresses microbes, which can limit the efficacy of this system. An overlay of mineral oil on the media can reduce desiccation and increase collection efficiency; however, little is known about the effect of this overlay on the diversity of culturable microorganisms collected. We tested the effect of the mineral oil overlay on biodiversity of readily culturable bacteria by collecting air samples inside a university building with an Anderson impactor. We compared control media to those overlaid with mineral oil. Colony forming units (CFU) were counted 24 and 48 hours after sampling and representative colonies were identified by MALDI-TOF. The number of CFUs averaged 17 per plate and did not differ significantly between plates overlaid with mineral oil and controls. The experiment was repeated several months later. This second run confirmed that a mineral oil overlay did not change the number of CFUs significantly, with an average of 8 and 9 CFU's for oil and control treatments respectively. The type of bacteria collected appeared to differ between treatments. *Bacillus* sp. and *Staphylococcus* sp. were relatively abundant in libraries generated from plates overlaid with oil and *Lysinbacillus*

fusiformis, *Actinobacter pittiti*, *Moraxella oleansis*, *Rothia nasimurium*, and *Aerococcus viridans* were present only in libraries generated from controls (without oil) . This suggests that overlaying media with oil may select for and against particular microbes.

UP 8: Observing the Functional Character of the *Gambusia affinis* Mucosal Microbiome

Madison B Cowdrey, Lindsey A Burcham, Jeffrey M Belanger, Javier A Gomez, & Todd P Primm

Department of Biological Sciences, Sam Houston State University

Next-generation DNA sequencing technology has allowed detailed discovery of the community composition of human and other microbiomes. However, much about the metabolic functions of microbial communities remains unclear. We use the skin microbiome of the fish *Gambusia affinis* as a model mucosal vertebrate microbiome. Community composition data from 16S gene sequencing has revealed the dominate bacterial genera in the microbiome. To characterize the functions of these organisms, we have isolated over 40 strains from the fish. We performed the Microgen GN A+B biochemical identification assays on 18 microbiome-derived strains, along with oxidase, motility, and Gram stains determinations. Strains were also plated onto ten different selective and differential medias in further understand growth capabilities and metabolism. Full 16S sequences from five strains were determined. Strains identified include *Aeromonas cavaie*, *Acinetobacter lwoffii*, and *Pseudomonas aeruginosa*. Most of these strains have been previously isolated from fish and/or freshwater by other investigators. These biochemically characterized strains are now a resource to explore how they interact with each other and with the fish host.

UP 9: Identifying Enterococcus Isolates in Corpus Christi Bay after Rainfall

Halie Davis¹ , Nicole C. Elledge² , Hailey Wallgren² , Sandra Marbach² , Daiyuan Zhang¹ , Jeffery W. Turner²

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Fecal indicator bacteria (FIB) are bacteria that are associated with the intestines of humans and other animals, and consequently, are indicative of fecal contamination. While the quest to find the most ideal indicator bacteria is still ongoing, several species have been accepted as suitable fecal indicators, including *Escherichia coli* and *Clostridium perfringens*. In marine environments, the identification and quantification of enterococci has become an EPA- and TCEQ-approved method of detecting fecal contamination. Unfortunately, in urban, agricultural, and industrial locations, FIB (and other contaminants) often build up in the environment over time and can be accidentally introduced to surrounding environments via stormwater runoff. For example, wastewater containing FIB can be introduced to the marine environment through the runoff of leaking sewage and storm drain systems. The overflow of this potentially contaminated water can disrupt the surrounding environment through the introduction of harmful microorganisms. In

particular, Corpus Christi Bay (CCB) has experienced a long history of elevated FIB levels. To determine the effect that stormwater runoff has on the enterococci population in CCB, we conducted a 14-month long study with the following objectives: 1) quantify the levels of enterococci before and after rainfall, 2) determine the ratio of *Enterococcus faecalis* and *Enterococcus faecium* before and after rainfall, and 3) test the antimicrobial-susceptibility of the *E. faecalis* and *E. faecium* isolates collected before and after rainfall. Preliminary results indicate significantly higher levels of enterococci (specifically, *E. faecium*) occurring after rainfall.

UP 10: Bacterial Assassins: An Approach to Combating Bacterial Diseases in Shrimp

Daniela De La Cruz, Dr. Joanne Rampersad

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Coastal shrimp farming in South Texas was a multi-million dollar industry that provided a major source of income, jobs and food resources to the region. In recent years, these shrimp farms have suffered due to shrimp mortality caused by outbreaks of Early Mortality Syndrome (EMS), a bacterial disease caused by *Vibrio* bacteria. A possible solution to this problem is a group of bacteria referred to collectively as *Bdellovibrio* and like organisms (BALOs), which attack and kill *Vibrio* species. The objective of this research was to collect a library of local BALOs and test their effectiveness in protecting shrimp against EMS. Our hypothesis was that BALOs are present in the Lower Laguna Madre (LLM) since *Vibrio* is ubiquitous. Samples were collected from different ecological niches along the LLM region and the following procedures were conducted for each sample: Amplify BALOs, Identify presence of BALOs, Purify & Store, and Test BALOs predatory effectiveness on *Vibrio* species.

UP11: Investigating Soil Samples for Antibiotic Producing Bacteria

Angelinda Maldonado

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Antibiotics are critical in clinical applications for treatment of human infections caused by pathogenic microbial species. However, in clinical settings, a prevalence of multi-drug resistant bacteria coupled with fewer new drug alternatives has directed research towards discovering new antibiotics. Our objective is to investigate soils potentially containing bacterial strains that synthesize antibiotics using soil samples from regions in Houston, TX. Strategic microbiology lab techniques were used to isolate bacterial colonies. Isolated soil bacterial colonies were used to make pure cultures and used for antimicrobial susceptibility testing to determine the production of antimicrobial compounds. Standardized protocols for antibiotic efficacy by using tester strains, of Gram-positive and negative bacteria, were used. Bacteria exhibiting antimicrobial properties will be explored using biochemical assays, 16S rRNA sequencing, differential staining and morphological characteristics. Thus far, we have isolated a few bacterial colonies from the soil samples which exhibit anti-microbial properties. During the preparation of

purified bacterial colonies, we were able to observe zones of clearance surrounding some bacterial colonies indicating their potential to inhibit growth. These observations are currently being investigated further. Additionally, we have initiated the characterization process by carrying out staining and other microbiological techniques. The results demonstrate the presence of both Gram positive and Gram-negative bacteria. Our goal is to investigate soil bacteria for their potential to produce new antibiotics and for clinical applications targeted against multi-drug resistant bacteria and characterize the isolates.

UP 12: Genomic identification and characterization of an antibiotic resistant *Ochrobactrum intermedium* isolate from the Amos Rehabilitation Keep

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In Port Aransas, Texas, the University of Texas at Austin Marine Science Institute (UTMSI) operates the Amos Rehabilitation Keep (ARK) program to rehabilitate and release injured turtles, birds, and other stranded marine organisms. When an injured or diseased turtle is brought in for treatment, it is placed in a holding tank that is recirculated with seawater and administered antibiotics. After the turtles are released back into the environment, the waste water from the holding tanks is disposed of directly into the surrounding soil. Given that exposure of microorganisms to low levels of antibiotics often leads to the development of antibiotic resistance, we hypothesized that soil from this site contained microbes with high levels of antibiotic resistance. To test this hypothesis, bacteria were isolated from soil samples and assayed for antibiotic resistance. One isolate exhibited resistance to tetracycline, ceftazidime, enrofloxacin, neomycin, streptomycin, novobiocin, erythromycin, and chloroamphenicol. That unknown bacterium was preliminarily identified as *Ochrobactrum intermedium* by SSU rRNA sequencing. To confirm the species assignment and investigate the genetic mechanisms underpinning resistance, the draft genome was sequenced with the Illumina HiSeq platform using 250-bp paired end chemistry. The genome of the unknown bacterium was assembled using SPAdes, resulting in an assembly of 55 contigs. The unknown bacterium was identified as *Ochrobactrum intermedium* (strain Tara1) based a genome-scale phylogenetic analysis of the *Ochrobactrum* genus and greater than 97% average nucleotide identity (ANI). Analysis of SEED subsystems in RASTtk revealed the presence of 54 genes encoding putative virulence, resistance, and defense factors. Previous studies have identified some *Ochrobactrum* species as emerging opportunistic pathogens resistant to a wide range of antibiotics. The results of this study illustrate the negative impacts of improper antibiotics disposal and advance understanding of *Ochrobactrum intermedium*.

UP 13: Bacterial Quantification and Source Tracking in Little Bay

Hailey R. Wallgren, Sandra M. Amend, Nicole C. Elledge, Jeffrey W.

Turner

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Little Bay in Rockport, Texas is a shallow lagoon that experiences limited exchange with the Gulf of Mexico and consequently has a high residence time. Due to its close proximity to the local wastewater treatment plant, residents have become concerned that Little Bay may act as a sink for wastewater effluent and stormwater runoff, which can transmit harmful pollutants and fecal-associated microorganisms to the surrounding aquatic environment. The associated environmental repercussions and health risks are expected to increase with urbanization, growing populations, and climate change. Remediation efforts have included the creation of artificial wetlands surrounding the wastewater treatment plant outfall and the construction of oyster reefs in the bay to attenuate the spread of potentially harmful pollutants. However, two of the routinely monitored Texas Beach Watch stations in Little Bay have shown elevated levels of fecal indicator bacteria (FIB), thus prompting a seven-month long bacterial-source tracking project in Little Bay. The main objectives of this study include 1) measuring and monitoring the concentration of enterococci, an EPA-approved FIB, 2) quantifying host-specific molecular markers to determine the most probable source of fecal pollution (ie, humans, canines, or gulls), and 3) characterizing the overall bacterial community composition through 16S rRNA analysis. Thus far, the concentration of enterococci at one site adjacent to the wastewater treatment plant outfall was on average 36 times higher than the EPA safety limit of 104 CFU 100 mL⁻¹ of water. All other sites showed average enterococci concentrations that were below the EPA limit, excluding the site where the outfall creek flows into Little Bay with average concentrations exceeding two times the EPA limit. The results of this study, upon its completion, will inform the city of Rockport of the main fecal contamination sources in Little Bay and guide future efforts for remediation.

UP 14: Effects of different carbon supplements on the growth kinetics of *Rhodobacter sphaeroides* under anaerobic-dark conditions

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Rhodobacter sphaeroides is a purple non-sulfur bacterium, and it belongs to α 3 subdivision of *Proteobacteria*. The complex genome of *R. sphaeroides* consists of multiple chromosomes, and harbors 30% of gene duplication. It grows in a variety of conditions, including aerobic (~20% O₂), semi-aerobic (~2% O₂), and photosynthetic (anaerobic and light) conditions. Previous experiments revealed that *R. sphaeroides* grows at a very low rate in anaerobic-dark conditions as oxygen is not present as a terminal electron acceptor in the culture. Alternatively, various compounds, like dimethyl sulfoxide (DMSO), nitrate or trimethyl-amine N-oxide (TMAO) may substitute oxygen as terminal electron acceptor and allowing for efficient utilization of other carbon sources. To test whether bacterial cell growth will increase in minimal media

supplemented with carbon sources under anaerobic-dark conditions, *R. sphaeroides* was grown using a number of supplemental carbon sources (acetate, butyrate, citrate, mannose, sorbitol, arabinose, pyruvate, maltose, or fructose) with and without DMSO. Growth kinetics, colony morphology, and colony forming units were analyzed. Results revealed that bacterial cultures grown with pyruvate or acetate exhibit enhanced cell density and colony forming units compared to the culture grown in only minimal medium in anaerobic-dark condition. However, there is no significant difference in colony morphology including color and texture of colonies. The viability and bacterial biomass production under anaerobic-dark condition as observed in current investigation will be useful as it can be employed to deplete oxygen level from the natural gas streams.

UP 15: Isolation, characterization and genomic exploration of ‘phiNASRA1’ an *Enterococcus faecalis* bacteriophage

Danial Nasr Azadani¹, Rob. J hatherill², Daiyuan Zhang² and Jeffrey W. Turner¹

Texas A&M University¹, Del Mar College²

Enterococcus faecalis is a Gram-positive bacterium that commonly inhabits the gastrointestinal tracks of humans and animals. A minority of strains are pathogenic and the emergence of drug-resistant strains is a growing health crisis. To address this crisis, the search for alternative treatments like bacteriophage (phage) therapy has been revitalized. In this study, a lytic *E. faecalis* bacteriophage was isolated from an in-flow water sample collected at a local wastewater treatment plant (WWTP). Transmission electron microscopy (TEM) and whole-genome sequencing showed that enterophage phiNASRA1 belongs to the Siphoviridae family of double-stranded DNA viruses. The phage is approximately 250 nm in length and its complete genome (40,139 bp, 34.77% GC) encodes 60 putative proteins. It demonstrated a short latent period (~20 min) and a high lytic efficiency (97.52%) using a streptomycin-resistant *E. faecalis* as the host. A phylogenetic comparison of phiNASRA1 with 28 closely related *Enterococcus* phages, from wastewater and sewage collected from municipal, hospital and agricultural environments spanning five countries, indicated that these phages were widely distributed and recalcitrant to spatial and temporal turnover. The phage’s narrow host range, short latent period and high lytic efficiency suggest that phiNASRA1 would be a promising candidate for the development of a phage therapy.

UP 16: The Fate of Dental Implant Osseointegration Compromised by Microbial Infection

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According to the American Dental Association, each year approximately five million dental implants are placed, of which 5-10% of total cases fail. Failure can occur when host mammalian cells fail to *win the race* for implant surface colonization against bacterial cells. Bacteria can cause early and late stage implant failures, both affecting the osseointegration of the implant. Early stage failures occur due to lack of osseointegration when the race to the surface is lost. Late stage failures occur after osseointegration is achieved but is later lost due to bacterial contamination. Multiple *Streptococcus* species are constituents of the healthy human oral flora; however, under some conditions they may become opportunistic pathogens. One early colonizer of dental implants that can inhibit adequate osseointegration and cause early stage failure is *Streptococcus oralis*. To better model dental implant failure under laboratory conditions, we aimed to design a reproducible co-culture model with an environment permissible for mammalian oral and bacterial cell survival. Previous reports demonstrated that pre-osteoblasts were not able to grow in media supplemented with more than 10% brain heart infusion (BHI). We examined viability in 5% BHI-supplemented tissue culture growth medium. In this co-culture model, MC3T3-E1 pre-osteoblasts were infected with different multiplicity of infection (MOI) of *S. oralis* ATCC 35037 in co-culture media (α MEM + 10% fetal bovine serum + 5% BHI). The viability of both mammalian and bacterial cells was quantified at one-, three- and seven-days post-infection using MTT colorimetric and colony forming unit assays (CFU), respectively. Quantifying this data, we observed pre-osteoblast viability maintained for the majority of MOI treatments. A loss in viability occurred for MOIs of 0.13 (-7 dilution) and MOI 0.013 (-8 dilution) on day seven; further trials will be done to investigate the variability in the outcomes of these treatments. Analysis of CFU loads showed the most significant decrease in *S. oralis* viability at MOI 1.3×10^4 (-1 dilution). Overall, each MOI treatment allowed permissible conditions for both mammalian and bacterial cell viability for laboratory co-culture, excluding the -7/8 trials. The co-culture methodology described here will be used for future experiments that further characterize the race for the surface of dental implant coatings.

UP 17: Isolation and Characterization, and efficiency study of the Novel Bacteriophage ‘VanGogh’

Hannah LaClair, John Ramirez, Daiyuan Zhang

Del Mar College, Corpus Christi TX, Department of Natural Sciences

Bacteriophage (Phage) is the type of virus to attack or kill their host bacteria. Phage is a very unique type of life form that has been studied for many purposes, including phage therapy, antibiotic resistant bacterial treatment, and recombinant DNA technology. There is usually high level of specificity between bacteriophage and its host, which make them a good alternative treatment for antibiotic resistant pathogenic bacteria like *Mycobacterium tuberculosis*. Since 1920's, phages have been using to control pathogenic bacteria but more phages need to be isolated to test on the efficiency to eliminate their hosts. The chosen bacterial host, *Mycobacterium smegmatis*, was used in this project because it is a non-pathogenic species that is close to *Mycobacterium tuberculosis*. A soil sample was collected from south Texas and enriched with *M. smegmatis*. A single phage, later named as ‘VanGogh’, was confirmed and purified by spot test and multiple round of streak test. Transmission electron microscope is used

to study the morphology of ‘VanGogh’ and genomic DNA was isolated and studied using restriction enzyme. A Lysogen of “VanGogh” was generated using spot test and long incubation, confirmed using patch test, and used for efficiency tests on its host. The life cycle of ‘VanGogh’ changed from lysogenic to more lytic with longer incubation. When compared to a phage database, the TEM image and restriction patterns suggest ‘VanGogh’ is classified as A cluster. The efficiency test result indicated that ‘VanGogh’ could eliminate 88.91% of *M. smegmatis* and should be considered to be a candidate to test on *M. tuberculosis*.

UP 18 Effects of nebulization with TLR agonist combination on foal alveolar macrophage cytokine production

Cory Mabry¹ , Jocelyne M. Bray¹ , Noah D. Cohen¹ , Diane C. Markesich² , Londa Berghaus³ , Angela I. Bordin¹

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Emergence of antibiotic resistance and the lack of effective alternative antimicrobials require novel approaches to control infectious diseases such as pneumonia caused by *Rhodococcus equi*, an intracellular pathogen of foals, human beings, and other species. Host-directed therapies present an alternative strategy by stimulating the host to fight against these infections without the need of an antimicrobial. Our approach was to use a Toll-like receptor (TLR) 2/6 and TLR9 agonist combination known as PUL-042 (Pulmotect, Inc, Houston, TX) to stimulate innate immunity in the lungs. Our primary goal was to determine the safety and the effect of nebulization with PUL-042 on the expression of inflammatory cytokines from foal alveolar macrophages obtained by broncho-alveolar lavage (BAL). Forty-eight newborn foals divided into 4 groups were nebulized with either water with 0.4% glycerol (diluent of PUL-042), or 3 doubling doses of PUL-042. A BAL procedure was performed before and 24 hr after nebulization at ages 2 days and, following 6 nebulization treatments, age 22 days. Alveolar macrophages from BAL fluid were cultured and infected *in vitro* with virulent *R. equi*. Control wells received media only. The supernatants were collected before and 48 h post-infection and analyzed using ELISA for the concentrations of IL-1 α , IL-10, IL-6, TNF α , and IFN- γ . Nebulized PUL-042 did not induce either adverse clinical effects and was considered safe to be administered to newborn foals at the tested doses. Results of cytokine concentrations are pending, but we expect to detect higher IFN- γ in foals nebulized with PUL-042 regardless of dose. IFN- γ is an important cytokine in protective response to infection against intracellular pathogens like *R. equi*.

UP19 : Human Macrophage polarization in response to *Mycobacterium leprae* DNA

Kristopher Van Huss, Alberto Marin, Marco Rodriguez, Eduardo Vasquez, Bo-young Hong, and Jorge Cervantes

Leprosy is an infection caused by *Mycobacterium leprae*. The disease is still endemic in many parts of the world, including South Texas. The clinical spectrum ranges from less severe Tuberculoid Leprosy, to a more severe Lepromatous Leprosy. Currently, it is believed that genomic strain differences in *M. leprae* do little in explaining this clinical presentation, and that host response is the major player in the disease progression. Here we evaluated *M. leprae* strain variation effect on human macrophages, and the role of macrophage polarization in response to *M. leprae* DNA. THP-1 monocytic cell line was differentiated into M1, M1 activated, or M2 macrophages using standard methodology. Cells were then stimulated with genomic DNA from three different *M. leprae* strains (NHDP, Br4923, and Thai-53). Polyethylenimine (PEI) was used to deliver DNA into the cells. RNAseq and transcriptome analysis was performed to evaluate the genes involved in the macrophage response to *M. leprae* DNA. Different sets of genes were expressed in all macrophage categories when they were stimulated with DNA from the three different strains. M1 activated macrophages showed the highest number of differentially expressed genes compared to M1 or M2. These included genes involved in inflammation, immune response, and cell regulation. Despite current thinking, differences between *M. leprae* strains seem to elicit a different macrophage response, suggesting that the host is not the only variable responsible for the clinical outcome of the disease. Genomic strain variability may also interplay in the process of infection. The role of macrophage polarization in the response and recognition of mycobacterial DNA seems important and could translate into better treatment strategies for the disease.

UP 20: Analysis of Induced Antibiotic Susceptibility in *Streptomyces griseus* Lysogens

Nicholas Mercado and Lee Hughes

Using the Kirby-Bauer Antibiotic Susceptibility protocol, comparisons of susceptibility were made between *Streptomyces griseus* ATCC 10137 and lysogens of this strain. So far, lysogens created by infection with *Streptomyces* phages LilBooBoo, a Cluster BB1 phage, and Animus, an unclustered phage, have been tested. *S. griseus* (LilBooBoo) showed a 31% increase in the zone of inhibition for Ciprofloxacin, a 33% increase for Polymyxin B, and a 79% increase for Tetracycline. *S. griseus* (Animus) showed a 35% increase in the zone of inhibition for Ciprofloxacin, a 40% increase for Polymyxin B, a 53% increase for Tetracycline, and induced apparent new susceptibility in Amoxicillin and Ampicillin with an increase in inhibition zone diameter from 0mm in the parent strain to 7mm and 10mm respectively. Both the parent and lysogen strains were fully resistant to Sulfamethoxazole and Streptomycin. Lastly, Triple Sulfa produced a very cloudy zone with an average diameter of 26mm throughout all tested organisms. The Kirby-Bauer method will continue to be used for analysis of susceptibility differences in *S. griseus* and lysogens from various clusters to determine if this increase in susceptibility is cluster specific or a general trend for *S. griseus* lysogens. Once data has been collected for a wide array of phages in different clusters, bioinformatic tools will be used to develop an understanding of this increase. While research is still in early development, it is possible that genes related to resistance in the parent strain are being directly affected or otherwise disrupted by the insertion of the prophage, or genes carried by the prophage are changing susceptibility of the host. It is

also possible that the change in zone size is due to phage induction. These possibilities will be tested once the initial data collection phase is complete.

UP 21: Characterization of Flagellar Structural Mutants of *Vibrio cholerae*

Savannah Munoz, Adrian Mejia-Santana, Mylea Echazarreta, Karl E. Klose,

University of Texas at San Antonio

The human enteric disease Cholera is caused by *Vibrio cholerae*, a Gram-negative bacterium with a comma-shaped body and a sheathed, monotrichous flagellum. Cholera is acquired through the consumption of contaminated water. *V. cholerae* colonizes the small intestine and expresses cholera toxin. Cholera toxin is an ADP-ribosylating toxin that causes massive fluid loss. The effects of the toxin are the hallmarks of this disease: severe diarrhea or “rice-water” stool, dehydration, muscle cramps, nausea and vomiting.

Most motile bacteria have flagella that share a majority of conserved subunits that make up the basal body, which extends from the cytoplasm across the periplasm to outside the cell, and the hook and filament, the long appendage that spins and provides motility. One unique aspect to the flagellum of *V. cholerae* (and other *Vibrios*) is the presence of a sheath around the filament composed of outer membrane. We hypothesize that the flagellar subunits associated with contact and penetration of the outer membrane may play a role in sheath formation. In this research, we focused on the genes encoding three flagellar structural proteins, FlgG (distal rod), FlgI (P-ring) and FlgH (L-ring). The *flgG* and *flgI* genes were deleted from the *V. cholerae* genome by homologous recombination. Both *flgG* and *flgI* strains were incapable of motility as assessed in soft agar plates. The nonmotile phenotype of the *flgG* strain was due to the lack of a flagellum, as determined by electron microscopy. Interestingly, the *flgI* strain showed decreased transcription of Class IV flagellar genes, while the *flgG* strain exhibited relatively normal Class IV gene transcription. Because initiation of Class IV gene transcription involves secretion of the anti-sigma factor FlgM through the flagellar apparatus, these results may implicate FlgI in this checkpoint control of flagellar synthesis.

UP 22: The role of carbon metabolism pathways in levofloxacin resistance in *Enterococcus faecalis*

Uyen Thy Nguyen; Karthik Hullahalli; Kelli L. Palmer

University of Texas at Dallas, Richardson, TX

Cells must go through the processes of replication, transcription, and translation in order to survive. During DNA replication, the double stranded DNA efficiently unwinds with the help of DNA gyrase. Levofloxacin (LVX), a fluoroquinolone antibiotic, binds tightly to DNA gyrase, preventing bacterial replication and resulting in cell death. *Enterococcus faecalis* is a Gram-positive bacterium that is normally found in gastrointestinal tracts. Infections by *E. faecalis* in hospital settings have been complicated by the rise in antibiotic resistance, leading to infections

that now have limited treatment options. LVX remains a potential therapeutic strategy for many *E. faecalis* infections, and it is of interest to study how these organisms respond to LVX-induced stress. Examining these responses would provide insight into mechanisms that allow *E. faecalis* to overcome the lethal effects of a clinically relevant antibiotic, which may lead to improvement in LVX therapy. Previously, it was shown that over 600 genes are differentially regulated by LVX-induced stress through RNA-sequencing analysis. A subset of highly-induced genes involved in carbon metabolism that have no known role in the context of fluoroquinolone resistance were examined to determine if they provide tolerance to LVX. By utilizing a CRISPR-Cas9 genome editing system, genes in five major carbon metabolism pathways were deleted. Deletions were made in combinations to examine if each individual pathway was sufficient alone or in conjunction with others to provide LVX tolerance. Remarkably, the minimum inhibitory concentrations of LVX for these mutant strains remained the same relative to the wild-type, indicating that these carbon metabolism genes have no effect in LVX tolerance. This finding broadly reveals that specific changes in gene expression induced by antibiotic can have no role in direct antibiotic tolerance.

UP 23: Utilizing *Caenorhabditis elegans* – *Enterococcus faecium* model to understand evolution of pathogenicity.

Alexey V. Revtovich¹, Barbara Murray², Natalia V. Kirienko¹

¹ Department of Biosciences, Rice University

² UT Health Division Director, Professor, Infectious Disease

The enterococci are a family of Gram-positive pathogens that includes both *Enterococcus faecalis* and *Enterococcus faecium*. Multi-drug resistant enterococci are considered as serious threat by the WHO and the CDC, particularly since antimicrobial resistance occurs faster than drug development. In the search for alternative strategies, virulence inhibition has drawn substantial attention, due to the ability of many hosts to tolerate bacterial colonization and growth. For enterococci in particular, commensal and pathogenic strains of *E. faecium* are distinguishable by a small number of virulence factors. As such, abolishing the activity of a few key targets is likely to restore the system to a commensal state. Identifying treatments that can achieve this goal is complicated by the fact that enterococcal infection models in vertebrates are slow, expensive, cumbersome, and ethically challenging. In contrast, *C. elegans* models are cheap, fast, simple, and retain a surprising amount of infection biology. Historically, efforts to generate a pathogenic model for *E. faecium* in *C. elegans* have not been successful. The only prior existing *C. elegans* – *E. faecium* model required anaerobic production of hydrogen peroxide by the bacteria. To solve this limitation, we developed the first high-throughput *C. elegans*–*E. faecium* model. It is independent of peroxide production and host killing occurs in ~4 d. In a pilot screen, we tested approximately 30 isolates provided by Dr. Barbara Murray’s lab (UT Health). Strains showed considerable variation (~3-fold) in virulence, which is consistent with variations in pathogenesis often seen for enterococcal strains. Importantly, human commensal strains were less virulent in *C. elegans* than clinical isolates. Our model also recapitulates pathogenic potentials observed in a murine peritonitis model. We anticipate that this model will be very useful for understanding virulence, and its evolution, in *E. faecium*.

UP 24: Antibacterial activity of 22 different snake venoms from families Viperidae and Elapidae

Oscar Sánchez, Montamas Suntravat, Elda E. Sánchez

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Bacterial infection has been and persists to be a major cause of disease and death worldwide. Antibiotics were thought to make this problem disappear. However, bacteria have evolved to become resistant to conventional therapeutics. The global emergence of antibiotic-resistant bacteria resulting from subsequent use and misuse of antibiotics has today become a major public health problem. Drug resistance in bacteria greatly increases patient morbidity and is linked to decreased clinical cure rates. The development of new, potent, and less toxic agents is an increasingly urgent medical need. In the last decade, a wide range of diverse, novel classes of natural antibiotics has been isolated from different snake species. This study aimed to investigate antibacterial activity of 22 different snake venoms against 6 medically relevant bacterial strains: *Staphylococcus aureus*, *Enterococcus faecalis* (Gram-positive bacteria) and *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Klebsiella oxytoca* (Gram-negative bacteria) using agar disk-diffusion and minimum inhibitory concentrations (MIC) methods. In vitro screening provides convincing evidence that several venoms have promising antibacterial effects against gram-positive and gram-negative bacteria. Interestingly, the bacterium *S. aureus* exhibited extreme vulnerability against all venoms examined. Venom from the Southern Pacific rattlesnake (*Crotalus oreganus helleri*) showed the most bactericidal activity against *S. aureus*, comparable to gentamicin (an antibiotic drug), with MIC values of 16 µg/ml. In addition, the preliminary result on the first purification of *C. o. helleri* venom on cation-exchange chromatography revealed the antibacterial activity of the first fraction isolated from the cation-exchange column. The present findings indicate that the *C. o. helleri* venom may contain anionic antibacterial components such as acid forms of L- amino acid oxidases and PLA₂ enzymes. The results will be useful for further identification and characterization of novel, potentially useful, antibacterial agents from snake venoms.

UP 25: Isolation and Lysogenic Study of a Novel Bacteriophage

Antonio Banda-Trejo, Lori Levy, Daiyuan Zhang.

Department of Natural Sciences, Del Mar College, Corpus Christi, TX

Bacteriophages are a type of viruses that infect bacteria and then replicate using the host systems. On the planet of earth, there are estimated more than 10³¹ of phages. While being greatly abundant, bacteriophages are also highly diverse and each phage attacks specific host bacteria and its close species. Due to phage's ability to target specific bacteria, it has been used as alternatives for traditional antibiotics that failed to treat antibiotic resistant pathogenic bacteria. A novel Mycobacterium bacteriophage named 'Blinn1' was isolated using enrichment protocols

from a soil sample collected from Sinton TX. After, genomic DNA of ‘Blinn1’ was isolated and used for a restriction enzyme digest and HTL lysate was used for TEM imaging. The lysogen was isolated using longer incubation of ‘Blinn1’ s spot test and then used for efficiency test. ‘Blinn1’ shows a lytic life cycle. The DNA digest pattern and TEM morphology both suggested that it’s a A cluster phage. ‘Blinn1’ has near 100% efficiency rate at killing off its host Mycobacterium smegmatis, implying that it can be a good candidate to develop treatment for M. tuberculosis, a close species to smegmatis

UP 26: The Isolation, Characterization, and Lysogenic Study of The Novel Bacteriophage ‘Lucyedi’

Alexis Trujillo, John Ramirez, Daiyuan Zhang

Natural Science Department, Del Mar College, TX

Bacteriophages are viruses that attack and kill their host bacteria. It has been estimated that there are over 10³¹ bacteriophages present on our planet and recently phages have been used for gene therapy and treatment for antibiotic resistant bacteria. In this study, isolation of a novel bacteriophage ‘Lucyedi’ began with a soil enrichment procedure followed by several experiments to characterize the phage using its bacteria host Mycobacterium smegmatis. A high titer lysate was harvested for phage genomic DNA isolation. The isolated DNA of ‘Lucyedi’ was used for restriction digest analysis and genomic sequencing. The phage morphology of “Lucyedi” was studied by uranyl acetate negative staining and transmission electron microscope imaging. The lysogen of “Lucyedi” was isolated by spot test with extra incubation and was used for phage efficiency study. The plaques of “Lucyedi” indicated a lytic life cycle at the time of isolation. The TEM images show “Lucyedi” contains a capsid with 60nm in diameter and a tail 160 nm in length. The restriction digest patterns suggested the “Lucyedi” genome contains multiple recognition sites for BamHI, ClaI, HaeIII and few sites for HindIII and EcoRI. Both the TEM image and restriction pattern imply that “Lucyedi” belongs to the cluster A. The lysogen efficiency test of “Lucyedi” indicated that 99.14% of the host Mycobacterium smegmatis could be destroyed by this bacteriophage, which makes “Lucyedi” a good candidate to develop a phage treatment for pathogenic Mycobacterium tuberculosis, a close species to M. smegmatis.

UP 27: Production of Competitive Factors by Enterococci

Authors: Smriti Verma, Dr. Valerie Price, Dr. Kelli Palmer

Department of Biological Sciences, The University of Texas at Dallas

Bacteria produce factors that help them compete with other organisms for nutrients. Examples of such factors are antibiotics and bacteriocins. Bacteriocins are antimicrobial peptides produced by bacterial cells which can kill other bacteria. Typically, the bacteriocin-producing organism encodes immunity against the bacteriocin to prevent self-killing. This toxic activity manifests as a zone of inhibition when a lawn of bacteriocin-producing bacteria is challenged

with another bacterial culture. We are interested in the competitive factors produced by the opportunistic human pathogens *Enterococcus faecium* and *Enterococcus faecalis*. These Grampositive bacteria natively colonize the human gastrointestinal tract, but high-level antibiotic resistance has emerged in some strains. The long-term goal is to identify novel antimicrobials with activity against antibiotic-resistant enterococci. In this experiment, we spotted cultures of *E. faecium*, *E. faecalis*, *E. gallinarum*, *Escherichia coli*, *Streptococcus oralis*, *S. mitis*, and *Pseudomonas aeruginosa* against lawns of enterococcal clinical isolates to determine if any killing activity was present. The presence of zones of inhibition on the plates indicated that bacteriocin and/or antibiotic action was present. Two strains of *E. faecium* as well as two strains of *E. faecalis* often showed zones of inhibition in response to a number of clinical isolates. Other interesting competition phenotypes were observed. Future research will involve studies to confirm what specific competitive factor(s) are causing the observed killing activities and the mechanisms by which they act. This line of research is useful to the health field because of its application in therapy development against antibiotic-resistant pathogenic bacteria.

2:30-3:00pm

Eugene and Millicent Goldschmidt Graduate Student Award:

Nicole C. Elledge, TAMU-CC, *Stormwater is a pulse disturbance that alters bacterial community composition in urbanized bays*, in the University Center's Ballroom 147C.

The coastal bays of Texas, where barrier islands limit exchange with the Gulf of Mexico and increase residence time, are sinks for stormwater runoff. This runoff transports pollutants and potentially harmful microorganisms to the surrounding aquatic environments. Moreover, the loading of stormwater runoff in coastal Texas is predicted to worsen with population growth, urbanization, and climate change. However, the impact of stormwater runoff on coastal marine ecosystems is largely unknown. Given that fecal waste is a constituent of stormwater runoff, we conducted a nine-month study focused on the measurement and analysis of fecal indicator bacteria (FIB) to assess the impact of stormwater runoff in coastal Texas. The major objectives of this study included 1) the quantification of enterococci bacteria, 2) the assessment of antimicrobial susceptibility among the enterococci isolates, 3) the quantification of human-, canine-, and gull-associated molecular markers, and 4) the characterization of the taxonomic composition of the bacterial community at large. Findings have shown that enterococci concentrations frequently exceeded the EPA recreational water quality criterion of 104 MPN 100 mL⁻¹, with the most elevated enterococci concentrations occurring immediately after rainfall. Storm events also promoted shifts in the taxonomic structure of the bacterial community, resulting in an overall decrease in bacterial diversity. Yet storm events were not associated with an increase of the abundance of human-, canine-, and gull-associated molecular markers. Instead, these markers were abundant throughout the course of the study with gull being the most abundant, followed by human, and canine. The omnipresence of the human-associated marker suggests that leaking sanitary sewers are a year-round source of bacterial pollution. Additionally, the absence of a correlation between the human marker and enterococci indicates that traditional EPA criteria for risk assessment are a poor proxy for human-associated fecal pollution.

3:00-5:00pm

Scientific presentations in 3 concurrent sessions (Phycology, Virology, and a mixed-topic Student Research Session) in the University Center's Ballroom 147C and 147D.

Session A: Phycology, Ballroom 147A

Session Chair: Deana Erdner, PhD

Institution: University of Texas Marine Science Institute

1. Structure and function of the microbiome of the toxic dinoflagellate *Alexandrium tamarense*

Deana Erdner¹ and Cecile Jauzein²

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Like all organisms, algae harbor specific microbiomes that can have both positive and negative effects on the algal partner. Our previous work with the toxic dinoflagellate *Alexandrium tamarense* has shown that the presence of bacteria can be associated with faster growth, larger cell size, higher photosynthetic efficiency, and greater cellular chlorophyll and toxin content. To characterize interactions between *A. tamarense* and its microbiome, we used two sub-cultures of a single genetic strain of this species, one bacterized (xenic) and one axenic, which show significant differences in morphology and physiology. Bacteria from the xenic strain were re-inoculated into the axenic strain, producing replicate “rexenic” strains. At 9 and 15 months, we characterized algal physiology and the community composition of both the algal-attached bacteria and those that were free in the medium. Parental microbiomes were stable over a long time scale (6 years). Despite differences in physiology between the two rexenic strains, their free bacterial communities were similar to each other, and distinct from the xenic parent. The attached bacterial communities of the rexenic strains were similar to the parent at 9 months, but had diverged by 15 months. About 15% of the taxa drive the majority of differences between xenic/rexenic and attached/free communities. *Arcticiflavibacter* is more abundant in native attached communities, while lower algal function is associated with higher abundances of *Marinobacter* in the attached fraction, and disturbed communities contain more *Roseobacter* in the free fraction. Native and disturbed communities also show clear differences in the abundance of orders within the Bacteroidetes. These results suggest that the algal microbiome composition is not resilient to severe disturbance. Nonetheless, structurally dissimilar communities can be functionally equivalent in terms of impact on the algal partner.

2. Bloom initiation of *Karenia brevis* on the Texas coast: Can it be predicted?

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Karenia brevis is the major harmful algal bloom-forming species in the Gulf of Mexico. Physical factors are known to play a key role in the initiation, maintenance, and termination of blooms of *K. brevis* and previous work has shown blooms appearing near the coast of Texas originate in the southern Gulf of Mexico. Knowing the possible origin of blooms, it may be possible to predict the arrival of *K. brevis* blooms along the coast of Texas. To test this idea, we combined a spatially explicit, individual-based model (IBM) for *Karenia brevis* with a satellite imagery ensemble model to identify patches of *K. brevis* that may serve as seed populations in the southern Gulf of Mexico. Potential seed populations were identified from satellite imagery, used to seed the IBM with individuals, and tracked from ~1 Jul through 31 Dec (~180 days). The arrival (or not) of cells at the coast of Texas was compared with a 10-year time series of abundance captured with an Imaging FlowCytobot (IFCB) at Port Aransas, Texas, USA. The IFCB captures images of microphytoplankton cells (10-150 μ m) and has provided early warning for 8 harmful algal blooms, including *K. brevis*. In 2015, a bloom of *K. brevis* was identified at Port Aransas on 14 Sept. Retrospective analysis of the satellite imagery indicated the presence of a potential patch of *K. brevis* north of the Yucatan Peninsula on 12 Jul. Cells were seeded in the Yucatan region and tracked forward in time. The first sustained presence of modeled cells near Port Aransas occurred between 7-18 Sept, dependent upon how the nutrient field was parameterized. Model runs utilizing a mixed layer depth-based nutrient field captured the timing of arrival better while those utilizing a salinity-based nutrient field appeared to better capture the bloom dynamics once the bloom had arrived. As the true nutrient field is much more complex than those used here, the incorporation of better nutrient fields could greatly improve future predictions and the potential for forecasting of blooms. Ultimately, the use of satellite imagery to identify potential seed populations for subsequent blooms in Texas looks promising but additional field testing is necessary.

3. Omics-based monitoring of *Prymnesium parvum* (Haptophyta) and its toxins

Schonna R. Manning

Department of Molecular Biosciences, UTEX Culture Collection of Algae, The University of Texas at Austin

Seasonal blooms of the golden alga, *Prymnesium parvum*, have caused extensive fish killing events coupled with the presence of potent toxins – complex polyketides called prymnesins. While *P. parvum* is euryhaline and occurs in the ocean, harmful outbreaks have been isolated to brackish and freshwater systems. Yet, little is known regarding the mechanisms of bloom formation and there are no reference standards available for the confirmation of their toxins. A suite of genomic, metabolomic, and transcriptomic analyses are presented that may be used to rapidly monitor this microalga and its toxins. Initially, multiplex PCR assays were developed for quantifying cells of *P. parvum* in cultured and environmental samples wherein sets of primers simultaneously amplified four species- and gene-specific products using isolated genomic DNA or whole cells. PCR products resolved by gel electrophoresis generated a diagnostic banding pattern, and molecular beacons were designed for the application of real-time quantitative PCR. Both methods

were capable of detecting 1 cell in 50 cycles. Methods were also developed for the co-enrichment of Type A prymnesins, *prym1* and *prym2*, from cell extracts and culture supernatants using C18 solid-phase extraction. Prymnesin fractions were semi-quantified using a chemifluorescence assay and ESI-MS metabolic fingerprinting. Intracellular prymnesins were consistent on a per-cell basis, but extracellular prymnesins were often higher in supernatants of stressed cultures, indicating that exotoxins are associated with cell death. More than 10 ions were detected in mass spectra that agreed with predicted isotopic distributions for intact prymnesins and related fragments. The most abundant ion was 919.88^+ m/z, representing the aglycone structure common to both molecules. Moreover, a *de novo* transcriptome assembly with 47,289 transcripts is reported; 32 different polyketide synthases were identified, although none were differentially expressed under our experimental conditions. Analyses revealed evidence of post-transcriptional regulation of polyketide production and the synergistic effects of putative hemolysins and fatty acids toward ichthyotoxicity.

4. Potential role of oil-degrading bacteria in the formation of a harmful dinoflagellate blooms after oil spills: evidence from culture experiments

Bum Soo Park, Deana L Erdner, Hernando Bacosa, Zhanfei Liu, Edward J. Buskey

University of Texas Marine Science Institute, Port Aransas, TX 78373, USA

The association between phytoplankton blooms and oil spills is still controversial despite numerous studies. Surprisingly, to date, there have been no studies on the effect of bacterial communities exposed to crude oil on phytoplankton growth, even though crude oil leads to variation in bacterial communities, and this variation can affect phytoplankton growth and species composition. In this study, to investigate the impact of altered bacterial communities exposed to crude oil on the growth of dinoflagellate, we exposed free-living bacteria isolated from a *Prorocentrum texanum* culture to crude oil (100 ppm) for a month, and then investigated the growth change in *P. texanum* after addition of these oil-treated bacteria. As a result, the growth rate and yield of *P. texanum* in bacterial treatment was clearly enhanced, compared to control. To gain more direct evidence for the role of oil-degrading bacteria in bloom formation, we isolated oil-degrading bacteria from sediment samples collected from oil-contaminated sites after the Texas City “Y” oil spill, and investigated variation in dinoflagellate growth after co-culture with single bacterial isolates. A total of seven oil-degrading bacterial cultures were established, and two bacterial cultures (C1-T3 and E1-Gal-T2) clearly enhanced the growth rate and yield of six dinoflagellate cultures; axenic *Amphidinium carterae* and *Peridinium sociale*, and xenic *Karenia brevis*, *P. gracile*, *P. minimum*, and *P. texanum*. To determine whether or not these bacteria can enhance dinoflagellate growth by releasing nutrients, nutrient limited medium was prepared by removing each one of the components (nitrogen, phosphorous, trace metals or vitamins), and C1-T3 and E1-Gal-T2 were inoculated into each nutrient limited media, containing *A. carterae* and *Pe. sociale*. These two bacterial cultures greatly enhanced the growth rate and yield of the two

dinoflagellates, regardless of any nutrient-limited media. Together with these findings, oil-degrading bacteria may enhance the growth of dinoflagellates and this growth enhancing activity may not be derived from nutrients released from the bacteria.

5. Microbial community structure differences in the hypersaline wind-tidal flats of Laguna Madre, TX, USA

Paul V. Zimba, I-Shuo Huang, Sergei Shalygin, Lee J. Pinnell, Jeffrey W. Turner

Texas A&M University Corpus Christi, Corpus Christi, TX

Virtually nothing is known about algal composition of the hypersaline wind-tidal algal communities of the Laguna Madre, Texas. These communities cover 42% of the aerial extent of this ecosystem and have an important role in overwintering bird use as this area is the terminus of the Central Flyway. Historically, two publications have described the community as composed of two species of cyanobacteria, with a few diatoms rarely encountered. Beginning in 2017-present, we have used classical and modern approaches to evaluate these mats. Methods have included single strain isolation of cyanobacteria, with 11 new to science taxa isolated. Mat communities are notoriously difficult to quantify species abundance accurately, so we initiated a genomic approach using HiSeq analysis of 16S RNA in mats. Over 63 distinct taxa were identified using this procedure. Information on these species, and the assemblages associated with the mats will demonstrate distinct community interactions.

6. Can Photosynthetic Yield be Used as an Indicator for Nutrient Limitation in *Microcystis aeruginosa* UTEX LB 3037?

Katherine A. Perri¹, Schonna R. Manning², & Gregory L. Boyer¹

¹Department of Chemistry, College of Environmental Science and Forestry, State University of New York, Syracuse, NY

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Harmful algal blooms have been widely linked to excessive phosphorus (P) loading to inland waters, but increasing evidence shows that growth can be limited by other nutrients, notably nitrogen (N) and iron (Fe). Deficiency in each of these nutrients differentially affects the structure of the cell's photosystem that can manifest as changes in photosynthetic yield, which is calculated as the ratio of variable to maximal fluorescence (F_v/F_m). Several instruments have been used to measure F_v/F_m as a rapid *in situ* gauge of nutrient deficiency, but there have been few comparisons of their sensitivity and ability to resolve specific nutrient stresses. In this study, we used controlled assays with the bloom-forming cyanobacterium, *Microcystis aeruginosa* UTEX LB 3037, grown under nutrient-replete (R), N-limited (LN), P-limited (LP), and Fe-limited (LFe) conditions. The

F_v/F_m was measured using three pulse-amplitude modulated (PAM) fluorometers and the more traditional method of using the photosystem inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). F_v/F_m is typically measured on samples that have undergone dark-adaptation to fully oxidize the photosystem prior to a saturating light pulse, but several PAM fluorometers are often deployed in the field without a dark-adaptation period (F_v'/F_m'). Our results showed no significant differences in F_v/F_m measurements among PAM fluorometers, but they were all significantly higher than the DCMU-based measurements for LFe cultures. By day 21, there were no significant differences in F_v/F_m between R and LFe treatments (0.37 ± 0.06 and 0.38 ± 0.15 , respectively), but these were significantly higher than the average yield in LP treatments (0.26 ± 0.05) measured by PAM. Similarly, the average F_v/F_m of LN samples was not significantly lower (0.30 ± 0.17) than R measurements (0.37 ± 0.06) up to day 14. Average F_v/F_m was not statistically different from F_v'/F_m' across all treatments, suggesting that dark-adaptation does not significantly bias measurements made from prokaryotic-dominated assemblages. These data suggest that PAM fluorometry alone cannot resolve nutrient limitation in *M. aeruginosa*.

Session B: Microbial Ecology, Ballroom 147C

Session Chair: Brandi Kiel Reese, Ph.D.

1. Evaluation of Acquired Antibiotic Resistance in *Escherichia coli* exposed to long-term Low Shear Modelled Microgravity and Background Antibiotic Exposure

Madhan R Tirumalai^a, Fathi Karouia^b, Quyen Tran^a, Victor G. Stepanov^a, Rebekah J. Bruce^c, C. Mark Ott^c, Duane L Pierson^c, George E. Fox^{a#}

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The long-term response of microbial communities to the microgravity environment of space is not yet fully understood. Of special interest is the possibility that members of these communities may acquire antibiotic resistance (AR). In this study, *Escherichia coli* cells were grown under low-shear modeled microgravity (LSMMG) conditions for over 1000 generations (1000G) in (i) steam-sterilized HARVs and in (ii) HARVs in which the sterilization was accomplished using chloramphenicol treatment between cycles. The steam-sterilized HARV grown strain (1000G) was compared with the strain grown in chloramphenicol treated HARV (1000G-BA). The sensitivity of the final 1000G and 1000G-BA strains to a variety of antibiotics was determined using Vitek analysis. The 1000G strain never acquired AR, and the cells acquired an adaptive advantage, a portion of which was genomic and as a result was maintained when the strain was returned to a shake flask environment for 30 generations. In contrast, the 1000G-BA strain acquired resistance to cefalotin, cefuroxime, cefuroxime axetil, cefoxitin, and tetracycline. Of these, resistance to cefalotin persisted for over 110 generations despite the removal of both LSMMG conditions and trace antibiotic exposure. Genome re-sequencing studies identified 17 changes in the 1000G strain,

while 25 changes were seen in the the 1000G-BA strain. At least one major and 4 minor changes that were seen in the 1000G-BA strain were directly linked with AR. Overall, LSMMG does not appear to alter the antibiotic stress resistance seen in other microbial ecosystems. The retention of AR (AR) in the 1000G-BA strain, suggests that similar persistence of microbial AR by other microorganisms could also occur. This is most likely to happen independent of the microgravity component. This is of particular concern, in the event of using antibiotics as cleaning agents to reduce the bioburden of microbes in the confined spaces of manned space flight missions. Finally, this study is restricted to just one Gram-negative non-pathogenic strain, namely, *E. coli* MG1655. Such long-term studies are relevant towards further exploring AR of the human (astronaut's) gut microbiome, of which enterobacteria (such as *E. coli*) are major components as well as Gram-positive organisms.

2. Molecular Detection of Fecal Indicator Bacteria and Human-Associated Bacteroidales in a Texas River impacted by Hurricane Harvey

Vikram Kapoor, Indrani Gupta, A.B.M. Tanvir Pasha, and Duc Phan

Department of Civil and Environmental Engineering, University of Texas at San Antonio, San Antonio, TX 78249, USA

Hurricane Harvey has caused unprecedented devastation to huge parts of southeastern Texas, particularly damaging the wastewater infrastructure resulting in release of sewage contamination into environmental waters. In response to Hurricane Harvey, a National Science Foundation RAPID project was launched to perform a comprehensive assessment of the microbial water quality in a Texas River impacted by the hurricane floodwaters. For this project, water samples were collected along the Guadalupe River during September–December 2017. In this presentation, we will share the results of the sampling, including the presence of fecal indicator bacteria, human-associated fecal genetic markers and pathogens, measured using qPCR and ddPCR assays. In general, results of this initial microbiological contaminant assessment will serve as baseline information for follow-on studies to monitor existing and emerging public health risks to residents of Texas and potential long-term environmental impacts on the water resources in the impacted regions.

3. Microbial symbiosis in a changing world: the effect of temperature on gut bacteria of the eastern subterranean termite, *Reticulitermes flavipes*

Rachel A. Arango^a, Sean D. Schoville^b, Camela Carlos-Shanley^{c*}

^aUSDA Forest Service, Forest Products Laboratory, Madison, Wisconsin, USA

^bUniversity of Wisconsin-Madison, Department of Entomology, Madison, Wisconsin, USA

^cTexas State University, Department of Biology, San Marcos, Texas, USA

Understanding the effects of environmental disturbances, such as shifts in global temperature, on insect biology is an essential component in developing predictions of distribution, abundance and ecology of these organisms in the future. Studies of thermal tolerance are one tool for answering

these types of questions, and when combined with observations of other physiological changes, can help elucidate how these types of adaptations are mediated. Here, subterranean termites, *Reticulitermes flavipes* were exposed to either low (15°C), medium (27°C), or high (35°C) temperatures. After four weeks, feeding, survival, cold tolerance (i.e. critical thermal minimum and supercoolingpoint), and shifts in gut/soil microbiota were evaluated. Results from this study suggest that exposure to high temperature had the greatest negative effect on overall termite fitness with a significant decrease in termite survival and thermal tolerance. Additionally, we observed reduced microbial diversity in high temperature termite gut samples compared to the low or medium temperature groups. Exposure to high temperature reduced termite survival and negatively affected microbial symbionts associated with termite-gut protists including Spirochaetes, Endomicrobia, and methanogenic Euryarchaeota. We are now investigating how the gut microbial community contributes to the physiological responses of termites to thermal stress. This is the first study to investigate the link between microbiome and thermal tolerance in social insect, with important implications for both pest management and species conservation.

4. Intraterrestrial Fungus: From Deep Sea to Deep Space

Brandi Kiel Reese¹, Morgan Sobol¹, Tom Metz², Tatsuhiko Hoshino³, Fumio Inagaki³

1 Texas A&M University-Corpus Christi

2 Pacific Northwest National Laboratory, Richland, Washington

3 Kochi Core Center, Kochi University, Japan

Unique stressors during space travel induce multiple biological responses. Spaceflight stress studies have focused on microgravity and radiation, both having the capacity to alter many metabolic pathways. Fungi have the capacity to degrade many recalcitrant compounds and can produce valuable metabolites including many antibiotics, making them a candidate for biotech space-based culturing efforts. A novel deep surface fungi, related to *Penicillium chrysogenum*, originally isolated from 127 meters below seafloor in the South Pacific Gyre on Integrated Ocean Drilling Program (IODP) Expedition 329 was grown onboard the International Space Station before being cryopreserved and returned to Earth. Control samples were grown on Earth with the same cultivation times and preservation methods. Terrestrial isolates of this lineage have been widely used in the production of penicillin and thus alterations of its metabolic capacity may have direct industry impact. Metatranscriptomes will be compared to determine the different effects of spaceflight. Efforts will be made to examine DNA repair mechanism, structural pathways, and antibiotic production mechanisms. Additional pathways will be examined based on resulting data sets.

5. Disturbance and recovery of the microbial communities in Galveston Bay following Hurricane Harvey and flooding of the Houston area

Jordan R. Walker, Samantha Setta, Jamie Steichen, Karl Kaiser, David Hala, Antonietta Quigg, and Jessica M. Labonté

Hurricane Harvey made landfall over the Texas coast as a Category 4 storm on August 25, 2017. During the subsequent five days, precipitation reached over 130 cm of rain onto the Houston area, causing city-wide flooding. Large amounts of freshwater from the floodwater/runoff inundated Galveston Bay, causing decreases in salinity and discharge of biolabile dissolved organic matter. We performed a transect from the mouth of the San Jacinto river to the Gulf of Mexico over the six weeks following the storm. Here, metagenomics was used to characterize the changes in diversity and metabolic potential of nanoplankton, prokaryotes and viruses. Our results show that the storm introduced soil, sedimentary, and freshwater bacteria, as well as induced stress in the microbial communities. Shortly after the storm, the bacterial community displayed more genes related to specific pathways (nitrogen, sulfur, aromatic compounds), virulence and stationary phase. Over the following five weeks, the community reverted back to a coastal community dominated by Cyanobacteria with a metabolic gene pool indicating fast turnover and high viral infection rates. Viruses went from a community dominated by ssDNA viruses shortly after the storm to a coastal assemblage constituted mainly of members from the Myoviridae family and cyanophages. This study shows that microbial communities can quickly recover from extreme events such as a hurricane or flooding event to maintain ecosystem equilibrium.

6. Metagenomic and metabolic microbiome analysis of native plant soils versus invasive plant soils and the effect of soil inoculation on plant growth

Brenda G. Rushing, Marissa Narvaez, and J. R. Valdez Barillas

Biology Program, Texas A&M University-San Antonio

Plant biodiversity decreases in areas where native plant species have been eliminated by invasive plants, and plant invasion may also affect microbial diversity of the area. Our study set out to determine the effects of two plants on the bacterial community of their surrounding natural soil, and whether providing native soil microbes might improve establishment of native species when in competition with an invasive plant. We analyzed the microbiomes present in the soil of a native Texas plant species, Sideoats grama, and an invasive species, Bermuda grass. We performed a metagenomics sequence analysis using the V4 region of 16S rRNA to determine bacterial community members within these two soil types. We also performed community-level physiological profiling (CLPP) using the Biolog EcoPlate™ system for carbon source utilization. Additionally, biomass of the two plant species grown in commercial soil alone, and commercial soil with either native or invasive soil added, were compared. Examination of the metagenomics data and the CLPP indicated that the soil surrounding the native Sideoats plants contained a more varied population of bacterial species with broader metabolic diversity than the Bermuda grass soil. The plant biomass data showed that both plants grew better individually when inoculated with their respective soils. When the plants were in competition in the same container, Sideoats grew better than Bermuda when treated with native Sideoats soil, suggesting that the plant may shift the microbial soil community in its favor, promoting Sideoats plant growth and potentially inhibiting Bermuda grass. These results point toward Sideoats grama as a good candidate for re-establishment of native plants in areas where Bermuda grass has become dominant.

Session C: Microbial Infectious Disease, Ballroom 147C

Session Chair: Richard Laughlin, PhD

Institution: Texas A&M University-Kingsville

***1. Vibrio cholerae* Induced Inhibition of *Candida albicans* Filamentation**

Mylea Echazarreta, Daniel Montelongo-Jauregui, Jose L. Lopez-Ribot and Karl E. Klose

South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio.

Vibrio cholerae colonizes the human gastrointestinal (GI) tract and expresses cholera toxin, which leads to the profuse watery diarrhea characteristic of cholera. The GI tract contains a wide variety of commensal microorganisms including bacteria, archaea and fungi, known as the microbiota. *V. cholerae* is known to interact and compete with bacteria, but interactions with eukaryotic organisms within the microbiota remain understudied. The pleiomorphic opportunistic fungal pathogen *Candida albicans*, is a member of the microbiota within the human GI tract. In this study, we have characterized the interaction of *V. cholerae* and *C. albicans*. We developed an in vitro microtiter plate model to study fungal-bacterial biofilms of *C. albicans* and *V. cholerae*. Biofilm formation was assessed using crystal violet staining and Scanning Electron Microscopy. We found that *V. cholerae* and *C. albicans* form mixed species biofilms in which the bacteria adhere tightly to the fungi. Moreover, results demonstrated that *V. cholerae* is able to inhibit filamentation of *C. albicans*, and thus inhibit *C. albicans* biofilm formation, in both mixed and pre-formed biofilms. To identify *V. cholerae* factors involved in this process, we co-cultured *V. cholerae* mutant strains lacking genes important for adherence, motility, quorum sensing, T6SS, chitin sensing, and biofilm formation in combination with *C. albicans*. These studies revealed a requirement for MSHA pili, flagella, the chitin sensor ChiS, and the QS regulator HapR for *V. cholerae* inhibition of *C. albicans* biofilms. Overall, our results suggest that *V. cholerae* senses chitin in the *C. albicans* cell wall, utilizes motility and the MSHA pilus to colonize the fungal surface, and then utilizes QS to inhibit *C. albicans* filamentation and biofilm formation. These studies may identify novel antifungal therapies to treat *C. albicans* infections.

2. Metabolic Control of Virulence Potential of the Agent of Lyme disease

J. Seshu

South Texas Center for Emerging Infectious Diseases (STCEID), Department of Biology, The University of Texas at San Antonio, TX-78249

Abstract: Lyme disease is the most common vector-borne disease in the US. The agent of Lyme disease - *Borrelia burgdorferi* (*Bb*) - is an extreme auxotroph with limited metabolic capabilities and relies on its hosts to acquire several nutrients. This spirochetal pathogen is transmitted to

mammalian hosts via the bite of infected ticks. How does the host-specific, pathogen metabolism influence its virulence potential during the tick and mammalian phases of infection? Phenotypic analysis of genetically defined mutants, pathway analysis and specific inhibitors of rate limiting enzymes have helped to dissect how *Bb* is able to navigate across highly divergent host environments. Translational implications of these fundamental studies directed at reducing the pathogen burden during various stages of the life cycle of *Bb* will be discussed.

3. A Whole Organism, Host-Pathogen Screening System Identifies Novel Therapeutic Leads

Natasha Kirienko

Department of Biosciences, Rice University, Houston, TX

Pseudomonas aeruginosa is a major pathogen, responsible for over 50,000 nosocomial infections per year, and is the main cause of mortality in patients with cystic fibrosis. This bacterium exhibits broad-spectrum resistance to classical antimicrobials and readily acquires additional resistance mechanisms, making treatment challenging. Additionally, new targets for classical antimicrobials have been waning for decades. To circumvent these difficulties, we have developed a high-throughput, high-content, live, whole-organism infection model using *Caenorhabditis elegans*. Whole organism-based screens exhibit several advantages over target-based screening methodologies, including dramatically increased probability of identifying druglike molecules that have low acute toxicity and high bioavailability. In addition, the use of a whole organism, in contrast to a cell culture system, allows for the complex interplay of biochemistry that occurs within the various tissues of an intact organism.

Using this platform, we screened approximately 85,000 compounds in duplicate for the ability to ameliorate infection. We identified approximately 200 molecules with curative effects, 70 of which were molecules with unknown functions. The majority of these compounds do not exhibit the characteristics of a classical antimicrobial. Instead, some small molecules appear to activate host immune responses while a small number of compounds functions by compromising either the biosynthesis or function of the *P. aeruginosa* master virulence factor pyoverdine. Pyoverdine is a siderophore that is dispensable for growth in the laboratory, but required for virulence in both mammalian infections and in *C. elegans*. Promisingly, these compounds are also capable of synergizing with conventional antimicrobials for a more effective treatment cocktail.

4. Cooperation versus competition in polymicrobial infections

Catherine A. Wakeman, Ph.D

Texas Tech University

Microorganisms demonstrate numerous types of community behaviors that range from cooperative to competitive interactions. These interactions can include coordinated efforts to build and maintain protective biofilm structures or active killing of competitors via the production of antimicrobial compounds. This spectrum of behaviors is often influenced by environmental conditions. The interactions between bacterial pathogens surviving within chronic polymicrobial infections are of particular interest to many researchers in both basic and translational sciences. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are well-studied opportunistic pathogens known to occupy sites of polymicrobial infection within the cystic fibrosis lung. The ability of *P. aeruginosa* to outcompete and kill *S. aureus* under standard laboratory conditions has been well established. However, recent data indicates that conditions of the host environment can reduce the production of competitive molecules in *P. aeruginosa* which may ultimately select for coexistence with *S. aureus* and other members of polymicrobial infectious communities. I seek to identify the genetic pathways that drive the switch from competition to cooperation in *P. aeruginosa* and to determine the potential benefits that *P. aeruginosa* gains from a cooperative lifestyle in the presence of *S. aureus* and other microbes during infection.

5. Precision editing of the gut microbiota ameliorates colitis and colorectal tumorigenesis

Wenhan Zhu

University of Texas Southwestern Medical Center

Episodes of intestinal inflammation are associated with changes in gut microbial communities, in particular an expansion of *Enterobacteriaceae* populations. This inflammation-associated dysbiosis is thought to contribute to disease progression in inflammatory bowel disease and colitis-associated colorectal cancer (CAC). Here, we use mouse models of colitis to investigate the mechanisms underlying inflammation-associated dysbiosis. Expansion of *Enterobacteriaceae* populations in the colitis model was driven by a disease-specific metabolic program. During inflammatory flares, electron acceptors were generated that facilitate a molybdoenzyme-dependent, respiratory metabolism in *Enterobacteriaceae* family members. Administration of the heavy metal tungsten inhibited bacterial molybdoenzymes in the gut microbiota and selectively prevented the inflammation-associated bloom of *Enterobacteriaceae* in mouse models of acute and chronic colitis. In contrast, tungstate treatment caused no overt changes in the microbiota composition under homeostatic conditions. Importantly, tungstate-mediated microbiota editing reduced the severity of intestinal inflammation in murine models of acute colitis. Oral administration of sodium tungstate also decreased gut colonization with pro-tumoral *E. coli* strains in the setting of chronic intestinal inflammation and CAC development. Restricting the bloom of *Enterobacteriaceae* decreased the tumor incidence in two models of CAC. We conclude that metabolic targeting of *Enterobacteriaceae* during intestinal inflammation is a suitable strategy to improve acute and chronic colitis and to prevent malignancies arising from gut microbiota dysbiosis.

6. Whole Genome Sequencing of Microbes During Disasters

Randall Olsen

Institute for Academic Medicine, Houston Methodist Hospital

Our laboratory has validated whole genome sequencing of microbes as a routine clinical test. It is used for taxonomic identification of difficult to identify or slow growing organisms, investigation of unusual or interesting infections, and investigation of possible outbreaks or nosocomial infections. Our experience using whole genome sequencing during disasters will be discussed.

Saturday, November 10th

9:30-11:30am STEM education and Bioinformatics demonstrations/presentations in concurrent sessions in the University Center's Ballroom 147C and 147D.

Session A: Big Data Science, Ballroom 147A

Session Chair: Jessica Labonté, PhD

Institution: Texas A&M University-Galveston

1. Leveraging metagenomics to explore marine sediments

Brett J Baker, Nina Dombrowski, Kiley W Seitz, Valerie de Anda, Lin-xing Chen, Laura Eme, Jonathan Lombard, Andreas Teske, Jillian F Banfield, Anja Spang, Thijs JG Ettema

Since many microbial taxa lack genomic representation, our understanding of their ecology, evolution, and physiological capabilities are limited. To overcome this problem, we have sampled anoxic environments around the world, including hot springs, estuaries, and deep-sea sediments (namely those associated with hydrothermal vent in the Guaymas Basin, Gulf of California). Metagenomic assembly and binning of these environments has resulted in the reconstruction of thousands of new genomes that comprise several distinct lineages that differ from those that have been described previously. Guaymas Basin sediments select for distinctive microbial communities that stand out by expansive biodiversity, we have obtained genomes belonging to 26 unique lineages on the tree of life; include 9 new phyla, from that environment alone. Analyses of the proteomes of the uncultured organisms are providing the first insights into their ecological roles and evolution histories. For example, we have obtained several genomes belonging to new archaeal superphylum, named "Asgard", which are revolutionizing our understanding of cellular complexity and eukaryogenesis. Recently genomes belonging to a new Asgard group, Helarchaeota, contains genes for alkane activation via alkyl-coenzyme M formation, and associated pathways for short-chain alkane (likely butane) oxidation. Genomes of another new phylum, named Brockarchaeota, are found in hot spring sediments around the world and appear to be involved in methylamine and methanol utilization. This effort has also increased the diversity of genomes in several previously characterized phyla. For example, newly characterized genomes of Korarchaeota indicate that these archaea are capable of autotrophic

sulfide oxidation. We are also leveraging this genomic-centric community wide comparison to understand how ecological roles are partitioned in marine sediments. Interestingly, a detailed examination of metabolic capacities within individual communities suggests that there is little redundancy for specific deep-sea niches between microbial populations. Overall, we are generating a more comprehensive genomic catalog of the ocean floor, which will provide a framework for resolving the biology of these communities.

2. Prediction and validation of host:pathogen protein-protein interactions from an *in vivo* model of infection

Richard C Laughlin¹, Jing Wu², Sara D Lawhon², Kenneth Drake³, L. Garry Adams^{2*}

1- Department of Biological and Health Sciences, Texas A&M University Kingsville, Kingsville, TX

2- Department of Veterinary Pathobiology, Texas A&M University, College Station, TX

3- Serologix LLC, Austin, TX

*- Presenting author

Infectious diseases involve complex and transient interactions between proteins and other molecules of the host and pathogen. Identifying and understanding the role of each of these interactions in disease and immune response is critical to understanding the molecular mechanisms of infection and immune response. To efficiently identify previously unappreciated host-pathogen protein-to-protein interactions (PPI), we have developed an *in silico* systems biology model based on data derived from an *in vivo* infection model. We used a machine learning computational approach to analyze host and pathogen transcriptome and proteome data to identify and prioritize plausible host-pathogen PPIs based on the strength of mechanistic evidence coupled with inference techniques employing prior biological interaction knowledge. From these data, we validated and confirmed predicted PPIs in both *in vitro* cellular model systems and an *in vivo* enteric challenge model in the natural bovine host, supporting the utility of computational discovery approaches in operational clinical or biodefense research initiatives. We describe our target discovery methodology, technical implementation, and experimental results. Our work demonstrates the potential for *in silico* interactome systems biology models to enable systematic identification and prioritization of novel interacting host-pathogen target pairs against pathogens, thus accelerating drug discovery and medical countermeasures research.

Session C: STEM Education, Ballroom 147C

Session Chair: Daiyuan Zhang, PhD and Linnea Fletcher, PhD

Institution: Del Mar College and Austin Community College

1. An observational exercise to prepare microbiology students for the unknown project

Richard C Laughlin

Department of Biological and Health Sciences, Texas A&M University Kingsville

Traditionally structured introductory Microbiology labs often end the semester with an independently preformed unknown identification project. While this project allows the students to put into practice the skills they have learned during the semester, it can also highlight weaknesses in their comprehension and obscure higher order learning tasks, such as analyzing and evaluating. To refocus students prior to the unknown project, they participate in a scientific analysis of colored and scented liquids. The samples are passed out to lab groups in sealed containers and they are asked to identify them based solely on observable properties. Students are asked make a falsifiable statement about the identification of their sample before continuing the investigation by noting other characteristics such as smell. A second round of hypothesis generation occurs before a final discussion and sample identification. This activity has 3 primary goals: 1) provide an overview and perspective on the scientific method, 2) practice analysis and critical evaluation of data, and 3) incorporate new evidence and modify working hypotheses.

2. Expand Undergraduate Opportunities By Creating Your Own Course-based Undergraduate Research Experience

Lee E. Hughes

University of North Texas

Course-based undergraduate research experiences (CUREs) provide opportunities for a group of students to engage in research with a faculty mentor in a classroom laboratory setting. CUREs expand the reach of undergraduate research experience beyond the small numbers of students who can be accommodated in apprentice-based research experiences. Several national programs have been developed to offer CUREs to students across the country, including the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program for isolating and characterizing bacteriophages and the Tiny Earth Network's search for antibiotic-producing microorganisms. While joining an existing project can be a great way to get started with CUREs, it is also possible to develop your own CURE for students that complements your existing research program. At the University of North Texas, we have developed several CURE topics under the course title "Advanced Research in Life Science". These topics include "Developmental Physiology", "Environmental Toxicology", "Plant Biochemistry, Cell, and Molecular Biology", and "Bacteriophage Genetics". In this presentation, I will discuss my experiences teaching the "Bacteriophage Genetics" CURE and provide advice on creating your own CURE based on your research expertise.

3. Undergraduate Research at Four-Year Colleges and Universities

Robert JC (Bob) McLean,

Texas State University, San Marcos TX

Many scientists get their inspiration to pursue a career in research and discovery through exposure to undergraduate research. While traditional coursework and lab exercises can build a

solid academic foundation for underlying concepts, student ingenuity and inquiry processes are best addressed in a discovery approach. Large class enrollments, coupled with institution research responsibilities and other “stuff”, do present a challenge of faculty time and resources. In this presentation, I describe several approaches that have been successful, including one strategy that incorporates undergraduate research as a major component of a senior-level course in microbial ecology. Students involved include undergraduates in biology and microbiology, related fields of chemistry and biochemistry, community college students, and even some precollege students

4. Using authentic research experiences to create bridges between community college and universities

Brandi Kiel Reese

Texas A&M University, Corpus Christi, TX

Participation in undergraduate research is a proven high-impact practice that promotes STEM learning. Opportunities in undergraduate research are available at both two-year colleges and four-year universities, but a lack of connectivity between institutes limits goals to 1) increase the number and diversity of STEM students transferring from 2-year to 4-year institutions, 2) improve students' STEM learning outcomes, and 3) prepare students for careers in tomorrow's STEM workforce. To promote these goals, students at Del Mar College were recruited to participate in a Summer Research Experience at Texas A&M University at Corpus Christi. The SRE used participation in research to promote engaged student learning and served as a bridge between institutes that eases transition from a two-year college to a four-year university. During the internship, the students had the opportunity to learn additional laboratory techniques as well as bioinformatics, skills that are highly marketable and transferrable to the workforce and graduate school. In addition, the students gained valuable professional development skills through weekly development meetings led by faculty mentors and outside guest speakers.

5. A summer SURE program to connect SEA-PHAGE and PARE at Del Mar College

Daisy Zhang

Del Mar College

Del Mar College (DMC) has been revising science education with authentic discovery-based research experiences by embedding authentic research (HHMI SEA-PHAGES and PARE) into core bioscience courses. The curricular reform has also followed the Vision and Change initiatives by focusing on the core concepts and competencies rather than memorizing extensive course content. The two discovery-based research programs into the bioscience classrooms are Assessing the Prevalence of Antibiotic-Resistance in the Environment (PARE) and Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES). Students gained experience on novel bacteriophage isolation and antibiotic

resistance bacteria study. During the last three summers, a new summer undergraduate research program (SURE) is developed based on our experience working with both PARE and SEA-PHAGE. During the summer research internship, students who took PARE course can use their discovered antibiotic resistant bacteria strain to isolate a bacteriophage, followed by the phage efficacy study and genomic annotation. The assessment data was collected by on-line CURE surveys post-SURE program.