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Graduate Oral Presentations

Medical Microbiology

GO1 Redefining antibiotic resistance in *Acinetobacter baumannii*

Muneer Yaqub, Chinmai Rudra, Zoe Du, Reagan Crow, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Acinetobacter baumannii infections pose significant human health concerns due to their increasing resistance to antibiotics, particularly Polymyxin E (colistin), a last-resort treatment. As colistin resistance rates are still low in *A. baumannii*, the mechanistic basis for colistin resistance in *A. baumannii* is unclear. To address this, we employed a machine learning model trained on clinical *A. baumannii* isolates, identifying 31 genes linked to colistin resistance. To assess their impact, we employed the Manoil AB5075 transposon library and conducted standardized antimicrobial susceptibility testing on each transposon mutant. None of the 31 *A. baumannii* mutants exhibited increased resistance to colistin when assessed based on their Minimum Inhibitory Concentration (MIC). However, further investigation into the relationship between these genes and resistance revealed intriguing results. Although MIC values showed no significant differences, we observed distinct adaptive fitness phenotypes among the mutants in response to colistin. Approximately 48% of the mutants displayed heightened fitness, while 26% exhibited decreased fitness when exposed to sub-inhibitory colistin concentrations. Moreover, we found that most mutants show alterations in membrane properties, biofilm formation, efflux pump activity, and oxidative stress response in the presence of colistin. These findings suggest that routine susceptibility tests based solely on MIC values may not fully capture A. baumannii's adaptive responses to colistin. Consequently, our study emphasizes the need for comprehensive assessments beyond MIC measurements to accurately define colistin resistance in *A. baumannii*. By revealing the intricate adaptive responses of *A. baumannii* to colistin, our research urges reevaluation of how we define antibiotic resistance in bacterial pathogens.

GO2 Cytotoxic rhamnolipid micelles drive acute virulence in *Pseudomonas aeruginosa*

Donghoon (Alex) Kang, Qi Xu, Natasha Kirienko

Rice University, Houston, TX, USA

Abstract

Pseudomonas aeruginosa is a multidrug-resistant, Gram-negative 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 secreted toxins and disrupts host iron and mitochondrial homeostasis. Here, we developed an in vitro human lung epithelial cell culture model to further elucidate the role of pyoverdine during infection. Conditioned medium from *P. aeruginosa* caused acute cell death and severe damage to the epithelial monolayer in a pyoverdine-dependent manner. Interestingly, this damage did not require host iron chelation nor production of known pyoverdine-regulated toxins. Instead, pyoverdine promoted the production of cytotoxic rhamnolipids. Chemical depletion of secreted lipids or genetic disruption of rhamnolipid biosynthesis was sufficient to abrogate conditioned medium toxicity. Importantly, the pyoverdine biosynthetic inhibitor 5-fluorocytosine was able to effectively limit rhamnolipid production and mitigate *P. aeruginosa* virulence in this model.

We also examined the structural properties of rhamnolipids by TEM and LC-MS and tested their activity against other host membranes. Secreted rhamnolipids assembled into micelles that were highly cytotoxic to murine macrophages, rupturing the plasma membrane and causing organellar (mitochondrial) membrane damage within minutes of exposure. These micelles were also capable of damaging erythrocytes, Gram-positive bacteria, and non-cellular models like giant plasma membrane vesicles. Most importantly, rhamnolipid production strongly correlated to *P. aeruginosa* virulence in several panels (cystic fibrosis, blood-stream infections) of clinical isolates. Altogether, our findings demonstrate that rhamnolipid micelles drive acute host damage during *P. aeruginosa* infections.

GO3 Uncovering the mechanism behind metronidazole inactivation in *Enterococcus faecalis* and its role in protecting metronidazole-susceptible bacteria

Muqaddas Amer[1], Jacob Rappazzo[1], Kezia Phillip[1], Michael Neugent[1], Jordan Owen[2], Ziqiang Guan[2], Julian Hurdle[3], Isaac Prah[3], Kelli Palmer[1]

[1]UTD, Dallas, TX, USA
[2]Duke University, North Carolina, TX, USA
[3]Texas A&M Institute of Biosciences and Technology, Houston, TX, USA

Abstract

The 5-nitroimidazole antibiotic metronidazole is used for the treatment of obligate anaerobic bacterial infections. *Enterococcus faecalis* is a Gram-positive commensal bacterium and opportunistic pathogen that typically resides in the gastrointestinal tract and is intrinsically resistant to metronidazole. We have determined that *E. faecalis* and some other enterococcal species inactivate metronidazole and other nitroimidazoles. The main goal of this study is to elucidate the molecular mechanism by which *E. faecalis* and other enterococci can inactivate metronidazole, as well as to verify if *E. faecalis* can contribute to the proliferation of metronidazole-susceptible bacteria by protecting them from metronidazole. By screening of transposon mutants and lipidomics, we found that the mechanism is menaquinone-dependent and that the Extracellular Electron Transfer (EET) pathway may be required for metronidazole inactivation. We further developed an efficient anaerobic co-culture assay to provide us with reliable and valid assessments if *E. faecalis* can protect metronidazole-susceptible bacteria, in this case *Escherichia coli*, in vitro. The results from our assay confirm that *E. faecalis* can protect *E. coli* from metronidazole in vitro, whereas transposon mutants with insertions in certain EET genes, as well as some other enterococci, i.e. *E. faecium* and *E. dispar*, have a diminished ability or are unable to protect. Our results suggest that *E. faecalis* in the human intestine may protect anaerobic pathogens from killing by metronidazole.

GO4 Environmental context alters antibiotic susceptibility: implications for clinical antibiotic susceptibility testing

Caroline Black, Catherine Wakeman

Texas Tech University, Lubbock, TX, USA

Abstract

While certain aspects of the host-pathogen interface are common to all sites of infection, other factors can be highly specific to the localized environment. For example, chronic infections are often polymicrobial with the specific microbial consortia present varying greatly between patients. Additionally, nutrient and atmospheric composition shifts between different sites of infection. Herein, we demonstrate that antibiotic susceptibility profiles of pathogens can dramatically shift with changes in the microbial consortium, oxygen levels, or carbon source availability. Specifically, we demonstrate that *Enterococcus faecalis* grown in a polymicrobial community containing other common wound pathogens (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*) demonstrated increased susceptibility to gentamicin due to heme cross-feeding allowing more gentamicin to enter the cell via altered proton motive force. However, performing the same AST in anaerobic conditions reversed this phenotype. When *E. faecalis* was grown in community with *A. baumannii*, it exhibited decreased susceptibility to cephalexin as *A. baumannii* likely produces a beta-lactamase allowing for the neutralization of the antibiotic. Further research demonstrated that environmental conditions, like available carbon sources, can also influence antibiotic susceptibilities. *P. aeruginosa* exhibits increased susceptibility to kanamycin when grown in minimal media containing glycerol, as compared to minimal media with malonate, but decreased susceptibility to ciprofloxacin. Overall, these results demonstrate that environmental conditions, such as community members and available nutrient sources, play a role in determining an individual bacterium's antibiotic susceptibility. By accounting for the infection environment when determining antibiotic susceptibilities, we can more effectively treat persistent infections, leading to improved patient outcomes.

GO5 Exploiting cooperation to fight resistant *Pseudomonas aeruginosa* infections *in vivo*

Emily Vanderpool[1,2], Alper Mutlu[3], Ashleigh Griffin[3], Kendra Rumbaugh[1,2]

[1]Burn Center of Research Excellence, Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX, USA
[2]Department of Immunology and Molecular Microbiology, Texas Tech University Health Sciences Center, Lubbock, TX, USA
[3]Department of Biology, University of Oxford, Oxford, UK

Abstract

Antimicrobial resistant infections present a pressing challenge, burdening individuals and straining the healthcare system. Their prevalence is on the rise, necessitating innovative solutions. Exploiting pathogen cooperation offers a promising avenue to render resistant infections susceptible to antibiotics that has yet to be validated experimentally. Therefore, in our study, we investigated if a *Pseudomonas aeruginosa* quorum-sensing cheat could sensitize a resistant population to antimicrobials, both in vitro and in vivo. Our findings confirm that the cheat invaded resistant populations, resulting in heightened antibiotic susceptibility. This breakthrough supports the viability of Trojan cheats as a therapeutic strategy against resistant infections, addressing a critical need in modern healthcare.

GO6 Gut microbiomes and a fermented soybean product: monitoring changes of microbial communities in human gut

Shannon Moncier, Brynna Miljenovich, Jordyn Waters, Ai-Vy Dinh, Woo-Suk Chang

The University of Texas at Arlington, Arlington, TX, USA

Abstract

The gastrointestinal tract is home to many complex and diverse microorganisms and their genetic material known as the microbiome, which is crucial to our overall health and well-being. Each person's microbiome is unique and can be influenced by many factors, such as age, gender, and diet. In this study, we used a fermented soybean product (FSP) called Cheonggukjang to find out if it affects the gut flora, such as an increase or decrease in particular microorganisms, and which microbial communities in the gut are affected when the change occurs. A total of 40 participants in the study submitted fecal samples before and after consuming FSPs. The fecal samples were homogenized, and genomic DNA (gDNA) was extracted, prepared, and shipped to the SeqCenter (www.seqcenter.com) for 16S rRNA sequencing with targeting the V3/V4 region. The sequences were analyzed using microbial composition at the phylum level, and alpha and beta diversity at the species level. As a result, three of the most abundant phyla, Firmicutes, Actinobacteria, and Bacteroidetes, were identified across the participants. In addition, we observed differences in alpha diversity amongst the group of participants, and an overall decrease in beta diversity.

GO7 *Borrelia burgdorferi* BosR binds small non-coding RNAs (sRNAs): Implications for borrelial post-transcriptional gene regulation and pathogenesis

Brittany Shapiro[1], Prashant Jaiswal[1], Taylor Van Gundy[2], Sourav Roy[3], Brandon Garcia[3], Meghan Lybecker[2], Jenny Hyde[1], Jon Skare[1]

[1]Texas A&M, Health, Bryan, TX, USA
[2]Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Disease, Ft. Collins, CO, USA
[3]East Carolina University, Greenville, NC, USA

Abstract

Post-transcriptional regulation is a mechanism that modulates protein levels in living systems in a dynamic manner. In the case of *Borrelia burgdorferi*, the etiologic agent of Lyme Disease, gene expression changes are a hallmark of the bacterium moving between the arthropod vector and infected hosts. However, the details of how *B. burgdorferi* adapts to host-specific signals are still being determined. Previously, BosR was identified as a global regulator that affects *rpoS*, a master switch for infectivity-associated mammalian-specific gene expression. These studies demonstrated that a *bosR* mutant dramatically reduced RpoS, decreasing global RpoS-regulation, including the virulence-associated *ospC* locus, which is essential for the establishment of mammalian infection. Recent data indicates that BosR also serves as a chaperone for small non-coding RNAs (sRNAs). We hypothesize that BosR-bound sRNAs provide an additional layer of regulation to modulate responses needed to adapt to their environment appropriately. Specifically, BosR-bound sRNAs are predicted to target mRNA transcripts, resulting in either their degradation or enhanced translation. This adds new BosR-mediated post-transcriptional regulation to borrelial pathogenesis. Herein, we demonstrate that BosR binds to several sRNAs, including SR0735. We genetically inactivated SR0735 and found that cells lacking this sRNA alter virulence-associated protein production, resulting in an attenuated mammalian infectivity phenotype. This suggests that SR0735 targets transcripts important for virulence-associated function(s). Characterizing the mechanisms of BosR::sRNA::mRNA interactions provides a new perspective regarding the exploitation of a post-transcriptional regulation scheme in *B. burgdorferi* to modulate regulatory responses required for optimal borrelial infectivity and pathogenic properties.

GO8 Host-specific adaptations in a ubiquitous zoonotic pathogen

Chahat Upreti, Kelli Palmer

UT Dallas, Richardson, TX, USA

Abstract

A key contributor to host-microbe interactions is the ability of a microbe to survive in multiple host species. This ability allows us to categorize microbes as specialists – colonizing a limited range, and generalists – that colonize a large variety, of hosts. In addition to gaining insights into bacterial physiology, studying host-associated bacteria from this perspective allows us to determine the likelihood of their zoonotic transmission. A key player associated with zoonotic transfer is *Enterococcus faecalis* – a Gram-positive bacterium ubiquitously found among humans, animals, and birds. Recent work looking at *E. faecalis* host preference has claimed it to be a generalist, although studies have also shown patterns of host preference across different sequence types. Additionally, most of these studies are limited in sample size, their spread across time, and host diversity – with a major bias towards human-origin strains. Here, we investigate over 1,600 *E. faecalis* samples isolated exclusively from non-human sources spanning a timescale of 1987-2023, to explore their host adaptation landscape. Phylogenetic analysis revealed genetic similarities in strains originating from the same host across time and geography. To further probe host preference, we examined genes enriched in specific hosts and found several, including an uncharacterized gene enriched in dairy isolates containing a WxL domain, known to mediate cell-surface binding. We analyzed the metabolic profiles of these strains and found that strains residing in pigs showed enrichment in genes responsible for polyphenol degradation. Together, our work elucidates potential host adaptation strategies employed by a ubiquitous pathogen to survive a variety of hosts.

GO9 Microbial Modulation of Aggression in Crickets

Christian Guerrero, Amanda Brown

Texas Tech University, Lubbock, TX, USA

Abstract

Across animals, the gut microbiome has long been investigated for its role in impacting host body functions. Microbiota can synthesize metabolites such as neurotransmitters, short-chain fatty acids, and other compounds that act on the brain. In mammals, we know microbiota can send signals to the brain via several routes involving the immune system, vagus nerve, and enteric nervous systems, but little is known about these signaling routes in invertebrates. Recent studies suggest that gut microbes modulate resistance and mortality in eusocial insects, including bees, but microbial modulation of behavior still needs to be investigated in insects. Our goal was to examine mechanisms by which gut microbiota modifies insect behavior. Using the aggressive repertoire of the cricket (*Acheta domesticus*), we tested the effects of altering the gut microbiome to track how dysbiosis shifts cricket fighting behavior. To change intestinal microbiota composition, we used *Bifidobacterium longum*, which we predicted would participate in the synthesis of γ-aminobutyric acid (GABA), and Vancomycin antibiotic treatment to diminish *Actinomycetota* while maintaining low bioavailability. Crickets were fought multiple times before and after treatments. We found that vancomycin-treated crickets tended to be more aggressive than probiotic-treated and control crickets. We also found that probiotic-treated crickets were less often the aggressor in the interaction. Future studies will measure metabolites and microgeography of neuroactive microbiota in these treatment groups to investigate mechanisms and pathways associated with these microbial changes modifying behavior to broadly understand unifying processes in the microbe-gut-brain axis of mammals and invertebrates.

GO10 Phenotypic effect of capsule deletion on *Streptococcus pneumoniae* sRNA mutant in the presence of zinc

Spencer Reiling[1], Nicholas De Lay[2]

[1]University of Texas at Arlington, Arlington, TX, USA
[2]UTHealth Houston, Houston, TX, USA

Abstract

Streptococcus pneumoniae causes several illnesses in humans including pneumonia, meningitis, and sepsis, and is becoming increasingly resistant to antimicrobial drugs. We have found that deleting small RNA genes (ccnA, ccnB, ccnC, ccnD, and ccnE) from this pathogen attenuates its virulence in a mouse model of invasive pneumococcal disease. An important virulence factor of *S. pneumoniae* is its variable capsular polysaccharide. Previous research has also identified the importance of zinc in protecting the host from *S. pnemoniae* infection. In larger concentrations, Zn(II) blocks manganese uptake and outcompetes Mn(II) in several enzymes, including superoxide dismutase, which leads to oxidative stress. Our goal was to identify the importance of capsule on *S. pneumoniae* Zn(II) resistance and the contribution of the Ccn sRNAs to capsule production. We first constructed clean knockouts of polysaccharide genes (cps2ABCDETFGH) in *S. pneumoniae* strain D39 and a derived ΔccnABCDE strain. We then tested the ability of these mutants and their parent strains to grow with and without 0.2mM Zn(II) by measuring OD_{620} over 8 hours. We then examined the amount of polysaccharide produced and measured the abundance of sugars present. These results were normalized to the level of total protein our strains synthesized. Our studies found that the Δcps2ABCDETFGH mutants produce far less polysaccharide as their capsular proficient parent strain and that deletion of capsule genes restores Zn(II) resistance in an *S. pneumoniae* strain that lacks ccnABCDE. This suggests that capsule affects the internal Zn:Mn ratio possibly by reducing Mn uptake and thus Zn resistance.

GO11 Thermal shifts drive structural adaptations of *Pseudomonas aeruginosa* biofilms in different environments

Alex Luecke[1], Karishma Bisht[2], Catherine Wakeman[1]

[1]Texas Tech University, Lubbock, TX, USA
[2]Princeton University, Princeton, NJ, USA

Abstract

Biofilms, a community of microbial cells encased in a substance called the Extracellular Polymeric Substance (EPS), are present in every biome on the planet including bodies of water, soil, industrial pipes, and human host. In chronic infections, biofilms pose a great threat due to their increase in tolerance to antibiotics and the host's immune response. As bacterial species transition from one environment to another, does the biofilm they form change as well? If so, how can these changes be exploited to improve our control of microbial growth?

To address these questions, we studied biofilms of the opportunistic pathogen *Pseudomonas aeruginosa*, which are known to thrive in a range of environments, including plant and human hosts. Specifically, we wanted to understand how the biofilm structure changes as the pathogen transitions from temperatures relevant to plant infection (23˚ and 30˚C) to warmer temperatures relevant to human infection (37˚ and 40˚C). In published studies, we have identified EPS changes associated with temperature shifts in *P. aeruginosa* PA14 and uncovered novel proteins important for biofilm formation at specific temperatures. Now, we are elucidating the function of two novel proteins important for biofilm formation exclusively at plant-associated temperatures but not human host-relevant temperatures and quantifying the temperature-responsive genetic expression of PA14 to ascertain any biofilm associated pathways that are upregulated/downregulated in response to temperature. Further studies of the temperature-dependent biofilms may elucidate novel drug targets unique to these phenotypic adaptations.

GO12 Investigating the effects of glufosinate on microbial communities in soil

Sarobi Das, Renee Ralston, Dr.Woo-Suk Chang

University of Texas at Arlington, Arlington, TX, USA

Abstract

Weeds are unwanted vegetation in agricultural fields that pose a significant threat to crop health and yield due to shared niches and resource depletion. Therefore, effective weed management is of importance in agriculture, and herbicides have been an efficient and cost-effective means of weed control. Among them, glufosinate has been brought to attention for being used as an alternative to glyphosate due to weeds' increasing resistance against glyphosate. Glufosinate is a nitrogen-based, broad-spectrum herbicide that is predominantly available in ammonium salt form and is largely produced by *Streptomyces hygroscopicus* and *Streptomyces viridochromogenes*. However, little is known about the effects of glufosinate on the dynamics of the soil microbiome. In this study, we conducted a mesocosm experiment to investigate the effects of glufosinate on microbial communities in soil. We also observed one-time vs. two-times of glufosinate applications in soil over a period of 30 days. Soil samples were collected at 0, 0.5, 3, 6, 6.5, 9, 15, and 30 days after treatment (DAT) for 16S rRNA gene sequencing and community analysis. In addition, we conducted an experiment to quantify the herbicide's persistence in the soil at 30 DAT. In conclusion, this study provides an insight into the impact of glufosinate on soil microbial communities and the efficacy of the herbicide over crop cycles.

Undergraduate Oral Presentations

UO1 Effects of a novel climate-smart approach associated with a drought-tolerant *Bradyrhizobium* inoculant on soybean cultivation in South Texas

Jordyn Waters, Angelica Ponce, Johnson Nguyen, Woo-suk Chang

University of Texas at Arlington, Arlington, TX, USA

Abstract

The soil bacterium *Bradyrhizobium japonicum* is an agriculturally important microbe because of its nitrogen-fixing capability and symbiotic relationship with the soybean plant (Glycine max). The *Bradyrhizobium*-soybean symbiosis results in the formation of root nodules where *B. japonicum* converts atmospheric nitrogen into ammonia, which the plant utilizes for growth. Thus, there is immense potential to reduce the use of synthetic nitrogen fertilizers by applying this bacterium as an inoculant in crop fields. Drought has an unfavorable effect on the plant, inhibiting the symbiotic relationship. Our laboratory previously isolated a Texas-native drought-tolerant strain, *B. japonicum* spp. TXVA, and optimized its application in soybean fields in the Mid-South. Here, we present our field study conducted in Port Lavaca, Texas, using a variety of soybean cultivars to measure the effectiveness of TXVA-associated climate-smart practices vs. non-inoculation controls. The analysis evaluated included root nodule comparisons, plant biomass, soil-physicochemical analysis, plant tissue analysis, and soybean yield. We hypothesize that the application of TXVA in South Texas will positively impact plant growth, nutrient availability, and therefore the final soybean yield.

UO2 Envelope stress responses functionally coordinate to maintain cell homeostasis in *Escherichia coli*

Ha Do[1,2], Anna Konovalova[2]

[1]University of Houston - Downtown, Houston, TX, USA
[2]University of Texas Health Science Center, Houston, TX, USA

Abstract

The envelope stress responses (ESRs) are essential pathways that maintain cell integrity and protect bacteria from antibiotics. Two critical ESRs that monitor outer membrane integrity in Escherichia coli are the Regulator of Capsule Synthesis (Rcs) and the extracytoplasmic σE response. Rcs and σE survey lipopolysaccharide (LPS) and outer membrane protein (OMP) biogenesis and are often induced under the same conditions. My project investigates potential regulatory feedback between them. Using σE-dependent transcriptional fusions, I showed that Rcs stimulates σE activity. Next, I focused on the mechanism underlying this stimulation. σE activity is regulated post-translationally by the anti-σ factor RseA, which is degraded in response to OMP defects, allowing free σE to regulate gene expression. My genetic epistasis analysis demonstrated that not only Rcs acts independently of RseA, but can also act synergistically with the RseA degradation pathway to stimulate σE activity.

σE - encoding gene, *rpoE*, forms an operon with *rseA*, and their expression is highly regulated. Previous studies implicated RcsB in controlling one of the minor *rpoE* promoters. I generated several transcriptional reporter fusions and showed that when Rcs is strongly activated, it indeed upregulates the expression of the *rpoE-rseA* operon. We hypothesize that by doing so, Rcs increases the amount of the σE protein that is made, while the RseA degradation pathway increases the fraction that is active, enabling a synergistic response. As LPS and OMPs are the major components of the outer membrane, this Rcs/σE regulatory feedback may help coordinate outer membrane homeostasis as a whole.

UO3 Building the urinary *Proteus mirabilis* pangenome

Serena Zhou[1], Braden Shipman[1], Belle Sharon[1], Phillipe Zimmern[2], Nicole De Nisco[1,2]

[1]Department of Biological Sciences, University of Texas at Dallas, Richardson, TX, USA
[2]Department of Urology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Abstract

Every year, hundreds of millions of individuals are affected by urinary tract infection (UTI). UTI significantly decreases quality-of-life and can cause severe complications such as sepsis and pyelonephritis. *Proteus mirabilis* is a Gram-negative bacterium that causes both complicated and uncomplicated UTI. We have previously shown that *P. mirabilis* degrades glycosaminoglycans (GAGs) that comprise the urinary tract mucosa, which we suspect are used as carbon source. Interestingly, we observed that urinary *P. mirabilis* isolates exhibit a high degree of variability in efficiency of GAG degradation and utilization. In light of these findings and the lack of focused genomics studies of urinary *P. mirabilis*, we aimed to perform an in-depth genomic analysis of *P. mirabilis* to analyze genetic variation within GAGase encoding genes and to discover genomic factors associated with urinary colonization.

We built a *P. mirabilis* genome database comprised of closed genomes of isolates collected from postmenopausal women with a history of rUTI, as well as publicly available high-quality complete and scaffold genomes. We used ResFinder to identify antibiotic resistance genes, as well as ANIclustermap to determine average nucleotide identity (ANI) and cluster related genomes. We grouped isolates by isolation source and location, and will build site-specific pangenomes that will be used to identify genomic factors associated with urinary colonization. Finally, because *P. mirabilis* still lacks a universal multi-locus sequence typing (MLST) database, we will use our core-genome analysis in combination with pyMLST to construct a *P. mirabilis* MLST database and devise a publicly available sequence typing scheme for the species.

Graduate Poster Presentations

Antimicrobials

GP1 Investigation of the role of soluble species in the antibacterial mechanism of zinc oxide against *Staphylococcus aureus*

Alexander J. Caron, Michael J. Delgado, Yuri Strzhemechny, Shauna McGillivray

Texas Christian University, Fort Worth, TX, USA

Abstract

As the burden of antibiotic-resistant infections continues to rise, there is a dire need for the development of new antibiotics to treat these infections. Recent developments in nanoscale engineering of metal oxide nanoparticles (NPs) have provided a new path forward for antibiotic development. In particular, zinc oxide NPs represent a promising antibacterial agent due to its suitable antibacterial ability, low toxicity to mammalian cells, and stability at room temperature. Despite work demonstrating its broad-spectrum application against diverse bacterial species, the antibacterial mechanism is unknown. Previous work has implicated the role of reactive oxygen species (ROS), toxic ionic species, and physical disruption of the cell envelope as possible mechanisms of action. To evaluate the role of these proposed methods, ZnO susceptibility assays using several S. aureus mutants were conducted. These assays demonstrated that the production of ROS and electrostatic interactions are not crucial to mediating ZnO NP toxicity. Additionally, we found that physical contact between ZnO NPs and the bacterial envelope was not necessary for ZnO-induced growth inhibition, implicating the role of a soluble species in the antimicrobial mechanism. These data coupled with investigation of the species that are present in the ZnO conditioned supernatant implicates accumulation of toxic Zn2+ released from the ZnO NP surface, but not ROS as vital to the antibacterial mechanism of ZnO NPs. Identification of this mechanism will allow the synthesis of ZnO NPs that possess chemical and morphological properties best suited for antibacterial efficacy.

GP2 Novel biofilm mutations promote minocycline resistance in *Acinetobacter baumannii*

Suman Tiwari, Hasan Raza, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Acinetobacter baumannii is a gram-negative human pathogen that poses significant challenges in healthcare settings due to its ability to acquire multidrug resistance. Minocycline is one of the few remaining effective antibiotics for treating *A. baumanni*i infections, yet resistance is beginning to emerge. Understanding the mechanisms underlying minocycline resistance is essential to preserve the antibiotics efficacy in the clinic. In this study, we utilized a machine learning prediction model to identify genes associated with minocycline resistance. Based on the predictions we selected 36 genes for further minocycline sensitivity analysis. We found that one of the mutants, ruvB, was 32-fold more resistant to minocycline than wild-type *A. baumannii* AB5075. The Ruv gene system, consisting of ruvA, ruvB, and ruvC, is typically involved in DNA repair and recombination processes. Prompted by the initial observation of high minocycline resistance in the ruvB mutant during screening, further evaluation revealed its pronounced ability to form robust biofilms. Interestingly, there was no difference in biofilm formation with either the ruvA or ruvC mutant compared to the wild-type *A. baumannii* AB5075 strain. Further MIC tests were conducted on all three mutants (ruvA, ruvB, and ruvC) using doxycycline and vancomycin, and intriguingly, only the ruvB mutant demonstrated resistance to these antimicrobial agents.

This study, through identifying an unknown role for the ruvB gene in promoting *A. baumannii* biofilm formation and minocycline resistance, highlights the importance of the essential interactions that occur between the drug, pathogen, and host that dictate clinical antibiotic efficacies.

GP3 Evolutionary trade-offs in nafcillin-resistant *Staphylococcus aureus*: collateral sensitivity to azithromycin and fitness changes

Namrata Dinesh Bonde, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Antibiotic resistance is one of the critical challenges in contemporary healthcare. The evolution of antibiotic resistance entails a complex and dynamic interplay between the bacteria and the selective pressures exerted by the antibiotic. The intricate processes of resistance evolution are often accompanied by evolutionary trade-offs, influencing bacterial growth and fitness, and response to other unrelated antibiotics. Understanding the mutational pathways driving evolution of antibiotic resistance, and the associated genetic trade-offs is paramount for devising effective therapeutic strategies, yet our current grasp of these intricacies remain unexplored. We therefore sought to define the evolutionary pathways of resistance to the β-lactam nafcillin in methicillin-resistant *Staphylococcus aureus* (MRSA) using Adaptive Laboratory Evolution (ALE). Our approach, conducted in physiologically relevant conditions, includes two distinct methodologies, one permitting continuous exponential growth and the other maintaining a 24-hour period to potentially allow entry into the stationary phase. Nafcillin-resistant strains obtained from these ALE experiments exhibit intriguing responses to macrolide azithromycin. Strains evolved in the exponential phase exhibit up to a 256-fold increase in azithromycin sensitivity, without incurring fitness changes. Conversely, strains evolved in 24-hour evolutionary periods display up to a 2-fold increase in azithromycin sensitivity. Interestingly, these nafcillin-resistant strains exhibit alterations in their growth patterns, indicating changes in their overall fitness. The study reveals a seesaw effect between nafcillin and azithromycin along with the fitness trade-offs incurred by *S.aureus* to gain nafcillin resistance. Future studies will explore the genetic mechanisms behind these changes and their role in host-pathogen interactions.

GP4 Uncovering seesaw interactions in *Acinetobacter baumannii*

Anna Evers, Lester Uy, Nicholas Dillon

University of Texas at Dallas, Richardson, TX, USA

Abstract

Antibiotic resistance is evolving at a rapid rate, presenting a growing critical health threat. This is especially exhibited in *Acinetobacter baumannii*, noted for its extreme tendency to develop multidrug resistance even among other ESKAPE pathogens. A promising strategy for combatting this unrestrained evolution is exploiting collateral susceptibilities, also called "seesaw interactions." Seesaw interactions are a phenomenon in which increasing resistance to one antibiotic causes an increased susceptibility to another antibiotic. Using published minimum inhibitory concentration (MIC) data, we created a model predicting seesaw interactions in *A. baumannii*. To test the model, we used adaptive laboratory evolution (ALE) to generate several lineages of *A. baumannii* resistant to one antibiotic and observed the resulting changes in MICs for other antibiotics. Confirming our predictions, we have found that the evolution of colistin resistance drives increased susceptibility to ciprofloxacin in *A. baumannii*. Interestingly, we have observed environmental differences, like nutritional and growth conditions, influence how seesaw interactions evolve. Ongoing genomic analysis aims to elucidate the underlying mechanisms associated with these observed shifts in antibiotic susceptibilities. Utilizing antibiotic seesaw interactions has the potential to slow the progression of resistance long-term and preserve the effectiveness of our current antibiotics.

GP5 Analyzing the interconnected relationship between virulence and antibiotic resistance in *Acinetobacter baumannii*

Nathaniel Thomas, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Acinetobacter baumannii is a highly drug-resistant Gram-negative bacterial pathogen. Although it is the #1 priority pathogen by the WHO, and a critical threat by the CDC, in the fight against antibiotic resistance, its virulence mechanisms are poorly understood. We therefore sought to examine the interplay between antibiotic resistance and virulence in three multi-drug resistant *A. baumannii* strains. We selected one laboratory (AB5075) and two unknown clinical isolates for analysis. The patient isolates were selected as they had disparate antibiotic susceptibility profiles, with one classified as pan-sensitive (PS) while the other was pan-resistant (PR) to clinical therapy. As reported, the PR clinical strain was resistant to a majority of antibiotics during our in vitro testing, while the PS was only resistant to colistin and carbapenems. However, the PR strain had attenuated virulence in mice while the PS strain was highly virulent. We next sought to understand the virulence differences of the three strains using a big data approach. Surprisingly, despite the differences in lethality between the murine pneumonia models, each strain produced a similar number of unique, but different, virulence factors. Our data indicates a clear tradeoff between virulence and antibiotic resistance in clinical strains of *A. baumannii*. By further studying these diametrically opposed strains in the host, we hope to gain a better mechanistic understanding of the interplay between virulence and resistance in *A. baumannii*.

GP6 Impact of choline-dependent growth on antibiotic susceptibility in *Streptococcus* sp. *mitis* 1643

Lily Tillett, Yahan Wei, Guan Chen, Kelli Palmer

University of Texas at Dallas, Richardson, TX, USA

Abstract

Streptococcus sp. 1643 is a gram-positive bacterium isolated from an endocarditis patient that produces type-IV lipoteichoic acid (LTA) polymers. LTAs decorate streptococcal membranes and are responsible for a variety of host-microbe regulatory roles, including resistance to antimicrobial compounds. These functions are regulated in part by the bonding of cationic moieties like choline that decorate the teichoic acid. *Streptococcus* sp. 1643 has been observed to grow independently of choline, but with an associated decrease in growth. In this study, we investigated how choline-independent growth impacts antibiotic susceptibility. To determine if there is altered antimicrobial susceptibility, *Streptococcus* sp. 1643 was cultured with and without choline in a chemically defined medium with the addition of a variety of antibiotics, including vancomycin, ampicillin, and gentamicin. S*treptococcus* sp. 1643 grown with choline had a 4-fold increase in MIC for both ampicillin and vancomycin. The resulting MICs were as follows: 2 ug/mL with choline compared to 0.5 ug/mL without choline for ampicillin, 1 ug/mL with choline, and 0.25 ug/mL without for vancomycin. Gentamicin-treated *Streptococcus* sp. 1643 had a MIC >8 ug/mL and no determined MIC for the no-choline group, but there was an observed decrease in growth. The resulting MICs support the conclusion that when lacking choline, the antibiotic susceptibility of *Streptococcus* sp. 1643 is lowered. This conclusion is supported across a range of antibiotics, highlighting choline's role in streptococcal physiology, and providing insight into treating antibiotic-resistant strains in the future.

GP7 Utilizing bicarbonate to improve treatment efficacies in cystic fibrosis

Ravali Arugonda, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Cystic fibrosis (CF) is a genetic disease that predisposes patients to severe *Pseudomonas aeruginosa* infections. After initial colonization, *P. aeruginosa* develops antibiotic resistance as it persists within the host, eventually killing the patient once lung function is inexorably compromised. CF disease arises from mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) which block the efflux of chloride and the biological buffer bicarbonate. We have previously found that bicarbonate can improve antibiotic susceptibilities against multi drug resistant bacteria, and prior studies have linked it to altered host responses. We hypothesize bicarbonate is a perfect host target that can be modified to improve treatment outcomes in CF patients. We conducted antibiotic susceptibility testing in both bacteriological and physiological media, revealing differential responses with various classes of antibiotics against *P. aeruginosa* specifically in physiologically relevant conditions. We have also performed macrophage killing assays to observe the impact of bicarbonate on the host response to *P. aeruginosa.* Surprisingly, bicarbonate was found to interfere with the macrophage efficiency in *P. aeruginosa* eradication. In the future, we plan to investigate the impact of varying bicarbonate concentrations on host-pathogen interactions and in vivo antibiotic efficacies. These studies highlight the potential for improvement of treatment efficacies in CF through modulation of host microenvironmental conditions.

GP8 Bicarbonate dictates the evolution of azithromycin resistance in *Pseudomonas aeruginosa* in cystic fibrosis

Tuhina Maity, Nicholas Dillon

University of Texas at Dallas, Richardson, TX, USA

Abstract

Cystic Fibrosis (CF), a hereditary condition affecting the respiratory system due to mutations in the CFTR gene, which deprive the lung of chloride and bicarbonate, resulting in chronic respiratory infections primarily caused by *Pseudomonas aeruginosa*. *P. aeruginosa* gains antibiotic resistance as infections progress, eventually killing the patient as all treatment options are exhausted. This study investigates how the conditions of the CF lung impact the evolution of antibiotic resistance in *P. aeruginosa*. We utilized adaptive laboratory evolution (ALE) to drive antibiotic resistance in multidrug-resistant *P. aeruginosa* strain P4 in either bacteriologic or physiologically relevant conditions. In bacteriologic medium, *P. aeruginosa* exhibited an impressive capacity to develop resistance to the macrolide azithromycin (AZM), becoming 128-fold resistant to the antibiotic over the course of 8 days. However, in physiological medium, P. aeruginosa was unable to evolve resistance to AZM over the same timeframe. Our prior work has found a role for the mammalian buffer bicarbonate, found within humans and our physiologic medium, but absent from the bacteriologic medium, in influencing antibiotic susceptibilities. Suspecting bicarbonate may be influencing the evolution of AZM resistance, we conducted ALE experiments in CAMHB supplemented with bicarbonate. Exogenous bicarbonate impaired the evolution of AZM resistance in *P. aeruginosa* in CA-MHB. In the future we will examine the genetic basis for bicarbonates' influence on the evolution of AZM resistance and we will evaluate the evolution of resistance in other macrolide antibiotics. This study highlights how CF host environmental factors influence the evolution of antibiotic resistance in *P. aeruginosa*.

GP9 Surveillance for reticuloendotheliosis virus and lymphoproliferative disease virus in Rio Grande wild turkeys of Texas

Dasire Brawley, Dustin Edwards

Tarleton State University, Stephenville, TX, USA

Abstract

Reticuloendotheliosis virus (REV) and lymphoproliferative disease virus (LPDV) are oncogenic and immunosuppressive avian retroviruses associated with neoplastic disease in galliform species. Birds infected with REV have an increase in coinfection susceptibility and have the potential to act as reservoirs for viral transmission for at-risk flocks, such as the Attwater's prairie chicken. Our objective was to continue a 10-year surveillance and determine changes in the prevalence of REV and LPDV in Texas counties. Dried blood spots from 85 Rio Grande wild turkeys (*Meleagris gallopavo intermedia*) from six Texas counties were provided by Texas Parks and Wildlife Department biologists and screened for proviral REV or LPDV DNA. The REV 3' long terminal repeat or the LPDV p31/CA gene was targeted by polymerase chain reaction. Positive samples were genetically sequenced, and nucleotide similarity was confirmed by BLASTn queries. Approximately 1% of individuals were infected with REV (1/85), and 36% were infected with LPDV (33/85), including one coinfection. The results from the screening showed a similar prevalence of REV compared to a previous survey conducted in 2018–2020; however, there was a significantly higher prevalence of LPDV. While we previously detected most LPDV infections in females, in the current survey, we detected a similar proportion of infections in males and females. Future directions include continued surveillance, whole genome sequencing of REV and LPDV, and gut microbiome analysis of infected and uninfected birds.

GP10 A quantitative PCR analysis of antibiotic-resistant genes (ARGs) in Lake Meredith, an important water reservoir in the arid Texas Panhandle region

Robyn Cuthbert, Quincy Nakamura, Erik Crosman, Maitreyee Mukherjee

West Texas A&M University, Canyon, TX, USA

Abstract

Background: Due to increased antibiotic use by agricultural and medical facilities together with decades of improper use of antibiotics, incidences of antimicrobial resistance (AMR) and multidrug resistance (MDR) within pathogenic bacterial populations are on the rise. This is a highly concerning global public health issue that needs tracking and monitoring of AMR/MDR within the environment so we can come up with potential solutions. Antibiotic resistance genes (ARGs), responsible for the spread of AMR/MDR, are of particular importance because they can spread rapidly among bacterial populations via horizontal gene transfer mechanisms. Lake Meredith is one of the most important water sources for the arid Texas Panhandle region, used as a vital resource for drinking, agricultural, and recreational purposes. The aim of this project was to identify and quantify the amount of specific ARGs in water collected from Lake Meredith over a period of four months.

Methods: Environmental DNA was extracted from water collected from six different sites in Lake Meredith between March – June 2023. Quantitative real-time polymerase chain reaction (qPCR) technique was utilized to quantify AMR genes (*intI-1*, *ermF*, and *tetA*) within these samples.

Results: Our results reveal high levels of ARGs across several samples collected from the lake. Overall, ARG levels were higher during the warmer months compared to the cooler spring months.

Future Directions: We plan to continue analyzing ARGs in Lake Meredith until the end of the year. Following this, we plan to examine the water for sources of such contamination utilizing molecular microbial source-tracking methods.

GP11 Analysis of growth characteristics and differentially expressed homologous genes in *Rhodobacter sphaeroides* under normal and simulated microgravity conditions

Weerakkody Ranasinghe[1], Eduardo Gutierrez[1], Zelaya Alyson[1], Sabrina Vazquez[1], Ashleigh Ogg[1], Hyuk Cho[2], Madhusudan Choudhary[1]

[1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX, USA
[2]Department of Computer Science, Sam Houston State University, Huntsville, TX, USA

Abstract

The term "microgravity" is used to describe the "weightlessness" or "zero-g" circumstances that can only be found in space beyond earth's atmosphere. *R. sphaeroides* is a gram-negative purple phototroph, used as a model organism for this study due to its genomic complexity and metabolic versatility. Its genome has been completely sequenced, and profiles of the differential gene expression were recently examined under aerobic, semi-aerobic, and photosynthetic conditions. In this study, we hypothesized that *R. sphaeroides* will show altered growth characteristics, morphological properties, and gene expression patterns when grown under simulated microgravity. To test that, we measured the optical density and colony-forming units of cell cultures grown under both microgravity and normal gravity conditions. Differences in the cell morphology were observed using scanning electron microscopy (SEM) images by measuring the length and the surface area of the cells under both conditions. Furthermore, we also identified homologous genes of *R. spheroides* using the differential gene expression study of *Acidovorax* under microgravity in our laboratory. Growth kinetics results showed that *R. sphaeroides* cells grown under microgravity experience a shorter log phase and early stationary phase compared to the cells grown under normal gravity conditions. The length and surface area of the cells under microgravity were significantly higher confirming that bacterial cells experience altered morphological features when grown under microgravity conditions. Differentially expressed homologous gene analysis indicated that genes coding for several COG and GO functions, such as metabolism, signal-transduction, transcription, translation, chemotaxis, and cell motility are differentially expressed to adapt and survive microgravity.

GP12 The effect of elevated atmospheric CO_2 on methanotrophic bacteria in upland soil

Sreemoye Nath, Calvin A. Henard

BioDiscovery Institute, Department of Biological Sciences, University of North Texas, Denton, TX, USA

Abstract

Methane (CH_4)- oxidizing bacteria, methanotrophs, uniquely consume CH_4 as a carbon and energy source and are a CH_4 sink in terrestrial and aquatic ecosystems. A subset of methanotrophs can also use carbon dioxide (CO_2) as a carbon source and exhibit improved growth in axenic culture when CO_2 is also supplied with CH_4. However, the effect of CO_2 on methanotrophs in the environment is unknown. In this study, we used 16s rRNA sequencing to compare the bacterial community composition in soil microcosms with differing atmospheric gas compositions (10% CH_4 in air, 10% CO_2 in air, and 10% CH_4/10% CO_2 in air). As expected, based on prior studies, we observed enrichment of extant Gammaproteobacterial *Methylococcaceae* and Alphaproteobacterial *Methylocystaceae* methanotrophs in microcosms supplied with CH_4. Notably, the CH_4/ CO_2 microcosms, but not the CO_2-only microcosms, exhibited a significant increase in *Methylococcus, Methylocystis,* and *Methylosinus* populations compared to CH_4-only microcosms, suggesting CO_2 also improves methanotroph growth and CH_4 metabolism in the environment. Isolates obtained from CH_4- or CH_4/ CO_2-enriched microcosms were predominately *Methylocystis* or *Methylosinus* spp. Several of the isolates showed improved growth with CH_4 and CO_2 compared to CH_4 only as the sole carbon source. In addition, we observed faster growth kinetics of a CH_4/ CO_2 microcosm Methylosinus isolate compared to the Methylosinus trichosporium OB3b type-strain, which may represent a novel, industrially relevant biocatalyst. This study underscores the role of methanotrophs in greenhouse-gas mitigation and enhances our understanding of their response to elevated CO2 levels, offering valuable insights for the advancement of carbon capture strategies.

GP13 Microbial March Madness: a CURE for student research opportunities

Jacqueline Miralles-Salazar[1], Nina Walker[2], Desiree Molinar[1], Joshua Reid[1], Catherine Wakeman[1]

[1]Texas Tech University, Lubbock, TX, USA
[2]South Plains College, Lubbock, TX, USA

Abstract

To gain research experience, undergraduates typically attempt to apply to the research programs of their professors. However, demand for these spots is high and the number of positions is limited. To solve this problem, a new modality of teaching has been implemented in multiple universities: “the Course-based Undergraduate Research Experience (CURE)”. Through CUREs, students are introduced to the research process in a classroom setting, build their critical thinking skills, and master lab techniques. The proven benefits of CUREs include enhancing the students’ awareness of what it means to be a researcher, increasing their likeness to stay in STEM careers, and pursue graduate school.

With the CURE developed herein, we anticipate that we will additionally observe an increased understanding of the more complex topics associated with the upper-level microbiology courses offered by our department. The Microbial March Madness CURE embraces the TTU love of NCAA basketball by allowing the students to prepare and experimentally test an NCAA-style March Madness bracket of microbes pitted against each other in different environments. The students will learn about the specific environmental adaptations of the different microbes in order to develop and test their own hypotheses about the environmental fitness of individual microbes. If their hypotheses are proven incorrect, they are given a chance to reevaluate the reasons for their experimental findings to improve their critical thinking skills. As a pilot study, we had 3 students working in the course, A high schooler, a community college student, and a senior from Biology.

GP14 Filling the cracks with bacteria

Jennifer Bracewell, Kelli Palmer

University of Texas at Dallas, Richardson, TX, USA

Abstract

Concrete production and repair are environmentally costly, being responsible for >8% of global CO2 emissions. Reducing environmental costs is needed to make global concrete production more sustainable. Microbially induced carbonate precipitation (MICP), or bioconcrete, is one approach to making concrete infrastructure more environmentally friendly. Autonomous repair by embedded bacteria can prolong the service life of concrete structures by sealing cracks, thus preventing the degradation of the interior components that provide strength to the structure. Bacteria, usually as spores, can be added to the concrete mixture. Upon cracking of the concrete, they regain metabolic activity, resulting in MICP. Current research often does not account for biological differences in various bacteria and their diverse responses to different environments which impacts the amount and quality of the bioconcrete produced. Since different applications have different requirements, standardizing an approach for comparison of bioconcrete production by different bacteria and under different conditions is necessary. We started with a procedure previously reported to quantify MICP by *Sporosarcina pasteurii*, the most frequently used model organism in bioconcrete research. From that procedure, we saw several aspects where quantification could be improved or the methods clarified. Therefore, we have created a revised protocol that reduces error and allows for comparison of bioconcrete produced by different bacteria or by the same bacteria in various conditions. This revised protocol will allow improved bioconcrete research as it clarifies the procedures needed for quantifying the amount of MICP to give a clear method of comparing the results of researchers across disciplines.

GP15 Differential gene expression of *Acidovorax* species under normal and microgravity conditions

Eduardo Gutierrez, Madhusudan Choudhary

Sam Houston State University, Huntsville, TX, USA

Abstract

Previous studies reported that several bacterial species under microgravity stress have been found to undergo a multitude of physiological responses such as increased growth, virulence, antibiotic resistance, and biofilm production. The current study examines the gene expression differences of *Acidovorax* (Strain ID: 1608163) under normal and microgravity conditions. *Acidovorax* is a gram-negative plant bacterium capable of infecting crops. Its genome has been completely sequenced. The hypothesis that “bacterial cultures grown under normal and simulated microgravity conditions will differentially express their genes” will be tested by comparing transcriptomic profiles of the bacteria between the two conditions. Acidovorax bacterial cultures were grown in minimal media under normal and microgravity conditions, and cells were collected during the mid and late log-phase of growth (48 and 72 hours). The total RNA was extracted and reverse transcribed, and then the DNA was sequenced using Illumina Mi-Seq. Results revealed that bacterial growth rates were significantly higher under microgravity conditions compared to the normal gravity conditions, however the cells were smaller in size when grown under microgravity. The results further demonstrated that the genes encoding several COG (cluster of orthologous group) and GO (gene ontology) functions were altered under microgravity. These findings will help understand the regulation of bacterial adaptive mechanisms under microgravity stress environment.

GP16 A computational approach to identify functional gene orthologs and molecular marker genes for two novel drought tolerant *Bradyrhizobium* strains isolated in Texas

Mohammad Kamruzzaman, Woo-Suk Chang

The University of Texas at Arlington, Arlington, TX, USA

Abstract

Bradyrhizobium japonicum, a nitrogen-fixing soil bacterium, can develop a symbiotic relationship with legume plants such as soybean. The successful outcome of the *Bradyrhizobium*-soybean symbiosis is the formation of root nodules where the bacteria fix atmospheric nitrogen into ammonia and provide this nitrogen source to the plant. However, this symbiosis is greatly affected by drought, one of the most severe abiotic stresses in the US soybean cultivation. Thus, developing a drought-tolerant soybean and its partner has been a growing concern. In our previous study, we isolated two drought tolerant *Bradyrhizobium* strains, TXVA and TXEA, which were isolated in Texas. In addition, the whole genomes of these two Texas-native strains have been sequenced. TXVA's genome contains 9,193,770 bp with 8,980 protein-coding genes, while TXEA's genome contains 9,339,455 bp with 9,158 protein-coding genes. In this study, we implemented a computational approach to identify functionally similar gene orthologs between the two strains. OrthoFinder was used to find and comprehend genes showing comparable activities in TXVA, TXEA, and seven other known rhizobia strains. To identify distinct molecular marker genes for TXVA and TXEA, we used the Pangenome pipeline. Additionally, we adopted the Phylogenetic Profiler for Single Genes (IMG, JGI) to identify unique genes in all compared genomes. The pangenome analysis revealed that a hypothetical protein (IMG gene ID: 2929624621) and a gene encoding Deoxycytidylate deaminase are unique for TXVA and TXEA, respectively.

GP17 Engineering the chromosome of the honey bee gut symbiont *Snodgrassella alvi*

A. H. M. Zuberi Ashraf, PJ Lariviere, Jeffrey Barrick

The University of Texas at Austin, Austin, TX, USA

Abstract

Snodgrassella alvi is a core member of the honey bee gut microbiome and also one of the first and most important colonizers of the honey bee gut. However, we still lack straightforward and reliable genetic tools for modifying the *S. alvi* chromosome. Homologous recombination is a DNA repair mechanism that can be harnessed for knocking out or inserting genes into a bacterial chromosome.

Objective: Insert fluorescent markers in the S. alvi genome through homologous recombination by electroporating plasmid DNA.

Methods: A neutral intergenic region between two non-essential genes was identified. Homology arms flanking this region, fluorescence cassettes, and antibiotic cassettes were amplified using PCR and cloned into an entry vector to make part plasmids, followed by combining all the part plasmids to make a full assembly plasmid with a ColE1 origin using the Bee Microbiome Toolkit. Electrocompetent *S. alvi* cells were electroporated using the full assembly plasmid and screened for selection.

Results: Successful transformants expressed fluorescent proteins from the genome and their insertions were verified by whole-genome sequencing.

Conclusion: Homologous recombination using plasmid DNA was successful in inserting expression cassettes in the targeted intergenic region in the *S. alvi* genome. When compared to expression from a multicopy plasmid, genomic expression of fluorescent markers was lower, as expected, but still resulted in enough signal for cell sorting applications. This approach provides an efficient way to engineer *S. alvi* by knocking out genes or inserting heterologous genes to study bee gene function and improve bee health.

GP18 Generation and characterization of a recombinant vaccinia virus with both decapping enzymes knocked out

Faithful Williams, Zhilong Yang

Texas A&M University, College Station, TX, USA

Abstract

Decapping enzymes are encoded in poxviruses and other nucleocytoplasmic large DNA viruses to moderate host and viral RNA levels during infection. The decapping activity is catalyzed by the nudix motif in the protein, which removes the 5' end m7G cap structure of eukaryotic mRNA by hydrolysis. The prototypic poxvirus, vaccinia virus (VACV), encodes two mRNA decapping enzymes: D9 and D10. Previous research has demonstrated the importance of these enzymes by showing a reduction in viral replication when the nudix motif is disabled by mutating the key amino acids (vD9muD10mu). However, our lab recently showed that VACV D10 colocalizes with mitochondria and that colocalization is also required for VACV replication, hinting at important functions outside of its decapping activity. Here we generated a recombinant VACV with both D9 and D10 knocked out (vDD9DD10). We compared the viral replication dynamics and plaque sizes between vDD9DD10 and vD9muD10mu. We found that vDD9DD10 showed a significant decrease in both plaque size and viral replication as compared to vD9muD10mu. This result suggests that the non-decapping activities of D9/D10 are also required for efficient VACV replication. The vDD9DD10 recombinant virus also provides a valuable tool to understand decapping enzyme function.

GP19 Functional analysis of the mycobacteriophage HicAB-like toxin-antitoxin system in prophage maintenance: a novel method proposal

Nicholas Mercado, Lee Hughes

University of North Texas, Denton, TX, USA

Abstract

With the ongoing threat of multidrug-resistant pathogens, there is a substantial need for alternatives to traditional drug therapies. One promising alternative is the use of bacteriophage therapy. A concern with the implementation of this therapy is the lack of a strong understanding of the relationship between host and bacteriophage. We propose a project outline with the specific aim of elucidating one specific component of the interplay between Mycobacterium smegmatis and mycobacteriophage Adephagia. This host is of exceptional relevance due to its relatedness to the pathogenic Mycobacterium tuberculosis. In this project, the role of the Adephagia HicAB-like toxin-antitoxin system will be assessed as it pertains to prophage maintenance in the host. Prior studies have provided some evidence that this toxin-antitoxin system may provide increased prophage stability when brought into the host cell. To test this hypothesis, multiple mutants will be used in a fluorescence-based assay to quantify levels of prophage maintenance in each system. These mutants include various knockouts allowing for the effects of missing toxin-antitoxin system components on prophage stability to be studied. Furthermore, each sample will be genetically engineered to express mCherry fluorescent protein following excision from the host genome. This novel method of quantifying prophage stability will be performed in tandem with the more traditional method of comparing plaques resulting from phage replication and subsequent cell lysis. Should the hypothesis be correct, we expect increased fluorescence (i.e., decreased prophage stability) in each system with an incomplete toxin-antitoxin system and will be able to quantify levels of instability.

GP20 *Paracoccus dentirificans* boosts growth of the methanotroph *Methylococcus capsulatus* in co-culture

Layla Dale, Calvin Henard, Jessica Henard

Department of Biological Science and BioDiscovery Institute, University of North Texas, Denton, TX, USA

Abstract

Methanotrophic bacteria (methanotrophs) can utilize methane (CH_4) as their carbon and energy source. This unique ability to utilize CH_4 has industrial potential, particularly in environmentally friendly biomanufacturing processes. Previous studies have shown that methanotrophs exhibit improved productivity metrics in mixed vs. monoculture, but the underlying mechanisms are poorly understood. In this study, we established a co-culture of the methanotroph, *Methylococcus capsulatus* str. Bath and the methylotroph, *Paracoccus denitrificans* Pd1222. We show that *M. capsulatus* and *P. denitrificans* can be cultivated at 37°C in methanotroph nitrate mineral salts medium supplemented with 20% CH_4 and 0.1% nutrient broth, establishing permissive co-culture conditions. Our results show that *M. capsulatus* reaches higher cell densities in co-culture with *P. denitrificans*. Notably, the observed increase in *M. capsulatus* biomass is not correlated to an increase in CH_4 consumption, improving overall methanotroph biomass yield from CH_4. These results suggest that *M. capsulatus* either utilizes alternative carbon sources in addition to CH_4 or alters its CH_4 flux to biomass in the presence of *P. denitrificans*. Collectively, this study supports that coculture may be a viable route to improve methanotroph productivity in CH_4 bioconversion processes.

GP21 Carbonic anhydrase expression improves carbon conversion efficiency in the biocatalyst *Methylococcus capsulatus* str. Bath

Spencer Lee, Jessica Henard, Sreemoye Nath, Calvin Henard

Biodiscovery Institute, Department of Biology, University of North Texas, Denton, TX, USA

Abstract

Methylococcus capsulatus is a methanotrophic bacteria capable of methane (CH_4) utilization that also encodes ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). Recent studies have shown the essentiality of both RubisCO and carbon dioxide (CO_2) for *M. capsulatus* growth; however, inorganic carbon metabolism in these bacteria remains poorly understood. Carbonic anhydrases (CAs) catalyze the interconversion of CO_2 and bicarbonate and have been shown to improve CO_2 conversion efficiency in phototrophs by colocalizing CO_2 production with RubisCO. In this study, we identifed that *M. capsulatus* encodes five distinct CA isoforms. Real-time quantitative PCR analyses showed that *M. capsulatus* expresses all isoforms during growth on CH_4 and increases transcription of the CA genes in response to a decrease in CO_2 availability. Bacterial growth was inhibited by the CA inhibitor acetazolamide (AZM), underscoring the importance of CAs for *M. capsulatus* metabolism. Further, KO strains deficient in individual CA isoforms exhibited significant growth defects suggesting that the CAs have important, non-redundant roles in *M. capsulatus*. Notably, we overexpressed each CA isoform and identified a strain with enhanced growth, reduced CO_2 evolution, and increased carbon conversion efficiency. These results further elucidate the proteins necessary for CO_2 assimilation in *M. capsulatus* and identify an improved biocatalyst for development of carbon negative biotechnologies.

GP22 Optimizing *ex vivo* culturing of the mouse intestinal microbiome

Carlos Canales[1], Janice Speshock[1], Jeffrey Brady[2], Max Sanderford[1]

[1]Tarleton State University, Stephenville, TX, USA
[2]Texas A&M Agrilife Research, Stephenville, TX, USA

Abstract

The gastrointestinal tract microbiome is full of an assortment of microorganisms that have an intricate relationship with the host and has such an impact on the host. Understanding the core microbiome, can lead to improving the host's overall health and can be beneficial nutrition wise. The objective of this study is to collect microorganisms from the fecal matter of six CD-1 mice and successfully replicate the microbiome *ex vivo* and indicate *in vitro* that they are active. Next, observe that the microorganisms react to processes that happen in the body, replicating by processing sugars and comparing the different treatment groups. To culture the microbiome, for each mouse, the fecal matter was homogenized and placed into different concentrations of sucrose aliquots and observed the changes before and after one week of incubation. Using the Qiagen RNeasy kit, we isolated the RNA and quantified using Qubit High Sensitivity RNA quantification kit and sent it for RNA sequencing. The 16S rRNA strands are used to observe comparisons in populations through MicrobiomeAnalyst. We worked with sucrose and saw success with different concentrations and observed an acidic effect of the bacteria present in the homogenate solution by pH measurement. It has already been proven that the gut microbiome can be replicated *ex vivo* the mouse gastrointestinal tract, further research in understanding the gut microbiome can be beneficial by gaining more information on other diseases caused by the gut microbiome.

GP23 Novel lipid discovery enabled by sequence statistics and machine learning

Priya Christensen[1], Jonathan Martin[1], Aparna Uppuluri[1], Luke Joyce[2], Yahan Wei[3], Ziqiang Guan[4], Faruck Morcos[5], Kelli Palmer[1]

[1]The University of Texas at Dallas, Richardson, TX, USA
[2]University of Colorado Anshutz Medical Campus, Aurora, CO, USA
[3]University of Texas Rio Grande Valley, Harlingen, TX, USA
[4]Duke University Medical Center, Durham, NC, USA
[5]The University, Richardson, TX, USA

Abstract

Many drugs and antimicrobial agents target the cell membrane. The cell membrane is composed of lipids which can be modified. One enzyme responsible for the modification of lipids is the multiple peptide resistance factor (MprF). MprF is composed of two domains: an N-terminal flippase and a C-terminal synthase. In *Streptococcus agalactiae* (Group B *Streptococcus*, GBS), MprF synthesizes both a novel cationic glycolipid, lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG) and the more common phospholipid lysyl-phosphatidylglycerol (Lys-PG). The identification of a new lipid, Lys-Glc-DAG highlighted a novel lipid substrate (Glc-DAG) for MprF. The goal of this study is to use amino acid sequence statistics across the MprF synthase domain family to identify other MprF enyzmes that synthesize novel lipids. Utilizing this approach, *Enterococcus dispar* MprF was identified for possible novel lipid substrate capabilities. Acidic Bligh-Dyer lipid extractions and comprehensive lipidomic analysis of *E. dispar* showed the synthesis of a novel cationic glycolipid, lysyl-diglucosyl-diacylglycerol (Lys-Glc_2-DAG), in addition to Lys-PG and Lys-Glc-DAG. This led us to further explore other *Enterococcus* species for Lys-Glc_2-DAG synthesis. All *Enterococcus* strains analyzed in this study were capable of synthesizing Lys-Glc_2-DAG. Notably, *E. faecalis* and *E. faecium* encode two *mprF* genes. We confirmed that one *mprF* allele confers synthesis of the lysine-modified lipids. In summary, an interdisciplinary approach combining computational, genetic, and lipidomic approaches identified a novel cationic lipid, Lys-Glc_2-DAG, produced by bacterial pathogens.

GP24 Investigating the role of lysine lipids in biofilm formation in group B *Streptococcus*

Aayushi Gaur, Kelli Palmer

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Group B *Streptococcus* (GBS) is a prominent gram-positive pathogen known for its ability to form biofilms which contributes to its virulence and resistance to antibiotics. GBS colonize the gastrointestinal and lower genital tracts. In GBS, the multiple peptide resistance factor (MprF) is responsible for the synthesis of two distinct lipids: the novel glycolipid lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG) and the well-known phospholipid lysyl-phosphatidylglycerol (Lys-PG). Lys-PG serves to reduce the overall negative charge of the bacterial membrane, thereby offering protection against cationic antimicrobial peptides (CAMPs). Lys-Glc-DAG contributes to bacterial uptake into host cells and disease progression. This work explores the hypothesis that these Lys-lipids play a significant role in GBS biofilm formation. To test this hypothesis, biofilm formation was assessed in two GBS strains, COH1 and CBJ111 - comparing wild type and *mprF* deletion mutant strains which lack Lys-lipids. The results showed significant variation in the biofilm forming capacity not only between the wild type and *mprF* mutant strains, but also between the two GBS strains. To support our data, we employed various image analysis by using Scanning Electron Microscopy and Confocal Imaging supplemented by Light Microscopy to visualize morphological variation in the biofilm structure. In conclusion, this work addresses the significance of Lys-lipids in GBS biofilm formation. By combining biofilm assays, image analysis, and optimization of growth conditions, we established the significant effect of Lys lipids in biofilm formation and further aim to unravel the complex interplay between lipid metabolism and gene expression leading to the development of novel strategies for controlling GBS infections.

GP25 Carbon dioxide metabolism in the methanotroph *Methylococcus capsulatus* Bath

Yao-Chuan Yu, Jessica M. Henard, Calvin A. Henard

Department of Biological Sciences and BioDiscovery Institute, University of North Texas, Denton, TX, USA

Abstract

The methanotrophic bacterium *Methylococcus capsulatus* Bath assimilates methane (CH_4) and carbon dioxide (CO_2), making it an ideal biocatalyst for upgrading anaerobic digestion-derived biogas consisting of a mixture of CH_4 and CO_2 greenhouse gases. Our prior results show that CO_2 is assimilated by the *M. capsulatus* ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), and both RubisCO and CO_2 are essential for bacterial cultivation. However, the CO_2-associated biochemical pathways are unknown. To further understand CO_2 metabolism and its interrelationship with CH_4 metabolism in *M. capsulatus*, we performed $^{13}CO_2$ tracing experiments and quantified the isotopic distribution of central metabolites via LC-MS/MS. Isotopomer labeling patterns identify significant overlap between inorganic and organic carbon biochemical pathways and show that a non-canonical Calvin-Benson-Bassham (CBB) cycle is functional in *M. capsulatus*. Consistent with prior studies evaluating enzyme activity in *M. capsulatus* whole-cell lysates, the labeling patterns support that *M. capsulatus* has an incomplete, bifurcated tricarboxylic acid (TCA) cycle. These insights will guide downstream metabolic engineering of *M. capsulatus* to optimize carbon uptake and conversion to products in this methanoautotrophic microbial chassis.

GP26 Two-sided: exploring bacterial and host aspects of biofilm dispersal in wound infections

Rebecca Schneider, Mark Gao, Kendra Rumbaugh

Texas Tech University Health Sciences Center, Lubbock, TX, USA

Abstract

Biofilm infections often complicate wound care by causing treatment-resistant infections. Microbes form biofilms as a survival strategy whereby they surround themselves with an antibiotic-tolerant extracellular polymeric substance (EPS) composed of proteins, extracellular DNA (eDNA), and carbohydrates. Emerging strategies have focused on the enzymatic breakdown of the EPS to increase the efficacy of current clinical treatments, including antibiotics and debridement. However, biofilm dispersal in the context of *in vivo* pre-clinical models deserves further investigation. We studied the impact of glycoside hydrolase (GH)-mediated biofilm dispersal on *Pseudomonas aeruginosa* (PA) phenotype and host response in our murine surgical wound model. We hypothesized that GH biofilm dispersal would increase PA virulence, leading to systemic infection and sepsis in acute wound infections. We evaluated the effects of dispersal on PA phenotype by assessing virulence changes including biofilm attachment and increased infectivity in the *Galleria mellonella* model. Furthermore, we gauged host response to dispersal by determining systemic dissemination to internal organs and assessing sepsis symptoms on a murine sepsis scale (MSS). Our results suggest that exposure to GH treatment can alter PA's virulence in acute infections, which contributes to widespread systemic dissemination and sepsis. However, the risk of dissemination and sepsis decreases significantly for older infections. These findings highlight the importance of both altered bacterial behavior and host immune response in determining the outcome of GH treatment and biofilm dispersal. Further studies will explore the impact of GH treatment on PA gene expression and wound healing.

GP27 Associations between human genetics and the chronic wound microbiome

Rebecca Gabrilska[1], Jacob Ancira[2], Clint Miller[3], Khalid Omeir[2], Craig Tipton[4], Joe Wolcott[3], Kendra Rumbaugh[1], Nicole Phillips[5], Caleb Phillips[2,6]

[1]Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX, USA
[2]Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA
[3]Southwest Regional Wound Care Center, Lubbock, TX, USA
[4]RTL Genomics, MicroGenDX, Lubbock, TX, USA
[5]Microbiology, Immunology & Genetics, University of North Texas Health Science Center, Fort Worth, TX, USA
[6]Natural Science Research Laboratory, Texas Tech University, Lubbock, TX, USA

Abstract

Chronic wounds are a burden to millions of patients and healthcare providers worldwide. With rising incidence and prevalence, there is an urgent need to address these non-healing wounds with novel approaches. Impaired wound healing has been shown to be associated with the wound microbiota, as multiple bacterial species are known to contribute to infection and delays in closure. Whether these microbial communities are shaped by host genetics is less understood. Microbiome genome-wide association studies (mbGWAS) can provide insight into host genetic factors that may influence bacterial community structure. Previous work has shown that the alpha diversity of the chronic wound microbiome is significantly associated with specific human genomic loci and healing. To compare wound microbiomes to human genomes, we performed a two-stage mbGWAS using a cohort of 517 patients. Briefly, patients with lower extremity, non-healing wounds were consented, and buccal and wound samples were collected then genotyped and sequenced for bacterial taxa, respectively. mbGWAS was then performed to test association of the relative abundances of multiple wound-relevant bacteria with patient genotype. We identified bacterial taxa that are repeatedly significantly associated with single nucleotide polymorphisms (SNPs) in the host genome, suggesting a correlation between patient genetics and specific bacterial taxa within chronic wounds. Future studies will involve translating these mbGWAS findings to functionally validate likely causal SNPs. Identification of correlated biomarkers may provide new mechanistic insight into microbe-host interactions and may serve as predictive risk factors to guide personalized management for chronic wound patients.

GP28 Global metabolic profiling of lytic KSHV infection of endothelial cells

Fatima Hisam, Erica Sanchez

University of Texas at Dallas, Dallas, TX, USA

Abstract

Kaposi's Sarcoma Herpes Virus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS) and a leading cause of cancer in AIDS patients. With limited treatment for KS, it is essential to understand the mechanics behind KSHV infection. KSHV undergoes latent and lytic viral phases in the host cell. Only a few viral genes are expressed during latency, however, during lytic replication, KSHV encodes for ~90 genes, including a viral G protein-coupled receptor (vGPCR), a chemokine-like receptor with homology to host receptors. vGPCR signaling has been shown to alter host cell survival, angiogenesis, and metabolism. Previous metabolomic analysis showed that KSHV latently-infected endothelial cells modulate central carbon pathways (glycolysis, fatty acid synthesis, and amino-acid metabolism). Drug inhibition of these metabolic pathways resulted in reduced survival of latently infected cells and a significant reduction in virion production during the lytic phase. However, no study has measured the global alterations in the host cell metabolome during lytic KSHV to date. We hypothesize that global host cell metabolism is modulated to support maximal lytic infection. To test this hypothesis, endothelial cells overexpressing vGPCR or undergoing KSHV lytic infection will be analyzed via mass-spectrometry for metabolite levels. We expect to see significant metabolite changes in central carbon metabolic pathways and potentially reveal novel pathways modulated by KSHV lytic infection.

GP29 Targeting ANO1 signaling to counter vGPCR-mediated anti-apoptotic effects

Anisha Reddy Konakalla, Erica Sanchez

University of Texas at Dallas, Richardson, TX, USA

Abstract

The oncogenic virus, Kaposi's Sarcoma-Associated Herpesvirus (KSHV) causes Kaposi Sarcoma (KS), a malignant cancer. Both latent and lytic stages of KSHV contribute to its pathogenesis. Modulating host cell transcription and ensuring cell survival are critical for the progression of KSHV infection. Viral G Protein-Coupled Receptor (vGPCR), expressed during lytic infection, notably induces angiogenesis, promotes cell growth, inhibits endothelial cell apoptosis, and influences host gene expression and cell survival signaling. The specific role of vGPCR in apoptosis regulation is still understudied. A better understanding of these mechanisms will contribute to the development of effective treatments for KS. We conducted an RNA-Seq study to evaluate host cell gene expression in vGPCR overexpressing (OE) endothelial cells. Several host cell transcriptional changes were measured, including the upregulation of anoctamin 1 (ANO1), a host calcium-activated chloride channel that is implicated in modulating apoptosis. Preliminary data shows that upon ANO1 knockdown, vGPCR overexpressing endothelial cells show an increase in cell death under apoptotic conditions. Our results show the host protein ANO1 is required for vGPCR-mediated inhibition of host cell death, expanding our understanding of the fundamental mechanisms of vGPCR. Currently, we are using ANO1 drug inhibitors and measuring cell viability and their impact on various cellular processes. We hypothesize that vGPCR-dependent ANO1 signaling mediates the anti-apoptotic pathway which contributes to the intricate network of cellular responses aimed at preserving cell survival. Our goal is to elucidate the molecular pathways involving vGPCR and ANO1, thereby suggesting ANO1 as a therapeutic agent against KS.

GP30 Phylogenomic framework and virulence profiles of *Escherichia coli* serotype O118

Irvin Rivera, Anwar Kalalah, Sara Konig, Mark Eppinger

The University of Texas at San Antonio, San Antonio, TX, USA

Abstract

Background and Significance: Infections with Non-157 Shiga toxin-producing *Escherichia coli* (STEC) are an increasing global health concerns. Among these are serotype O118 isolates, which the CDC linked to 57 human cases in 2016, and infections have been on a steep incline. The aim of this study was to provide a comprehensive analysis of the pathogenome features and relationships found in this serogroup based on all currently publicly available genomes.

Materials and Methods: Genomes were retrieved from NCBI GenBank and imported into Ridom SeqSphere+ for (targeted and core genome) MLST. Virulence and prophage inventories were identified using Virulence Factor Database (VFDB) and PHASTER, respectively. The pangenome was determined and classified with Roary and Scoary.

Results and Conclusions: In total we analyzed 218 genomes that feature five H-serotypes. Among these H2 and H16 form the intimin (eae)+ STEC pathogroup, while the (eae)- non-STEC pathogroups are made up by H10, H11, and H51. The STEC carry toxins stx1a, 2a, 2c alone or in combination of two, along with suballeles (β and ε). A cgMLST-based tree was inferred from the core and accessory inventory of 4160 genes. Its topology unveiled the clustering of STEC and non-STEC groups according to their H-groups. For the STEC we further noted a correlation between respective H-serotype and eae-subtypes (H2/eae-ε, H16/eae-β). The global gene reservoir was computed at 20768 genes, and further analysis allowed insights into the common, shared and strain-specific gene content and virulence determinants of the different *E. coli* pathogroups that make up in this lineage.

GP31 Elucidating CRISPR-Cas defense against antibiotic resistance plasmids in *Enterococcus faecalis*

Tahira Amdid Ratna, Alejandro Barros, Kelli Palmer

University of Texas at Dallas, Richardson, TX, USA

Abstract

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 via 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 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.

GP32 Elucidating the synthesis and function of a novel glycophospholipid in *Pseudomonas aeruginosa*

Fabiha Zaheen Khan[1], Kelli Palmer[1], Ziqiang Guan[2]

[1]University of Texas at Dallas, Richardson, TX, USA. [2]Duke University, Durham, TX, USA

Abstract

Pseudomonas aeruginosa is a Gram-negative bacterium that causes secondary chronic infections in cystic fibrosis (CF) patients. *P. aeruginosa* can cause a range of other opportunistic infections and is able to effectively dominate in a polymicrobial community. Bacteria, in general, have been found to modify their membrane lipids in response to external stress. Likewise, *P. aeruginosa* possesses a range of membrane phospholipids, which can be modified to protect against cationic antimicrobial peptides (CAMPs). An Acidic Bligh-Dyer lipid extraction conducted on *P. aeruginosa* PA14 revealed a previously uncharacterized glycophospholipid, aminohexosyl-phosphatidylglycerol (AH-PG), in mass spectrometric analysis. The genes likely responsible for AH-PG production were found in a three gene operon containing a glycosyltransferase, a deacetylase and an MprF-like flippase. Previous literature studies in *Clostridoides difficile* identified a similar three gene organization responsible for resistance against daptomycin and bacitracin. When the putative deacetylase gene was disrupted, AH-PG production by *P. aeruginosa* was lost. *P. aeruginosa* also encodes the multiple peptide resistance factor (MprF), which is responsible for aminoacylation of phospholipids and flipping of them across the membrane. In *P. aeruginosa*, MprF uses phosphatidylglycerol (PG) as substrate, to make significant levels of alanyl-phosphatidylglycerol (Ala-PG) in acidic stress conditions. We found that when *mprF* was interrupted, there was an increase in AH-PG in an acidic environment (pH = 5.5), indicating a compensatory mechanism for the loss of Ala-PG. Hence, we hypothesize that AH-PG may be playing a role in protecting *P. aeruginosa* from CAMPs and similar antimicrobials, along with Ala-PG.

GP33 Role of EF1797, a hypothetical protein associated with daptomycin resistance, and its link to a novel glycolipid in *Enterococcus faecalis*

Aparna Uppuluri[1], Ziqiang Guan[2], Kelli Palmer[1]

[1]The University of Texas at Dallas, Richardson, TX, USA
[2]Duke University Medical Center, Durham, TX, USA

Abstract

Enterococcus faecalis is a gram-positive bacterium that causes endocarditis and other infections. The enterococcal MprF, an integral membrane protein, synthesizes the recently identified, novel cationic glycolipids lysyl-diglucosyl-diacylglycerol (Lys-Glc2-DAG) and lysyl-glucosyl-diacylglycerol (Lys-Glc-DAG), as well as the modified phospholipid lysyl-phosphatidylglycerol (Lys-PG). Enterococci acquire antibiotic resistance upon repeated exposure to antimicrobials like daptomycin (DAP), a last-resort antibiotic used to treat vancomycin-resistant enterococci. In previous studies in *E. faecalis* V583, three highly DAP-resistant (DAP-R) strains were generated by in vitro evolution. These strains had been characterized by genome sequencing, in which mutations in cardiolipin synthetase and a range of other genes were identified. In this study, we analyzed these strains by comprehensive lipidomics. This analysis revealed significant reduction in Lys-Glc2-DAG levels and other cationic lipid changes in the DAP-R strains compared to wild-type V583. We determined that mutations in EF1797, encoding a hypothetical protein, confer dramatic changes in Lys-Glc2-DAG levels. In summary, we identify a potential role for cationic glycolipids in adaptive pathways to daptomycin resistance, and a potential function for the hypothetical protein EF1797.

GP34 Redefining antibiotic resistance in *Acinetobacter baumannii*

Muneer Yaqub, Chinmai Rudra, Zoe Du, Reagan Crow, Nicholas Dillon

The University of Texas at Dallas, Richardson, TX, USA

Abstract

Acinetobacter baumannii infections pose significant human health concerns due to their increasing resistance to antibiotics, particularly Polymyxin E (colistin), a last-resort treatment. As colistin resistance rates are still low in *A. baumannii*, the mechanistic basis for colistin resistance in *A. baumannii* is unclear. To address this, we employed a machine learning model trained on clinical *A. baumannii* isolates, identifying 31 genes linked to colistin resistance. To assess their impact, we employed the Manoil AB5075 transposon library and conducted standardized antimicrobial susceptibility testing on each transposon mutant. None of the 31 *A. baumannii* mutants exhibited increased resistance to colistin when assessed based on their Minimum Inhibitory Concentration (MIC). However, further investigation into the relationship between these genes and resistance revealed intriguing results. Although MIC values showed no significant differences, we observed distinct adaptive fitness phenotypes among the mutants in response to colistin. Approximately 48% of the mutants displayed heightened fitness, while 26% exhibited decreased fitness when exposed to sub-inhibitory colistin concentrations. Moreover, we found that most mutants show alterations in membrane properties, biofilm formation, efflux pump activity, and oxidative stress response in the presence of colistin. These findings suggest that routine susceptibility tests based solely on MIC values may not fully capture A. baumannii's adaptive responses to colistin. Consequently, our study emphasizes the need for comprehensive assessments beyond MIC measurements to accurately define colistin resistance in *A. baumannii*. By revealing the intricate adaptive responses of *A. baumannii* to colistin, our research urges reevaluation of how we define antibiotic resistance in bacterial pathogens.

Undergraduate Poster Presentations

General Microbiology

UP1 Antibiotic-resistant 'superbugs' in Lake Meredith, a premier water reservoir in the Texas Panhandle region

Quincy Nakamura, Robyn Cuthbert, Bentli VeneKlasen, Kara Ramirez, Cassie Dorsett, Jessica Case, Erik Crosman, Maitreyee Mukherjee

West Texas A&M University, Canyon, TX, USA

Abstract

Background: As humans misuse antibiotics, the microflora within the human body become resistant to these antibiotics that eventually make their way into the sewer systems and then the environment. We are now seeing global cases of bacterial infections unable to be treated with any currently known antibiotics. Because resistant strains of bacteria can be deadly and can be spread among even unrelated bacteria via horizontal gene transfer, it is important to measure and create a representative dataset that can be used to help create public policy to help mitigate the spread of antimicrobial and multidrug resistance. Lake Meredith is the most important water resource in the Texas Panhandle region used widely for recreational and drinking water sources. In this study we analyzed antimicrobial resistance and multidrug-resistant bacteria in Lake Meredith over a period of seven months.

Methods: Between March – August 2023, we collected water samples from six different sites in Lake Meredith, Texas. *E. coli* strains was isolated and then analyzed for resistance to eight different antibiotics – erythromycin, ampicillin, amoxycillin, sufamethoxazole, cephalothin, imipenem and tetracycline utilizing the Kirby Bauer Disc Diffusion Method.

Results: Many isolates showed high antimicrobial resistance, especially in warmer months likely to an increase in biomass and human traffic. Erythromycin resistance was highest, about 92.8% of all isolates were resistant to erythromycin. Similarly, high amounts of multidrug resistance were observed.

Future Directions: We plan to continue collecting data from Lake Meredith and analyze seasonal and spatio temporal variations of AMR and MDR bacteria in the lake.

UP2 An analysis of the levels of microbial contamination in Lake Meredith, a vital water resource in the Texas Panhandle region.

Kara Ramirez, Quincy Nakamura, Bentli VeneKlasen, Robyn Cuthbert, Cassie Dorsett, Erik Crosman, Maitreyee Mukherjee

West Texas A&M University, Canyon, TX, USA

Abstract

Background: Pathogenic contamination of water sources is increasing globally. Several water bodies across the state of Texas have been deemed unsuitable for human use due to the presence of high levels of microbial fecal contamination. Lake Meredith is a vital water resource in the Texas Panhandle region, used for drinking water and several recreational activities by residents, e.g., boating, swimming, fishing, camping, etc. The aim of this project was to analyze the occurrence, distribution, and abundance of *E. coli* and Enterococcus, two indicator organisms for fecal contamination, within Lake Meredith across a seven-month period.

Methods: We collected water samples across 6 sites in Lake Meredith each month between March-August 2023. These samples were immediately analyzed for the presence and amount of fecal indicator bacteria (FIBs) following the standard EPA methods 1603 (*E. coli*) and 1600 (Enterococcus). Colonies were enumerated for colony forming units (CFU) per 100 ml of water sample.

Results: Our results reveal substantially high amounts of both *E. coli* and Enterococcus contamination across all sites in Lake Meredith. Levels of contamination were higher in the summer months compared to spring months, especially following high rainfall events. Many of the samples analyzed fell well beyond the EPA set standards.

Future Directions: We plan to continue with the collection and data analysis until the end of the year. Following this, we plan to examine the sources of the contamination observed in the lake using molecular-based microbial source tracking methods.

UP3 What is that in the air of Longview Texas: a fungal focus

Jordan Angeles, Jayden Dennis, Jordan Davault, Josh McLoud

LeTourneau University, Longview, TX, USA

Abstract

Rationale: The atmosphere contains a tremendous diversity of airborne fungi including both unicellular yeast and multicellular mycelial fungi; fungal species may produce allergens or be plant pathogens or be opportunistic pathogens to immunocompromised individuals. Airborne mycelial fungi have been characterized extensively by spore trap and culture-based sampling externally to East Texas. This investigation was the first survey of viable airborne fungi in Longview, Texas.

Methods: Beginning 11th September 2023, a single stage impaction sampler with Malt Extract Agar culture medium was exposed for 1 minute at 1 meter above the ground on Longview LeTourneau University campus. Sample collection occurred on Mondays and Fridays each week at noon with two collections per day. Following 3-4 days incubation, each isolate was identified by morphology of sporangium or spores or cells. Yeast isolates were subclassified by pigment because yeast cells lack morphological distinction. Observed colonies were transformed into colony forming unit per cubic meter (CFU/m³) for each culture plate, sample.

Results: Eight taxa of fungi were isolated, this included *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Penicillium*, white-yeasts, and pink/orange-yeasts. Presence of mycelial fungi were 100% and yeasts were 88% of samples. Most frequent airborne fungi included (1) *Cladosporium* 100% (2) Unidentified Fungi 100% (3) *Aspergillus niger* 88% with respective peak concentrations of 7,058, 789, and 117 CFU/m3.

Conclusion: Trends in our data for mycelial fungi agreed with previous investigations; yet the frequency and diversity of yeasts was novel. Future investigations will focus on temporal trends in the culturable airborne fungi.

UP4 Impact of the COVID-19 pandemic on microbiology undergraduate students' academic performance at the University of Texas at Arlington

Mohamed Abdalati, Whitney Tholen, Ph.D.

University of Texas at Arlington, Arlington, TX, USA

Abstract

The COVID-19 pandemic has brought unprecedented challenges to higher education, impacting students' academic performance. This study investigates the effects of the pandemic on microbiology undergraduate students at the University of Texas at Arlington (UTA). A retrospective cohort study design was employed, analyzing academic records of 3,059 microbiology undergraduate students from 2020 to 2022. Results indicate a significant decline in academic performance for both cohorts of general and nursing microbiology students during spring 2020 and 2022. A literature review explored the relationship between socioeconomic status (SES) and academic performance during the pandemic. Literature shows students of lower economic status experienced more educational disparities. This may have impacted many students at UTA. This study reveals the COVID-19 pandemic significantly impacted students in higher education. Findings emphasize the need for tailored interventions and equitable education strategies to support students navigating unforeseen disruptions.

UP5 Differential membrane permeability of LL-37 resistant uropathogenic *Escherichia coli*

Eesha Desai[1], Namrata Sawant[1], Swami Sadanala[1], Philippe Zimmern[2], Nicole De Nisco[1,2]

[1]University of Texas at Dallas, Richardson, TX, USA
[2]UT Southwestern, Dallas, TX, USA

Abstract

Recurrent urinary tract infection (rUTI) has become difficult to treat due to rising antibiotic resistance. The antimicrobial peptide LL-37 is a critical component of the human innate immune response to UTI. Uropathogenic Escherichia coli (UPEC) strains that are resistant to LL-37 have been shown to cause more severe UTIs. Our lab has recently determined the minimum inhibitory concentration (MIC) of LL-37 against several UPEC strains isolated from women with rUTI and we have observed significant resistance among these isolates.

Cationic LL-37 targets UPEC by binding to anionic lipopolysaccharide (LPS) in outer membranes (OM), forming pores in the lipid bilayer leading to lysis. OM modification is a strategy used by Gram-negative bacteria to resist cationic antimicrobial peptides. This study aims to measure the effect of LL-37 on the membrane permeability of phylogenetically diverse UPEC strains isolated from women with rUTI. We used an ethidium bromide (EtBr) uptake assay to measure the membrane permeability of LL-37 resistant and LL-37 sensitive UPEC strains in response to treatment with sub-inhibitory concentrations of LL-37. EtBr is an intercalating dye that fluoresces when wrapped around DNA and concentrates in the cytoplasm. Fluorescence increases over time as EtBr accumulates inside the cell and reaches a steady state. Using this assay, we can determine if decreasing membrane permeability may be a mechanism utilized by LL-37-resistant strains. We hypothesize that LL-37 treatment will substantially increase the permeability of UPEC strains with lower LL-37 MICs and that highly LL-37-resistant UPEC strains will not demonstrate significant changes in membrane permeability.

UP6 The impact of biological medium on antimicrobial peptide resistance among clinically isolated uropathogenic *Escherichia coli* strains

Swami Sadanala[1], Namrata Sawant[1], Eesha Desai[1], Philippe Zimmern[2], Nicole De Nisco[1,2]

[1]University of Texas at Dallas, Richardson, TX, USA
[2]University of Texas Southwestern Medical Center, Dallas, TX, USA

Abstract

Urinary tract infection (UTI) is one of the most common bacterial infections globally and primarily affects women. In response to infection, the bladder epithelium produces LL-37, a cationic antimicrobial peptide (CAMP) that hinders bacterial growth and is a critical component of the innate immune response to infection. However, some strains of uropathogenic *Escherichia coli* (UPEC), the main causative agent of UTI, are resistant to LL-37. Our group previously identified LL-37 resistant UPEC strains using standard laboratory conditions of lysogeny broth (LB) medium in an ambient atmosphere. However, because LL-37 acts at the bladder mucosal surface and antimicrobial efficacy is known to be impacted by growth medium, we sought to expand our study of LL-37 resistance in diverse UPEC strains to conditions more relevant to the urinary environment using Artificial Urine Medium (AUM) and pooled urine. An important distinction between AUM/urine and LB, for example, is the presence of anionic compounds such as sulfate, bicarbonate, and trifluoroacetate in AUM and urine that may interact with the cationic LL-37 and alter interactions between the peptide and UPEC. To identify potential differences in LL-37 susceptibility of UPEC strains between LB, AUM, and urine, we determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of LL-37 in LB, AUM, and urine for 7 UPEC strains. To differentiate effects that may be specific to LL-37 we also determined the MICs and MBCs of Polymyxin B and Colistin, CAMPs with a similar mechanism to LL-37, in all three media conditions.

UP7 Detection of pathogens in wastewater through Next Generation Sequencing

Amna Elbadawi, Hannah Allie, Janine Yost, Michelle Crum

UT Tyler SOM- PHLET, Tyler, TX, USA

Abstract

Background: One of the greatest hurdles to outbreak response, as exemplified by the COVID-19 pandemic, is disease monitoring and reporting. The rapid evolution of SARS-CoV-2 variants and lack of adequate testing information makes it difficult to track and assess the evolution of the pathogen in a timely manner; hence hindering public health response. Currently, the Public Health Laboratory of East Texas (PHLET) service area includes 35 counties within East Texas. Most of which are rural in nature and are underserved, making them especially vulnerable to exposure to rapidly emerging diseases. Therefore, PHLET established the first wastewater-based epidemiology program to monitor SARS-CoV-2 levels in the community. Additionally, PHLET also completed the validation of NGS for rapid detection of SARS-CoV-2 variants circulating within the population near Smith and Gregg counties.

Objectives: The primary aim of this project is to evaluate clinical validation study of SARS-CoV-2 and perform a retrospective analysis of wastewater-based epidemiology data. This will serve as an important and educative basis for our secondary goal, which is to monitor circulating SARS-CoV-2 in East Texas through regional wastewater samples.

Methods: The method followed the standard amplicon-based Illumina MiniSeq system. For the validation phase, over 40 clinical samples containing SARS-CoV-2 were sequenced.

Conclusions: Overall, PHLET has validated NGS and plans to expand to other pathogens such as influenza and respiratory syncytial virus (RSV) detection. In the future, we will work towards increased sharing and coordination with our local partners to implement appropriate public health measures in response.

UP8 Using fluorescence in situ hybridization to detect the bladder microbiome in women with urge urinary incontinence

Mark Nguyen[1], Ujjaini Basu[1], Nazema Siddiqui[2], Nicole De Nisco[1,3]

[1]The University of Texas at Dallas, Richardson, TX, USA
[2]Duke University Medical Center, Durham, NC, USA
[3]The University of Texas Southwestern Medical Center, Dallas, TX, USA

Abstract

Over the last decade, it has been conclusively shown that a microbiota exists within the urinary tract (i.e., urethra and bladder) of healthy women. It has been hypothesized that disruption of this urinary microbiome (urobiome) may impact susceptibility to disorders of the urinary system. Although the composition of the urobiome has been described in both healthy women and those with urologic disorders like incontinence and urinary tract infection (UTI), the spatial organization of the urobiome is unknown. Previously, our group has used 16S rRNA fluorescence in situ hybridization (FISH) to visualize bacterial communities within the bladder of postmenopausal women with recurrent UTI. In this study, we use 16S rRNA FISH to visualize bladder associated bacteria in 34 bladder biopsies. Following informed consent, bladder biopsies from 34 patients were classified into four groups based on their medical history of urge urinary incontinence (UUI) and insulin resistance (IR): UUI only (n = 13), IR only (n = 12), UUI and IR (n = 8), and Healthy (n = 1). Thus far, we have detected bladder bacteria in 77% of the UUI only cases, 67% of the IR only cases, 63% of the UUI and IR cases, and none in the control case using a universal probe that labels all bacteria. To determine the taxonomic identity of these bladder-associated bacteria, we also used genus-specific 16S rRNA probes. We have successfully applied *Escherichia* and *Staphylococcus* probes and are currently developing *Enterococcus*, *Lactobacillus*, and *Streptococcus* probes to distinguish both commensal and pathogenic bladder-associated bacteria.

UP9 Bacteria in bath towels and their pathogenic relationships with human skin microbiome

Ri-Qing Yu, Alan Lizarraga, Nelly Soria

University of Texas at Tyler, Tyler, TX, USA

Abstract

The face and bath towels as the major household textiles could contribute to the transmission of antibiotic-resistant bacterial pathogens and infectious viruses. This study hypothesizes that, without proper sanitization, towels for bathing or face-washing may contain a variety of microbes and secondary contamination source of antibiotic-resistant bacterial pathogens that undermines public health. This study has been conducted via microbiology laboratory discussing human skin microbiome since 2021 with the methods including plate streaking isolation, strain purification, genomic DNA extraction, gel electrophoresis, PCR of 16S rRNA genes, DNA purification, sequencing, and bioinformatics analyses. Results Spring 2021 investigation 30 students sample showed total 47 microbial isolates obtained. Bacterial isolates dominated by *Staphylococcus*-related species, having largest cluster (37 isolates) on the phylogenetic tree, 14 species isolated from the towels and 23 species from skin. The major clade included the pathogen species of *S. epidermidis*, *S. aureus*, *S. warneri*, *S. haemolyticus*, and *S. hominis*. Two isolates, *Bacillus* and *Roseomonas*, one isolate from *Nosocomiicoccus*, *Exiguobacterium*, *Micrococcus*, *Klebsiella*, *Stenotrophomonas*, *Acinetobacter*. Fall 2021 samples of 60 students showed the total 78 Staphylococcus-related isolates were dominated group microbes in towels (29), skin (49). *Bacillus* was second largest cluster (4 from towels, 3 from skin). Two isolates were each from *Micrococcus* and *Enterobacter*, one isolate from *Paenibacillus*, *Corynebacterium*, and *Pseudomonas*. Further exploration on the human skin and bath towel microbiome for three semesters from 2022 to 2023 are under processing. Results from this study provide scientific evidence and basis for establishing potential hygiene strategies for the public in the future.

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