# Abstracts

# Texas ASM Branch Fall Meeting 2021

Hosted by Texas State University, San Marcos TX 78666

## Keynote Lecture:

**Esther Babady** Ph.D., D (ABMM), FIDSA, F(AAM) – Director of the Clinical Microbiology Service, Memorial Sloan Kettering Cancer Center, New York City

Title: **COVID-19 Testing: Innovation in the Midst of a Pandemic**

Abstract: This presentation will discuss the evolution of molecular diagnostics for SARS-CoV-2 testing at a cancer hospital from the beginning of the pandemic to current state. In addition, it will highlight how clinical laboratories constantly innovated to manage, increase and maintain accurate diagnostic and variants surveillance testing for SARS-CoV-2 throughout the pandemic.

## 1A – New Frontiers in Microbiology session

**Nicholas Dillon** Ph.D., Dept. of Biological Sciences, The University of Texas at Dallas

Title: **Bicarbonate modulates antibiotic activities**

Abstract: Accurate prediction of antibiotic susceptibility *in vitro* is a cornerstone of modern infectious disease care. However, current antimicrobial susceptibility testing often fails to accurately estimate the efficacy of antibiotics in patients as the standardized bacteriologic medium used is not reflective of host conditions. Antimicrobial susceptibility testing in a more physiologically relevant medium has previously permitted the discovery of antibiotics with unrealized activity against MDR pathogens, highlighting the essentiality in incorporating host conditions into the testing paradigm. Expanding upon these findings we have sought to identify antibiotics impacted by susceptibility testing conditions and define the mechanistic basis for their altered activities. We have uncovered differential responses to more physiologically relevant media spanning within and between antibiotic classes. While some classes are not affected, macrolides, tetracyclines, and fluoroquinolones are impacted both positively and surprisingly, negatively. Contrasting the composition of the two media led to the identification of the key component dictating the observed differential antibiotic activities, the biological buffer bicarbonate. These studies showcase the importance of understanding the microenvironmental conditions which impact an antibiotics true clinical efficacy.

**Davida Smyth**, Department of Life Sciences, Texas A&M San Antonio

Title: **Moving beyond SARS-CoV-2 surveillance: How can Next-Gen Sequencing support wastewater-based epidemiology?**

Since the emergence of the COVID-19 pandemic, there has been an unprecedented interest in identifying ways to monitor and measure SARS-CoV-2 viral transmission. Many groups have focused on developing wastewater-based surveillance methods. By targeting wastewater, large populations of individuals can be monitored at different levels of scale, in a passive, non-invasive, and affordable way. By gathering information about fecal shedding of the virus in all persons, including both symptomatic and asymptomatic individuals, viral trends in wastewater can be compared to trends observed from

sequenced clinical specimens. The combination of clinical and wastewater-based sequencing can reveal the virus's response to treatment, drugs, and the vaccine in large populations.

Wastewater epidemiology for SARS-CoV-2 has two aims: (1) to monitor the abundance of the virus in wastewater samples over time, and (2) to determine the relative amounts of mutations associated with variants of concern (VOC) that are of interest to healthcare professionals. This presentation will focus on efforts to perform Next-Gen Sequencing (NGS) of the SARS-CoV-2 virus from wastewater using both targeted and whole genome amplification approaches. Our team leveraged our prior experiences and knowledge to overcome challenges associated with varying viral concentrations as well as viral genome fragmentation (degradation) that are characteristic of wastewater samples to perform NGS and analysis successfully. Finally, other challenges and barriers to viral NGS wastewater analysis will be discussed in relation to potential future pandemics.

**Elizabeth Skellam**, PhD Department of Chemistry, BioDiscovery Institute, University of North Texas, Denton TX

Title: **Fantastic fungi, complex chemistry, and exciting enzymes**

Abstract: Microorganisms are prolific producers of secondary (specialized) metabolites that are not thought to be essential for life but offer a competitive advantage to the producing organism by acting as virulence factors, signaling molecules, or specifically inhibiting the growth of other organisms. These biologically active molecules are also a significant source of pharmaceuticals, agrochemicals, and other chemicals used commercially. Here, I will briefly introduce some examples of biosynthetic gene clusters we have identified and investigated in fungi and the unexpected enzymes and engineering examples we have discovered along the way.

## 1B – Infectious Diseases session

**Julian Hurdle**
Associate Professor, Institute of Bioscience and technology, TAMU, Houston , TX, USA

**Title**: Mechanisms and impact of antibiotic resistance in Clostridioides difficile.

**Abstract**

Evolution by *Clostridioides difficile* to resist killing by the antibiotic therapies vancomycin and metronidazole, is increasingly recognized as an underlying cause of reduced treatment success. However, there remain considerable knowledge gaps on the associated mechanisms of resistance. This seminar will discuss how *C. difficile* evolved resistance to CDI therapies, focusing on metronidazole that is no longer recommended by IDSA/SHEA as a first-line drug. Furthermore, it will be described how *C. difficile* co-opted the cofactor heme to resist killing by metronidazole and how this mechanism influenced the global spread of epidemic strains. The clinical significance of reduced susceptibility to metronidazole will also be discussed, based on patient cohorts from the Texas Medical Center.

**Jennifer K. Spinler**

**Title:** Whole genome sequencing of SARS-CoV-2 viral genome variants in the pediatric population

Jennifer K. Spinler[1,2,3], Jessica Runge[1,2,3], Sabeen Raza[1,2,3], Alamelu Venkatachalam[1,2,3], James Dunn[1,3], Ila Singh[1,3], James Versalovic[1,2,3], Sridevi Devaraj[1,2,3], Ruth Ann Luna[1,2,3]
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**Abstract :**
The coronavirus disease 2019 (COVID-19) pandemic is the first time in history scientists across the globe united through technology to freely share timely genomic sequence data that informed public health decision making. Global sequencing efforts have produced and made publicly available over 4 million severe acute respiratory syndrome virus 2 (SARS-CoV-2) whole-genome sequences. SARS-CoV-2 has undergone sustained rapid genetic evolution captured by near-to-real-time global genomic epidemiology enabling the detection of genomic variants that not only impact the clinical course of COVID-19 infection, but also the practical aspect of SARS-CoV-2 diagnostics and therapeutics. Our Texas Children's Microbiome Center (TCMC) moved rapidly to efficiently adapt and deploy whole genome sequencing-based SARS-CoV-2 variant identification for assessing the impact of SARS-CoV-2 in children at Texas Children's Hospital (TCH). As the largest children's hospital in the world located in the largest global medical complex (Texas Medical Center), TCH receives over 10 million unique patient visits per year. Since the beginning of the pandemic, TCH has diagnosed >28,000 pediatric patients with COVID-19 and treated >2,100 of these patients in hospital. The TCMC has successfully sequenced >1,400 SARS-CoV-2 positive specimens from ~1,300 patients for variant tracking. Live genomic surveillance of SARS-CoV-2 at TCH has provided valuable data to inform patient care practices and infection control while concurrently accumulating a valuable data set for assessing the impact of SARS-CoV-2 variants on disease severity in children.

**Jayhun Lee.**
Assistant Professor, Department of Microbiology and Molecular Genetics, McGovern Medical School at UTHealth (Houston, TX)

**Title**
The esophageal gland mediates host immune evasion by the human parasite *Schistosoma mansoni*

**Abstract**
Schistosomes are parasitic flatworms that cause schistosomiasis, a major neglected tropical disease that affects over 200 million people globally. Their complex life cycle involves multiple body plans as they switch between asexual (in a molluscan host) and sexual (in a mammalian host) reproduction. As such, their successful propagation requires stem cells to undergo frequent transitions in their cycling and differentiation during parasite homeostasis and reproduction. As infectious larvae (cercariae) arise from snails, a handful of stem cells packed inside the larval body serve as a likely origin for intra-mammalian parasitic development. However, how these early stem cells contribute to organogenesis remains unknown. Surprisingly, we found that the esophageal gland, an anterior accessory organ of the digestive tract, develops before the rest of the digestive system, and prior to blood feeding, suggesting that it may play a role in processes beyond nutrient uptake. To investigate the function of the esophageal gland, we characterized *Sm-foxA (foxA)*, a gene encoding a forkhead-box transcription factor, that is highly enriched in the esophageal gland. Knockdown of *foxA* completely blocked development and maintenance of the gland, without affecting other somatic tissues, as well as parasite viability, reproduction, and behavior *in vitro*. Intriguingly, schistosomes lacking the gland died after

transplantation into naïve mice, while they were able to survive in immunodeficient mice lacking B-cells. Furthermore, feeding of GFP-expressing immune cells revealed that the gland-lacking parasites fail to lyse ingested immune cells within the esophagus before passing them into the gut. Finally, comparative transcriptomic analysis followed by preliminary RNAi screening reveal several esophageal gland proteins that are important for proper degradation of ingested host immune cells. Together, our results unveil a novel immune-evasion mechanism mediated by the esophageal gland, which is essential for parasite survival and pathogenesis.

**David Greenberg**
Professor, Department of Internal Medicine – Infectious Disease, UTSW-Dallas-TX.
**Title:** Developing Pseudomonas-Specific Antibiotics Utilizing Antisense

**Abstract:**
Gram-negative pathogens are becoming increasingly resistant to many antimicrobials. This can be especially problematic in patients who suffer from chronic infections or are immunocompromised. Multidrug-resistant *Pseudomonas aeruginosa* has been identified by the Centers for Disease Control and Prevention as a serious threat. *P. aeruginosa* causes healthcare associated infections in a variety of clinical settings and hosts, but is particularly devastating in patients with cystic fibrosis (CF). We have been interested in using antisense molecules called PPMOs as potential therapeutics in these infections. These molecules block messenger RNA and prevent the formation of the target protein. Targeting the essential genes *acpP*, *lpxC* and *rpsJ* with PPMOs are active both *in vitro* and *in vivo*. We will discuss taking a pathogen-specific drug development approach for treating Pseudomonas infections.

## 2A – Graduate Student – Medical Microbiology session

G1. **Ileana Corsi**, Soumita Dutta, Ambro van Hoof, Theresa M. Koehler. UT-Health-Houston "AtxA-Controlled Small RNAs of *Bacillus anthracis* Virulence Plasmid pXO1 Regulate Gene Expression in trans"

Abstract: Small regulatory RNAs (sRNAs) are short transcripts that base-pair to mRNA targets or interact with regulatory proteins. sRNA function has been studied extensively in Gram-negative bacteria; comparatively less is known about sRNAs in Firmicutes. Here we investigate two sRNAs encoded by virulence plasmid pXO1 of Bacillus anthracis, the causative agent of anthrax. The sRNAs, named "XrrA and XrrB" (for p¬XO1-encoded regulatory RNA) are abundant and highly stable primary transcripts, whose expression is dependent upon AtxA, the master virulence regulator of B. anthracis. sRNA levels are highest during culture conditions that promote AtxA expression and activity, and sRNA levels are unaltered in Hfq RNA chaperone null-mutants. Comparison of the transcriptome of a virulent Ames-derived strain to the transcriptome of isogenic sRNA-null mutants revealed multiple 4.0- to >100-fold differences in gene expression. Most regulatory effects were associated with XrrA, although regulation of some transcripts suggests functional overlap between the XrrA and XrrB. In silico analysis revealed complementarity between XrrA and the 5' UTR of seven mRNA regulatory targets, suggesting base-pairing interactions. A translational fusion of one of these targets, the secreted metalloprotease InhA1, to GFP suggests XrrA-mediated regulation of protease translation. Finally, in a mouse model for systemic anthrax, the lungs and livers of animals infected with xrrA-null mutants had a small reduction in bacterial burden, suggesting a role for XrrA in B. anthracis pathogenesis. Future work will focus on the molecular basis for sRNA function, including investigations of potential RNA and/or protein interacting partners of XrrA and XrrB.

G.2 **James McLellan**, William Sausman, Kirsten K. Hanson. UT-San Antonio "Single-cell quantitative bioimaging of *Plasmodium berghei* liver stage translation"

Antimalarial drug resistance poses a critical threat to the treatment and prevention of Plasmodium infections, highlighting the need for new multistage drugs and drug targets, along with assays to speed their identification. Plasmodium growth and maturation is causally dependent on protein synthesis, a complex cellular process essential throughout the lifecycle, which has an abundance of potential drug targets. To identify compounds capable of inhibiting protein synthesis, agnostic to target type or identity, we developed an assay visualizing and quantifying translation in single Plasmodium berghei liver stages during intracellular growth in human hepatoma cells. Incubation with a functionalized puromycin analog, followed by fluorescence-based click chemistry and automated microscopy allows the visualization and quantification of the nascent proteome in both host and parasite simultaneously. This approach allows us to document changes in translation intensity throughout liver stage development, and in response to addition of known pan-eukaryotic and Plasmodium-specific translation inhibitors, demonstrating that parasite translation status is not necessarily coupled with that of its host cell. Flexible pre-treatment and competition assay modalities allow discrimination between direct and indirect inhibitors of parasite protein synthesis. Concentration-response data were generated for a select set of compounds, with differences in translation inhibition efficacy, as well as potency, seen for compounds with known mechanisms of action. This approach, harnessing quantitative bioimaging to assay protein synthesis in single parasites during development in translating host cells, may prove useful for addressing core biological questions, as well as drug discovery, in a range of intracellular pathogens and infection systems.

G.3 **Donghoon (Alex) Kang**, Alexey V. Revtovich, Carolyn L. Cannon, Natalia V. Kirienko. Rice University "5-Fluorocytosine Synergizes with Gallium Nitrate to Inhibit *Pseudomonas aeruginosa*"

Pseudomonas aeruginosa is a multidrug-resistant pathogen that causes life-threatening infections in immunocompromised patients. One key virulence factor in this pathogen is the siderophore pyoverdine, which not only provides the bacterium with iron, but also regulates the production of several secreted toxins. We recently demonstrated that pyoverdine can also directly exert virulence against Caenorhabditis elegans and murine macrophages by translocating into host cells, disrupting iron and mitochondrial homeostasis. Due to a combination of these functions, pyoverdine production is necessary for P. aeruginosa virulence during murine lung infection and is a clinically important target for new drug development.
One such therapeutic is the biosynthetic inhibitor 5-fluorocytosine (5-FC). We demonstrated that 5-FC attenuates pyoverdine-dependent virulence against a variety of hosts without overtly affecting bacterial titer, consistent with an antivirulent mechanism of action. However, 5-FC also synergizes with the antipseudomonal agent gallium nitrate to inhibit bacterial growth. This is likely due to pyoverdine's ability to sequester the metal, preventing it from reaching cytoplasmic targets. Interestingly, even in the presence of gallium, 5-FC largely functioned as an antivirulent. Spontaneously resistant mutants that emerged in the presence of both drugs were resistant to gallium but remained sensitive to 5-FC, carrying mutations in the HitAB ferric iron transporter as well as a putative ATP-binding protein in this system. We expect these drug-adapted populations to remain less virulent during Ga/5-FC treatment due to pyoverdine inhibition. These results suggest that Ga/5-FC may be a promising drug combination to target both pathogen virulence and growth during multidrug-resistant pseudomonal infections.

G.4 **Jordan Wolfkill**, David Silva, Hailey R. Wallgren, Boris Ermolinsky, Jeffrey W. Turner, Daniele Provenzano. UT-Rio Grande Valley and Texas A&M Corpus Christi. "Expression of cold shock

transcriptional regulator CaspA and the putative virulence-associated protein VacB/RNaseR restores growth at low temperature in pandemic O3:K6 serotype *Vibrio parahaemolyticus*"

Vibrio parahaemolyticus is the leading cause of seafood borne gastritis worldwide, partly resulting from clonal expansion of the highly pathogenic O3:K6 serotype. Genomic analysis of environmental V. parahaemolyticus 03:K6 strains isolated from the Pacific Northwest (PNW) led to the discovery of a novel, predominant ecotype harboring a deletion of a genetic locus spanning from VP1884 through VP1890, found previously to be transcriptionally upregulated upon bacterial culture at cold temperatures. VP1889 is annotated as cold shock transcriptional regulator CspA, and VP1890 as a putative virulence-associated protein VacB/RNaseR. We deleted the VP1884-VP1890 locus from clinical O3:K6 laboratory strain BAA-239 and complemented cspA and vacB/rnaseR with a plasmid vector to understand the dominance of the O4:K6 serotype V. parahaemolyticus as the primary clinical serovar causing vibriosis in the PNW over the last several decades. Allelic exchange with the lacZ reporter gene from V. cholerae and the tetR selection marker from pBR332 was employed to generate a DVP1884-VP1890 strain to test the hypothesis that cspA and vacB/rnaseR affect growth kinetics at cold temperatures. The ΔVP1884-ΔVP1890::lacZ/tetR strain displays no growth defect at 30°C but does at 10°C. The growth defect of the ΔVP1884-ΔVP1890::lacZ/tetR strain is partially complemented by cspA alone and; interestingly, over complemented by expression of both cspA and vacB/rnaseR. These results suggest that the VP1884-VP1890 genetic locus codes two genes essential for growth at temperatures standardized for shellfish storage. This may be contributing to the unusual pattern of dominance by the O4:K12 serotype V. parahaemolyticus as the principal clinical strain in the PNW.

G5. **Megan Burch** and Jeremy Bechelli. Sam Houston State University "Development of a SYBR Green-Based RT-qPCR for the Detection and Quantification of Lone Star Virus"

Lone Star virus (LSV) is a newly characterized tick-borne bandavirus with pathogenic potential as indicated by infection and cytopathic effect in human and non-human primate cell cultures. However, there are no detection methods available to identify and monitor LSV in vitro. Here we describe the development of a SYBR green-based RT-qPCR assay for the detection and quantification of LSV. Primers were developed for the M segment of the tri-segmented genome and were initially tested for amplicon formation and non-specific binding. Portions of the LSV genome were cloned into a plasmid and propagated in competent Escherichia coli to obtain the template for a standard curve. Amplicon formation of the developed primers indicated that a single product was formed of the expected size of 152 base pair with a consistent melting temperature (Tm) of 82°C. The limit of detection for the assay was less than 10 copies/µl of the viral genome. Specificity testing revealed slight cross-reactivity with four related viruses (Heartland virus, La Cross virus, Jamestown virus, Crimean-Congo Hemorrhagic Fever virus); however, the Tm for the related viruses was either below the threshold or dissimilar to the previously indicated Tm for LSV. Standard curve analysis showed the efficiency of the primers was 96.3%-102% with an R2 value of 0.992-0.996 and a slope of 3.276-3.363. For reproducibility analysis, the interassay coefficient of variation (CV) was 0.471%-1.108%, and the intra-assay CV was 0.110%-4.203%. This data suggests that the SYBR green-based RT-qPCR assay for the M segment of LSV is highly sensitive, specific, and reproducible.

G6. **Chahat Upreti** and Kelli Palmer, UT-Dallas "The clinic vs the farm: exploring differences in genomic attributes of *Enterococcus faecalis* sourced from human and animal niches"

Global efforts to combat antibiotic resistance stress the need for an approach that includes human, animal, and environmental perspectives. Based on its role as an adaptive defense mechanism against

horizontal gene transfer in bacteria, CRISPR-Cas is a possible tool to address this crisis. However, most studies in this area primarily study clinically-derived strains. As a result, the prevalence of CRISPR-Cas in bacteria from agricultural sources and its efficacy in those environments is poorly understood. Here, we perform a comprehensive analysis of 1,985 publicly available Enterococcus faecalis genomes for CRISPR-Cas diversity, antibiotic resistance profile and genome size, based on source of isolation (human vs animal). We found that (a) E. faecalis genomes from human sources were significantly larger than those from animal ($p<0.0001$), (b) genomes from both sources showed similar trends in CRISPR-Cas type: CRISPR1-Cas (25.7% in human vs 25.6% in animal), CRISPR3-Cas (6.7% vs 8.3%) and (c) tet(M) and erm(B) were the most common antimicrobial resistance genes in both groups. Examination of CRISPR spacer profiles from these different environments revealed reduced redundancy and higher target diversity in spacers from animal-sourced E. faecalis as compared to their human counterpart. Among divergent viral targets we saw stark contrast between the two groups, with unique spacers from animal-sourced E. faecalis targeting staphylococcal phages while the human-sourced spacers primarily targeting streptococcal phages. Together, our work shows for the first time a comprehensive picture of agriculturally derived E. faecalis genomic landscape in contrast with its clinical counterpart, making a case for its further targeted exploration.

## 2B Graduate Student – General Microbiology session

G7. **Trusha Parekh**, Sneha Narvekar, Stephen Spiro. UT-Dallas "New insights into the regulation of methylotrophic metabolism in *Paracoccus denitrificans*"

Background: Paracoccus denitrificans uses methanol or methylamine as sources of energy generating formaldehyde in the periplasm. Formaldehyde is oxidized to formate in a glutathione-dependent pathway. Expression of the genes required for C1 metabolism is regulated by a sensor-regulator pair, FlhSR. Co-expressed with FlhSR is a previously uncharacterized FIST-domain protein tentatively named FlhT.
Methods: Single in-frame deletion mutations were constructed in flhR, flhS and flhT. Growth on methanol and methylamine was measured; and compared to growth on succinate and choline (which generates formaldehyde in the cytoplasm). Complementation tests were performed with the corresponding genes expressed from a plasmid and promoter activities were assayed using promoter-lacZ reporter fusions.
Results: We have confirmed previous reports that both FlhS and FlhR are required for growth on methylamine or methanol and have now shown that FlhT is also required. In agreement with previous studies, we found that flhS and flhR mutants cannot utilize choline on solid media. However, we find that some growth is possible in liquid media, in a pattern that is consistent with the accumulation of a toxic intermediate (likely to be formaldehyde). Interestingly, the flhT mutant grows normally on choline (in contrast to its phenotype on C1 substrates).
Conclusion: We hypothesize that FlhT interacts directly with FlhS and serves as the signal sensing component of the FlhSR signal transduction pathway, with formaldehyde the likely signal. Interaction of FlhT with formaldehyde would be a novel role for a FIST domain protein and would add new complexity to the regulatory circuit controlling C1 metabolism.

G8. **Samuel Tye**, Desiree Moore, Katherine Bockrath, Camila Carlos-Shanley. Texas State "Impact of Captivity on the Microbiome of the endangered Comal Springs Riffle Beetle, *Heterelmis comalensis*"

The Comal Springs riffle beetle, Heterelmis comalensis, is an endangered aquatic beetle which has been difficult to reproduce in captivity. Since, the gut microbiome of insects plays a significant role in their

lifecycle, characterizing the gut microbiome of H. comalensis would help explain the difficulty rearing them. In this study, two distinct hypotheses are being tested to elucidate the link between their microbiome and their difficulty reproducing in refugia. First, I hypothesize that the microbiome is significantly different between wild and captive larval and adult H. comalensis. Shotgun metagenomic sequencing was used to determine the gut microbiome composition in captivity at two separate facilities, and wild H. comalensis larvae and adults. This identifies differences between juvenile and adult organisms from the wild and refugia where they are being studied, preserved, and rehabilitated. Differences were not only observed between wild and captive organisms, but also compared between captive organisms at both refugia. I also hypothesized that Staphylococcal contamination from human researchers could impact the larval microbiome and affect their lifecycle. To test this, captive larvae were raised in three groups: a non-inoculated control, inoculated with Bacillus, and with Staphylococcus isolated from captive beetles. Observed mortality during the Staphylococcus exposure experiment was: 33% from the Staphylococcus group, 13% from the Bacillus group, and 73% from the non-inoculated group. Combining shotgun metagenomic sequencing and experimental manipulation of the microbiome will help elucidate the role of microbiome in H. comalensis health and will provide insight into why these organisms have trouble pupating in refugia.

G9. **Caroline Black**, Catherine Wakeman, Ph.D., Allie Clinton Smith, Ph.D., M(ASCP)CM
Texas Tech "Polymicrobial Communities in Chronic Wounds Impact Antibiotic Susceptibilities"

Recent advances in sequencing technologies have demonstrated that many chronic infections are polymicrobial in nature. In polymicrobial communities, multiple species interact and can synergize activities, leading to decreased antibiotic efficacy and worse patient outcomes. Despite this knowledge, hospital laboratories assess antimicrobial susceptibility based on monomicrobial suspensions. This project investigates the role a polymicrobial community plays on shifts to antimicrobial efficacy using four clinically relevant wound pathogens (Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Enterococcus faecalis). Monomicrobial versus polymicrobial assessment demonstrated impacts to antibiotic efficacy; both increases and decreases in sensitivity were observed depending on the condition. This demonstrates that current clinical methods which focus on determining the monomicrobial causative agent of disease for determining antibiotic susceptibility may not fully represent the clinical environment. Acknowledging a microorganism's role in its community is crucial to more effectively treating persistent infections and improving patient outcomes.

G10. **Feroz Ahmed**, Katie N Kang, Jacob Gray, Waldemar Vollmer, Joseph M Boll
UT-Arlington "Characterizing a role for outer membrane lipoproteins in remodeling the cell envelope of *Acinetobacter baumannii*"

The lipid A precursor of LPS/LOS was canonically thought to be essential for Gram-negative survival. However, Acinetobacter baumannii (Ab) inactivates lipid A biosynthesis to gain resistance to the last line antimicrobial, colistin. We do not have a comprehensive understanding of how Ab survives without LOS (LOS-) to develop colistin resistance but found that lipoproteins are enriched in the LOS- outer membrane (OM). Using transposon mutagenesis, we determined that a putative LD-transpeptidase, LdtK, is essential for LOS- Ab fitness. We also found that LdtK catalyzes OM lipoprotein attachment to peptidoglycan. Proteomic analysis of peptidoglycan attached proteins showed two putative lipoproteins (denoted as Lpp1 & Lpp2) were attached to peptidoglycan in wild type but not Dldtk. Notably, both lipoproteins encode C-terminal lysine residues, which are attachment sites for LD-transpeptidase-dependent covalent attachment of lipoproteins to meso-diaminopimelic acid in peptidoglycan stem peptides. Physically tethering the OM to peptidoglycan via lipoproteins likely stabilizes the cell envelope

when LOS is not produced. Analysis of Dlpp1 and Dlpp2 increased production of OM vesicles relative to wild type, which supports a model where lipoprotein attachment fortifies the OM barrier in a LdtK-dependent manner. Transcriptomics analysis suggests that lpp1 and lpp2 are regulated by the BfmRS two-component system, which senses cell envelope stress and induces protective mechanisms. We found that Lpp1 is expressed in growth and stationary phase, while Lpp2 is only expressed in stasis, suggesting separate roles in OM assembly. Together, our studies show that in response to OM defects, Ab lipoproteins increase cell envelope stability.

G11. **Paxton T. Bachand**, Lee J. Pinnell, Nicole C. Powers, Sara Tominack, Kenneth C. Hayes Michael S. Wetz, Jeffrey W. Turner, Texas A&M-Corpus Christi "Analysis of a Brown Tide Harmful Algal Bloom Microbiome"

Aureoumbra lagunensis algae blooms (a.k.a., brown tides) pose a serious threat to environmental health through disrupting ecosystem processes. Specifically, blooms impact seagrass and fisheries' habitat through sunlight attenuation and dissolved oxygen depletion. In the Laguna Madre, where brown tides occur near annually, studies have described how physical parameters (e.g., temperature, salinity) and nutrients (e.g., nitrogen, phosphorous) affect bloom dynamics; however, the microbiological factors affecting bloom dynamics are largely unknown. In this study, the role of microbes was assessed through the completion of three objectives: 1) identifying microbial communities (16S rRNA gene sequencing) associated with bloom and non-bloom study sites, 2) identifying enriched or uniquely associated bloom taxa, and 3) correlating changes in environmental parameters with variations in the bloom-associated microbial community. These objectives were completed by sampling a brown tide bloom (N = 6 sampling events) in a hypersaline creek of a reverse estuary. Samples of bloom and non-bloom water were taken in duplicate for six weeks, where the bloom site's A. lagunensis cell counts were greater than 800,000 cells per mL. Bloom formation and maintenance were strongly correlated with salinity (28.4 - 64.8 ppt). Forty-two bacterial and three archaeal phyla were identified, representing over 1,500 potential species. Bacteroidia (17.9%), Gammaproteobacteria (16.5%), and Alphaproteobacteria (12.9%) were the most abundant classes comprising bloom samples. Importantly, the HAB community was enriched with environmentally significant taxa (e.g., Rhodobacterales, Oxyphotobacteria, Planctomycetes). We hypothesize location, salinity, and organic nutrients were responsible for community patterns, as Principle Component Analysis (PCoA) samples clustered based upon these variables.

G12. **Meaghan Rose**, Tien Doan and Woo-Suk Chang. UT-Arlington "Characterization of a *Bacillus megaterium* strain isolated from *Aeschynomene indica* stem nodules: Nod-factor independent nodulation"

The formation of root nodules on leguminous plants by nitrogen fixing bacteria, known as rhizobia, is an important symbiotic mechanism that has been utilized for nutrient cycling in agricultural systems. The most well studied model of these relationships involves soybean crops and their Bradyrhizobium symbionts. The current understanding of this symbiosis dictates that the bacterial symbiont must produce signal molecules known as "Nod-factors" to facilitate the invasion and infection of the host plant's root cortical tissues. However, a few cases involving the legume Aeschynomene indica and their bacterial symbionts demonstrate an ability to form nodules independently of Nod factors. To better understand this unique Nod Factor-independent nodulation system, we isolated bacterial symbionts from both stem and root nodules of Texas native A. indica plants. Of the seven unique isolates obtained, one in particular demonstrates the most consistent nodulation ability despite lacking Nod Factor-related genes. This isolate, tentatively identified as Bacillus megaterium via 16S rRNA sequencing, has undergone plant growth chamber experiments as well as molecular analysis to observe and characterize

its nodulation ability. This unique nodulation mechanism is not yet fully understood but has the potential to open the door for the induction of nodules and nitrogen fixation across genera of both plants and bacteria, specifically non-legume crop plants such as cotton, corn, wheat, and rice and their symbiotic bacteria.

## 2C Undergraduate Students Oral Session

U1. **Tony Jha**, Jovinna Mendel, Hyuk Cho, Madhusudan Choudhary. Sam Houston State University and Cal Berkeley, "Prediction of Bacterial sRNAs using Sequence-Derived Features and Machine Learning"

Small RNAs (sRNAs) are 50-500 nucleotide long, noncoding RNA sequences that play an important role in regulating transcription and translation within a bacterial cell. As such, it is important to identify sRNA sequences within an organism's genome to understand the impact of these RNA molecules on cellular processes. Recent bioinformatic approaches using machine learning models have been applied to predict sRNAs within bacterial genomes. In this study, we investigate the importance of features and datasets with individual, group, and combined features of sRNAs. Specifically, we make use of the sRNA sequence features previously observed in Barman et. al.1, along with additional sequence-derived features tested by Tang et. al.2 to identify a compiled, robust feature set for detecting novel sRNAs. Furthermore, we employ six classification (i.e., supervised learning) algorithms and measure the performance in terms of Accuracy and Area Under Curve (AUC) when applied to the Salmonella typhimurium LT2 and Escherichia coli K12 genomes. In summary, we determined that AdaBoost and XGBoost paired with a larger feature set produce more accurate and consistent results. Both algorithms were able to utilize the characteristics latent in the combined features of sRNAs. As future work, we plan to extend the current approach to other existing sRNA sequence datasets as well as employ deep learning algorithms.

U2. **Filemon Tan**, Liyang Zhang, Natasha Kirienko. Rice University "Competitive Interactions between *oprD*-mutant and Wild-type *P. aeruginosa*"

Mutation of the OprD porin protein in the Gram-negative pathogen Pseudomonas aeruginosa results in carbapenem resistance. Commonly, antimicrobial resistance is energetically costly, causing fitness disadvantage in absence of antibiotic. However, several recent publications indicated that oprD mutants have fitness benefits compared to wild-type bacteria. This project aimed to investigated four hospital-isolated strains of oprD-mutant Pseudomonas in vitro and in a Caenorhabditis elegans model. In a series of colony forming unit experiments, this study observed that three oprD strains had decreased ability to colonize C. elegans compared to PA14 (wt oprD control), yet two of those three strains could still out-compete PA14 if mixed with it. Importantly, oprD mutant strains had less virulence that PA14, and infection with the mixed bacteria resulted in significantly increased survival of C. elegans, compared to PA14 alone. This study then proceeded to investigate a potential mechanism for PA14 inhibition by measuring PA14 growth in the presence of cell-free supernatant of oprD-mutant cultures, or in the presence of live bacteria. Data indicated that different oprD mutants may have different strategies to outcompete wild-type bacteria.

U3. **Christian Miller**, Alyssa Russel, Jeremy Bechelli. Sam Houston State University "Inhibition of autophagy in human microvascular endothelial cells during Colorado tick fever virus infection"

Colorado tick fever virus (CTFV) is the responsible agent for the acute febrile illness Colorado tick fever (CTF) can manifest mild to severe symptoms in humans including meningitis, encephalitis, and bleeding disorders. Despite the clinical significance, the understanding of mechanisms that induce CTFV pathology remains largely unknown. Transcriptomics analysis of CTFV infected human microvascular endothelial cells (HMEC-1's) showed increases in total mRNA expression of autophagy-associated genes including p62/SQSTM1 and BECN1, and downregulation of genes including ULK1 and WIP12, which suggested an interaction between CTFV infection and autophagy in HMEC-1's. Further investigation at the protein level through Western Blot analysis showed a reduction in the ratio of light chain 3 form II (LC3-II) to light chain 3 form 1 (LC3-I) and a significant increase in p62, highlighting an overall decrease in autophagosome formation and inhibition of degradative autophagy. Data also showed increased protein expressions of pAkt (Ser473), mTOR (Ser2448), and pp70S6K (Thr389), which highlighted a potential pathway by which CTFV downregulates autophagy through modulation of Akt/mTOR/p70S6K signaling. Cell viability analysis utilizing 3-MA and chloroquine (CQ), known inhibitors of autophagy, showed a decrease in cell survival during CTFV infection. Furthermore, rapamycin, a known inducer of autophagy, showed an increase in cell viability when compared to infection with autophagy inhibitors as well as infection alone. Collectively, our data suggest that autophagy is downregulated in response to CTFV infection in HMEC-1's to subvert the cellular innate immune response and avoid degradation.

U4. **Sadie Ruesewald**, Christian Peterson, Shannon Perry, Camille Condron, and Woo-Suk Chang UT-Arlington "The effect of a drought-tolerant *Bradyrhizobium* bio-fertilizer on soybean growth in a temperature gradient greenhouse"

The soybean symbiont, Bradyrhizobium japonicum, fixes atmospheric nitrogen (N2) into a more usable form such as ammonia (NH3) in specialized organs called root nodules. B. japonicum cultures have been applied as inoculants (i.e., bio-fertilizers) to soybean fields and its applications have been shown to be beneficial to soil health, plant growth, and final yield. However, heat and drought as part of global warming, present a huge impediment to this application due to inhibition of nitrogen fixation by killing off the symbiont. Our lab has developed a novel molecular marker system to test intrinsic drought resistance leading to the isolation of a Texas-native drought-tolerant strain, B. japonicum sp. TXVA. In this study, our lab collaborated with the USDA at North Carolina to examine effects of the TXVA strain on soil health and plant growth under the environmental conditions of ambient temperature, ambient +2°C, and ambient +4°C. Parameters examined include soil health via physiochemical testing at planting, R2 stage, and harvest as well as assessment of plant vitality by measuring height, dry plant weight, nodule size, number and weight, total leaf area, and final yield. We hypothesize that application of the TXVA strain will effectively induce symbiotic nitrogen fixation in spite of temperature increases. In addition, we expect that this bio-fertilizer will induce positive microbial community interactions that manifest in final yield.

U5. **Saoirse Disney-McKeethen**, Seokju Seo, Heer Mehta, Yousif Shamoo. Rice University "Evolution of *P. aeruginosa* to colistin in a microfluidic emulsion reveals distinct evolutionary trajectories from those observed in traditional batch environments"

Antibiotic resistance is a global health crisis. Colistin is often the last resort antibiotic used to treat infections of P. aeruginosa, a gram negative pathogen that is the leading cause of death in patients with CF, but resistance has already been observed in clinical settings. Understanding mechanisms of resistance could allow for the design of novel therapies or new antimicrobial strategies to combat resistance. In vitro experimental evolution typically evolves resistance to an antibiotic by passaging cells in bulk cultures, leading to loss of less fit, low frequency mutants that can still provide a successful

evolutionary trajectory. Additionally, batch methods do not offer a large degree of control over parameters such as starting population size, spatial separation and total number of doublings. Microfluidic emulsion is a method with the potential to offer far greater control over environmental parameters, and can be used to select for mutants with characteristics such as slower growth that are disadvantaged in a traditional bulk experiment. Our project aimed to adapt P. aeruginosa to colistin in a microfluidic environment and to compare the mutation types and frequencies observed in droplets with those observed in a traditional bulk flask evolution experiment through longitudinal whole genome analysis of individual populations. We found that while both flask and microemulsion environments recapitulated common resistance mutations found in clinical settings such as inactivation of phoQ and pmr genes, there were significant differences in both the population dynamics and in what evolutionary trajectories of resistance were favored between flask and microemulsion environments.

U6. **Taylor M Ranson**, Marilynn E Barton, Robert JC McLean. Texas State "The Effects of *Escherichia coli* central metabolism mutants on planktonic growth and biofilm formation"

The Keio collection of Escherichia coli contains 3985 deletion mutants derived from an E. coli K12 strain, BW25113. We investigated the impact of three central metabolism deletion mutants including edd (which impacted the Entner-Doudoroff pathway), pgi (which impacted glycolysis), and gnd (which impacted the pentose phosphate pathway). A microtiter plate was used to determine planktonic growth and degree of biofilm formation of the three mutants as well as the wt strain (BW25113) when cultured in three different concentrations of LB broth. Motility tests were also performed. Of the four strains tested, the edd knockout mutant had the highest planktonic growth and biofilm formation in full strength (100%) and half strength (50%) LB. Unexpectedly, the pgi knockout mutant had the greatest motility and highest biofilm formation in the lowest concentration (25% LB) of media. To our knowledge, this is the first investigation of the impact of central metabolism mutants on Escherichia coli biofilm formation.

## 3A – Postdoctoral Presentations

PD1. **Renee Fleeman**, Luis Macias, Jennifer Brodbelt, Bryan Davies. – UT Austin "Refining our understanding of antimicrobial peptide mediated disruption of the *Klebsiella pneumoniae* capsule"

Multi-drug resistant Klebsiella pneumoniae bacterial infections are a major threat to human health as mortality rates are steadily on the rise. One of the defining characteristics of K. pneumoniae is a robust polysaccharide capsule that aids in resistance to the human immune system. There have been few efforts to determine how to bypass this barrier that protects K. pneumoniae. To this end we have discovered a novel mechanism of peptide disruption of the capsule barrier of K. pneumoniae. Characterizing the interactions of our synthetic alpha helical peptide using circular dichroism and native mass spectrometry we revealed loss of peptide structure is associated with the formation of peptide:polysaccharide aggregates. We found the peptide aggregation with capsular polysaccharide is accompanied by removal of the capsule layer surrounding K. pneumoniae. Analog analysis led to the discovery that although cationic charge plays a large role in these interactions, tryptophan residues can increase the aggregation abilities of this synthetic peptide. Further work revealed that native host defense peptides also aggregate with K. pneumoniae capsule. In particular, the polyproline helical peptide Bac7 (1-35) can interact with both capsule and biofilm polysaccharides. The interaction of Bac7 (1-35) with biofilm polysaccharides weakens a preformed biofilm of hypervirulent K. pneumoniae. These findings reveal host defense peptide binding of extracellular polysaccharides may be a mechanism to protect against K. pneumoniae infections rather than a route to their inactivation.

PD2. **Kristen Engevik** 1, Melinda A. Engevik 2, Lori D. Banks 1,3, Alexandra Chang-Graham 1, Jacob L. Perry 1, Joseph F. Petrosino 1,3, Joseph M. Hyser 1,3

1 Department of Molecular Virology & Microbiology, Baylor College of Medicine, Houston, TX;
2 Department of Regenerative Medicine & Cell Biology, Medical University of South Carolina, SC;
3 Alkek Center for Metagenomic & Microbiome Research, Baylor College of Medicine, Houston, TX
– Baylor College of Medicine

"Rotavirus infection causes specific changes in the intestinal microbiome"

Background: When enteric viruses enter the intestine, they encounter a dynamic microbial community. Currently, it is not well understood how the small intestinal microbiota influences rotavirus infection. We hypothesized that certain microbes would influence rotavirus infection.
Methods & Results: To address this question, rotavirus-infected neonatal mice were examined for changes in bacterial community dynamics by 16S rRNA sequencing, host gene expression by qPCR, and histology by immunostaining. 16S sequencing revealed significant and distinct changes in ileal communities in response to rotavirus infection, with no significant changes for other gastrointestinal compartments. Rotavirus significantly decreased ileal Lactobacillus and increased Bacteroides and Akkermansia after one day post-infection. Since Bacteroides and Akkermansia degrade mucus, we performed immunostaining and observed a loss of mucus-filled goblet cells at day 1, with recovery occurring by day 3. No changes were observed in MUC2 mRNA indicating that the mucus was being secreted. Rotavirus infection of mucus-producing cell lines and human intestinal enteroids stimulated release of stored mucus granules, suggesting that rotavirus alone can stimulate mucus release. Mucus glycans can bind rotavirus and serve as a decoy for epithelial glycans. We reasoned that mucin-degradation might alter the protective nature of mucus. Incubation of mucus with Bacteroides or Akkermansia members resulted in significant glycan degradation, which altered the binding capacity of rotavirus in silico and in vitro to MA104 cells.
Conclusions: Taken together, these data suggest that the response to and recovery from rotavirus-diarrhea is unique between sub-compartments of the GI tract and may be influenced by mucus-degrading microbes.

PD3. **Starla G Thornhill** 1,2, Jiseon Yang 3, Jennifer Barrila 3, Sandhya Gangaraju 3, Richard Davis 3, Cheryl A. Nickerson 3, C. Mark Ott 4, Robert J. C. McLean 1.
1-Texas State University, 2-Texas A&M San Antonio, 3-Arizona State University, 4-NASA-JSC.
"Bacterial adhesion and corrosion in space flight"

The International Space Station is a built environment that has been continuously inhabited since November 2000. Living with the crew are the microorganisms carried to the ISS as normal astronaut flora and by accidental introduction in supplies. These microorganisms have established biofilms in the water recycling system (WRS) that recycles urine and ambient humidity to provide drinking water to the ISS crew. Biofilms in the WRS can serve as a reservoir for opportunistic pathogens, including Escherichia coli and Pseudomonas aeruginosa, and can also induce clogs and corrosion damage on the stainless steel components. To investigate biofilm formation, silver disinfection susceptibility, and microbially induced corrosion in space flight, an experiment was launched to the ISS on SpaceX CRS-21 in December 2020 and returned to Earth on SpaceX CRS-21 and SpaceX Crew-1. To model biofouling in the WRS, mixed-species biofilms of E. coli and P. aeruginosa were cultured in artificial urine on 316L stainless steel using a specialized BioCell apparatus. Space flight-cultured biofilms in artificial urine show consistent

morphology and coverage as ground-based controls, and there are indications that silver disinfection may be more effective in space flight than on Earth. Preliminary corrosion analysis data indicates that corrosion on 316L stainless steel is primarily driven by electrochemical changes, rather than by microbial induction, and may be more extensive in space flight than on Earth. Additionally, the inclusion of silver fluoride as a disinfectant may increase pitting corrosion on stainless steel. The presence of biofilms in space flight water filters is unavoidable and may pose significant risk to the inhabiting crew as a source of infection or by causing failure of the WRS. Characterizing the microbial response to silver disinfection in flight will allow for improved development of water recycling systems and disinfection protocols for long-term manned space flight missions.

PD4. **Firas Midani**, Heather A Danhof, James Collins, Colleen K Brand, Kevin W Garey, Robert A Britton. Baylor College of Medicine
"Automated analysis of microbial growth reveals phenotypic diversity of *Clostridioides difficile*"

Clostridioides difficile is a gram-positive spore-forming pathogen that recently has become the most common nosocomial infection in the developed world. C. difficile is a genetically diverse species and distinct ribotypes are overrepresented in both human outbreaks and animals. Mass use of trehalose in food manufacturing coincided with the emergence of two epidemic ribotypes, which have a heightened ability to utilize this sugar as a carbon source. We aimed to identify whether carbon substrate utilization by C. difficile isolates explains the distribution of ribotypes in the state of Texas and the novel emergence of ribotype 255. We developed a framework for the rapid analysis of carbon substrate utilization with Biolog Phenotype Microarray carbon source plates and designed a new software, Analysis of Microbial Growth Assays (AMiGA), for modelling microbial growth curves. Using this integrative approach, we profiled clinical isolates collected through an active surveillance network in Texas. Clinical isolates generally clustered by ribotype based on their carbon substrate utilization. Ribotypes dominant in Texas (RT027 and RT014-020) exhibited higher area under the growth curve on carbon substrates commonly metabolized by C. difficile. Our analysis identified several substrates, such as leucine, melezitose, sorbitol, and trehalose, that are differentially metabolized by distinct ribotypes. It also showed that ribotype 255 grows faster than most ribotypes on several carbon substrates. Finally, animal-associated ribotypes exhibited higher total growth on simple sugars than human-associated ribotypes. Ongoing work will continue to profile additional isolates and validate substrate-based fitness advantages with genomic verification, molecular characterization, and competition assays.

PD5. **Yahan Wei**, Ziqiang Guan, Kelli L. Palmer – UT Dallas "Utilization of the host-derived metabolite glycerophosphocholine for phosphatidylcholine biosynthesis in mitis group streptococci"

Significant human pathogen Streptococcus pneumoniae belongs to the mitis group streptococci (MGS). Other MGS such as S. mitis and S. oralis are major human oral commensals and opportunistic pathogens that cause bacteremia and infective endocarditis. S. pneumoniae, S. mitis and S. oralis produce the membrane lipid phosphatidylcholine (PC) by scavenging host-derived metabolites lyso-PC and glycerophosphocholine (GPC), a process that is likely to influence host-MGS interactions. However, the MGS pathway(s) for utilizing lyso-PC and GPC for PC biosynthesis is unknown, nor is it known whether these metabolites have other roles in MGS physiology and pathogenesis. This study used lipidomic, transcriptomic, and bioinformatic methods to study GPC utilization in MGS. PC production in MGS was detected as early as 15 minutes after GPC exposure. Transcriptomic (RNA-Seq) analysis found no genes significantly regulated after 15 minutes exposure. No differences in growth were observed between MGS grown with and without GPC. These results suggest that GPC uptake is likely through a constitutively expressed transporter, and that the production of PC is not required for optimum growth

of MGS in vitro. Two putative GPC substrate binding proteins were predicted in S. mitis: SM12261_RS08975 and SM12261_RS02380. Though transcription of both genes were detected in RNA-seq, double deletion does not abolish the production of PC from GPC, suggesting S. mitis utilize a novel system for GPC uptake. Further transporter candidates are under discovery with bioinformatic analyses. Overall, our work describes progress in identifying the mechanistic basis for PC biosynthesis by host metabolite scavenge in the MGS.

PD6. **Chetanchandra Joshi**, Amy Mora, Paul Felder, Indira Mysorekar – Baylor College of Medicine
"The NRF2/Keap1/p62 Pathway Governs the Host Response to Urinary Tract infections"

Objective: Investigate redox stress response in urothelial cell defense against invading Uropathogenic Escherichia coli (UPEC) during Urinary Tract Infection
Methods: Bladder carcinoma 5637 cell lines, henceforth referred to as urothelial cells, were used as an in vitro model of UPEC infection. The oxidative stress response of urothelial cells post UPEC infection was measured using Reactive oxygen species (ROS) specific dye H2DCF-DA. At different time intervals post-infection, protein and mRNA were analyzed to dissect the temporal response of urothelial cells. At fixed time post-infection, the UPEC expulsion rate and intracellular UPEC load were estimated. Consequently, we developed KEAP1-deficient cells using CRISPR-Cas9 displayed over activated NRF2. in vitro data was validated using Nrf2-/- mice. Finally, Dimethyl fumarate (DMF), an FDA-approved NRF2 inducer, was tested in vitro and in vivo for its effect on UTI course.
Results: We report a whole suite of mechanisms the host uses to expel UPEC from bladder epithelial cells and based on the mechanism, we identify a potential new treatment strategy to improve bacterial clearance and UTI outcomes. We show that the NRF2 pathway is activated in response to UPEC-triggered ROS production, reduces ROS production, inflammation, and cell death, promotes UPEC expulsion, and reduces bacterial load. In contrast, loss of NRF2 leads to increased ROS production, bacterial burden, and inflammation, both in vitro and in vivo. NRF2 promotes UPEC expulsion by regulating transcription of the RAB GTPase, RAB27B. Finally, Dimethyl fumarate, an FDA-approved NRF2 inducer, reduces inflammatory response, increases RAB27B expression, and lowers bacterial burden in urothelial cells and in a mouse UTI model.
Conclusions: Our results demonstrate that activation of the NRF2 pathway is necessary for augmented innate host defense i.e. RAB27B transcriptional upregulation and bacterial expulsion along with other benefits like lowered ROS and increased urothelial health. Our work suggests a model wherein orchestration between recognition of UPEC, p62 mediated KEAP1 engagement, and NRF2 activation ensures urothelial defense against invading UPEC. Given these important roles of NRF2 in urothelial response to UPEC, future efforts should be focused on identifying NRF2 or KEAP1 modulators such as DMF, and ROS regulators in the management of UTIs. Our findings elucidate mechanisms underlying the host response to UPEC and provide a new strategy to combat UTIs.

## Goldschmidt Awardee sessions

**Lauren Howe-Kerr**, Rice University "Dynamics of symbiont-infecting dinoRNAVs across a reef-wide thermal stress event"

Viruses are implicated in coral health and disease, yet the dynamics of infection by an individual viral lineage have not yet been linked to colony fates across a reefscape. To address this challenge, we repeatedly screened >50 colonies of the reef-building coral *Porites lobata* for positive-sense single-stranded RNA 'dinoRNAVs' that putatively infect the dinoflagellate symbionts of corals (family Symbiodiniaceae). Our sampling spanned three reef zones in Mo'orea, French Polynesia over three years

and included timepoints before, during, and after a period of elevated ocean temperatures. Amplicon sequencing of Symbiodiniaceae communities (D1-D2 region of the 28S large subunit nuclear ribosomal RNA gene) and associated dinoRNAV consortia (major capsid protein) revealed diverse dinoRNAV communities that vary with colony identity, sampling location, and reef environment. DinoRNAV diversity was highest in the shallow fringing reef, and communities were most variable during the peak of thermal stress. Taken together, dinoRNAVs may play a role in coral holobiont stress response, ultimately influencing coral health trajectories under global change.

**Kathryn L Campbell**, Texas A&M-Galveston "Lifestyles and Metabolic Contributions Of Viruses In The Various Environments Of A Hydrothermal System"

Hydrothermal systems host a diverse range of environments and conditions of temperature and pH that support an equally diverse microbial population, which is impacted by viruses that serve as drivers for both ecological functions and evolutionary changes. In March/April of 2019 samples were taken aboard the R/V Atlantis at 9°50'N segment of the East Pacific Rise (EPR). The main focus was collecting a variety of viral communities and included samples of microbial mats (4 samples) and the neutrally buoyant plume (5 samples) using manned deep-ocean submersible DSV-2 Alvin and CTD casts to assess the role and impact of the viral community. Microbial mat samples were induced with Mitomycin C (1µg/ml), while between 80-120L of 0.22 µm filtered seawater was concentrated using tangential flow filtration for the plume samples. Both sets of samples were sequenced using Illumina technology the results of which were processed using BBtools, MEGAHIT, PRODIGAL, VirSorter2 and VIBRANT for quality control, assembly, annotation and viral identification, respectively. Resulting populations exhibited a largely lytic lifestyle with comparable numbers of auxiliary metabolic genes (AMGs). The microbial mat samples contained AMGs invested in metabolic processes such as various sugars, sulfur, lipid, vitamin and cofactor metabolisms. The plume sample AMGs were primarily invested in amino acid metabolism. Overall, while both viral populations utilize similar lifestyles and contribute to metabolic processes the mat viral populations seem to have more of a role than the plume viral populations in microbial metabolic functions.

## Friday Keynote Presentation

**Peter J Christie**. McGovern Medical School, Department of Microbiology and Molecular Genetics, UT-Health, Houston

### Mechanistic and structural advances in our understanding of bacterial conjugation

Dr. Christie's laboratory investigates the mechanisms of action and structures of bacterial type IV secretion systems (T4SSs). The T4SSs are a phylogenetically and functionally diverse group of translocation systems exploited by many species of bacteria to deliver DNA or effector proteins to bacterial or eukaryotic target cells during infection. A large T4SS subfamily, the conjugation systems, contribute to genome evolution and widespread dissemination of antibiotic resistance genes and virulence determinants among pathogens. Another subfamily, the effector translocators, mediate transfer of effector proteins to aid in bacterial colonization of the human host. Our structure - function studies are presently focusing on the F plasmid-encoded conjugation system, which is responsible for dissemination of F plasmids widely among members of the Enterobacteriaceae. Recent advances in structural definition of the F plasmid-encoded and other T4SSs have been driven by a collaboration with Dr. Bo Hu, an Assistant Professor also in the Department of Microbiology and Molecular Genetics at McGovern.

Besides these ongoing basic science studies, the lab is developing T4SSs as programmed delivery systems to mitigate infection and disease progression.

## Poster Abstracts:

### Graduate Students:

GP 1 **Analisa Narro**, Erin Brown, Heidi Kaplan, Catherine Ambrose. UT-Health Houston. "Bacteriophage-Containing Biodegradable Microsphere Technology to Treat Osteomyelitis"

The rise in antimicrobial resistant (AMR) infections is a growing concern and bacteriophage provide a unique biological approach. Concerns have been raised on the ability to deliver active bacteriophages using degradable drug-delivery systems. In manufacturing poly(lactic-co-glycolic acid) (PLGA) microspheres, the two steps that present the greatest possibility for phage inactivation are contact with the organic solvent dichloromethane and lyophilization. As studies have shown lyophilization is less likely to result in a significant loss of phage lytic activity, in this study we investigated protocols for microsphere manufacture. Two bacteriophages were tested for their ability to lyse a clinical Staphylococcus aureus isolate, known as UAMS-1, obtained from an osteomyelitis infection. PLGA microspheres were manufactured according to a water-oil-water protocol with two methods evaluated. The first included adding a 2.5 x 10^10 phage/ml solution directly to a PLGA-dichloromethane mixture. In the second method, the 2.5 x 10^10 phage/ml solution was added to a polyvinyl-alcohol solution before adding this mixture to the PLGA-dichloromethane solution. Both methods were evaluated using an elution-assay, and the eluent was collected after 24hr, 72hr, and 7 days. For method-1, a total of 2.0 x 10^7 phage eluted after 7 days, yielding a 0.6% entrapment efficiency. For method-2, a total of 5.1 x 10^5 phage eluted after 7 days, yielding a 0.02% entrapment efficiency. Method-1 eluted a statistically greater number of active phage and had a greater entrapment efficiency ($p=0.012$). Future studies should determine the effectiveness of phage-containing PLGA microspheres in in vitro biofilms and in in vivo animal models.

GP2 **Tiffany Lujan**, Faith Cox, Kristin Sefcik, Jesse Meik, Jeff Brady, and Dustin Edwards. Tarleton State University "Characterization of the Microbiome of the Barton Springs Salamander"

Amphibians are suffering a global decline with urbanization being a large contributor. The Edwards Aquifer provides water for the city of Austin, Texas, as well as a habitat to the federally endangered Eurycea sosorum. The Austin Nature and Science center is a permanent facility where salamanders are held for a captive rearing with the aim of reintroduction into Barton Springs. We hypothesize that the microbiome of host captive salamanders differs from that of wild salamanders and could be contributing to negative effects on host fitness. The skin microbiota of amphibians is a vital organ that regulates different physiological processes and has been shown to impact overall host fitness by providing protection against fungal pathogens. Microbial community composition between captive and wild populations of amphibians varies dramatically in species richness and abundance. In partnership with the City of Austin, we collected skin fecal, substrate, and water samples from salamander habitats to characterize the differences in microbial communities between captive and wild salamanders. DNA was extracted from the samples and amplified using bacterial 16S rRNA and the ITS region of fungal rRNA tags using Earth Microbiome Project primers. Amplicons then had sequence specific barcoding primers attached during index PCR. Index products were pooled to equimolar concentrations and purified using Pippin Prep. Amplicons were sequenced at Texas A&M Genomics and Bioinformatics Services. Sequence analysis was conducted USEARCH pipeline that assigned operational taxonomic units (OTUs) based on GreenGenes. The OTU table was clustered at 97% similarity, filtered to remove rare OTUs, and normalized.

GP3 **Jacob Zulk**, Marlyd Mejia, Vicki Mercado, Mallory Ballard, Emmaline Heckmann, Belkys Sánchez, Barbara Trautner, Anthony Maresso, Katy Patras. Baylor College of Medicine. "Uropathogenic E. coli resistance to phage ES17 decreases bacterial fitness"

As with antibiotics, bacteria can become resistant to phage. While several resistance mechanisms have been documented, no groups have evaluated the effects of resistance in the urinary tract environment. We hypothesized bacterial resistance to phage would come at the cost of reduced bacterial fitness and decreased colonization of the urinary tract. We isolated uropathogenic E coli (UPEC) resistant to phage by challenging UTI89, a well-characterized cystitis isolate, or DS566, a catheter-associated UTI clinical isolate, with phage ES17 in liquid culture for 18 hours. Through whole-genome sequencing, we discovered UPEC evaded killing by ES17 by mutating lipopolysaccharide (LPS). These UPEC grow worse than their parental strains in urine and poorly colonize the murine urinary tract. Ongoing research will determine frequency of phage resistance *in vivo* and the impact of LPS mutations on UPEC's ability to establish intracellular reservoirs. In total, these findings suggest phage resistance negatively impacts bacterial fitness in the urinary tract, which could be exploited during UTI phage therapy.

GP4 **Bradley Himes**, Adrian Mejia-Santana, Karl E. Klose. UTSA "Mutations in Regulatory Histidine Kinase FlrB Enhance Motility in Vibrio cholerae with a single FlaA Flagellin Subunit"

Motility of the Gram-negative bacterium Vibrio cholerae contributes to virulence and environmental persistence. Motility is mediated by a single polar flagellum, composed of five flagellin subunits (flaABCDE) in the filament, however only flaA is required for motility. Flagellar gene expression is organized into a transcriptional gene hierarchy consisting of 4 temporal classes of flagellar genes. FlrB and FlrC control Class Ⅲ gene expression, which comprise basal body/hook components and include the FlaA flagellin. FlrB is a histidine kinase that phosphorylates FlrC, which in turn activates σ54-dependent transcription at Class III promoters. A V. cholerae strain lacking four (of five) flagellin genes (ΔflaBCDE) is surprisingly non-motile. Selection for spontaneously motile strains in this background resulted in mutations localized to the N-terminus of FlrB, mostly within a Per-Arnt-Sim (PAS) domain, which were identified by Whole Genome Sequencing. To gain insight into the function of FlrB, ten of these mutations were reconstructed in both wildtype and ΔflaBCDE backgrounds. Motility phenotypes were measured by soft agar motility assays, and the mutations could be divided into two phenotypes based on swarm appearance in soft agar. Western immunoblot analysis of whole cell lysates demonstrated low levels of FlaA in the ΔflaBCDE parent, which increased in the flrB mutant strains. Interestingly, measurement of flaAp-lacZ activity in these strains did not reveal major differences in flaA transcription due to the flrB mutations. Our results suggest that FlrB may be involved in post-transcriptional regulation of FlaA expression, which would illuminate a novel activity of this regulatory protein.

GP5 **Vaidehi Pusadkar**, Rajeev K. Azad, UNT "Multi-omics based identification of gut microbiome COVID-19 signatures"

COVID-19, caused by SARS-CoV-2, results in respiratory and cardiopulmonary infections. There is an urgent need to understand not just the pathogenic mechanisms of this novel disease, but also its impact on the physiology of different organs and the microbiome. Multiple studies have reported the influence of COVID-19 on gastrointestinal microbiota, such as, in promoting dysbiosis (imbalances in the microbiome) and other consequences following the disease progression. Deconstructing the dynamic changes in microbiome composition and their impact on genetic mechanisms and functional pathways at the systems level remains a challenge. Motivated by this problem, here we performed an integrated multi-omics (metagenomics and metatranscriptomics) biomarker identification study for deciphering the

impact of COVID-19 on gut microbial composition, and the functional repercussions post-infection. Metagenomic studies of COVID-19 patients presented here demonstrate the complex effects leading to the rise in opportunistic pathogens as well as the depletion of beneficial bacteria. This has a pronounced effect on the functional components of the gastrointestinal microbiome. Several gene targets and their correlation with other genes were identified from a gene co-expression network constructed using metatranscriptomic samples of COVID-19 patients. Functional assessment of these biomarkers resulted in the identification of the perturbed metabolic pathways in the progression of COVID-19. Overall, this study provided insights into the complete gut microbiome shifts occurring in COVID-19 patients, shining a new light on the compositional, expression, and functional changes.

GP6 **Stephanie Beane**, Luis Grado, Jeremy Bechelli. Sam Houston State University. Infection of Human Endothelial Cells with Colorado Tick Fever Virus Stimulates Cyclooxygenase 2 Expression and Vascular Dysfunction

Colorado tick fever virus (CTFV), a tick-borne double-stranded RNA virus, is the causative agent of Colorado tick fever (CTF). CTF is generally self-limiting; however, severe manifestations can include meningitis, hemorrhagic fever, and meningoencephalitis. The mechanism of CTFV mediated pathology is currently unknown, including mechanisms underlying CTFV infection-associated vascular damage. Cultured human endothelial cells are highly susceptible to infection and respond by altering regulatory cytokines, and ultimately undergoing apoptosis. Cyclooxygenase-2 (COX-2) is a known mediator of inflammation and facilitates the synthesis of prostaglandins. In this study, infection of HMEC-1s showed robust induction of COX-2 but no effect on COX-1 using transcriptomics and qPCR. Additionally, cell viability measured during infection with COX inhibitors showed an increase in cell survival when treated with indomethacin, a potent non-selective inhibitor of cyclooxygenase enzymes. Angiopoietin-1 (ANG-1) and angiopoietin-2 (ANG-2) are biomarkers produced during vascular dysfunction during infections; Tie-2 is an endothelial receptor involved in inflammation and vascular leakage. qPCR analysis showed an increased ANG-2/ANG-1 ratio and Tie-2 expression at 12- and 24- hours post-infection (hpi). Current data suggests CTFV induces pathological characteristics of vascular activation and dysfunction evidenced by enhanced COX-2 expression and skewed ANG-2/ANG-1 ratio, and cyclooxygenase may be important during CTFV infections through increased cell viability with cyclooxygenase inhibition. Furthermore, infection of primary human umbilical vein endothelial cells demonstrates upregulation of COX-2 protein at 4-, 12-, and 24-hpi. In this study, we uncover specific biomarkers for CTFV-induced vascular dysfunction and inflammatory responses, highlighting potential therapeutic markers for the treatment of this neglected tick-borne disease.

GP7. **Ricardo A. Martinez**, Anwar Kalalah, Armando Rodriguez, Sara S.K. Koenig, Mark Eppinger. UTSA Shiga-Toxin Phages Regulate Virulence and Metabolism in Enterohemorrhagic Escherichia coli of the O157:H7 Serotype

Background: Infections with foodborne Enterohemorrhagic Escherichia coli (EHEC) O157:H7 can cause severe and potentially life threatening human illness. Phage-borne Shiga-toxin (Stx) production is a virulence hallmark of Stx-producing E. coli (STEC). Atypical non-shigatoxigenic isolates that either never acquired Stx-phages or lost them secondarily are referred to as Lost Shiga-toxin (LST) isolates. In this study, we examine the impact of Stx-phages on global gene expression in the background of the two EHEC O157:H7 isogens, TT12A (Stx+) and TT12B (Stx-). Methods: Transcriptomes were recorded under non-induced and mitomycin-C-induced conditions, which precede and result in Stx production. Total RNA was extracted and enriched for mRNA using the PureLink RNA and MICROBExpress kits, respectively. Libraries were sequenced on the Illumina NextSeq 500 platform. Differentially Expressed

Genes (DEGs) were identified, characterized and visualized using a custom pipeline on the Galaxy platform and were further analyzed using Degust. Results and conclusions: Transcriptomic profiling of the isogenic strains revealed altered regulatory networks affecting both aerobic/anaerobic metabolism as well as other lineage-specific virulence factors. Overall, our data endorse a significant role for the carried Stx-phages in the modulation of bacterial host genes beyond Stx production.

GP8. **Cheyenne Ziegler**, Claude Sinner, Xianli Jiang, Uyen Thy Nguyen, Kelli Palmer, Faruck Morcos. UT-Dallas "Two Component System Specificity Model Detects Noncognate Interactions for Antibiotic Resistance Pathways and Other Two Component Pathways "

Two component systems (TCS) are ubiquitous in bacteria, both intrinsically and extrinsically when encoded by plasmids, and are responsible for antibiotic resistance, nutrient signaling, and other environmental responses. TCS are signal transduction pathways in which a histidine kinase (HK) senses an environmental signal and transduces the signal by phosphorylating a response regulator (RR). Once the RR is phosphorylated it can carry out several functions, often acting as a transcription factor. While TCS interactions primarily occur between a HK and its cognate RR, many functionally relevant noncognate interactions have been identified, suggesting that TCS pathways are frequently nonorthogonal. To predict noncognate interactions, we developed an encoded specificity model that utilizes sequence information and applied it to 6,676 organisms. The results showed robust ability to detect both cognate partners and known noncognate interactions. In addition to detecting naturally occurring noncognate partners, the model can assess the change in specificity upon mutation in TCS proteins. This allows the interaction to be probed or engineered in silico and helps identify how signaling networks can reconfigure when nodes in the network mutate. Furthermore, we demonstrated our encoded specificity model can successfully identify crosstalk interactions between intrinsic and extrinsic TCS during antibiotic treatment, uncovering a mechanism for synergistic antibiotic resistance. Thus, the model also provides insights on how components behave synergistically in the presence of multiple stressors. Expanding the usage of this model to other signaling families may continue to further our understanding of TCS networks and the phenotypes that result from nonorthogonal interactions.

GP9. **Qi Xu**, Alex Kang, Emily Zhou, Natasha Kirienko. Rice University. "Cytotoxicity of membrane vesicles from *Pseudomonas aeruginosa*"

Pseudomonas aeruginosa is a type of Gram-negative opportunistic human pathogen. It has developed resistance towards many commonly used antibiotics, which makes standard-of-care treatments less effective. According to the 2019 AR Threats Report, multidrug-resistant P. aeruginosa caused about 32,600 infections among hospitalized patients and 2,700 estimated deaths in the United States in 2017. Our lab has previously found that the bacteria filtrate has toxicity towards murine macrophages. This cytotoxicity could be associated with the membrane vesicles (MVs) produced by P. aeruginosa. Better understanding the role that MVs play in cytotoxicity will enable us to elucidate the mechanism behind their cytotoxicity.

Here, I first established a purification pipeline for these vesicles. This is based on protein precipitation and density gradient, which shows a high reproducibility. The cytotoxicity of purified vesicles was verified in a variety of cells. Meanwhile, the characteristics of these vesicles were further investigated using multiple biochemical and cell biological methods, including particle measurement via NanoSight, protein analysis via SDS-PAGE, and fluorescence microscopy. In addition to the standard lab strain PA14, I expanded the strain choices to clinical isolates from pediatric patients with cystic fibrosis, in order to better understand vesicle production and the role of MVs in infection. This work may partially explain

the cellular damage that occurs during P. aeruginosa infections, and eventually help us to lower the pathogenicity and even prevent the potential infection.

GP10 **Marlyd Mejia**, Samantha Ottinger, Korinna Ruiz, Katy Patras. Baylor College of Medicine. "The germ free murine vaginal tract is restrictive to the colonization of human vaginal microbes"

Perturbation of the vaginal microbiome has been associated with adverse pregnancy outcomes, increased susceptibility to sexually transmitted infections, and local inflammation. Considering the 25% incidence of vaginal microbial dysregulation in women across the nation, an understanding of how endogenous microbiota respond to or protect against pathogen exposure is of increasing importance. Currently, conventional murine models have been used to test causality of vaginal pathogens in a host environment. However, the murine vaginal tract is not fully representative of microbial interactions that occur in the human female since it is dominated by Staphylococcus succinus rather than Lactobacillus, which is the dominant microbe of the human vaginal tract. Therefore, a translational model is needed to assess in vitro microbial dynamics. We generated a humanized vaginal microbiota (HVM) mouse model using clinically derived vaginal communities to inoculate germ free mice orally and vaginally. Microbial analysis using 16S rRNA sequencing at three timepoints was accompanied by cultivation of murine passaged vaginal microbes. We found that Lactobacillus did not stably colonize the murine vaginal tract when introduced in a community; rather, Enterococcus and Enterobacter became the dominant species. Additionally, we observed that vaginal microbes differentially colonize the gut, which displayed a more even community than in the vaginal tract. At both sites, microbial communities stabilized by seven weeks post inoculation as analyzed via the Bray-Curtis Distance metric. While the model is still not an accurate representation of the human vaginal microbiome, we demonstrate the selectivity of mice in receiving human vaginal microbiota.

GP11 **Craig Schindewolf**, Kumari Lokugamage, Michelle N. Vu, Bryan A. Johnson, Dionna Scharton, Jessica A. Plante, Kenneth S. Plante, Birte Kalveram, Stephanea Sotcheff, Kari Debbink, Matthew D. Daugherty, Andrew Routh, Vineet D. Menachery. University of Texas Medical Branch
"Nsp16 mutation of SARS-CoV-2 sensitizes virus to type I interferon and attenuates pathogenesis"

Understanding the molecular basis of innate immune evasion by severe acute respiratory syndrome (SARS)-CoV-2 is of immense importance for informing the next wave of therapeutics. We investigate the role of the nonstructural protein 16 (nsp16) of SARS-CoV-2 in pathogenesis. Nsp16 is a putative ribonucleoside 2'-O methyltransferase (MTase), an enzyme that catalyzes the transfer of a methyl group to mRNA as part of the capping process. In the case of SARS-CoV-2, nsp16 may conceal viral RNA from cap-sensitive host restriction. We show that SARS-CoV-2 that contains a mutation in a conserved residue of nsp16 is attenuated both in vitro and in vivo. Additionally, we show in vitro that the nsp16 mutant is more sensitive to exogenous type I interferon and replicates more poorly in type I interferon-competent cell lines. We further show that knocking down IFIT1, an interferon-stimulated gene that senses a lack of 2'-O methylation, helps restore fitness to the nsp16 mutant. Understanding the contribution of nsp16 to SARS-CoV-2 virulence could inform future therapeutic development or serve as the basis for an attenuated vaccine.

GP12 **Samantha Ottinger**, Marlyd E. Mejia, Larissa Neves, Kathryn A. Patras. Baylor College of Medicine. "Cultivation of vaginal microbial communities to screen for pathogen resistance"

The human vaginal microbiome is a complex ecosystem linked to health and disease states. Healthy vaginal communities provide protection from urinary tract and sexually transmitted infections, as well as

adverse reproductive outcomes, such as preterm birth, neonatal sepsis, and infertility. Our work aims to harness these protective effects as an alternative to antibiotics in the face of rapidly increasing drug resistance and non-targeted impacts on the endogenous microbiota. However, little work has been done to cultivate human vaginal communities and assess their therapeutic potential to treat urogenital infections and inflammatory conditions. Here, we optimize cultivation of human vaginal communities and exam their ability to inhibit urogenital pathogens in vitro. Using healthy human donor vaginal swab samples, we tested various cultivation conditions, including media, oxygen concentration, and agitation. These results identified two optimal cultivation conditions. One condition maximizes culture density (~3 x 109 CFU/mL) and colony morphologies (median 2 [1-4] morphologies per community), while the other condition simulates the nutritional environment of human vaginal fluid. Using these conditions, we identified three unique communities that inhibit growth of Group B Streptococcus, a urogenital pathogen that causes urinary tract infections and neonatal sepsis. These studies enable ongoing efforts to increase scale and throughput of human vaginal community cultivation in continuous flow systems termed mini bioreactor arrays (MBRAs). Vaginal community cultivation in MBRAs will enable further identification and characterization of communities with therapeutic potential. This work is critical towards our goal of identifying non-antibiotic treatments for urogenital infections.

GP13 **Calvin Tran**, Quentin DiPasquale, Starla G. Thornhill, Robert JC McLean. Texas State University "Nutritional preference of a novel *Exiguobacterium* species"

Members of the genus Exiguobacterium are Gram-positive bacilli that are often extremophiles. A novel Exiguobacterium species (A1) was isolated from a laminarly-flowing region of the San Marcos River, which has previously been identified as an organism which may display enhanced survival in the spaceflight environment. Exiguobacterium A1 shows a preference for low fluid shear growth when cultured in the lab but is difficult to propagate using conventional culturing methods. Exiguobacterium A1 was grown in Reasoner 2A (R2A) and Luria-Bertani (LB) media in both low shear modeled microgravity (LSMMG) and normal gravity conditions to identify if there was a nutritional preference in these conditions. When grown in LB medium, Exiguobacterium A1 displayed more growth in normal gravity when compared to LSMMG. When grown in R2A medium, Exiguobacterium A1 saw a shift in growth between 6 hours and 12 hours, with normal gravity having greater growth after 12 hours than LSMMG. LSMMG plays a small role initially when growth in R2A media, but overall, Exiguobacterium A1 did not show a preference in growth under LSMMG.

GP14 **Rachel Porter**, Javier A. Gomez, Megan Burch, Luis M. Lopez Salazar, Alyssa Russell, Sebastian Juarez-Casillas, Grant Means, Aaron Lynne, Jeremy Bechelli. Sam Houston State University. "Bacterial Community Profiling of Ixodes scapularis ticks from Western New York, USA"

The microbial community composition of disease vectors, including ticks, is an area of growing interest due to its ability to transmit a diverse array of human pathogens resulting in Lyme disease, anaplasmosis, and ehrlichiosis. We examined the diversity of bacteria associated with the blacklegged tick (Ixodes scapularis) by sequencing the hypervariable region three (V3) and four (V4) of the bacterial 16S rRNA gene originating from ticks collected from Cattaraugus County, New York across life stages (larvae, nymphs, and adult males and adult females). Sequencing generated 598 ESVs (exact sequence variants) that were assigned to 195 taxa. The microbiome across all life stages was dominated by gram-negative proteobacteria, specifically Rickettsia species closely matching Ixodes Rickettsial endosymbionts. Rickettsial abundance decreased as ticks matured, and adult females had significantly more Rickettsia than adult males (81.3% and 32.8% respectively). We detected Anaplasma species in adults (16.67%) and nymphs (75%) by 16s sequencing and confirmed A. phagocytophilum in 62% of

nymphs and 14.58% of adults using primers for msp2. The findings of our study confirm previous data about the I. scapularis microbiome conducted in other geographical regions and provide insight into the microbial diversity and pathogen burden of I. scapularis in Western New York that is applicable for a One-health approach for monitoring and prevention of tick-borne disease transmission.

GP15 **Arash Jafarzadeh**, Sina Vedadi Moghadam, Akanksha Matta, Jeffrey Hutchinson, Samer Dessouky, and Vikram Kapoor. UTSA
"Evaluation of the effectiveness of the roadside vegetation located in Edwards Aquifer recharge zone in the removal of heavy metals"

Impervious surfaces in Texas have increased faster than any other states in the U.S. in the past few decades. Heavy metals run off are of great concern due to their negative impacts on habitats and human health. Conventional remedial methods recognize the hydrological factors mostly related to precipitation. Low impact development (LID), however, considers other constituents of the hydrological cycle. The six toxic heavy metals including cadmium, copper, lead, nickel, chromium, and zinc as well as two metals for monitoring purposes including iron and magnesium have been selected to evaluate the efficiency of the vegetation in removing heavy metals. The samples are collected using the ISCO autosamplers located at the inflow and the outflow of the basin. The collected samples are then analyzed using ICP-OES. The results have indicated that retention basins can decrease the concentration of heavy metals to some extent. Iron was decreased in most cases, and zinc was decreased greatly if not entirely. Nickel and chromium, though very low concentrations were detected, were also decreased or removed. Copper in most cases showed lower concentration in the outflow. Magnesium and lead did not change significantly possibly due to the geological and structural characteristics of the basin. Cadmium was only detected in two events which its concentration was below the detection limit. This study verified the importance of the vegetation in the removal of heavy metals within the Edwards Aquifer zone. This study will also consider the microbial activities to further investigate the importance of LID.

GP16 **Tahira Amdid Ratna**, Alejandro Barros, Dennise Palacios Araya, Moutusee J. Islam, Kelli L. Palmer. UT-Dallas "CRISPR-Cas defense against antibiotic resistance plasmids in *Enterococcus faecalis*"

Enterococcus faecalis is a gram-positive bacterium and a natural inhabitant of the mammalian gastrointestinal tract. E. faecalis is also an opportunistic pathogen and is associated with life threatening infections like bacteremia and infective endocarditis. E. faecalis can acquire resistance to a wide range of antibiotics by horizontal gene transfer (HGT). Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems can provide sequence-specific defense against HGT. From previous studies, we know that CRISPR-Cas in E. faecalis can provide sequence-specific anti-plasmid defense during agar plate biofilm mating in vitro and in the murine intestine in vivo. However, these studies were conducted using only one model antibiotic resistance plasmid harbored by a model laboratory donor strain. CRISPR-Cas anti-plasmid activity has not been assessed in a multiplasmid system using clinical isolate plasmid donors. This is important because antibiotic-resistant E. faecalis clinical isolates typically possess multiple (up to 7) resistance plasmids that may interact with their host and with each other to impact CRISPR-Cas efficacy. In this study, using a combination of laboratory model strains and clinical fecal surveillance isolates, we found that the number of plasmids in an E. faecalis donor impacts antibiotic resistance gene transfer frequency by conjugation. We also found that CRISPR-Cas confers different levels of defense against a plasmid donor strain harboring varying numbers of plasmids. This study provides insight about the interplay between plasmids and CRISPR-Cas. This work is also important for designing new therapeutic interventions that limit HGT among bacterial pathogens.

GP17 **Sujina Manandhar**, Benjamin Dawson, Carmen Montana, and Madhusudan Choudhary. Sam Houston State University. “Effects of Mercury on Microbiome Composition in Trinity River, Texas”

Heavy metal contamination in freshwater ecosystems has become a serious issue impacting ecological, environmental, and human health. Over a decade, the Trinity River in Texas has become polluted with heavy metals, especially mercury (Hg) through various natural and anthropogenic sources. The coal-burning, especially used for electricity generation in the coal-fired power plants are the prime source of mercury pollution in Texas. The inorganic Hg undergoes biotransformation mediated by some anaerobic microorganisms present in aquatic ecosystem to form a highly neurotoxic organic methyl mercury (MeHg). These MeHg are readily bioaccumulated and biomagnified to various trophic levels. In this study, we examined how the microbiome contained in sediments of the Trinity River varied along a gradient from upstream site (less urban/ industrialized development) to downstream sites (high to intermediate development) which are influenced by mercury deposition. We hypothesized that Hg concentration will vary from upstream to downstream sites and this Hg variation will be reflected in the microbiome composition. Soil sediments were collected from four different sites, namely Jacksboro, Downtown Dallas, Oakwood, and Romayor along the Trinity River.
The total mercury concentration in the soils samples was determined using Cold vapor atomic absorption spectroscopy (CVAAS). The microbiome analysis was performed by sequencing the V3 and V4 regions of 16S rDNA and analyzed using QIIME2. The result of mercury analysis revealed a higher concentration of Hg (0.447µg/Kg) further down to the south in Romayor. We can infer that power plants such as Big Brown power plants, NRG Texas, and Trinidad power plants located along the Trinity River contribute to Hg inputs to river which flows with water stream and ultimately settle to sediments. The microbiome analysis results revealed that Proteobacteria as the most abundant phylum across all sites. The Deltaproteobacteria and Firmicutes are the primary mercury methylators predominantly found in the downstream contaminated sites. These groups of bacteria utilize their specific genes, the HgcA and HgcB during the process of biotransformation, contributing to mercury pollution in freshwater ecosystem. These findings suggest that microbial community patterns and structure are influenced by mercury contamination along this river gradient.

GP18 **Braden Hanson**, Amanuel Hailemariam, Dwight Baker, Sargurunathan Subashchandrabose. Texas A&M University Department of Veterinary Pathobiology. “Novel small molecule inhibits the growth of Uropathogenic E. coli in the presence of copper.”

Urinary Tract Infections (UTI) are one of the most globally treated diseases. As consequence, the risk of antimicrobial resistance is climbing at an alarming rate. UTI causing pathogens can range from gram-negative strains (Escherichia coli, Proteus Mirabilis, Klebsiella pneumoniae, etc.) to gram-positive pathogens (Staphylococcus Saprophyticus, Staphylococcus aureus). The uropathogenic E. coli strain CFT073 ΔtolC was grown in LB media containing 25µM $CuSO_4$ and was screened against a small library consisting of 51,098 different molecules. With the initial hit count of 584 molecules a secondary screen (dose-responsive) and tertiary screen (copper-dependence) reduced our number of hits to 136 and 12, respectfully. Of the 12 compounds only 1 molecule was commercially available, had zones of inhibitions that responded in a copper dependent manner, and was able to not only inhibit the mutant strain but also the wild-type strain. The compound’s chemical properties meet all criteria for the Lipinski Rule of 5.

GP19 **Soham Sengupta**, Rajeev K. Azad. University of North Texas. “APP: A new tool for comparative genomics based detection of alien genes in bacterial genomes”

An important problem in bacterial genomics is to catalog genes acquired through the evolutionary process of horizontal gene transfer. Both comparative genomics and sequence composition based methods have often been invoked to quantify horizontally acquired genes in bacterial genomes. Comparative genomics based methods rely on the availability of completely sequenced genomes of both close and distant relatives, and therefore, the confidence on their predictions increases as the databases become more enriched in completely sequenced genomes. Owing to the sheer volume of complete genomes of bacteria archived in the NCBI database, a reassessment of alien genes is necessary based on the information-rich resources currently available. We revisited the comparative genomics approach and developed a new algorithm for alien gene detection. Our algorithm, APP – Alienness by Phyletic Pattern, compared favorably with the existing comparative genomics based methods and is capable of detecting both recent and ancient transfers. APP is a user-friendly command line based tool that incorporates taxonomy and a new algorithm that employs BLAST to efficiently catalog putative horizontally acquired genes in bacterial genomes by searching for atypical distribution of a query gene within species, genus, and family groups it belongs to. It can be used as a standalone tool or in concert with other complementary algorithms for comprehensively cataloging alien genes in bacterial genomes. The source code of APP is publicly available at https://github.com/sohamsg90/APP-Alieness-by-Phyletic-Pattern.

GP20 **Casey Hughes Lago** and Deborah Threadgill. Texas A&M University. “Comparative Analysis of Secretion Systems in *Campylobacter rectus*”

Campylobacter rectus is a gram-negative, anaerobic bacteria strongly associated with periodontitis. It is also shown to cause various extraoral infections and is linked to adverse pregnancy outcomes in human and murine models. C. rectus and related oral campylobacters including C. showae, C. concisus, C. gracilis and C. curvus have been termed as “emerging campylobacter species” because infections by these organisms are likely underreported. Currently, there are only three publicly available genomes published for C. rectus, including one complete genome (ATCC 33238), and two WGS (OH2158 and SRR9217431). Up to this date, no comparative methods have been used to analyze more than single C. rectus strains. This lack of genomic information prevents exploration of intraspecific genetic variability and evolution and limits our ability to study pathogenesis. We sequenced nine new C. rectus sequences and used comparative methods to identify regions of interest. A focus was put on the type-3 secretion system (T3SS), type-4 secretion system (T4SS), and type-6 secretion system (T6SS) because these protein complexes were shown to be important for pathogenesis in other campylobacter species. All genomes were assembled, annotated, and estimated to be >99% complete in the Pathosystems Resource Integration Center (PATRIC). The pangenome of these strains consists of 2885 genes. All isolates have T3SS and T6SS hallmark proteins, while five of the isolates are missing a T4SS system. 21 prophage clusters were identified across the panel of isolates, including four that appear intact. Additionally, significant genomic islands were found, suggesting regions in the genomes underwent horizontal gene transfer.

GP21 **Adeline Supandy**, Heer Mehta, Truc T. Tran, William R. Miller, Cesar A. Arias, Yousif Shamoo. Rice University. “Combinatorial Evolution of *Enterococcus faecium* to Daptomycin and Fosfomycin”

Background/Goals. Daptomycin (DAP) is currently being used as the last resort drug against vancomycin-resistant enterococci (VRE). However, rising resistance to DAP has pushed combination antimicrobial therapy forward as a treatment option for VRE infections. DAP and Fosfomycin (FOS) combination arose as a promising method. FOS, a PEP analog, targets MurA, the enzyme involved in the first step of bacterial cell wall synthesis. Despite targeting cell membrane, resistance to DAP in enterococci has been

shown to also involve changes to cell wall. As such, evolution to this drug combination may also provide insight into possible interactions between cell wall and cell membrane synthesis while under antibiotic stress. To study this, E. faecium HOU503 was evolved to DAP and FOS individually and in combination through flask transfer adaptation.

Results. DAP and FOS combination exhibit indifferent/additive activity against HOU503, but adaptation to DAP-FOS combination took significantly longer compared to single antibiotic adaptation (18 vs 8 days in average). HOU503's resistance to DAP-FOS was achieved through independent mutations that conferred resistance to each drug individually. FOS resistance was mediated through changes in cell wall synthesis and PEP flux while DAP resistance was achieved largely through changes to cell membrane lipids compositions and fluidity.

Conclusions. The addition of FOS can prolong the efficacy of DAP and significantly extend the timeline to resistance in vitro. Genomics data showed that there were no genetic drivers involved in DAP-FOS resistance that could undermine the combinatorial approach. Instead, HOU503 evolved mutations in separate independent pathways to become DAP-FOS-resistant.

GP22 **Mohammad Kamruzzaman**, Hemachandra Ishanka, Sarbjeet Niraula, Meaghan Rose, Woo-Suk Chang. UT-Arlington. "Role of extracytoplasmic function (ECF) sigma factors in desiccation stress response of *Bradyrhizobium japonicum*, a nitrogen-fixing symbiont of soybeans"

Bradyrhizobium japonicum can fix atmospheric nitrogen into ammonia through a symbiotic association with soybeans. Although the symbiotic nitrogen fixation (SNF) is beneficial to sustainable agriculture, the two partners are susceptible to abiotic stresses such as drought (i.e., desiccation stress). Several drought-tolerant soybean varieties have been developed; however, the absence of corresponding symbiotic rhizobia limits the optimal SNF, specifically under drought conditions. Extracytoplasmic function (ECF) sigma factors are a group of transcription factors that sense signals from the extracytoplasmic compartment and respond to abiotic stresses. In this study, we selected three ECF sigma factors blr2203, bll3014, and bll2628 because they were related to heat shock and desiccation stress in the previous gene expression study. To confirm their functions in B. japonicum USDA110, we constructed knockout mutants for these individual genes using site-specific mutagenesis. The blr2203 mutant showed less survivability than the wild type up to 30 h of desiccation. However, when desiccation time extends, the susceptibility of the mutant did not appear to be drastic compared to that of the wild B. japonicum USDA110. A similar result was observed for the bll3014 mutant, indicating that other genes may compensate for the function of the bll3014 gene for a prolonged duration of desiccation. Mutagenesis for bll2628 is currently underway and we will examine its survivability and other physiological characteristics such as desiccation stress response and symbiosis with soybeans.

GP23 **Sarah Owen**, Rajesh Balaraman, Jeremey Bechelli. Sam Houston State University. "Colorado Tick Fever Virus Mediated Apoptosis in Human Endothelial Cells"

Colorado tick fever virus (CTFV), the causative agent of Colorado tick fever (CTF), is a member of the Family Reoviridae and genus Coltivirus. Symptoms of CTF are characterized by sudden biphasic fever, headache, myalgia, petechial rash, and photophobia, while severe forms of the disease can include meningoencephalitis, hemorrhagic fever, and death in children. However, the mechanisms underlying CTFV induced pathology and severe complications remain unknown. Our previous work indicated that CTFV induces apoptosis in HMEC-1 cells. To gain a better understanding of CTFV-induced apoptosis, we investigated the mechanisms of apoptosis initiation in HMECs during CTFV infection. We first analyzed

the expression of key death receptors and ligands during CTFV infection in HMECs by qPCR and observed significant increases in gene expression for TRAIL and its receptors, DR4 and DR5, at 24 hours post infection (hpi). We then analyzed the protein expression of TRAIL in infected cells by western blot and observed a significant increase of protein expression at 24 hpi. We also analyzed the protein expression of BID and observed a decrease in full length BID at 24 hpi, indicating BID activation. Additionally, we analyzed the protein expression of caspase-9 by western blot and observed a decrease in full length caspase-9 at 48 hpi, indicating caspase-9 activation. Overall, our data suggests that the intrinsic and extrinsic pathways are activated to initiate apoptosis during CTFV infection in HMEC-1 cells. Further studies will examine if inhibition of these pathways will reduce CTFV-induced apoptosis in HMEC-1 cells.

GP24 **Priya Christensen**, Luke Joyce, Ziqiang Guan, Kelli Palmer. UT-Dallas. "Expression of diverse streptococcal MprFs and lipid hydrolase in Streptococcus mitis."

Streptococcus agalactiae (GBS) is a gram-positive pathogen that colonizes the gastrointestinal and lower genital tract. In GBS, the multiple peptide resistance factor (MprF) synthesizes a novel lipid, lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG), and the well-known lipid lysyl-phosphatidylglycerol (Lys-PG). Lys-PG reduces the negative charge of the membrane, protecting bacteria from cationic antimicrobial peptides. Additionally, GBS encodes a predicted hydrolase upstream of mprF. In Enterococcus faecium, this hydrolase is responsible for the turnover of Lys-PG. This project has two goals: to determine whether other MprF proteins from other streptococci also synthesize Lys-Glc-DAG, and whether the GBS hydrolase turns over both Lys-Glc-DAG and Lys-PG. Streptococcus mitis was chosen as a heterologous host for this study since it does not natively encode mprF and does not natively synthesize Lys-Glc-DAG or Lys-PG. Candidate MprF proteins from other streptococcal species (S. downei, S. orisratti, S. ferus) with high identity to GBS MprF were identified by BLASTp. These genes and the GBS hydrolase gene were inserted into a plasmid using Gibson assembly and transformed into S. mitis. Ongoing experiments include growth curves in THB and chemically defined medium, fluorescamine screening, and lipidomics analyses.

GP25 **Sarbjeet Niraula,** Christian Peterson, Mary Muhibi Asmaty, Sarobi Das, Sadie Ruesewald, Woo-Suk Chang. UT-Arlington. Effects of a drought-tolerant bio-inoculant on the soybean rhizosphere microbiome

Bioinoculants, microorganisms that promote plant growth and productivity, are a promising alternative to the chemical fertilizers in agricultural fields. Their use is rapidly increasing because of the nutrient solubilization activity and environment-friendly nature. However, little is known about their impacts on native soil microbiomes. In the previous study, we isolated a novel drought-tolerant Bradyrhizobium inoculant in South Texas. In this research, we evaluate the effects of the bioinoculant on the microbial community of bulk soil, rhizosphere, and root nodules of the soybean plant in the field condition. The field experiment was performed at a drought prone site at Yoakum, TX. Soil samples were collected during 7-weeks for evaluation of soil physicochemical properties and the microbiome analysis was done based on 16S rRNA (V3-V4) sequencing. We observed reduced evenness and a shift in rhizosphere bacterial communities in response to the inoculum treatment. The bioinoculant increased the proportions of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere, including other plant growth promoting rhizobia (PGPR) as well as the host endosymbiont B. japonicum. Similarly, it showed beneficial effects on the co-occurrence pattern of the bacterial community. Additionally, the proportion of mutually exclusive interactions and clustering coefficient was decreased, whereas the modularity and density of the network were increased. Our results suggest that this novel bioinoculant not only improves the crop yield but also enhances the quality of beneficial microbiomes in the

rhizosphere. Also, this study provides evidence of the mechanism by which the bio-fertilizer interacts with local bacterial communities to enhance plant growth.

## Undergraduate Poster Abstracts

UP1 **Austin De La Garza**, Anastasia Guseva, Breanna Edinger, and Daisy Zhang. Delmar College. "The Isolation and Characterization of the Bacteriophage 'Zaideros'"

The first bacteriophages were discovered in the early 20th century by a scientist named William Twort, about two years after initial discovery, another scientist named Felix d'Herelle realized bacteriophages had potential to infect bacteria. One of the first phage therapeutic applications took place in 1919 when d'Herelle and colleagues administered a bacteriophage cocktail to a few patients diagnosed with dysentery which may have led to their recovery. Isolation of the novel bacteriophage "Zaideros" began with a soil sample collection followed by an enrichment procedure to ensure adequate phage concentration for analysis which lead to the goal of characterizing an isolated phage using its bacterial host Mycobacterium smegmatis. A high titer lysate is produced and harvested for genomic DNA isolation. The restriction enzyme digest and genomic analysis were conducted using isolated genomic DNA. The phage morphology of "Zaideros" was observed with the use of uranyl acetate preparation and electron microscopy. The lysogen of 'Zaideros' was isolated from spot test with extra incubation and used for host survivability studies based on "Zaideros" infection. The plaques of 'Zaideros' may indicate a temperate life cycle at time of isolation. The TEM images depict 'Zaideros' presenting a capsid 80 to ~100nm in diameter and a tail of ~250 - 260nm in length. Lastly, efficiency studies concluded that "Zaideros" demonstrated 38% effectiveness towards host Mycobacterium smegmatis. This indicates that "Zaideros" may not be a proper candidate for phage therapy but may provide further insight on research regarding phages that share similar clusters.

UP2. **Francis Villarreal**, Michael Wilson. Texas State University. "SARS CoV-2 Variant Analysis Reveals Potential Evolutionary Constraints that Predict Essential Elements of the Viral Genome"

The global effort to control the SARS CoV-2 pandemic has resulted in an unprecedented, world-wide effort to sequence viral isolates as a public health tool for tracking the transmission of different variants. This massive compilation of genomic viral sequence data is a valuable scientific resource, curated by the GISAID database. We asked whether the types and frequencies of mutations that accumulate in different variants could reveal protein domains that are essential for viral propagation and transmission. We analyzed a dataset posted by Chen, J., Gao, K., Wang, R., and Wei, G that tabulated all point mutations in open reading frames of SARS CoV-2, as of June 9, 2021. We developed a Synonymous Enrichment Index (SEI) to compare the frequency of synonymous and nonsynonymous point mutations across all positions in the genome in order to identify loci that are more likely to have synonymous point mutations and less likely to have nonsynonymous mutations in comparison to other loci across the genome. The SEI pinpointed several genomic loci that have excellent potential for diagnostic marker development. RT-PCR primers and probes designed to amplify these loci have low mutation rates across the global repertoire of recently circulating viruses. Notably, the encoded protein domains of these loci are likely to be essential for viral replication or transmission because nonsynonymous mutations are poorly tolerated. One such locus (nucleotides 26,500-27,100) includes the three transmembrane domains of the membrane glycoprotein (M), which is proposed to help mediate SARS-CoV-2 membrane fusion and exploit the host cell's Er-Golgi intermediate compartment.

UP3. **Kezia Philip**, Michael Neugent, Jordan Owen, Julian Hurdle, Kelli Palmer. UT-Dallas. "Uncovering the Mechanism of Metronidazole Resistance in *Enterococcus faecalis*"

Enterococcus faecalis are gram-positive bacteria that normally colonize the gastrointestinal tract of humans. E. faecalis is also a problematic causative agent of several nosocomial infections, including urinary tract infections, bacteremia, surgical site infections, and endocarditis. E. faecalis are intrinsically resistant to metronidazole, a nitroimidazole antibiotic that remains a front-line choice for treatment of infections caused by anaerobic intestinal bacteria such as Clostridioides difficile. The main goal of this study is to underpin the elusive molecular mechanism behind E. faecalis resistance to metronidazole. Previous literature reported that E. faecalis and other enterococci can degrade metronidazole. We confirmed these results with gas chromatography-mass spectrometry. We further developed a simple and efficient spectrophotometric assay that provides a reliable and valid measurement of metronidazole in culture supernatants. The results from our assays confirm that E. faecalis can degrade metronidazole, whereas Enterococcus faecium cannot. Interestingly, E. faecalis does not appear to degrade 4-nitrobenzoic acid, which also has a nitro group, indicating some specificity in the degradation mechanism. In current work, we are deleting the 4 nitroreductase genes encoded by E. faecalis, with particular focus on type I oxygen-insensitive nitroreductases, to characterize their potential roles in metronidazole degradation.

UP4. **Marivel O. Escamilla**, Anastasia Guseva, Breanna Edinger, Daisy Zhang. Delmar College. "The Isolation and Characterization of the Bacteriophage "MariaIsabella7"

Bacteriophage is a virus that infects then replicates itself inside its host bacteria and are the most abundant form on earth. It has been estimated that there are over 1031 bacteriophages present on our planet and more and more phages have been used as vectors for gene therapy in addition to treatment for the infection caused by antibiotic resistant's. In this study, isolation of a novel bacteriophage "MariaIsabella7" began with a soil enrichment procedure, followed by several experiments to characterize the isolated phage using its bacteria host Mycobacterium smegmatis. A High-titer lysate Assay was created for phage genomic DNA isolation and five restriction enzymes. The restriction digest analysis was conducted using the isolated DNA. The phage morphology was determined by uranyl acetate negative staining and transmission electron microscope imaging. It showed characteristics of having both lytic and temperate morphologies and different plaque sizes. The lysogen was isolated from a spot test to produce mesa. The TEM images show "MariaIsabella7" contains a capsid with 60 nm in diameter and a tail 100 nm in length. The restriction digest patterns suggested the genome contains multiple recognition sites for the following enzymes BamH I, Cla I, and Hae III, but not for Hind III and EcoR I. The lysogen efficiency test indicated that 95% of the host could be destroyed by this bacteriophage, which makes "MariaIsabella7" a potential candidate to study phage treatment for tuberculosis.

UP5 **Robyn Alba**, Calvin Henard. University of North Texas. "Cloning and heterologous expression of a putative oxoacid::ferredoxin oxidoreductase from *Methylococcus capsulatus*."

Methylococcus capsulatus is a methanotrophic bacterium with the unique capacity to use both methane ($CH_4$) and carbon dioxide ($CO_2$) as carbon sources, but the metabolic pathways mediating $CO_2$ transformation are incompletely understood. Recent $^{13}CO_2$ tracing experiments indicate M. capsulatus rapidly produces α-ketoglutarate from $CO_2$, a tricarboxylic acid (TCA) cycle metabolite. Based on these results and bioinformatic analysis of carboxylating enzymes encoded by M. capsulatus, we identified a

putative oxoacid::ferredoxin oxidoreductase (Ofo) that could catalyze the carboxylation of succinyl-CoA to α-ketoglutarate. To characterize the catalytic properties of the enzyme, we cloned and heterologously expressed a 6xHis-tagged variant of the M. capsulatus Ofo in E. coli. Ofo expression experiments were conducted iteratively under varying IPTG concentrations and incubation temperatures to determine optimal protein expression parameters. The results indicate that optimal Ofo expression conditions are obtained by inducing protein expression during logarithmic growth (OD600 = 0.5) with 100 µM IPTG followed by cultivation at 16˚C for 20 hours. These data will guide future experiments targeting the purification and biochemical analyses of Ofo.

UP6

UP7 **Camille Sturges**, Yahan Wei, Kelli Palmer. UT-Dallas. "Heteroexpression of acyl transferase genes in mitis group streptococci"

Gram-positive bacteria Streptococcus mitis and S. oralis are common colonizers of human oral cavity and opportunistic pathogens that are among the leading causes of bacteremia and infective endocarditis (IE). Previously, both S. mitis and S. oralis were found to be able to synthesize phosphatidylcholine (PC) via scavenging extracellular lipid species such as lyso-PC and glycerophosphocholine (GPC), a process that is uncommon in bacteria. In human, Lyso-PC is not commonly present, as it is quickly metabolized into other phospholipids; as a comparison, GPC is commonly present in both blood and saliva and functions as a storage vessel for choline, an important nutrient for humans. To produce PC from GPC or Lyso-PC, acylation is needed. S. mitis and S. oralis contain three acyl transferase genes: plsX, plsY, and plsC, whose functions in PC biosynthesis have not yet been verified, and thus, the main goal of this study. Aiming at verifying the functions of the pls genes in PC biosynthesis, we heterologously expressed and purified mitis group streptococcal acyltransferases and test whether they could reconstruct the PC generation process. Specifically, we amplified the coding regions of the pls genes from Streptococcus sp. 1643, a mitis/oralis strain isolated from IE patient, inserted the amplicon separately into plasmid pET-28a(+) between the positions of XhoI and NcoI cutting sites, constructing 3 plasmids that individually express C-terminal His-tagged PlsX, PlsY, and PlsC. The successful constructed plasmids were verified with Sanger sequencing; and then transformed into Escherichia coli BL21 DE3 pLys for protein expression. Following future steps are completing the expression assay, purification of the proteins, and reconstruction of the PC biosynthetic process in vitro.

UP8 **Sri Snehita Reddy Bonthu**, Lauren E Boggs, Belle M Sharon, Philippe E Zimmern, Kelli L Palmer, Nicole J De Nisco. UT-Dallas and UT-Southwestern. "Growth phenotype assessment of clinical urinary strains of *Enterococcus faecalis* in artificial urine medium"

Enterococcus faecalis, a Gram-positive bacterium, emerges as an increasingly frequent cause of Urinary Tract Infection (UTI). UTIs are a significant healthcare burden affecting millions of people around the world annually. While E. faecalis natively inhabits the human gastrointestinal tract, persistence in the urinary tract despite environmental pressures suggests E. faecalis urine isolates may have acquired genetic adaptations facilitating growth in this environment. Studies of E. faecalis urinary fitness are limited and focus on blood isolates or oral isolates (OG1RF) grown in pooled urine. Further, experimentation in animal models of UTI is also most commonly performed with OG1RF. However, it is unclear if these commonly utilized isolates are phenotypically representative of urinary E. faecalis strains. In this study, we investigate the growth phenotypes of clinical urinary E. faecalis isolates in physiologically relevant media and culture conditions. This will provide insight into the heterogeneity of growth phenotypes between E. faecalis strains isolated from different anatomical niches and allow

identification of a representative model strain of urinary E. faecalis. We established a protocol for the assessment of growth rate and yield evaluating OG1RF in three types of culture media: modified Artificial Urine Medium (AUM), Tryptic Soy Broth (TSB), and Brain-Heart Infusion (BHI) broth. Our preliminary data indicates that OG1RF does not exhibit the same growth phenotype as clinical urinary isolates in modified AUM. Urinary isolates on average grow more rapidly and to higher densities than OG1RF, suggesting that OG1RF may not be the optimal model strain of E. faecalis in UTI research.

UP9 **Irene N Hau**, Neha V. Hulyalkar, Vivian H. Nguyen, Belle M. Sharon, Nicole J. De Nisco*. UT-Dallas. "Gain of function cytolysin variant expressed by clinically isolated urinary *Enterococcus faecalis*"

Enterococcus faecalis can cause serious nosocomial infections, of which urinary tract infection is the most common. Cytolysin, an enterococcal exotoxin and bacteriocin encoded by the cyl operon, has been reported to target various mammalian cells, including erythrocytes, macrophages, and neutrophils, as well as bacteria. Cytolysin is active against human, rabbit, and horse erythrocytes due to their high membrane phosphatidylcholine (PC) content, but sheep erythrocytes (SE) have been reported to be resistant.
We identified a urinary E. faecalis isolate, C33, that lysed SE, a phenotype not previously observed. To identify putative cytolysin genes, we resolved the complete, closed genome of C33 via hybrid assembly of Illumina and Oxford Nanopore reads. Genome mining identified the cyl operon on a pAD1-lineage plasmid. Gene deletion using markerless gene exchange approach confirmed the dependence of SE hemolytic activity on the cyl operon. Additionally, SE hemolysis became more pronounced as environmental oxygen was reduced, suggesting that expression of the C33 cyl operon may be regulated by oxygen tension. Since cytolysin has been previously reported to target bacterial cells, we hypothesized that the C33 cytolysin variant may be important for competition with the urinary microbiota. We observed that E. faecalis C33 inhibited the growth of Enterococcus raffinosus and Streptococcus parasanguinis isolated from the same urine. Additionally, under anaerobic conditions, C33 showed increased survival against Staphylococcus capitis, which was highly inhibitory in aerobic conditions. Future work will determine the mechanism underlying the C33 cytolysin gain-of-function against SEs and the urinary microbiota.

UP10 **Lauren Samudio**, Breanna Edinger, Anastasia Guseva, Daisy Zhang. Delmar College. The Isolation and Characterization of the Bacteriophage "Cerberuss"

Bacteriophages (named by Felix d'Herelle) are virus specific to bacteria in the way that it attacks and kills only the bacteria host. They are quite durable in any environment, in fact there is around 1031 bacteriophages present on Earth. Phages are quite important in the scientific community. They can be used for cloning, mutation, gene therapy, and treatment for antibiotic resistant bacteria. In this study, the isolation of bacteriophage 'Cerberuss' began with a soil enrichment procedure followed by several experiments using the bacteria Mycobacterium smegmatis as host. A high titer lysate was harvested for phage genomic DNA isolation. The restriction digest analysis was conducted using the isolated DNA. The phage morphology of 'Cerberuss' was studied by uranyl acetate negative staining and sent for transmission electron microscope imaging. The lysogen of 'Cerberuss' was isolated from a spot test with extra incubation and was used for phage efficiency on its host Mycobacterium smegmatis. The plaques of 'Cerberuss' indicated a lytic life cycle at the time of isolation. The TEM images show 'Cerberuss' contains a capsid with 70nm in diameter and a tail 290nm in length. The restriction digest patterns suggested the 'Cerberuss' genome contains multiple recognition sites for BamH I, Cla I, but not very many noticeable in Hae III, Hind III, and EcoR I. The lysogen efficiency test of indicated that 24.44% of

the host Mycobacterium smegmatis could be destroyed by the bacteriophage “Cerberuss”, which makes it a good candidate for study gene therapy using phage vectors.

UP11 **Justin Daniel Segura**, Anastasia Gruseva, Breanna Edinger and Daisy Zhang. Delmar College. The Isolation and Characterization of the Bacteriophage ‘JustinDaniel’

A bacteriophage is a virus that specifically infect bacterium in order to replicate. Bacteriophages are among the most common and diverse entities found on earth, it’s estimated there are more than 1031 bacteriophages on the planet making them the most abundant organism in the biosphere. Phage therapy, also known as bacteriophage therapy, has been used to treat antibiotic-resistant bacterial infections. Phage ‘JustinDaniel’ began with a soil enrichment procedure, followed by several experiments to isolate the phage by using the bacterial host Mycobacterium smegmatis. A high titer lysate experiment was produced for phage genomic DNA isolation. The structural morphology of ‘JustinDaniel’ was captured by uranyl acetate staining for transmission electron microscope imaging. The lysogen of ‘JustinDaniel’ was isolated from a spot test plaque and used for further efficiency studies. The plaques of ‘JustinDaniel’ indicate a lytic life cycle at the time of isolation, with TEM images of ‘JustinDaniel’ consisting of a tail length of 220nm and a capsid of 80nm in diameter. The restriction digest patterns suggest that ‘JustinDaniel’ genome contains particularly well known prokaryotic endonucleases. The lysogen efficiency of ‘JustinDaniel’ indicated that near 100% of the host Mycobacterium smegmatis may be lysed using this bacteriophage; suggesting that it may pose as a potential candidate to develop a phage treatment for Mycobacterium tuberculosis infection, a close species to Mycobacterium smegmatis.

UP12 **Bryce Jones**, A. Gutierrez-Cano, J. Macario, S. Martin, K. Boundy-Mills, C. Edwards. St. Edward’s University. Growth of *Saccharomyces cerevisiae* 09-448 Under Industrially Relevant Stressors

Finding more sources of mass for ethanol production increases the efficacy of biofuels; one such source is known as pectin-rich biomass. Pectin-rich biomass is normally treated as a waste product. One yeast strain, known as 09-448, is able to break down pectin and free the sugars that yeast can then ferment. In using 09-448, the enzymes normally needed to break down pectin are no longer needed, making the process more cost effective. In this study, the yeast strain 09-448 is compared to an industrial Active Dry Yeast strain (ADY) as a control to compare how 09-448 is able to survive under industrial conditions and understand its limitations. Industrial conditions tested were pH, ethanol concentrations, and their efficiency in using different sugars as a carbon source. To quantify the growth rates, a plate reader was used to compare the rates of growth of the respective strains under these stressful conditions, taking the OD600 every 15 minutes. ADY tended to grow better than 09-448 in industrial conditions, with better generation times and higher optical densities. In conjunction, 09-448 was grown separately in stressful conditions in an attempt to help it along in its evolution to adapt to industrial conditions; 09-448 was able to begin adapting better to stressor conditions. Such results demonstrate that normally discarded biomass could be used as a source of biofuel. Increasing ethanol production from the fermentation of corn alone is unsustainable, and new biomass sources can make the concept of renewable biofuel a more attractive alternative to fossil fuels.

UP13 **Kinsey Morris** and Angela Mitchell. Texas A&M. “Lipid Substrate for the Synthesis of Phospholipid-Linked Enterobacterial Common Antigen”

Gram negative bacteria exhibit extreme levels of impermeability towards environmental stressors, particularly antibiotic entry, due to the possession of an impervious outer membrane (OM).

Enterobacterial common antigen or ECA, a carbohydrate antigen conserved throughout all Enterobacterales envelopes, widely contributes to membrane integrity and permeability. ECA can further be differentiated into three types: ECALPS, ECACYC, and ECAPG. The predominant linear form of ECA on the cell surface is ECAPG, which consists of an ECA polysaccharide chain covalently attached via phosphodiester linkage to a diacylglycerol. In synthesis of ECAPG, the ECA polysaccharide chain is transferred from an isoprenoid carrier to a phospholipid anchored within the inner membrane, and yet the substrate which facilitates this reaction remained unknown. Reporter assays, ECA Western Blots, and WGA stainings were performed to narrow down the substrate for this inner membrane transfer reaction. These experiments specifically investigated the following phospholipid biosynthetic genes: cdsA, pgpA, clsC and pssA. clsC and pssA displayed low amounts of ECAPG production and a slightly increased ECA promoter activity, thus these genes were considered off pathway from the lipid substrate in question. cdsA showed copious amounts of ECAPG production with normal promoter activity, thus it was determined to be upstream of the lipid substrate. Finally, pgpA displayed increased amounts of ECAPG production but showed slightly decreased promoter activity, thus this gene was determined to immediately upstream of the substrate, indicating the substrate is phosphatidylglycerol. Future directions include performing these experiments with deletion and depletion strains to verify phosphatidylglycerol as the lipid substrate.

UP14 **Utkarsh Singh**, Anne Gaillard, and Madhu Choudhary. Sam Houston State University.
Identification of programmed cell death genes in *Chlamydomonas reinhardtii*

This research proposal focuses upon unicellular green alga Chlamydomonas reinhardtii, which has been experimentally used as a powerful model to dissect various biological processes at a unicellular level. Throughout the past decade, within these unicellular eukaryotes and prokaryotes, evidence of programmed cell death has been identified as response to apoptosis-like signals within multicellular animals. The exact form of the programmed cell death pathway seems to have evolved as a result of evolutionary divergence; however, it is evident that the pathways have been conserved to a certain degree. For example, p53 like protein, Ehp53, was identified within unicellular Entamoeba histolytica. Furthermore, protozoan Leishmania donovani and mutant Saccharomyces cerevisiae strain cdc48 have both shown signs of programmed cell death in terms of key indicators such as DNA fragmentation and chromatin condensation, both key stages within apoptosis induced cell death. Chlamydomonas reinhardtii also shows these indicators when placed under high-stress conditions, displaying responses similar to apoptosis within animals.

The objective of this study is to identify whether protein homologs of p-53 and other genes related to PCD are present within Chlamydomonas reinhardtii. Since p53 is an essential regulator for programmed cell death within multicellular eukaryotes as a response to stress, we hypothesize that p-53 homologs or p-53 itself will be found within C. reinhardtii using PSI-BLAST and PHI-BLAST protein searches. We further hypothesize that the programmed cell death genes previously identified within C. reinhardtii will contain conserved domains in comparison to human p53.

## Faculty and Other Professional Poster Abstracts

FP1 **Sheuli Zakia**, Sandy Zhang, Janet Gonzalez, Cristine Clement, Manfred Philipp. Laredo College, Weill-Cornell Medical Center, Pelham High School, LaGuardia Community College, Lehman College & the Graduate Center, the City University of New York
“A New Phylogenetic and Sequence Analysis of metallo-β-Lactamases”

Metallo-β-lactamases are important causative agents of bacterial resistance to β-lactam antibiotics. In contrast to lactam resistance associated with Class A, C, and D β-lactamases, there is a paucity of clinically-accepted treatments for lactam resistance that is related to the expression of metallo-β-lactamases.

Metallo-β-lactamases (Ambler Class B) are generally quite uniform among very different bacterial species, indicating rapid spread of their genes among those species. One hundred protein structure depositions are available for the NDM-1 β-lactamase. However, the VAST database (https://www.ncbi.nlm.nih.gov/Structure/vastplus) shows that there are many other closely related structures in the pdb databases that show very high three-dimensional positional alignment values (over 200 out of 231 amino acids) but very low sequence identity. These variant proteins show positional RMS deviations as low as 1.9 Angstroms at as little as 22% sequence identity, an RMS value close to the experimental error of the structure determinations.

This study examines the structural variations and phylogenetic relationships among these proteins.

FP2 **Matthew Barker** and Chiung-Yu Hung. UT-San Antonio. "*Galleria mellonella* model for the study of *Coccidioides* infection, virulence and antifungal drug susceptibility test"

Coccidioides immitis and Coccidioides posadasii are dimorphic fungal pathogens that can cause a life-threatening respiratory disease in both healthy and immunocompromised individuals. Our goal is to establish a cost-effective Galleria mellonella larva model for high throughput screening of Coccidioides virulence factors and potential drug candidates. The larvae are able to survive at 37 ºC and they require no special housing equipment, make them suitable for studies of human infectious diseases. G. mellonella larvae are susceptible to Coccidioides infection with a lethal dose around 5x105 viable spores administrated by the haemocoel route. Notably, Coccidioides spp. can covert to spherules within the Galleria larvae to complete their parasitic life cycles that simulate in mammalian hosts. The larvae were monitored for fungal burden, melanization and mortality for a period of 7 days postchallenge. Results showed that the melanization score peaked at 2-3 days postchallenge with a mean of survival for 4 days. We further apply this model to screen a Coccidioides mutant library that is created by random Ti plasmid integration. We identified four Coccidioides mutants that displayed various degrees of reduction in their melanization and mortality. These mutants demonstrated similar reduced virulence in murine models of pulmonary coccidioidomycosis. Furthermore, we assessed therapeutic efficacy of known antifungal drugs administrated 2 hr postchallenge using this newly established larva model. Two clinically approved antifungal drugs are effective against a potentially lethal challenge with Coccidioides spores. These findings suggest that G. mellonella is a useful in vivo model of coccidioidomycosis and convenient for pre-screening assays for the identification of fungal virulence factors and novel antifungal drugs.