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ABSTRACT BOOK

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Abilene Christian University
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UNDERGRADUATE ORAL PRESENTATIONS

UO 1: Diversity of Cluster M Phages: A Comparative Analysis of Minor Tail Protein Phamilies

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Abstract

Bacteriophage minor tail proteins (MTPs) play an important role in phage tail assembly. These proteins are essential for phage adsorption, a prelude to host infection, and could serve as reliable tools for bacterial pathogen recognition. For host infection and host range to be better understood, it is warranted to investigate the sequence and structural diversity of MTPs. Previous research focused on cluster F phages, whereas ours is focused on cluster M phages. Gene products of the two phams closest to the lysin A gene where most diversity was observed based on Phamerator color codes were comparatively analyzed. 77 MTPs were observed in the 15 currently annotated phages within cluster M, two of which were analyzed per phage, giving a total of 30 genes compared across subclusters M1, M2, and M3. The two amino acid sequences of each phage were convocated. Clustal Omega and MEGA11 were used to construct maximum likelihood cladograms. Subclusters M1 and M2 grouped in their own clades, with M3 coming in between. Differences in sister taxa were observed, due to differences in amino acid sequence ordering within the clades. Three-dimensional protein folding models were also constructed using SWISS-MODEL, and minor structural differences were observed. Next steps should include expanding this investigation to understand MTP diversity and structural conservation across a wide range of clusters in relation to host range. The potential for using phage minor tail proteins for pathogenic bacterial identification to replace the time-consuming culture methods should also be investigated.

UO 2: Isolating Discrete Life Cycle Stages of a Cockroach-Infecting Gregarine for Gene Expression Analysis

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Abstract

Gregarines are a subclass of apicomplexan protists that parasitize the intestinal epithelia of nearly all invertebrate clades. *Blabericola migrator* is a species of Gregarine that solely infects Madagascar Hissing Cockroaches. Gregarines' closest relatives, Cryptosporidians, infect vertebrates and cause serious diarrheal disease in humans. Gregarines' similar lifestyle and life cycles to Cryptosporidians suggest that the largely understudied parasites may serve as a model for these human infectious parasites. Gregarines have distinct internal stages: the sporozoites, trophozoites (attached and detached), and those in the sexual stage of syzygy. To date, transcriptomic analysis has only been completed on Gregarines from a mixed internal stage population, which does not distinguish between the specific genes expressed at each internal stage; therefore, to develop stage-specific Gregarine transcriptomes, we isolated three subpopulations of internal stage Gregarines: attached trophozoites, detached trophozoites, and those in syzygy. RNA was isolated from 4 biological replicates of each subpopulation and submitted for mRNA sequencing. Each subpopulation's transcriptome was compared to one another and to the published mixed internal stage's transcriptome to identify how gene expression is altered throughout the maturation of internal Gregarine parasites. Gene cluster analysis measured the variation among subpopulations and determined unique groupings for each subpopulation. The detached trophozoite subpopulation had the most abundance amongst its biological replicates most likely due to the biological variation of the subpopulation. Currently, gene ontology pathways are being analyzed. Once completed, these detailed findings will provide vital information regarding stage-specific biology of the parasite and its interactions with its host.

UO 3: Analysis of Tetracycline Resistant Bacteria Within the Lake Fort Phantom Hill Watershed

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Abstract

Tetracycline resistance genes have been reported to be abundant in bacteria in environments impacted by agriculture, including manure treated soils. Abilene, Texas is surrounded by ranchlands and lies completely within the Fort Phantom Hill Watershed. We hypothesized that tetracycline resistant coliforms could be present throughout the watershed. To investigate our hypothesis, replicated samples were taken from the sediment of 7 sites within the watershed between June 2022-Aug 2022. Following enrichment in buffered peptone water, samples were serially plated on MacConkey agar supplemented with tetracycline (16 µg/mL). To date, we isolated tetracycline resistant coliforms and non-coliforms from 6 out of 7 sites. Tetracycline resistant non-coliform isolates included *Pseudomonas* and *Serratia* species, while resistant coliforms included *Escherichia* and *Klebsiella*. We also screened 120 tetracycline resistant isolates for cefotaxime and nalidixic acid susceptibility. Eight isolates cultured from different sites throughout the watershed were resistant to all three antibiotics: 7 *Pseudomonas* strains and 1 strain of *Escherichia coli*, designated WHS-109. We completed an additional screen of *E. coli* isolates from the same site as WHS-109 for susceptibility to sulfamethoxazole, ampicillin, and kanamycin. Our results indicated WHS-109 is also resistant to ampicillin and kanamycin, while all other isolates were susceptible. To determine the mechanism of tetracycline resistance, we completed multiplex PCR for 7 *tet* genes. We determined WHS-109 contains the *tetB* gene, while other *E. coli* isolates at the site contain *tetA*. Further research will investigate additional genetic differences and similarities of tetracycline resistant isolates throughout the watershed and the composition of plasmids observed.

UO 4: The Effect of Lanthanum on Growth and Methanol Metabolism in *Paracoccus denitrificans*

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Abstract

Paracoccus denitrificans is a non-obligate methylotroph that can grow on methanol as a sole source of energy. Regulatory proteins MxaXYZ control the transcription of *mxaF*, which encodes a calcium-dependent methanol dehydrogenase. The *P. denitrificans* genome also encodes XoxF, a predicted lanthanide-dependent methanol dehydrogenase. β-galactosidase assays in methanol media were used to assess the activity of *lacZ* reporter fusions to the *xoxF* and *mxaF* promoters. Results suggest that growth on calcium-containing media fostered considerable activity of the *mxaF* promoter and minor activity of *xoxF*, while growth on lanthanum-containing media limited their activities. This may imply lanthanum has an inhibitory effect on MxaXYZ's regulatory activity. β-galactosidase assays in lanthanum-containing methanol media were then conducted to measure the activity of a *mxaZ* promoter-reporter fusion to test this theory, and lanthanum appears to inhibit its function as well. While growing the cultures in an aerobic environment, cell aggregation was observed with higher concentrations of lanthanum, possibly indicating that similar results would occur with cells grown in a static environment.

Biofilming, when cells grow in thin layers on surfaces, is one such static environment. Mutant strains lacking *bapBCD*, a Type 1 Secretion System, and *bapA*, a secreted protein that assists biofilming in *P. denitrificans*, were grown in calcium or lanthanum-containing media. Results indicate lanthanum impedes biofilming and promotes cell aggregation. Furthermore, calcium-dependent biofilming in methanol-grown cultures seems to utilize a non-BapA and non-BapBCD-dependent pathway. This finding, coupled with lanthanum's effect on methylotrophic growth and regulation, suggests lanthanum likely directly inactivates multiple calcium-requiring enzymes/pathways.

UO 5: Role of ClpX in the Stress Response and Virulence of *Bacillus anthracis*: Protease or Chaperone?

Lillian Wilson, Vuong Do, Shauna M. McGillivray
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Abstract

Anthrax is a lethal infectious disease caused by the bacterial pathogen *Bacillus anthracis*. Our lab studies the virulence and antibiotic resistance of *B. anthracis* and we have identified a chromosomal gene *clpX*, as an important virulence factor, as its loss increases susceptibility to cell-envelope targeting antibiotics such as penicillin, daptomycin, and the antimicrobial peptide LL-37. ClpX is an ATPase that can act autonomously as a chaperone, or with a proteolytic core, ClpP, to degrade proteins. To investigate the mechanism ClpX uses, a plasmid p*clpX*^{I264E} was designed with a mutation in *clpX* (I264E) that prevents ClpP binding and inhibits the formation of the ClpXP protease but does not disrupt the chaperone activity of ClpX. We used this to create 4 strains in the unencapsulated Sterne strain: wild-type and *ΔclpX* containing the empty inducible plasmid pUTE657, complementation plasmid with the non-mutated *clpX* gene (*ΔclpX* +p*clpX*), and the mutated plasmid (*ΔclpX* + p*clpX*^{I264E}). Prior research done on these strains confirmed that ClpX relies on protease activity in antimicrobial stress; however, our goal was to assess its response in other environmental stressors such as acid stress, heat stress, and its virulence *in vivo* with the *Galleria mellonella* infection model. We find that that the protease activity of ClpX is important for all of these stresses. These results build on our earlier understanding and demonstrate that formation of the ClpXP protease is critical, and any future development of drugs targeting the ClpX system should focus on protease formation rather than chaperone activity.

UO 6: RT-qPCR Analysis of Target Genes in Multi-Drug Resistant *Pseudomonas aeruginosa* Clinical Isolates

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Abstract

Pseudomonas aeruginosa is a Gram negative, opportunistic bacterium capable of causing a number of acute illnesses in humans. *P. aeruginosa* readily evolves resistance to virtually all antibiotic classes in a multifactorial fashion that makes this pathogen difficult to treat.

In this study we collected clinical samples of *P. aeruginosa* in order to analyze local resistance trends by assessing expression of five constitutive genes: ampC, mexR, mexZ, nfxB, and oprD. **We hypothesize** that there will be trade-offs in resistance gene expression due to co-regulatory mechanisms of different genes. Our findings may help to illuminate the multifactorial nature of multi-drug resistance in *P. aeruginosa.*

We used RT-qPCR to assess RNA expression of aforementioned genes and found Pearson's Correlation Coefficients of 0.947 for ampC vs mexR expression and 0.862 for ampC vs nfxB expression in thirteen local clinical isolates. Furthermore, our preliminary data shows ampC overexpression in all tested isolates and we are in the process of expanding our cohort to evaluate this trend through statistical analysis. We also performed MIC microdilution for four β-lactam antibiotics to assess the relationship between ampC expression level and degree of resistance. Regression analysis and Pearson's Correlation found no statistical relationship between these variables indicating that expansion of the gene targets under consideration is necessary to better characterize other drug resistance determinants.

As we expand our cohort, we will begin DNA and RNA sequence analysis in an effort to gain insight into the observed genetic expression data and to understand the co-regulatory mechanisms at work.

GRADUATE ORAL PRESENTATIONS

GO 1: Identification of Multiple *Salmonella* Serotypes in Beef Cattle Samples using CRISPR-SeroSeq

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Abstract

Culture-based *Salmonella* isolation techniques culminate in selection of 1-3 presumptive colonies, which are then serotyped. In a mixed population containing multiple serotypes, this approach typically only detects the most abundant serotype and those outnumbered remain undetected. CRISPR-SeroSeq is an amplicon-based sequencing approach that uses the native CRISPR spacer sequences in *Salmonella* to quantify multiple serotypes in a sample. We used CRISPR-SeroSeq to evaluate *Salmonella* positive fecal, lymph node, and hide samples from cattle and compared the serotyping results with those identified by culture methods.

Levent et al. (2019) collected fecal, hide, and lymph node samples from beef cattle and used culture methods to isolate and then serotype *Salmonella*. Thirty of the *Salmonella* positive samples were selected for enrichment and analysis by CRISPR-SeroSeq. We isolated the total genomic DNA from the enriched cultures and performed CRISPR-SeroSeq in two sequential PCR steps. The first PCR targeted the CRISPR regions, and the second added dual index barcodes to facilitate multiplexed sequencing. DNA libraries were pooled in equimolar amounts and sequenced on an Illumina NextSeq.

The original study isolated a single serotype per sample: Cerro (15/30), Anatum (7/30), Lubbock (4/30), Montevideo (3/30), and Newport (1/30). CRISPR-SeroSeq identified an average of 1.5 serotypes per sample with 5 serotypes total (Anatum, Mbdanka/Lubbock, Montevideo I, Newport II, Cerro). Multiple serotypes were identified in 43.3% (13/30) of all samples and 50% (6/12) of lymph node samples. Anatum was the most identified serotype in 67% (20/30) of samples and Cerro was the second most identified serotype (11/30).

GO 2: Characterization of Novel Zinc Oxide Resistant *Staphylococcus aureus* Mutants

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Abstract

As the number of antibiotics in development dwindles and antibiotic resistance continues to rise, there is a need for novel, non-traditional antibiotics such as zinc oxide nanoparticles (ZnO NPs). While the broad-spectrum antimicrobial properties are well established, the mechanism of action is still unknown. Previous work has proposed that reactive oxygen species (ROS), toxic Zn^{2+} ions, and electrostatic interactions with the cell envelope may be implicated in the mechanism. To evaluate which of these mechanisms are involved, we characterized the physical and genetic properties that confer resistance to ZnO NPs in three novel ZnO resistant strains of *Staphylococcus aureus* (ZnO^R). These strains possess comparable growth rates and are at least four times more resistant than the parental strain against ZnO NPs acquired from multiple sources. This suggests that all ZnO NPs, regardless of morphology, size, or method of synthesis share a mechanism of action. We found that cell charge, measured by cytochrome c, was not different between the parental and resistant strains, indicating that electrostatic interactions with the membrane are not involved in the mechanism. Additionally, the ZnO^R strains shared a similar susceptibility to H_2O_2, a ROS commonly suggested to be generated by ZnO. We have also found that internalization and physical contact with the bacterial envelope are not necessary for ZnO mediated growth inhibition suggesting that ZnO produces a soluble species that is responsible for the antibacterial action. Future work includes sequencing the genome of the parental and ZnO^R strains to identify mutations that led to gain of resistance.

GO 3: The Characterization of the Potential Iron-Acquisition Gene *dUTPase in Bacillus anthracis*

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Texas Christian University, Fort Worth, USA

Abstract

Bacillus anthracis is the causative agent of the fatal disease anthrax, and its virulence is of great interest due to its potential as a biological weapon. *B. anthracis* causes disease by both escaping immune defenses and acquiring nutrients. A necessary extracellular nutrient that pathogens must acquire from its host is iron. To discover novel genes that are essential for for iron acquisition, we screened transposon mutants in iron-deficient media with hemoglobin as the sole source of iron. We further prioritized the mutants discovered in our in vitro screen by assessing for attenuated virulence using our in vivo *G. mellonella* infection model. We found one mutant that has a disruption in the first gene of a two-gene operon containing putative *dUTPase* and *aminopeptidase* genes known as 9F12 Tn. Neither of these genes have been previously linked to iron acquisition. To confirm the role of the *dUTPase* gene in the observed 9F12 Tn phenotype, we created an independent insertional mutant in the dUTPase gene (dUTPase IM). We found that both of our mutants, 9F12 Tn and dUTPase IM, could not use hemoglobin as a source of iron. We also found that *G. mellonella* injected with 9F12 Tn and dUTPase IM had higher survival rates than those injected with the parent strain. Our results indicate that the *dUTPase* gene is necessary for iron-acquisition and virulence in *B. anthracis*. This study furthers our understanding of iron acquisition in a bacterial pathogen and increases our knowledge of how *B. anthracis* causes disease.

GO 4: *E. coli* Phylogenetic Groups Differentially Invade Uroepithelia Suggesting Alternate Entry Mechanisms

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Abstract

Urinary pathogenic E. coli (UPEC) strains cause 65-75% of urinary tract infections (UTIs), ~75% of these UPEC are phylogenetic group B2, while the remaining 25% are groups A, D, and B1. UTIs require bacterial attachment, entry, and replication within epithelial cells forming intracellular bacterial colonies (IBCs). IBC formation has been studied using the model UPEC UTI89 which requires pili for entry. However, several in vitro transcriptomic analyses have shown significant strain-dependent differences in bacterial appendage gene expression, which suggested alternate entry mechanisms.

We hypothesize that the phylogenetic skew seen in the clinic is dependent on the groups' innate abilities to form IBCs. To examine this, IBC formation assays were performed using UPEC and non-pathogenic E. coli (NPEC) strains from groups A, B2, and D in two different model urothelial cell lines. Group B2 bacteria formed 20X the IBCs compared to groups A or D, supporting clinical observations. Surprisingly, one group B2 NPEC strain formed more IBCs than the UPEC strains in one cell line. This observation prompted a comparative transcriptomic analysis which showed variable, and strain dependent appendage expression supporting multiple mechanisms of entry. Examination of human toll like receptor (TLR) gene expression in the cell lines showed differential expression of TLR5—recognizes bacterial flagella—which correlated to the higher invasiveness of flagella overexpressing strains. Our results illustrate differences in the E. coli phylogenetic groups' abilities to form IBCs skewing towards group B2. We suggest that the host-pathogen interaction varies between individuals and significantly contributes to infection outcomes.

GO 5: Evaluation of KKF768, a Live Attenuated Vaccine, Against Pneumonic Tularemia

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Abstract

Francisella tularensis subsp. *tularensis* (*Ftt*) is a highly virulent bacterium that causes tularemia. *Ftt* is classified as a Category A select agent due to the ease of transmission, the low infectious dose, and the high mortality associated with the pneumonic form of the disease. Currently, there are no licensed vaccines, rendering the general population at risk from the illicit use of *F. tularensis* as a bioweapon. We previously developed a novel vaccine platform based on *Francisella novicida* (*Fn*), which shares high genetic similarity to *Ftt*, but has low virulence for humans. In this study, we evaluated the attenuated *Fn-ΔiglD* expressing *Ftt* OAg (KKF768) vaccine in mouse and rat pulmonary *Francisella* challenge models. Intradermal immunization of BALB/c mice induced high titers of serum antibodies against KKF768 and T cell (Th1/Th17) mediated immunity. KKF768 provided greater than 90% protection against intranasal challenge with *F. tularensis* subsp. *holarctica* strain LVS. We further evaluated the KKF768 vaccine against highly virulent *Ftt* SCHU S4 strain in Fischer 344 rats, exhibiting similar sensitivity to *Ftt* strains as that reported for humans. Similarly, KKF768 vaccination induced both humoral and cell-mediated immunity and provided greater than 80% protection against a *Ftt* intratracheal challenge. These studies suggest that KKF768 vaccine is effective against pulmonary tularemia. The assessment of long-term vaccine protection in rats is ongoing. Current available data from these studies indicate that KKF768 vaccination induced sustainable antibody titers at 8 months after booster shot in rats.

EDUCATION SESSION

ES 1: Effects of Applied Practice on Microbiology Comprehension & Testing Anxiety in a First-Year Medical School Block

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Abstract

First-year medical students (MS1) are expected to comprehend a large amount of material in a limited time span. This pressure can lead to increased testing anxiety and decreased long term information retention. Previous studies have indicated that the provision of resources such as practice multiple-choice questions (MCQ) can aid in addressing both issues. Additionally, MCQs that are higher order promote inference rather than just recognition skills. In 2021, a survey of 52 MS1 students at TTUHSC who completed the General Principles (GPX) block, which includes general microbiology, indicated 76.95% would have found MCQ quizzes a useful resource, and 88.5% indicated additional MCQs would alleviate testing anxiety. To address this need, additional MCQ quizzes consisting of first and second order questions were developed and provided to the next MS1 cohort. Exam performance both overall and on specific exam questions linked to the practice questions was then assessed. Additionally, students were surveyed regarding the utility of the questions and effects of testing anxiety. Our results indicate that utilization of the MCQ resource resulted in better exam performance on specific questions and reduced testing anxiety. In general, MS1 students found the resource helpful for both content comprehension and anxiety reduction.

ES 2: Is the Traditional Doctoral Level Course Format Effective for Learning by all Graduate Students?

Jolene Ramsey
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Abstract

Courses designed for delivery at the graduate level are often based on discussion of niche primary literature or derivation of field-specific principles or skills. In contrast, the undergraduate courses most graduate students completed in their immediate prior educational experience are broadly knowledge-based. Despite this distinction, few faculty are trained in effective pedagogy for graduate courses. Additionally, the microbiology field is replete with resources on best practices for undergraduate teaching, but evidence-based approaches to graduate education, especially at the Master's level, are not as common. Many faculty structure graduate courses they teach similar to their own graduate experience. Striking the right balance between fostering independent scholarship in graduate students and using proven student-centered learning strategies is essential to train highly competent biology professionals. In this discussion, attendees will be invited to discuss effective strategies for teaching in classrooms with Master's students, doctoral students, or a blend of students in various degree programs

ES 3: Simple Techniques to Promote Positive Learning Environment in the Microbiology Classroom

Joni H. Ylostalo
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Abstract

Positive learning environment is known to have an important effect on student learning. How to promote this can be often challenging especially in a mix majors microbiology classroom. I will be discussing my experiences and innovations in classroom techniques that involve all student in a positive manner while achieving rigorous assessments and effective learning. For example, I will be discussing how quizzes can be used not only as assessments but also as a method to promote student buy-in utilizing die roll, "lifeline", and birthday bonus. The positive atmosphere generated in my microbiology classroom generates a fun and caring environment for learning.

ES 4: Using a One Health Assignment as a Final Project in a Microbiology Course

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Abstract*

The One Health initiative is a comprehensive strategy that seeks to understand the balance between the
human, animal, environmental domains and how each affects the health of the others. A One Health project is presented here that can be used as a final formative and summative assignment for undergraduate students enrolled in a microbiology course. Students learn about the initiative and then choose topics of relevance. They then synthesize concepts learned throughout the course and new information about the One Health initiative. An assessment rubric is provided that can be modified to a variety of different project types, not just papers, as described here. This One Health assignment helps students learn the importance of the microbiology concepts addressed in the course and also their real-world implications.

* Huddleston JR. Using a One Health Assignment as a Final Project in a Microbiology Course. J Microbiol Biol Educ. 2022 Aug 15;23(3):e00077-22. doi: 10.1128/jmbe.00077-22. PMID: 36532214; PMCID: PMC9753676.

UNDERGRADUATE POSTER PRESENTATIONS

Environmental Microbiology

UP 01: Determining if Extracts of *Eysenhardtia polystachya* and *Pinus maritima* can Combat Antimicrobial Resistance

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Abstract

Antimicrobial resistance (AMR) causes more than 35,000 deaths annually in the United States, so new treatments are needed. In this study, parts of two different plants were examined for their antibacterial activity: the bark of *Eysenhardtia Polystachya* (palo azul) and *Pinus maritima* (pine).The barks were extracted and were then tested for antimicrobial activity using a Kirby-Bauer disk diffusion assay against *E. coli lptD4213*-. The average zone of inhibition was 13mm for pine bark and 11mm for palo azul. A minimum inhibitory concentration assay (MIC) was performed, to better quantify the antimicrobial activity and showed that pine bark had an MIC of 320 ug/ml while the MIC of palo azul was 1280 ug/ml. To investigate the mechanism of action (MOA) of the extracts, bacterial cytological profiling (BCP) was performed. BCP involves treating the bacterium with an antimicrobial substance and then visualizing the changes in shape that are different based on which part of the cell is targeted. Visualization was via confocal microscopy using FM4-64 to show the membranes and DAPI to see the DNA. The treated cells changed shape but additional replication will be required to fully analyze the results. In the future, the cell shapes can be quantified and compared with the known MOA of known antibiotics. New treatments are crucial when AMR infections are increasing. Plant substances can be characterized and developed into possible alternative treatments to AMR microbes.

UP 02: The Analysis of *Punica granatum* and *Terminalia chebula* for Antimicrobial Activity

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Abstract

In the U.S., over 2.8 million people acquire and 35,000 people die from antimicrobial-resistant (AMR) infections. The overuse of antibiotics is one of the leading causes of AMR. There is a demand for creating new treatments to reduce the impact of AMR infections. *Punica granatum* (pomegranate) and *Terminalia chebula* (black myrobalan) extracts were evaluated to determine if they displayed antimicrobial activity. The pomegranate rinds were blended with water, filtered, and dried by rotary evaporation. Black myrobalan powder was extracted in methanol, filtered, and dried by rotary evaporation. For both, the solid was resuspended in water to a concentration of 100 mg/mL. Extracts were tested for antimicrobial activity by using the Kirby-Bauer Disk Diffusion assay against *Escherichia coli lptD4213-*, a strain that contains a mutation that causes a permeable lipopolysaccharide layer. The average zone of inhibition for the pomegranate extract was 10.75 mm and for the black myrobalan extract this was 7.75 mm. To quantify the antimicrobial activity, a minimum inhibitory concentration (MIC) assay was conducted and indicated that both extracts had an MIC of 2000 ug/ml. Bacterial cytological profiling was used to identify the mechanism of action (MOA) of the extracts. Cells were treated with each extract and the observed morphological changes were compared to untreated bacteria and bacteria treated antibiotics with known MOAs. By characterizing the beneficial properties of plants, future therapies against AMR infections can be improved.

UP 03: Identification of Antibiotic Resistant Cave Microbes Isolated from the Deepest Cave in Texas

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Abstract

Natural underground structures are reservoirs for many bacterial species. Bacterial cave microbes are of interest to research due to their drastically different environment compared to surface bacteria, which could lead to entirely different mechanisms of development, defense, and resistance. Our lab was able to obtain strains from Sorcerer's Cave, the deepest cave in Texas. This cave is privately owned and is documented as being rarely explored. This would suggest that the cave microbes have not been exposed to common antibiotics or resistance plasmids seen in pathogenic bacteria treated by medicine. However, previous tests showed a few strains to be highly resistant to several antibiotics including ampicillin, kanamycin, cefotaxime, rifampicin, nalidixic acid, sulfamethoxazole, and tetracycline. This study completes a catalog of 96-well plate resistance assays of 316 total isolates. Isolates were identified to genus by sequencing the 16S rRNA gene. This is the first report to identify the genera of antibiotic resistant isolates from Sorcerer's Cave.

UP 04: Tracing Antimicrobial Resistance and Virulence Factors of Enteric Bacteria Obtained from a Year-Long Sampling of Canada Geese (*Branta canadensis*)

Elizabeth Bocanegra-Nunez, Zaniya Medlin, Elizabeth Machunis-Masuoka, James Masuoka
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Abstract

Antimicrobial resistance (AMR) greatly impacts patient treatment. Wild animals, including migratory birds, can act as reservoirs for antimicrobic-resistant organisms. Tracking resistance within these populations is therefore important as novel resistance genes can be introduced into the local environment via migration. We are attempting to trace the spread of AMR gut bacteria from migrant gulls (family *Laridae*) to non-migratory Canada Geese (*Branta canadensis*) inhabiting a small campus lake. Temporal changes in genetic variability could signify the introduction of new genes coding for AMR, toxins, and other virulence factors. We hypothesize that overall genetic and pathotype diversity of bacteria isolated from geese will increase or change when gulls are present. A total of 243 bacterial isolates preliminarily identified as *E. coli* were obtained from 120 separate goose feces collected over 1 year. Repetitive-element (REP) and enterobacterial repetitive intergenic consensus (ERIC) PCR-based genetic fingerprints demonstrated consensus bands across all or most isolates as well as band variation within and between individual birds. *E. coli*-specific multiplex PCR was used to amplify known virulence factors. Analysis of these results suggest that some isolates are not *E. coli*. Further, at least some of the isolates confirmed as *E. coli* are nonpathogenic. Work on the remaining isolates is ongoing. The results of this study will uncover temporal patterns in *E. coli* diversity and help us understand the role migrating birds play in spreading antimicrobial resistance.

UP 05: Use of *Staphylococcus aureus* to Study Indoor Air Transmission of Microbes

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Abstract

As SARS-CoV-2 continues to mutate and spread, it is imperative that the scientific community continue research on reducing exposure and transmission for the general public. The Centers for Disease Control and Prevention (CDC) have clear guidelines for vaccines, masks, social distancing, and personal hygiene. However, the CDC guidelines for building capacity and room capacity are not as well defined. This study aims to look at Staphylococcus aureus and use it as a model to determine how airflow, air filtration, as well as levels of occupancy, affect the levels of microbes in the air. To test the hypothesis air samples of various rooms were taken with varying levels of occupancy. Temperature and humidity were recorded to see if they had any effect on the variances of the samples that were obtained. The level of occupancy of a room was determined by making a ratio of the number of occupants in a room to the area of a room. The samples were incubated for 24-48 hours, and the number of presumptive Staphylococcus colonies was counted. Preliminary results show a strong correlation between the number of colonies and CO2 in the room and genotyping of the colonies obtained is underway.

UP 06: Antimicrobial-Producing Bacterial Strains Isolated from Sorcerer's Cave, Texas

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Abstract

Antibiotic resistance and the overuse of antibiotics are on the rise and research is being directed towards finding new solutions to these issues. Sorcerer's Cave is a previously unexplored cave which is now understood to contain a diverse ecosystem of bacteria. These bacteria compete for limited resources some of which have adapted to produce antibiotic compounds. Of over 400 test strains collected from the Sorcerer's Cave, 36 strains were found to be antibiotic producing after preliminary testing. These strains were then furthered analyzed as growth inhibitors to Escherichia coli and/or Staphylococcus aureus using a well plate procedure. As a result of this testing a preliminary report of the inhibition efficiency of these microbes was created. These strains are also being assayed for the phenazine-1-carboxylic acid (PCA) gene which is often present in microorganisms with antimicrobial producing capabilities.

UP 07: Prevalence of Vibrio in Sea Spray Aerosols during a Saharan Dust Storm

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Abstract

Aerosols from sea spray make up the majority of atmospheric particle levels in coastal areas. Sea spray aerosols can act as a vehicle for the transport of bacteria over long distances. Therefore, sea spray could have important implications for public health and the spread of infectious diseases if pathogenic bacteria can survive and remain viable in sea spray for extended periods of time. *Vibrio* are a Gram-negative genus of bacteria found in marine environments worldwide, particularly in warmer climates. Although many species are not pathogenic, several species cause illness in humans (e.g., *V. parahaemolytics, V. cholerae, V. vulnificus*) and marine life. Due to climate change and increasing sea surface temperatures, the geographic range of *Vibrio* is expanding, leading to an increase in cases of *Vibrio*-associated illness worldwide. Extensive research has increased our understanding of *Vibrio* in aquatic habitats, but the possibility of atmospheric transport and survival has yet to be determined. The primary objective of this study was to determine if *Vibrio* could be cultured directly from the atmosphere. Additionally, as Saharan dust storms are known to contribute to marine *Vibrio* blooms, we investigated the impact of a Saharan dust storm on the prevalence of atmospheric *Vibrio*. Results demonstrate a direct correlation between presumptive *Vibrio* colony forming units (CFUs), sulfur dioxide, and particulate matter ($PM_{2.5}$ and PM_{10}), as well as an inverse correlation between CFUs and nitrogen dioxide. Sea spray transport of *Vibrio* could have a major impact on its biogeography and further disrupt human and environmental health.

UP 08: The Transmission of Multi-Drug Resistant Enteric Bacteria Between Migrant and Non-Migrant Populations of Waterfowl

Dylan Graupmann, Elizabeth Machunis-Masuoka
Midwestern State University, Wichita Falls, USA

Abstract

Improper antibiotic use and the exposure of bacteria to antibiotics in the environment is a significant concern. Understanding the mechanisms behind increased spread of antibiotic resistance, particularly multi-drug resistance (MDR), is vital. A non-migratory population of Canada Geese (*Branta canadensis*) inhabits Sikes Lake, Midwestern State University, Wichita Falls, TX. Each year there is an influx of migratory gulls (family *Laridae*) colonized with bacteria acquired from their summer habitats. To determine if migrant gulls could be introducing MDR organisms into the resident goose population, we undertook a year-long survey of goose fecal samples. We hypothesize that there will be an increase in detectable MDR in resident birds during times of high gull traffic/migration. Samples were collected monthly from June 2021 – May 2022. A total of 243 bacterial isolates were obtained from 120 separate fecal samples. The first 72 isolates have been screened using the Kirby-Bauer assay. All 72 isolates are either erythromycin^{R} or erythromycin^{I}. Of these 72 isolates, 16 MDR organisms (~22%) were identified. All 16 are erythromycin^{R} and of the 16, 6 are also ampicillin^{I}, 9 are ampicillin^{R}, and 1 is tetracycline^{R}. Notably, the analyzed samples represent the off-peak season for gull migration (June – August). MDR isolates are being further characterized by metabolic screening, protein profiling, REP/ERIC PCR, and multiplex PCR for pathotyping. Once completed, our year-long study may provide evidence for the role of migrant and resident bird populations in the maintenance of resistance in the environment.

UP 09: Application of Endophytic Microbes to Increase Drought Tolerance in Peanuts

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Abstract

To successfully produce a crop, peanut farmers often have to combat various biotic and abiotic stressors. Drought stress is episodic and often severe in Texas. Reducing water use from the Ogallala Aquifer is also an important goal for Texas producers. More sustainable practices must be developed to produce peanuts with less inputs of water, fertilizers, and pesticides. By studying peanut endophytes, a better understanding of biological applications in peanuts will be achieved. Endophytes are microbes that live in internal plant structures. Some endosymbionts can enhance host plant resistance towards abiotic and biotic stressors. In this project, endophytic microbes were first isolated from surface-sterilized peanuts and plated on potato dextrose agar media. Once endophytic growth was observed, a pure isolated colony was obtained. Isolates were then inoculated into peanut plantlets where screening was performed to identify changes in drought tolerance. Based on results from the screening process, endophytes that increased drought resistance underwent 16s ribosomal DNA sequencing and biochemical characterization in order to identify these specific bacterial endophytes for further application in a field setting.

UP 11: Metabolic Plasticity Observed in Multi-Drug Resistant Enteric Bacteria Obtained as Part of a Year-Long Study of Canada Geese (*Branta canadensis*)

Joshua de Waal, Austin Groth, Jacob Turnbow, Elizabeth Machunis-Masuoka
Midwestern State University, Wichita Falls, USA

Abstract

Antimicrobial resistance, including multi-drug resistance (MDR), has been shown to exist in wild animal populations, especially birds. The extent to which this resistance is maintained is largely unknown. We are conducting a year-long survey of a non-migratory population of Canada Geese (*Branta canadensis*) residing at Sikes Lake, Midwestern State University, Wichita Falls, TX, looking for MDR. A total of 72 of 243 bacterial isolates obtained from 120 separate fecal samples have been screened. Of the 16 MDR isolates found, all are erythromycin^{R} and of these, 6 are also ampicillin^{I}, 9 are ampicillin^{R}, and 1 is tetracycline^{R}. MDR should be costly to maintain in organisms not exposed to antibiotic. We hypothesize that organisms will lose metabolic plasticity or change protein expression profiles to compensate for the MDR phenotype. Metabolic screening using a modified IMViC and sugar fermentation panel is being used to both aid identification of bacterial species as well as visualize phenotypic variability. Thus far, 25% of isolates show variable indole production and 23.6% show variable MRVP. Further testing is required to determine if variability represents species diversity or metabolic diversity. To examine protein profiles, soluble protein was obtained from lysed cell pellets and 20 µg by mass was run on 12% SDS-PAGE. Variability in whole-protein banding patterns (protein profiles) was observed across the MDR isolates, both in terms of the presence/absence of bands and band intensity. Overall, these initial findings appear to support the hypothesis that MDR organisms compensate metabolically to support the resistant phenotype.

Bacteriophage Microbiology

UP 12: Isolation of Nephthys from a *Microbacterium foliorum* Culture

Jessica Blakely, TimYee Leung, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Microbacteriophage Nephthys was collected from the soil along the Bosque River bank at Stephenville City Park in Stephenville, TX (32.2160768 N and -98.2023418 W). A direct isolation and plaque assay was performed by washing the soil in PYCa liquid media and centrifuging at 250 rpm for 2 hours. The supernatant was passed through a 0.22-µL syringe filter and incubated with the host bacterium Microbacterium foliorum in a soft agar overlay for 24 hours at 29°C. A single 1-cm diameter lytic plaque formed. Additional plaque assays with two rounds of 10-fold serially-diluted supernatant in a phage buffer produced a single, well-isolated lytic plaque. A portion of the plaque was diluted 1:1000 and incubated with the host as before on agar plates, which were flooded to produce a bacteriophage high volume lysate of 28.7×1011 pfu/mL. DNA was extracted using a modified zinc chloride precipitation method. High-titer lysate was placed on a 300-mesh copper grid, negatively stained with uranyl acetate, and imaged by transmission electron microscopy to show that microbacteriophage Nephthys has siphovirus morphology with an approximate capsid diameter of 53.33 nm and a tail length of 126.67 nm. Microbacteriophage Nephthys was archived at Tarleton State University and the Pittsburgh Bacteriophage Institute.

UP 13: Isolation and Annotation of Cluster AW Bacteriophage MrAaronian

Ian Haines[1], Jessica Blakely[2], Ashley Branson[1], Diana Estrada[1], Rebeca Fernandez Robles[1], Katelyn Fitzgerald[1], Shelby Jeffers[1], Timyee Leung[1], Jasmine Munoz[1], Ashley Olivos[1], Anayeli Ramirez[1], Caressa Smith[1], Justin Spere[1], Idaleth Tavarez[1], Isabella Wood[1], Ethan Zavala[1], Marlee Goppert[1], Dustin Edwards[1]
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Abstract

Arthrobacter globiformis is a common soil bacteria with the ability to degrade pollutants. Further characterization of bacteriophages that infect Arthrobacter could have potential applications in selective bioremediation. Arthrobacteriophage MrAaronian was directly isolated by washing moist soil from a flowerbed in Poughkeepsie, New York with peptone-yeast-calcium (PYCa) liquid media, passing the supernatant through a 0.22 µm filter, and incubating with a host culture of A. globiformis B-2979. Following two rounds of serial dilutions and plaque assays on PYCa agar plates to isolate the bacteriophage, MrAaronian formed small, lytic plaques. Negative-staining transmission electron microscopy showed MrAaronian has siphovirus morphology with an approximate tail length of 285 nm and capsid diameter of 67.5 nm. Bacteriophage DNA was extracted and sequenced to 1,325-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina Next-Generation Sequencing to determine a double-stranded DNA genome of 54,509 base-pairs containing a 9-base sticky overhang. Whole-genome sequence analysis using Starterator, GeneMark, and Phamerator software revealed MrAaronian has 51.7% G+C content and 86 protein-coding genes transcribed rightwards. Using HHPred and BLASTp database queries, putative functions for genes encoding structural proteins, an endolysin, and nucleases including three HNH endonucleases, VRR-Nuc domain protein, and a Cas4 exonuclease were determined. MrAaronian is a cluster AW bacteriophage and most closely related to Microbacteriophages Michelle and Stayer.

UP 14: The Isolation and Characterization of the Bacteriophage “Percy17”

Amber Hernandez, Daiyuan Zhang
Del Mar College, Corpus Christi, USA

Abstract

Bacteriophages are viruses that kill and selectively target bacteria. Phages are the most abundant life-form on earth, there are about 10^{31}phages worldwide. In modern biotechnology, they have been proposed as alternatives to antibiotics for many antibiotic resistant bacterial strains.

The isolation and characterization of novel bacteriophage "Percy17" began with a soil sample collection followed by the enrichment of the bacterial host, Mycobacterium smegmatis. After identifying the presence of potential phage, a titer assay was performed to determine the concentration of plaque forming units (pfu). Then, the phage's genomic DNA was isolated and purified before undergoing restriction digest analysis and sequencing. Individual phages were observed via uranyl acetate negative staining and transmission electron microscopy (TEM) imaging. The presence of lysogen was confirmed by a spot test.

The plaques of "Percy17" appeared to be lytic during isolation. Through the restriction digest analysis, it showed that this genome has confirmed recognition sites for all enzymes tested: BamHI, ClaI, EcoRI, HaeIII, and HindIII. The TEM images shows “Percy17” has a tail length of ~160nm and a capsid with a diameter of 60nm. "Percy17" is published at Phages. DB and future study include genomic DNA sequencing and lysogen assay.

UP 15: Isolation of Besitos from a *Microbacterium foliorum* Culture

Shelby Jeffers, Rebecca Fernandez-Robles, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Microbacteriophage Besitos was isolated from moist soil collected in Weatherford, Texas (GPS coordinates 32.2578 N, 97.7226 W). Samples were washed with peptone-yeast extract-calcium (PYCa) liquid media before being filtered and incubated with *Microbacterium foliorum* at 29°C in a soft agar overlay. After 24 hours, 2-mm clear plaques formed. Microbacteriophage Besitos was isolated by two rounds of picking a single, well-separated plaque, followed by 10-fold serial dilutions in phage buffer and plating with *M. foliorum* as before. High-titer lysates of 1.44×10^8 pfu/mL were prepared by flooding webbed plates. High-titer lysate on a 300 copper mesh grid was stained with 1% uranyl acetate and imaged by transmission electron microscopy to show that Microbacteriophage Besitos had a siphovirus morphology with an approximate capsid diameter of 50 nm and a tail length of 95 nm. Bacteriophage genomic DNA was extracted using a modified zinc chloride method and characterized by restriction enzyme digestion with HaeIII, NspI, SalI, MseI, and SacII. Microbacteriophage Besitos was archived at Tarleton State University and the Pittsburgh Bacteriophage Institute.

UP 16: Isolation and Comparative Analysis of Sequenced Mycobacteriophages Discovered in East Texas During 2021-2022

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Abstract

Since 2015, LeTourneau University has participated in the SEA-PHAGES program. During this period, 114 phages have been isolated, and 37 sequenced. This study examined the diversity of the bacteriophages isolated in East Texas over the years 2021-2022. During this period, 19 phages were isolated using *Mycobacterium smegmatis* mc^2155 as a host, 8 of which were sequenced. Modified enriched isolation and DNA extraction methods were used, which resulted in a high (>85%) phage isolation and 100% DNA extraction success rates these past two years. Whereas the same protocols were followed over the study period, differences were observed in morphotypes, life cycles, GC content, number of tRNA's, and genome sizes of the sequenced phages. Collectively, the 8 sequenced phages were classified across five subclusters, namely A4, A11, C1, M1, and P1. Even within the same subcluster, there were differences between the individual phages, showcasing the biodiversity among the genomes. The GC content for 2021 averaged ~64.3%, which was slightly higher than that of 2022, at ~62.2%. The average genome size for 2021 (83,844 bp, range 46,673 - 155,280 bp) was also higher than that of 2022 (73,072 bp, range 51,055-81,156 bp). All the phages were temperate, except Ikeloa, which had a lytic life cycle. All the temperate phages had the Siphoviridea morphotype, while the lytic Ikeloa had a Myoviridea morphotype. It is noteworthy that while these phages were discovered in the same area and underwent the same isolation method, they still displayed diverse characteristics.

UP 17: The Isolation and Characterization of Bacteriophage 'Doctrina'

Alexa Kuchar
Delmar College, Corpus Christi, USA

Abstract

Bacteriophages are viruses that attack and kill their host bacteria. It has been estimated that there are over 10^31 bacteriophages present on our planet and more and more phages have been used as vectors for gene therapy and treatment for antibiotic resistant bacterial infections.

In this study, isolation of a novel bacteriophage 'Doctrina' began with a soil enrichment procedure followed by several experiments to characterize the isolated phage using its bacteria host Mycobacterium smegmatis. A high titer lysate was harvested for phage genomic DNA isolation. The restriction digest analysis was conducted using the isolated DNA. The phage morphology of 'Doctrina' was studied by uranyl acetate negative staining and sent for electron microscope imaging. The lysogen of 'Doctrina' was isolated from spot test with extra incubation and confirmed by patch test.

'Doctrina' displays lysogenic life cycle. The restriction digest patterns suggested the 'Doctrina' genome contains multiple recognition sites for *BamHI, ClaI, EcoRI, HaeIII* and *HindIII.* TEM image suggested the phage tail is estimated 200nm and capsid is around 50nm in diameter. Future study include genomic sequencing and lysogeny efficacy test.

UP 18: From Discovery to Bioinformatics: The Story Behind Phage Glaske16

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Department of Biology & Kinesiology, LeTourneau University, Longview, TX, USA

Abstract

Antibiotic-resistant bacteria (ARB) are becoming an increasing concern. One of the alternative methods for treating ARB-afflicted individuals is phage therapy. This makes phage discovery and characterization ever more important. Mycobacteriophage Glaske16 was isolated from a soil sample collected at LeTourneau University in Longview, TX (332.465 N, 94.727778 W), on August 22, 2022. The enrichment method was used, with Middlebrook 7H9 medium and *Mycobacterium smegmatis* mc^2155 as the host. Phage presence was confirmed using the spot test. Glaske16 was then purified using 10-fold serial dilutions through three rounds of plating. Web plates were used to amplify the titer of the pure phage. A high titer lysate of 4.9×10^9 pfu/mL was used for DNA extraction, TEM imaging, and archiving. Whereas temperate phages typically produce turbid plaques, Glaske16 surprisingly produced clear plaques (average diameter 1.5 mm, range 1 - 2 mm) after 48 h at 37°C. Negative-stain TEM showed Glaske16 to be a siphovirus with an isometric capsid measuring ~67 nm (range 60 -70 nm) in diameter and a long, flexible, non-contractile tail measuring ~320 nm (range 310 - 320 nm) in length. Genome sequencing was done at the University of Pittsburg, using an Illumina MiSeq sequencer. Annotation was done using DNA Master, PhagesDB, NCBI, HHPred, and Phamerator. Sequence data showed Glaske16 to belong to subcluster M1 with an 11 bp 3' sticky overhang (ACCTCCTGCAA), genome size 81156 bp, and 61.6% G+C content. Glaske16 adds to our inventory of phages (114 so far) with potential for treating ARB infections.

UP 19: Isolation of DJDoc from a *Microbacterium foliorum* Culture

Jasmine Munoz, Diana Estrada, Marlee Goppert, Dustin Edwards
Tarleton State University, Dublin, USA

Abstract

Microbacteriophage DJDoc was isolated from a soil sample collected at the base of an ant-infested tree in Stephenville, TX (GPA coordinates 32.20387º N, 98.12513º W). Soil samples were washed with PYCa media and filtered with a 0.22-µm filter to directly isolate bacteriophages. The filtered supernatant was plated on a top agar plate with the host bacteria Microbacterium foliorum and incubated at 28°C for 48 hours, which resulted in small plaques. Bacteriophage DJDoc was isolated through 2 rounds of serial dilution. High-titer lysate at 1.5×1010 pfu/mL was used to prepare transmission electron microscopy (TEM) grids by staining with 1% uranyl acetate. TEM imaging showed that microbacteriophage DJDoc has siphoviral morphology. The approximate capsid diameter was 30 nm and the tail length was 63 nm. DNA extraction was performed by a modified zinc chloride method, and restriction enzymes used for gel electrophoresis were HaeIII, NspI, MseI, and SacII.

UP 20: Isolation of Milagros from a *Microbacterium foliorum* Culture

Ashley Olivos, Anayeli Ramirez, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Microbacteriophage Milagros was isolated from an abandoned pig corral in Dublin, TX (GPS coordinates 32.0676038°N, -98.3084757°W). The soil sample was washed with 2 mL of PYCa liquid media and filtered with a 0.22-µm syringe filter to collect bacteriophages. The supernatant was incubated with the host bacteria Microbaterium foliorum in a soft agar overlay at 28°C. After 24 hours, microbacteriophage Milagros formed a small plaque, around 0.5 cm in diameter. The bacteriophage was isolated after two rounds of 10-fold serial dilution. High-volume lysates were prepared from webbed plates for transmission electron microscopy by reverse staining with 1% uranyl acetate. Microbacteriophage Milagros had siphovirus morphology with an approximate capsid diameter of 35 nm and a tail length of 110 nm. Bacteriophage genomic DNA was extracted using a modified zinc chloride method and characterized by restriction enzyme digestion with HaeIII, NspI, MseI, and SacII. The results showed that the restriction enzymes did not cut anything, this could be due to methylation.

UP 21: The Isolation and Characterization of the Bacteriophage ‘NOVA 535’

Kaylee Scullion, Daiyuan Zhang
Del Mar, corpus christi, USA

Abstract

Bacteriophage is virus that infect a bacterium and destroy most of its host. There are about 10^31 phage particles on the planet, they can be found anywhere especially from soil bacteria. Bacteriophages can be used as vectors for gene therapy treatment for antibiotic resistant bacterial infections.

In this study isolation of a novel bacteriophage nova 535 began with soil enrichment procedures followed by experiments to characterize the isolated phage using bacteria host Mycobacterium smegmatis . A high titer lysate was harvested for phage genomic DNA isolation. The restriction digest analysis was conducted using the isolated DNA. The phage morphology of nova 535 was studied by uranyl acetate negative staining and transmission electron microscope imaging. The lysogen of nova 535 was isolated from spot test with a longer incubation time.

Future study of this phage include genomic DNA sequencing and phage efficiency study on its host.

UP 22: Isolation of DaddyP from a *Microbacterium foliorum* Culture

Justin Spere, Ethan Zavala, Marlee Goppert, Dustin Edwards
Tarleton State University, Stephenville, USA

Abstract

Bacteriophage DaddyP was isolated from soil collected 5 cm below the surface under a water tank on a farm outside of Carbon, Texas. The bacteriophage was isolated and incubated with *Microbacterium foliorum* NRRL-24224 SEA. It was incubated in PYCa media for 48 hours at 29°C. After two rounds of serial dilution and plaque assays, two lytic plaques of roughly 20 mm in diameter formed. Additionally, the titer was found to be 3.6×10^8 pfu/ml. Following a negative-staining transmission electron microscopy, we determined that microbacteriophage DaddyP had a siphovirus morphology with an average capsid diameter of approximately 44 nm and an average tail length of approximately 118 nm. Microbacteriophage genomic DNA was extracted by a modified zinc chloride precipitation method and incubated with restriction enzymes. Following agarose gel electrophoresis, the microbacteriophage DNA was found to be undigested by SalI, SacII, and NspI, indicating possible DNA modifications. Bacteriophage DaddyP was archived at the Pittsburgh Bacteriophage Institute and Tarleton State University.

UP 23: Isolation and Classification of the Phage ‘Kamisha’

Kami Tiemann, Daiyuan Zhang
Del Mar College, Corpus Christi, USA

Abstract

Bacteriophages are a class of bacteria that act like parasites to bacteria. Phages are the most abundant form on the planet, amounting to 10^31 phages worldwide, all measuring as small as 100 to 200 nm. Their morphology consists of the capsid end with the genetic material and the tail end which is tube shaped and used to attach the phage to its host and inject its DNA.

In this study, the isolation of the phage 'Kamisha' began its journey with a soil enrichment procedure, using its host, M. Smegmatis . After performing streak/spot tests to successfully isolate a phage plague, a high titer lysate was harvested and utilized for isolating sufficient phage DNA. Once the DNA concentration was determined, the sample was then prepared for restriction enzyme digest and the final TEM imaging. TEM images provide "Kamisha's' morphology. An additional spot test was also performed to isolate lysogens and mesa.

The plaques of 'Kamisha' were indicated as lytic the time of its isolation. The TEM images concluded the phages' approximate capsid size width to measure 40nm and its tail length 190nm. Although the restriction digest fragments were not well resolved in the gel, several faint recognition sites appear to be present in ClaI and HaeIII. Further evidence for existing recognition sites at BamHI, EcoRI, and HindIII are possible. The isolated phage is published at Phages. DB. Future study will include genomic sequencing and lysogen assay.

Pathogenic Microbiology

UP 24: Shiga Toxin Production and Regulatory Pathways in *Escherichia coli* O113: H21 with Disparate Disease Manifestations

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Abstract

Background: Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of serotype O113:H21 produce a potent phage-borne cytotoxin responsible for severe human disease. In previous works, we reported that sequence type ST-820, an Australian strain linked to severe human disease, produces significantly more Stx than ST-223 isolates found dispersed outside Australia. However, the underlying cause of elevated Stx-production capability remains unknown. Here we present the global transcriptome survey for the two prototypical ST strains, EH41 (ST-820) and 4 (ST-223) as basis to investigate ST-specific differences in gene expression and regulatory pathways associated with Stx-production. **Methods:** Cultures were grown under non-induced and phage-mobilizing conditions in LB and LB+mitomycin C media, with the latter activating the SOS-response, followed by mRNA isolation (PureLink RNA and MICROBExpress kits). RNASeq libraries were prepared using the KAPA Stranded Total RNA-Seq kit and sequenced on the Illumina NextSeq500 platform with 150 cycles. Differentially expressed genes (DEGs) were identified in Galaxy and Degust, along with affected pathways using Pathway Tools. **Results and conclusions:** The key SOS-response regulator, *recA*, was upregulated in both MMC-treated cultures, though only EH41 showed a statistically significant increase in *stx2a*, which is mirrored in its Stx-production. In induced cultures of strain 4, the mobilization of a lytic phage found adjacent to Stx2a prophage appears to cause dramatically reduced cell viability relative to strain EH41 and subsequently lowers toxin production. In summary, our data reveals significant transcriptome-level differences that are intimately associated with the strains' pathogenic potential, which cannot be simply inferred from static genome comparisons.

UP 25: Coevolution of *Pseudomonas aeruginosa* and *Drosophila melanogaster* in a Chronic Infection Model and the Red Queen Hypothesis

Caleb Biney, Mary Geddie, Drew Stenesen, and William L. Cody
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Abstract

Pseudomonas aeruginosa is a ubiquitous, opportunistic pathogen and frequent cause of chronic infection in the immunocompromised. Additionally, it is also the leading cause of morbidity and mortality in people with the genetic disorder cystic fibrosis, where the growth of biofilms within the inner walls of the lungs gives it a greater resistance to antibiotics and the body's immune response. In this study we developed a modified *Drosophila melanogaster* oral feeding model to examine the Red Queen Hypothesis and better understand host-pathogen coevolution over multiple generations using three different treatment groups: host evolution, pathogen evolution, and coevolution. Here, host survivability is presented through the third generation. Additional generations will provide a better understanding of host-pathogen interactions, virulence mechanisms necessary during chronic infection, and the evolution of host resistance mechanisms.

UP 26: Estimating the Bacterial Bioburden of Chronic Wound Infections using a Handheld Bacterial Imaging Device

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Abstract

Chronic wound infections pose a clinical concern because they often lead to high rates of mortality and morbidity. The MolecuLight d:X is a handheld bacterial imaging device that has been developed to detect the autofluorescent properties of most clinically relevant species of bacteria. The d:X device has recently been redesigned that allows for the fluorescent light source to exhibit variable light intensity. The purpose of this study is to determine the minimum threshold of detection for the bacterial bioburden; the hypothesis is that higher intensity of light will allow for detection of bacteria at lower concentrations compared to lower intensities of light. To test this, we conducted a time course experiment using the wound pathogen Staphylococcus aureus by taking fluorescent images over 24 hours of growth to determine at what time point individual colonies transitioned from signal negative to signal positive with the d:X device. From there, we determined how many bacterial cells are required for a colony to transition to signal negative to signal positive. Taken together, these data should demonstrate the environmental conditions required for detection of bacteria using the MolecuLight d:X device. The goal of this project is to develop a system where wounds can be 'graded' on their bacterial bioburden using the d:X device to estimate the concentration of bacteria in a chronic wound in real-time at the bedside, independent of time-consuming culture-dependent methods. This would allow for physicians to treat chronic wounds more readily and improve overall patient outcomes.

UP 27: Prevalence and Impact of *Staphylococcus aureus* Small Colony Variants in the Chronic Wound Environment

Eleanna Carris, Emily Pham, Landrye Landrye Reynolds, Isaiah K. George, Klara C. Keim, Nicholas Sandford, Allie Smith
Texas Tech University, Lubbock, USA

Abstract

A novel phenotype of *Staphylococcus aureus* (SA) known as Staphylococcus aureus Small Colony Variants (SA-SCV) are typically associated with chronic infections. SA-SCVs exhibit increases in biofilm production and altered antimicrobial susceptibility, making them clinically important. SA-SCVs also demonstrate altered colony morphology and biochemical reactions compared to the wild-type *S. aureus*, making them difficult to detect and identify in routine diagnostic procedures. Evidence suggests SA-SCVs contribute to patient morbidity and mortality due to these difficulties in diagnosis. This study seeks to determine the prevalence of SA-SCVs in chronic wound specimens and examines diagnostic procedures used to identify them. In collaboration with the Southwest Regional Wound Care Center, we obtained chronic wound specimens and screened them for SA-SCVs using in-house procedures. We then submitted the specimens the diagnostic laboratory at Covenant Medical Center and compared results using their gold standard diagnostic procedures. This study screened 13 chronic wound specimens, and 7 screened positive for harboring potential SA-SCVs using culture-dependent techniques. Confirmatory testing will be conducted using next-generation sequencing in collaboration with RTLGenomics (Lubbock, TX).

UP 28: Analyzing Differential Gene Expression in a Cockroach-infecting Gregarine

Emerie E. Danson, Mia Sanchez, Saraya AlSaffar, Charles Hauser, Daniel A. Gold
St. Edward's University, Austin, USA

Abstract

Blabericola migrator is a parasite that exclusively infects the intestinal tract of Madagascar Hissing Cockroaches. *B. migrator* belongs to a subclass of Apicomplexan parasitic protists called gregarines, an incredibly diverse but poorly understood group that infects invertebrates. It is a promising candidate to serve as a model organism for *Cryptosporidium spp.*, related parasites that can cause severe diarrheal disease in humans. *B. migrator* has a complex life cycle with many distinct life cycle stages that are distinguished by different sets of expressed genes. Our goal is to identify genes unique to specific stages to provide molecular stage markers, identify possible targets for drug treatment, and to elucidate host-pathogen interactions. mRNA transcripts were collected during three internal stages of *B. migrator*'s life cycle: attached to host intestinal cells, detached from host cells, and a reproductive phase called syzygy. After sequencing the transcripts, multiple transcriptomes were built using the NCGAS *de novo* transcriptome assembly pipeline, then merged into a single transcriptome in order to maximize coverage. Using the R package DESeq2, we analyzed differential expression of RNA between these different life cycle stages, yielding lists of up-regulated and down-regulated genes for each stage comparison. We ran BLAST to find homologs of these genes in *Cryptosporidium spp.*, then identified associated gene ontologies and biological pathways with *Cryptosporidium spp.* database searches. Genes found to be associated with parasite metabolism and those containing predicted signal peptides are initial candidates for future investigation.

UP 29: Identifying Novel Mutants with Increased Susceptibility to H_2O_2 and Reduced Virulence in *Bacillus anthracis* Sterne

Luke Hamilton, Victoria Adeleke, Zach Rouseau, Lauren Callaghan, Taylor Kelly, Shauna McGillivray
Texas Christian University, Fort Worth, USA

Abstract

Bacillus anthracis is a gram-positive bacterial pathogen that causes the deadly infectious disease anthrax. *B. anthracis* contains over 5,000 chromosomal genes, and we believe there are unidentified chromosomal genes important for virulence. Our lab constructed a transposon mutant library with random disruptions in the *B. anthracis* Sterne genome to screen for novel virulence factors, and we have previously identified two virulence genes, *clpX* and *yceGH,* using this library. In this screen, we used H_2O_2, a reactive oxygen species involved in innate immune defense, and screened around 1000 mutants. We obtained three mutants that were susceptible to hydrogen peroxide *in vitro*: 11F11, LV1, and LV2. To determine whether they also had phenotypes *in vivo*, we infected *Galleria mellonella* to study their virulence in an invertebrate animal infection model. LV2 showed reduced virulence in the *in vivo* survival assay, and all three mutants showed reduced virulence in the *in vivo* competition assay. I have determined the site of the transposon insertion in 11F11 and LV1, and the transposon has inserted in the genes for catalase and a collagenase-like protein, respectively. I am currently creating an independent insertional mutation in LV1 to confirm that the observed phenotypes are linked to the disruption of the collagenase-like protein. Future directions include creating a complementation plasmid for LV1 and determining the insertion site of LV2. The findings of this research could be used as potential therapeutic drug targets and will offer insight into the mechanisms that *B. anthracis* uses for its pathogenesis.

UP 30: Growth and Cell Viability in *Escherichia coli* O113: H21 with Disparate Disease Manifestations

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Abstract

Background: Shiga toxin (Stx)-producing *Escherichia coli* (STEC) of serotype O113:H21 causes life threatening human renal disease. This lineage can be partitioned into two major Sequence Types: ST-223 with a global distribution and ST-820 restricted to Australia. The latter group has been linked to severe clinical cases, while ST-223 isolates rarely cause human disease. To gain further insights into their marked virulence potential, we surveyed available genomes for group-specific signatures and phenotyped representative cultures. **Methods:** Twenty strains were grown in LB and under phage-mobilizing conditions in LB+mitomycin C. To assess viability, we followed growth for 12 hrs in a microplate reader assay. Colony-forming units along with the number of produced Stx-phages were determined after 6 hrs of incubation. Pangenome analyses with Roary identified the common, shared, and unique genes. Strain relatedness was inferred from cgMLST (Ridom SeqSphere+). **Results and discussion:** The global gene reservoir is comprised of 6514 genes, among these are 4043 core and 1078 shell genes; 4429 (ST-820) and 4367 (ST-223) loci are conserved in each group. ST-820 consists exclusively of high-level Stx producers with the exception of strain EH53, showing a 100-fold decrease. Its Stx_{2a}-phage and insertion site are indifferent. However, EH53 is highly susceptible to MMC, reflected in its impaired growth and reduced cell viability. Future investigations will be directed to study the mobilization of a lytic phage unique to EH53 that may cause the altered growth phenotype. Collectively, the data shows a direct correlation between growth, cell viability and strains individual Stx-production capabilities.

UP 31: An invertebrate model using *Galleria mellonella* to study the innate immune response to *Staphylococcus haemolyticus*

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Abstract

Staphylococcus haemolyticus is a normal inhabitant of healthy skin and is also an emerging cause of nosocomial infections in the immunocompromised. *S. haemolyticus* infections often lead to bacteremia, septicemia, peritonitis, endocarditis, and various other outcomes. The goal of this project was to establish an invertebrate infection model for studying the innate immune response to *S. haemolyticus* infections using the wax moth larva (Galleria mellonella). We have found that injecting wax worm larvae with 106 to 107 cfu/mL of *S. haemolyticus* consistently induced a rapid response and larval death within 24 hours at the higher dose range. We have characterized the general histopathological changes associated with infection and have started to characterize the general innate immune response as well as the hemocyte response to S. haemolyticus. Our findings support the usefulness of this model as an initial screening tool for systemic microbial infections.

UP 32: Phenotypic Characterization of *Nakaseomyces glabrata* Clinical Isolates from the Human Colon

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Abstract

The contributions of commensal fungi to human health and disease are not fully understood. *Nakaseomyces glabrata* (formally *Candida glabrata*) is an opportunistic pathogenic fungus that commonly colonizes the human gastrointestinal (GI) tract. *N. glabrata* has recently been classified as a high priority pathogen by the World Health Organization (WHO) due to its increased antifungal resistance and high mortality rates. Here, we describe the phenotypic characterization of three *N. glabrata* clinical isolates (DI22-110, DI22-111, DI22-112) from the human colon of three distinct patients. We show that while there are no significant differences in growth at 37 °C in YPD media or DMEM (supplemented with 10% FBS and 1% NEAA), there is a significant difference in their biofilm forming capabilities with clinical isolate 111 being a hyper-biofilm former with high levels of extracellular matrix. Measurement of β-D-glucan content on the fungal cell wall shows a trend towards a higher content for isolates DI22-111 and DI22-112. Further, clinical isolates DI22-111 and DI22-112 also display decreased sensitivities to a variety of stressors including SDS, caffeine, and calcofluor white compared to 110 and two WT controls. These findings suggest that clinical isolates DI22-111 and DI22-112 have developed distinct mechanisms for adaptation to their environment, potentially translating to differences in antifungal susceptibility. Characterizing these mechanisms of adaptation will allow us to better understand *N. glabrata* biology and develop novel therapeutic interventions.

UP 33: Investigating the Mechanism of *Pseudomonas*-induced Activation of Epidermal Growth Factor Receptor *in vitro*

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterial pathogen that causes pulmonary infection and inflammation among other organ systems. *P. aeruginosa* is associated with increased morbidity and mortality in immunocompromised individuals, particularly those with cystic fibrosis (CF). *P. aeruginosa* elastase (PE) is one of the primary virulence factors of *P. aeruginosa*. Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase that is involved in physiological processes such as cell proliferation and host inflammatory response. Our working hypothesis is that PE activates human structural lung cells immune response through interactions with EGFR. While our published research confirmed this notion, the mechanism(s) of protein-protein interaction is not yet clear. In this project, we sought to investigate the hypothesis that PE-induced activation of EGFR occurs by direct protein-protein interactions. To investigate this premise, we utilized the A549 cell line, western blot analysis, fluorescent microscopy, and Proximity Ligation Assays (PLA) for *in situ* detection of PE-EGFR interactions. Hybridization of PLA probes occurs only in close proximity, resulting in a fluorescence emission that is visualized with a fluorescence microscope. The emission of PLA signals was observed in cells that received PE treatment with the added primary antibodies against both PE and EGFR. Our results indicate that PE activates EGFR by a direct protein-protein interaction, without a secondary external messenger. Understanding the implications of the findings of this study will support the development of a novel treatment that will work against PE-induced pulmonary inflammation through EGFR.

UP 34: Defining the Spatial and Temporal Dynamics of the Urogenital Microbiome in Postmenopausal Women

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Abstract

Urinary tract infection (UTI) is among the most common infections in the United States and primarily affects women.[1] The prevalence of UTI generally increases with age, and recurrent UTI (rUTI), defined as 3 UTIs in the past year, doubles in women over 65.[1] The differential incidence of rUTIs in pre- and post-menopausal women postulates that hormone status and the urogenital microbiome may be key to rUTI susceptibility.[2] Because little is known about the spatial and temporal dynamics of the female urogenital microbiome in healthy post-menopausal (PM) women, this study seeks to define temporal changes in the urinary, vaginal, and peri-anal microbiomes associated with estrogen hormone therapy (EHT) in PM women with no UTI history.[2] Patients selected for the study donated urine and swabs at 4 time points. At each visit, a wet and dry swab were collected from the vagina and the peri-anal region, followed by clean-catch urine. Genomic DNA was extracted from dry swabs and microbiome composition was determined via 16S rRNA amplicon sequencing of the entire 16S gene using PacBio sequencing for species-level resolution. The wet swabs were cultivated by a recently published Advanced Culture method with PCR and Sanger sequencing of the 16S rRNA gene for species identification.[2] To date, full taxonomic profiles have been generated for 6 patients and over 400 isolates have been cultivated and identified.

1. Medina M & Castillo-Pino E. Ther Adv Urol. 11 (2019)

2. Neugent M, Kumar A, et al. Cell Reports Medicine (2022)

UP 35: Investigating the Unique Cyan Fluorescence in *Pseudomonas aeruginosa*

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Abstract

Chronic wound infections are of clinical concern as they often lead to high rates of mortality and morbidity. The MolecuLight *i:X* is a handheld bacterial imaging device, designed to detect the auto-fluorescent characteristics of most clinically relevant species of bacteria. The *i:X* device causes most species of bacteria to exhibit red fluorescence, due to the production of exoproduct porphyrins. One of the most significant contributors to the pathogenicity of chronic wounds is the pathogen *Pseudomonas aeruginosa* and interestingly, this organism exhibits an additional unique cyan fluorescence signature. There is over a 90% positive predictive value that when a chronic wound exhibits cyan fluorescence with the *i:X* device, the wound will harbor *P. aeruginosa*. In order to determine the source of the unique cyan fluorescent signature, a transposon mutant library and corresponding complimented mutants of *P. aeruginosa* were utilized to isolate which genetic factor(s) is responsible for the observed cyan phenotype. Clinically, this could allow for real-time detection of *P. aeruginosa*, which would be the first technology of its kind and could greatly improve treatment outcomes.

UP 36: Regulatory Effects of Loss of ClpX on the *msrA* Chromosomal Gene in *Bacillus anthracis*

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Abstract

Bacillus anthracis is the causative agent of anthrax. Previously, our lab identified the *clpX* gene as critical for virulence in *B. anthracis*. The *ΔclpX* mutant exhibited decreased cell wall integrity and increased susceptibility to cell-envelope active antibiotics. ClpX is one component of the intracellular caseinolytic protease ClpXP that degrades multiple proteins including transcriptional regulators. To understand changes in gene expression in *ΔclpX*, a microarray comparing WT and *ΔclpX* was conducted. This project focuses on *msrA*, an upregulated gene in *ΔclpX*. MsrA is an antioxidant enzyme that reduces methionine-S-sulfoxide to methionine but also impacts cell wall strength in *S. aureus*. This study will determine if loss of the *msrA* gene impacts antibiotic susceptibility. We hypothesized that since *ΔmsrA* is upregulated in *ΔclpX*, *ΔmsrA* would exhibit the opposite phenotype. Surprisingly, we find that *ΔmsrA* has significant growth inhibition in the presence of penicillin. However, we do not find susceptibility with other antibiotics, such as daptomycin, nor does it appear to be more susceptible to other clpX-related stress responses such as heat or acid stress. Future research will test *ΔmsrA* susceptibility to additional antimicrobials, such as the antimicrobial peptide LL-37 and the antibiotic vancomycin, as well as *ΔmsrA* virulence in vivo with the *Galleria mellonella* infection model. We will also complement *ΔmsrA* to confirm the phenotypes are due to loss of the *msrA* gene. This research is important as it aids our understanding of bacterial defenses and may provide new drug targets to help combat rising antibiotic resistance.

Molecular and Genetic Microbiology

UP 37: Reversion from Erythromycin Resistance to Erythromycin Susceptibility in *Escherichia coli*: Genetic Changes

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Abstract

The development of antibiotics, along with development of vaccines and sanitation systems, has been a major contributor to the improvement of human health, particularly over the last century. However, increasing resistance to these same antibiotics is threatening our ability to effectively treat infectious diseases. Alternatives to the drugs themselves or alternative strategies for using these drugs are needed to address this growing threat. One alternative-use strategy could be a rotating schedule of antibiotics. Bacteria resistant to one antibiotic can revert to being susceptible to it when grown in the presence of another. Previously, an erythromycin-resistant strain of *E. coli* showed increased susceptibility after growth in the presence of subinhibitory concentrations of tetracycline. For this project, we hypothesized that reversion was due to mutations in the erythromycin resistance genes and that these mutations will be different each time the experiment is performed. We repeated the tetracycline treatment experiment and generated a second isolate with increased susceptibility to erythromycin. Genomic DNA from the original strain and both revertant strains was extracted and submitted for whole genome sequencing. Comparison of these genomic sequences will determine where the genetic changes have occurred. Analysis of these changes will help us discern patterns of mutation and, we hope, provide insights into improved antibiotic stewardship.

UP 38: Role of Reactive Oxygen Species Formation in the Antimicrobial Action of Zinc Oxide

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Abstract

Staphylococcus aureus is the causative agent of many skin infections and the leading cause of death due to infectious disease in the United States. Additionally, *S. aureus* is known to rapidly gain antibiotic resistance, as seen with methicillin resistant *Staphylococcus aureus* (MRSA). Zinc oxide (ZnO), a nontraditional antibiotic, demonstrates antimicrobial action against *S. aureus*. While the exact mechanism of ZnO antibacterial action is currently unknown, production of reactive oxygen species (ROS) is a commonly proposed mechanism. We find that *S. aureus* Δ*katA*, a mutant susceptible to hydrogen peroxide (H_2O_2) due to a deletion in the catalase gene, exhibits comparable growth to wild type *S. aureus* in ZnO. This suggests that production of H_2O_2 is not vital to the antimicrobial action of ZnO. To further test this, we generated a ZnO resistant mutant (ZnO^R) that demonstrates less susceptibility to ZnO. We find that the ZnO^R mutant demonstrates comparable growth to wild type *S. aureus* in H_2O_2, making H_2O_2 production an unlikely toxicity mechanism of ZnO. To evaluate the role of ROS besides H_2O_2, susceptibility of ZnO^R and wild type *S. aureus* to two other ROS, bleach and paraquat was evaluated. We are currently investigating whether N-Acetyl-Cysteine (NAC), a compound that stimulates production of antioxidants and is protective against a wide range of ROS, protects *S. aureus* from ZnO mediated toxicity. Our data suggests that ROS formation is not the dominant mechanism of antimicrobial action by ZnO and future studies should focus on other potential mechanisms of action.

UP 39: Comparative Analysis of Cluster A Actinobacteriophages

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Abstract

Bacteriophages (or phages) are viruses that infect only bacteria. Viruses that specifically infect bacterial hosts in phylum Actinobacteria are called Actinobacteriophages. Research shows that bacteriophages are increasingly important to the scientific community for purposes like phage therapy and bioremediation. Comparative genomics is important because it can reveal evolutionary relationships between organisms, show what genes are conserved between organisms, and unveil unique sequences in specific genes. The purpose of this research was to comparatively analyze the genomes of the phages discovered at LeTourneau University in the largest Actinobacteriophage cluster, A. Various bioinformatics software and databases were utilized in this endeavor, including BLAST Ring Image Generator, DNA Master, Geneious Prime, HHPred, NCBI, phagesDB, Phamerator, and Vista Tools. This analysis highlights genes that are conserved across the cluster, such as endonucleases, exonucleases, and immunity repressors that are expected to be found in temperate phages. It also focuses on genes that were found only in some phages in Cluster A, notably integrase, ParA- and ParB-like dsDNA partitioning proteins. Such differences within the cluster are important to identifying which phages may be able to infect different bacteria more efficiently and be more effective in phage therapy for antibiotic resistant bacterial infections. In this work, notable differences within cluster A are discussed as well as how genomic analyses can help predict phage efficacy for therapy purposes.

UP 40: Laboratory Evolution of Patient Derived and Non-Pathogenic B2 *Escherichia coli*

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Abstract

About 50% of women will have at least one urinary tract infection (UTI), a bacterial infection predominantly caused by *Escherichia coli* (Al-Badr, 2013). Of the phylogroups of *E. coli*, group B2 is associated with ~50% of UTIs (Schreiber, 2017). Our current understanding of UTIs and their outcomes relies on the physiology of the bacteria and their host. Pathogenic *E. coli* are highly adaptable, and the transcriptome of recently isolated strains are likely to change and lose virulence properties during passage in lab media.

We describe the results of serial passage of two patient-derived strains, KE4 and KE21, in three different media: high protein: LB, balanced carbohydrate and protein: glucose-tryptone (GT), and rich cancer cell media: Leibovitz's L-15. Afterwards, the expression of global transcription factors (*arcA* and *phoP*) and a supercoiling sensitive gene (*glnA*) were assessed to identify alterations in the transcriptome. All three passages through the standard laboratory media, LB, led to increases in *glnA* expression consistent with that of increased supercoiling and decreases in expression of *arcA*. Loss of *arcA* expression was anticipated since ArcA represses the TCA cycle, and the mechanism of energy transduction proceeds through the TCA cycle during LB media growth. Our results suggest transcriptomic restructuring leading to altered gene expression and a suppression of the TCA cycle repressor, *arcA*, all occur in under 72 hours of growth. These results impact how we should approach media selection for recently isolated pathogens.

UP 41: Codon Usage Bias: What does Mycobacteriophage Glaske16 Contribute to the Story?

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Abstract

The phenomenon where specific codons are used more often than other synonymous codons during translation of genes is referred to as codon usage bias, and it varies within and among species. Since codon usage bias can impact gene expression and cellular function, it is important to investigate whether the non-random use of synonymous codons is observed in phages. Mycobacteriophage Glaske16 was selected for codon usage bias analysis using DNA Master's Bias Table function. The Bias Table included all genes and tRNAs within Glaske16's genome. Percentages were calculated for each codon and compared with previously examined codon bias in phylum Actinomycetes. Similarities were observed between Glaske16 and its isolation host *Mycobacterium smegmatis mc^2 155*. Codon bias percentages in phage Glaske16, from the highest to the lowest, were: Lys codon AAG (96%), Phe codon UUC (91%), Tyr codon UAC (89.5%), Glu codon GAG (86%), Asn codon AAC (86%), Ile codon AUC (83%), Cys codon UGC (78%), His codon CAC (77%), Asp codon GAC (72%), Leu codon CUG (63%), Gly codon GGC (54%), Pro codon CCG (51%), Thr codon ACC (48%), Arg codon CGC (46%), Val codon GUG (42%), Ser codon UCG (41%), and Ala codon GCC (38.5%). This analysis presents the observed codon usage bias for Glaske16 compared to its isolation host and other phages, which broadens our understanding of codon usage bias in bacteriophages.

UP 41: Comparative Analysis of the Reliability of Various Software for Topological Prediction of Bacteriophage Membrane Proteins

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Abstract

Membrane proteins (MPs) play essential roles in cellular function, including energy generation, transmembrane transport, cell signaling, and bacteriophage (phage) infectivity and replication. Thus, they are essential proteins to identify in phage genome annotation because deciphering the relationships between their sequences and functions could increase our knowledge of phage biology and the utility of phages in therapy, bioremediation, wastewater, and food industry biocontrol. Previously, the SEA-PHAGES program recommended using TMHMM and/or SOSUI software, but inconsistencies in results have been observed. Our aim was to investigate the reliability and efficiency of various software, including DeepTMHMM, TOPCONS, CCTOP, SOSUI, Philius, and Phobius in detecting MPs in subcluster M1 phages. All hypothetical proteins, endolysins, and holins were analyzed for the presence of putative transmembrane domains (TMDs). A comparative analysis of the number of TMDs and signal peptides detected per gene and efficiency of the software was done. Efficiency was determined by recording the average time each software took to run. Additionally, the lengths of the analyzed amino acid sequences were recorded to determine whether there is a manual method of saving time by ruling out the likelihood of a MP in a sequence. Per preliminary data, all software (except Philius) detected at least one TMD in the holin gene. Overall, SOSUI took the shortest average (1 sec) run time. This study describes the application of the collected data to elucidate the reliability and efficiency of the programs for detecting MPs and discusses some potential applications for phage membrane proteins.

UP 43: Analysis of the Regulatory Controls of the Alanine-Synthesizing Transaminases (*alaA* and *alaC*)

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Abstract

The pyruvate transaminases, *alaA* and *alaC*, are two of the three main enzymes that synthesize the amino acid alanine, an essential molecule in peptidoglycan that assists bacteria in structural maintenance of their walls and in protein synthesis. Familiarizing ourselves with the alanine biosynthesis pathway could therefore provide discernment to these important processes in bacterial physiology and provide potential methods for inhibiting resistance to cell wall targeting antibiotics.

Hypothesizing that different mechanisms control the AlaA and AlaC enzymes—responding to either cell wall stress or protein stress individually—an experiment was designed to explore the control of *alaA* and *alaC* expression. Reporter gene fusions were constructed of the *alaA* promoter to a green fluorescent protein (GFP) and transformed into three strains: W3110, *alaA* knockout, and *alaC* knockout. These constructs allowed for the comparison of gene expression when grown in various carbon sources and antibiotics.

Results showed that glucose and alanine induced *alaA* expression throughout all strains, but highest overall expression was in the media containing valine, specifically in the *ΔalaA* strain. This was attributed to the stringent response, which is induced by excessive amounts of valine in E. coli; the difference in *alaA* and *alaC* expression when grown in this media led us to believe the AlaA enzyme was responding to protein stress. Growth with sublethal levels of penicillin linked the AlaC enzyme to cell wall stress. We are now focusing on identifying regulatory proteins for *alaA* and *alaC* genes.

UP 44: Characterization of Genes Essential for Hydrocarbon Degradation in *Pseudomonas aeruginosa*

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Abstract

Thousands of gallons of aviation fuel spills are reported from across the nation each year, which can enter the underground water and pose detrimental threats to our health. The severity of such contamination can be alleviated through biostimulation efforts, which involves utilizing existing microorganisms in the soil in order to break down contaminants. Here we show that Pseudomonas aeruginosa, a metabolically versatile and highly adaptive bacteria, is one of the most abundant jet-fuel-degrading microorganisms found at the jet fuel spill sites. The ability of P. aeruginosa to degrade hydrocarbons comes from the Alkb1 and Alkb2, monooxygenases that catalyze the insertion of oxygen into the hydrocarbon chain. Expression of alkb1 and alkb2 is poorly understood and only occurs in the presence of hydrocarbons and absence of other carbon sources. Identification of genes involved in the alkb1 and alkb2 expression is crucial to maximizing the efficiency of hydrocarbon degradation in real world applications, where other hydrocarbons are present. In this study, 20 essential genes for accelerated JetA aviation fuel degradation were identified, and insertional inactivation mutants of these genes were created for further characterization.

UP 45: Establishing a UV Mutagenesis Technique using *Saccharomyces cerevisiae* 09-448

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Abstract

Yeast is a eukaryotic fungi that is commonly used for industrial processes. Phaff Yeast Culture Collection Strain Saccharomyces cerevisiae UCDFST 09-448, hereafter referred to as 09-448, is a strain capable of breaking down pectin rich biomass into oligosaccharides and free fermentable sugars, which can be used for the production of biofuel. In this study, a UV mutagenesis procedure was established to generate 09-448 mutants that can tolerate high ethanol concentrations to evaluate stress tolerance. To perform the UV mutagenesis experiment, several things needed to be determined. The exponential growth phase of 09-448 was determined by incubating 09-448 at 30C for 18 hours and measuring OD600 every hour, with 09-448 entering the exponential phase at 10-14 hours. Next, a standard curve correlating cell density to OD600 using cell counting was built to determine the OD600 of 108 cells/mL of 09-448, with an OD600 of 1.3 being equivalent to 108 cells/mL of 09-448. The optimal UV exposure length was established based on a UV dose dependent percent survival curve. The survival curve was generated by exposing 09-448 cells to UV for up to 6 minutes, sampling every 30 seconds. Lastly, ethanol selection plates were developed for mutant identification. 100 proof ethanol was added to TSA before plates were poured and solidified. A spot plate technique was used to evaluate the toxicity of the ethanol plates for future mutant selections, with ethanol concentrations of 8-10% being used. These experiments enabled the development of a UV mutagenesis protocol for future use.

UP 46: Effect of Wall Protein Glycosylation on *Candida albicans C*ell Surface Hydrophobicity

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Abstract

Candida albicans, a commensal fungal microorganism colonizing the gastrointestinal and vaginal tracts, is an opportunistic pathogen that can cause mild to severe disease. To colonize the host, *C. albicans* cells must adhere to host tissues. An important factor in the initial steps of adherence is cell surface hydrophobicity. Hydrophobic cells are more adherent than hydrophilic cells. Surface hydrophobicity correlates with conformation of surface-localized glycoproteins. Protein glycans comprise a primary branch, connected to the polypeptide backbone, with secondary and tertiary branches extending from the primary. Tertiary branches are composed of ß-1,2-oligomannosides and linked to secondary branches through a phosphodiester bond. Previous work demonstrated that hydrophobic cells have longer tertiary branches than do hydrophilic cells. We hypothesized that this glycosylation change directly influences surface hydrophobicity due to longer ß-1,2-oligomannosides interacting with like chains in the same or adjacent proteins. This interaction causes proteins to fold over or aggregate, leading to the observed surface hydrophobicity. To test this hypothesis, we assayed the hydrophobicity of wild-type *C. albicans* and mutant strains defective in various steps of tertiary branch glycosylation. Initial results suggest that limiting the length of the tertiary branches does not affect hydrophobicity. Thus, it appears that glycosylation changes correlate with, but do not directly influence, hydrophobicity levels. We are currently examining additional strains to ensure that our results are not strain-specific. The results of this work will help us understand how *C. albicans* interacts with host tissue and successfully colonizes the human host.

UP 47: A Proposal for Improved Genome Annotation Following a Comparative Look at Gene Calls in Mycobacteriophage Subcluster M1 Over a 14-Year Period

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Abstract

Accurate phage genome annotation is crucial to understanding phage biology and potential applications such as phage therapy. However, not all annotators are experts in phage biology, structural biology, chemistry, biochemistry, or proteomics. This opens opportunities for mis-identifying genes. Once called, functions get propagated. Challengingly, not all genes in the same pham necessarily have the same function. This is because some pham members may lack particular domains that others have, making them have different functions, although parts of their genes may show homology and have similar hits in HHPred with fellow pham members. This study examined annotation data in subcluster M1 phages over a 14-year period (2009-2022) to identify inconsistencies in gene calls, with the aim of proposing solutions for accurate gene calling. Various bioinformatics software and databases were utilized, including DNA Master, PhagesDB, NCBI, HHPred, and Phamerator. While annotating subcluster M1 Mycobacterium phage Glaske16 for example, BLASTp data from PhagesDB and NCBI revealed notable inconsistencies in function calls of genes in the same pham as the gene at 39487-40347 bp across the subcluster, even among genes with ≥99% homology. Such inconsistencies could be indicative of a larger annotation problem, namely, individual annotators heavily relying on synteny or BLAST results to make the final decision on gene function. Data on the accuracy of gene calling in the subcluster M1 over a 14-year span will be discussed, and a simple outline that should help improve the accuracy of phage gene calling in the future will be provided.

UP 48: Therapeutic Potential of Mycobacteriophage MazG Pyrophosphohydrolases

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Abstract

In bacteria, the MazG-like nucleotide pyrophosphohydrolase gene controls a toxin-antitoxin system which can trigger programmed cell death through the hydrolyzation of the (p)ppGpp alarmones. At increased levels, alarmones enable bacteria to stop replicating and enter metabolic quiescence, thereby enhancing their survival under stressful conditions, including antibiotics. Could more knowledge about the "MazG" gene in mycobacteriophages provide insights into potentially using it as an antibiotic treatment enhancer in phage cocktails to overcome multidrug resistant bacterial infections? This research sought to explore more about this gene across mycobacteriophage clusters at sequence and structural levels. In this study, the MazG gene was found in mycobacteriophages of cluster X, subclusters A2, A11, M1, and singleton phage DS6A. A comparative analysis of MazG amino acid sequences was done in phages L5 (A2), Sham4 (A11), Glaske16 (M1), Nebkiss (X), DS6A (singleton), and *Mycobacterium tuberculosis* using Multalin and BLASTp in NCBI and PhagesDb. BLAST revealed substantial inter-cluster diversity in MazG gene homology. The sequences were also run in Phyre to determine protein structural symmetry. Each gene possessed tertiary protein structure synteny with the *M. tuberculosis* nucleoside triphosphate pyrophosphohydrolase with MazG domains. Further research into MazG gene activation is necessary to determine its therapeutic potential, as this gene is usually activated only when bacteria are under stress. If the MazG-like nucleotide pyrophosphohydrolase gene could be activated, bacteriophages could possibly be used in viral cocktails as complementary therapy to treat tuberculosis and other antibiotic resistant infections.

UP 49: Effect of Tryptophan and its Derivatives Indole and Serotonin on *Candida albicans* Germination

Francine Pascal, James Masuoka
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Abstract

Candida albicans is a species of yeast commonly found in the human gastrointestinal and reproductive tracts. Although usually commensal, *C. albicans* can, under certain conditions, become invasive and cause disease. *C. albicans* is dimorphic, having unicellular yeast and filamentous hyphal forms. Germination, the transition between the yeast and hyphae forms, is an important virulence trait and one influenced by quorum-sensing molecules such as farnesol. Another *C. albicans* quorum-sensing molecule, called MARS, was reported to have similar effects, but was structurally different from farnesol. However, MARS did have structural similarities to serotonin, also shown to inhibit germination. Since *C. albicans* does not secrete serotonin, it is unlikely to be MARS. Serotonin is derived from tryptophan, as is indole – proposed to be a signaling molecule even between kingdoms. We hypothesized that MARS is indole and that serotonin is acting as an analogue of indole. The present study investigates the effects of subinhibitory concentrations of indole, serotonin, and tryptophan on *C. albicans* germination. Preliminary results suggest that indole inhibits *C. albicans* germination and this effect occurs within the first 30 minutes of indole addition. Current efforts are extending these experiments to include testing several strains of *C. albicans* along with the serotonin and tryptophan trials. These results will help us understand how *C. albicans* interacts with the human host and the other microbes making up the mucosal microbiome and may provide insights into ways by which we can maintain *C. albicans* in a commensal state.

UP 50: Human Plasma Like Medium (HPLM) Downregulates Antiviral Pathways to Promote MHV68 Titer

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Abstract

Human Plasma Like Medium (HPLM) is a novel cell culture media designed to mimic the physiological conditions of human plasma. Although recent studies have explored HPLM in conjunction with cellular metabolism, there is little documentation regarding its effects on viral replication. Viral DNA is sensed by antiviral sensors like cGAS or TLR9 that lead to Type I Interferon (IFN) production. Interferon-β (IFN-β) is a Type I IFN that induces transcription and activation of IFN-stimulated genes (ISGs), which can have direct antiviral properties. In this project, we investigated whether HPLM could impact viral titer in bone marrow derived macrophages (BMDMs) and if there are effects downstream of antiviral signaling pathways. We differentiated BMDMs in four media conditions with macrophage colony stimulating factor (M-CSF), which differentiates haematopoietic progenitors into macrophages, and infected them with murine gammaherpesvirus-68 (MHV68) to examine the immune response against a DNA virus. Our data demonstrates that BMDMs differentiated and grown in HPLM had a tenfold higher viral titer than those grown in Dulbecco's Modified Eagle Medium (DMEM). In addition, in HPLM-treated cells we observed a twofold downregulation of Ifnb1 and Isg20, two genes activated downstream of antiviral pathways. These results suggest that a decrease in antiviral signaling, leading to decreased IFN-β, could be responsible for the increase in viral replication. Our findings highlight the importance of using HPLM to accurately measure viral titer, as traditional media may lead to non-physiological stimulation of antiviral pathways. In addition, we hypothesize that the effects of HPLM extend to multiple antiviral pathways.

GRADUATE POSTER PRESENTATIONS

GP 01: IL-26 Increases Sensing of *Borrelia burgdorferi* DNA by Human Toll-like Receptor 9

Kedzie Arrington, Andre Taylor, Jen Griffin, Jorge Cervantes
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Abstract

Background
IL-26 has shown an antimicrobial effect in the control of *Borrelia burgdorferi* (Bb) growth *in vitro*, as well as in degradation of Bb DNA. It also enhances the anti-borrelial response of human macrophages. The addition of IL-26 to bacterial DNA promotes better sensing and production of Type I IFNs by human dendritic cells.

Objective
We aim to explore the effect of IL-26 in human toll-like receptor (TLR)-9 activation upon recognition of Bb DNA.

Methods
We utilized a single-receptor cell system, HEK-Dual™ hTLR9 cells (Invivogen), which harbors two reporter plasmids for NF-κB and IL-8 signaling pathways. Bb DNA was exposed to increasing concentrations of IL-26 in monomeric or dimeric form, and then used to stimulate the cells for 4 hours. TLR-9 ligand CpG was used as a control.

Results
We observed that NF-kB and IL-8 activation was maximal when cells were stimulated with Bb DNA previously treated with 5 uM of IL-26 monomer or 1 uM of IL-26 dimer. The same was observed for IL-8 activation upon CpG stimulation. However, NF-kB activation decreased upon stimulation with CpG treated with either form of IL-26.

Conclusions
Our study shows enhanced NF-kB and IL-8 activation upon recognition of IL-26-treated Bb DNA by TLR9. Increased NF-kB activation does not occur with IL-26-treated TLR9 ligand CpG, suggesting a difference in TLR9 recognition of the relatively G+C-rich oligonucleotide sequences from Bb DNA. These findings shall prompt further studies on the interaction of IL-26 and Bb DNA and its therapeutic potential in Bb-exposed human tissue.

GP 02: Phage Lysis Inhibition Is Phruitful Procrastination

Michael Awuah, Cody Martin, Adam Tomaszewski, Tes Sullivan, Qori Emilia, Steven Tran, Jason Snowden, Jake Chamblee, Ryland Young, Jolene Ramsey
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Abstract

Bacteriophage (phage) infections signify imminent death for their bacterial hosts. Infections by these viruses typically complete in an hour and terminate via lysis, an explosive release of viral progeny. Lysis in double-stranded DNA phages involves proteins that target and destroy each layer of the bacterial cell envelope. In well-studied phages of *E. coli* like N4, a phenomenon known as lysis inhibition (LIN) delays host killing and therefore release of viral progeny. An N4 infection cycle lasts more than five hours. Consequently, its burst size is increased, yielding 10 to 100 times more virion particles per infected cell. The active use of LIN to regulate the amount of viral progeny is of great interest in phage therapy, treatment of antibiotic-resistant bacterial infections using phages. Our goal is to understand the signals underpinning the LIN process in N4 and to recapitulate it in other phages to increase yield for applications in therapy. In this study, we use complementation assays to demonstrate experimentally the function of predicted N4 lysis proteins. We also show that the minimal N4 gene set required for host cell lysis is sufficient to induce LIN and propose a model of lysis and its regulation by LIN in N4. Future studies will use the information obtained about LIN signaling studies in N4 to reconfigure other phages for higher yields.

GP 03: Investigating the Effects of Dietary and Management Modifications on *Salmonella enterica* Populations in Harvest-Ready Beef Cattle

Yesica Botero[1], Kasi Schneid[2], Kendall L. Samuelson[2], John T. Richeson[2], Gizem Levent[1]
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Abstract

Salmonella enterica is a significant foodborne pathogen that can be found both in ready-to-harvest cattle and in feedlot environments. Dietary interventions and feeding schedules may influence the prevalence of *Salmonella* in cattle and feedlots. Our longitudinal randomized controlled study aimed to investigate the effects of high-acid (ACID) or control (CONT) diets with regular (REG) or erratic (ERRA) feeding schedules on *Salmonella* prevalence in cattle and in feedlots. Cattle (n=288) were weight-blocked and randomized into treatment pens (n=48) and received either of the 2*2 treatments until 56 days of the feeding period when the fecal (n=288) and pen-soil (n=96) samples were collected. *Salmonella* was isolated using standard methods. A multi-level logistic regression model incorporating fixed effects of treatments and a random effect for pen was used to estimate treatment effects on *Salmonella* prevalence. Overall, fecal, and pen-soil *Salmonella* prevalence and 95% CIs were 0.21 (0.16-0.26) and 0.44 (0.35 -0.55), respectively. Marginal predicted *Salmonella* prevalence in cattle feces versus pen-soil samples, respectively, and their 95% CIs were as follows: CONT-NORM 0.25 (0.14-0.36) versus 0.37 (0.17-0.58); CONT-ERRA 0.20 (0.10-0.31) versus 0.33 (0.14-0.54); ACID-NORM 0.16 (0.07-0.26) versus 0.54 (0.33-0.75); and ACID-ERRA 0.23 (0.13-0.34) versus 0.54 (0.33-0.75). Our preliminary findings showed no main treatment effects, or their interactions, on the *Salmonella* prevalence (P≥0.40). With this ongoing study, we aim to investigate the extended effects of the treatments up to slaughter (112+ days). Additionally, the clonal distribution of *Salmonella* isolates, including serotype, MLST, cgMLST, and antibiotic resistance patterns will be evaluated using whole-genome sequencing.

GP 04: Antimicrobial Activity of Over-the-Counter Ear Drops Containing Chamomile

Soroush Farsi, Shahrukh Chaudhry,, Ahmed Khan, Cervantes Jorge
Paul L Foster School of Medicine, El Paso, USA

Abstract

Chamomile (Matricaria chamomila) is a plant with known anti-inflammatory, analgesic, and antimicrobial properties. Homeopathic drops containing chamomile extract are often used for ear pain and chronic ear infection.

We aimed to evaluate the antimicrobial effect of over-the-counter available eardrops containing chamomile against the growth of two organisms causing otitis externa, Staphylococcus aureus and Pseudomonas aeruginosa.

Liquid cultures of S. aureus and P.aeruginosa were exposed to increasing concentrations of Earache Relief (Similasan, USA) eardrops containing extract of chamomile flower (10%, 20%, and 30%). Colony forming units (CFUs) were evaluated at three exposure time points.

We observed a reduction in the number of P.aeruginosa CFUs when the bacteria were exposed to any of the three concentrations of the chamomile drops as early as 5 minutes, with maximal reduction upon exposure to the 30% concenration at 45 minutes. Reduction of S.aureus CFUs, on the other hand, was observed for all three concentrations as maximal in the 5 minutes of exposure.

Few studies have suggested that chamomile essential oils could have antimicrobial properties. Our findings suggest that over-the-counter eardrops containing chamomile extract could potentially be used as a non-prescription treatment for mild cases of otitis externa.

GP 05: Chamomile Eye Drops Antimicrobial Activity against *Staphylococcus aureus*

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Abstract

Homeopathic drops are sometimes used as a complementary treatment for conjunctivitis, an inflammation or infection of the conjunctiva, the thin membrane that covers the white part of the eye and the inner surface of the eyelids. Eye drops containing chamomile (Martricaria chamomilla) have shown to have antioxidant and anti-inflammatory activities, allowing for protection against cell death and ameliorate wound healing. Many researchers have studied the antimicrobial activity of chamomile oil.

We aimed to test if commercially available eye drops containing chamomile would have an antimicrobial effect against Staphylococcus aureus, a main causative organism or bacterial conjunctivitis.

A liquid culture of S.aureus was exposed to increasing concentrations of eye drops (Manzanilla Sophia Herbal Eye Drops) for 5, 15, and 45 minutes. Colony forming units grown on TSA agar plates were assessed after 18 hours.

We observed a marked reduction in the number of S. aureus CFUs upon exposure to any of the three conditions (i.e., 10%, 20%, 30% v/v) which was retained through all time points.

The plant has previously shown an antimicrobial effect against S. aureus. Chamomile tea eye washing, however, can induce allergic conjunctivitis. Our findings may suggest a potential use of an over-the-counter medicine for Staphylococcal conjunctivitis.

GP 06: Differential Gene-Expression of *Acidovorax* under Normal and Microgravity Growth Conditions

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Abstract

Microorganisms grown under different stress conditions, such as heat, pH, UV radiation, and nutritional starvation differentially express their genes. These differences in gene expression produce cellular adaptive responses through specific mechanisms under specific conditions. However, bacteria grown under microgravity stress have not been fully investigated beyond the model bacterial species. Previous studies reported that bacteria 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 under normal and microgravity conditions. Acidovorax is a gram-negative plant pathogen capable of infecting crops. The null hypothesis "Bacterial cells grown under normal and microgravity conditions express the same pattern of gene expression profiles" was tested by comparing the transcriptomic data. Acidovorax bacterial cultures were grown in minimal medium under normal and microgravity conditions. Bacterial cells were collected during the log phase of growth (at 48 and 72 hours), 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 are smaller in size when grown under microgravity. The result from this study validated the previous finding that over hundreds of genes involving membrane transport, carbohydrate metabolism, DNA replication, and energy production were significantly altered, up- or down-regulated. These findings will further help understand the regulation of bacterial adaptive mechanisms including pathogenicity of this plant pathogen.

GP 07: Novel *Flavobacterium* sp. Isolated from Creek Bed in Dallas, Texas.

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Abstract

The genus *Flavobacterium* contains several pathogenic bacterial species that afflict the fish farming industry and cause severe economic losses (Declercq 2013). Establishing variations in motility, cell-cell associations, and biofilm formation between pathogens and non-pathogens could help to identify virulence factors present in the genus.

A novel species of *Flavobacterium* was isolated from a creek bed near Dallas, Texas and was characterized using various phenotypic and genetic approaches. After analyzing the 16S ribosomal DNA sequence and full genome, it was determined by NCBI Blast alignment that the most genomically similar strain to this species is F. denitrificans JS14-1 (~97.5%). Average Nucleotide Identity (ANI) analysis performed within KBase suggests the most similar species by this metric is *F. ginsenosidimutans* at 93.27% identity, which fails to meet a previously defined 95% identity species-demarcation threshold. These analyses together establish this isolate as a new species.

LC-MS/MS confirmed the presence of a novel lipid, likely a phosphorylated sulfobacin A. Sulfobacin A is known to impact gliding motility and seems to be a von Willebrand factor receptor antagonist (Vences-Guzmán 2021), so an alteration in this lipid could impact these virulence functions. Preliminary growth data and TEM imaging also show differences in cell aggregation between this isolate and established *Flavobacterium* pathogens, which suggests the novel isolate could have an altered mode of biofilm formation.

Going forward, this species will be further compared genomically and phenotypically to the model strain *F. johnsoniae* and established pathogen *F. columnare* to expand upon these differences and their ties to virulence.

GP 08: Role of *sigM* and *glpF* on Antimicrobial Resistance and Virulence in *Bacillus anthracis*

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Abstract

Bacillus anthracis is a gram-positive bacterium that causes the deadly anthrax disease. ClpX is a subunit of ClpXP protease that is known to be essential in virulence as well as providing resistance to cell-envelope targeting antibiotics such as penicillin, daptomycin, and the antimicrobial peptide LL-37. While *clpX* is critical for virulence in *B. anthracis*, it is unlikely to be directly mediating the effect. Hence, our lab investigated the genes that are differentially expressed in the Δ*clpX* mutant compared to the wild type *B. anthracis* through microarray analysis. We found 119 genes that were highly differentially expressed in the Δ*clpX* mutant. In this study, we focused on two genes *sigM* and *glpF*, which are downregulated in the Δ*clpX* mutant, because *sigM* and *glpF* confer resistance to cell-wall targeting antibiotics in the closely related gram-positive bacterial species, *Bacillus subtilis* and *Staphylococcus aureus* respectively. We wanted to determine whether loss of *sigM* and *glpF* will lead to similar phenotypes as loss of *clpX* in *B. anthracis* Sterne. We found that both mutants are more susceptible to penicillin, although in a growth phase dependent manner, and neither gene is critical for daptomycin resistance. Future studies will examine the susceptibility of these mutants to LL-37 and other stressors such as acid and heat stress. Complementation of these mutants will serve to further support the importance of these genes for the roles we examined. This research will aid in understanding the mechanism of antibiotic resistance and virulence in the ClpX regulatory network in *B. anthracis*.

GP 09: Investigating Novel *Streptomyces* bacteriophage Endolysins as Potential Antimicrobial Agents

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Abstract

As the post-antibiotic era arises, alternative strategies for control of bacterial infections are needed. Endolysin, a protein encoded by a phage gene, can lyse the bacterial peptidoglycan (PG). We, thus, are interested in exploring novel endolysins from *Streptomyces* phages as only a few of them have been experimentally characterized. Using several bioinformatics tools, we identified 9 different groups from 250 *Streptomyces* phages putative endolysins. We have selected seven putative endolysins of interest, including those from NootNoot gp34 (transglycosylase), Nabi gp26 (amidase), Araceli gp35 (CHAP), Tribute gp42 (PGRP-LysM), Wentworth gp29 (PGRP-PG binding-like), LazerLemon gp35 (CHAP), and FrodoSwaggins gp4 (zinc peptidase). We hypothesize that [1] the putative proteins of interest may have the ability to degrade PG at a different site. [2] endolysins with different domain architectures may have different bactericidal effectiveness against ESKAPE pathogens. So far, four plasmid constructs, LL35pET (with LazerLemon gp35), Nt34pET (with NootNoot gp34), Nb26pET (with Nabi gp26), and Tb42pET (with Tribute gp42) have been verified by plasmid sequencing. The predicted amidase from phage Nabi has been shown to exhibit PG-degrading activity when using the micrococcal-PG-embedded acrylamide gel (zymography). Interestingly, NootNoot gp34 was toxic to *E. coli* BL21(DE3) during protein expression. Currently, we are in the process of functional testing (zymography and colorimetric hydrolysis assay) for the other proteins. Furthermore, we plan to use mass spectrometry to identify and analyze the degraded PG products after treated by each enzyme. Lastly, antimicrobial resistant ESKAPE pathogens will be used to test the spectrum of the endolysins.

GP 10: Microbial Diversity of the Ganges River- A Bird's-Eye View

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Abstract

The Ganges River is a major source of livelihood for millions of people in India, however, it is polluted with sewage, industrial-wastes, and agricultural runoffs affecting the health of people, the economy, and the environment. Previous studies demonstrated that microbial community structure could be altered in response to anthropogenic stressors. This study is a preliminary microbiome analysis of domestic wastewater, Ganges water, and Ganges soil-sediment. The null hypothesis "Domestic wastewater, Ganges water, and Ganges soil-sediment exhibit a similar pattern of microbial compositions" was tested using microbiome data obtained from those three samples. Bacterial DNAs were isolated and used to amplify V3-V4 16S-regions. Metagenomic sequence library was prepared, and subsequently sequenced using Illumina Mi-Seq. Sequence analysis was performed on QIIME 2 platform, and the taxonomic assignment was made using RDP classifier. Results revealed that the microbial community structures at these three sites, both at the phylum and the genus levels were different. These three sites shared some bacterial phyla while each of these sites contained unique bacterial phyla and genera. All three sites shared *Proteobacteria* and *Bacteroidetes*. Both wastewater and Ganges water shared *Actinobacteria*, while wastewater and Ganges soil-sediment shared *Firmicutes*. Furthermore, wastewater contained unique genera, like *Lactococcus* and *Pseudomonas*, Ganges water contained unique genera, like *Actinobacter* and *Rhodobacter*, and Ganges soil-sediment contained unique genera, like *Bacillus* and *Nocardia*. The wide range of diversity included several soil and aquatic bacteria, including free living, and human and plant pathogens, thus examining these unique microbiomes thoroughly will be important for future endeavors.

GP 11: The Effects of *Escherichia coli* Central Metabolism Mutants on Planktonic Growth and Biofilm Formation

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Abstract

The Keio collection of *Escherichia coli* contains 3985 single gene deletion mutants derived from an *E. coli* K12 strain, BW25113. We investigated the impact of three central metabolism deletion mutants including *edd* (which impacted the Entner-Doudoroff pathway), *pgi* (which impacted glycolysis), and *gnd* (which impacted the Pentose Phosphate pathway). A microtiter plate was used to determine planktonic growth and density of biofilm formation of the three mutants as well as the wt strain when cultured in LB broth. CFU and MIC tests were also performed. Flow cells and silicone disks were used to analyze the biofilms with confocal and fluorescence microscopy. Of the four strains tested, the *pgi* knockout mutant had the highest planktonic growth, *edd* had the densest biofilm formation, *gnd* had the greatest biofilm CFUs, and wt BW25113 had the greatest planktonic CFUs. To our knowledge, this is the first investigation of the impact of central metabolism mutants on *Escherichia coli* biofilm formation.

GP 12: Microbial Source Tracking of *Escherichia coli* in Ambient Waters through Next Generation Sequencing with Utilization of the β-Glucuronidase Gene and Propidium Monoazide Treatment

Ariel Denise Robles[1], Davida Smyth[1,]
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Abstract

Microbial source tracking (MST) is a suite of methods that were developed to detect fecal contamination and track the source of fecal matter in environmental waterways through the usage of biological and chemical indicators. Environmental waterways face potential contamination of microbes from fecal matter causing a major threat to human health as it can harbor a variety of dangerous human and animal pathogens. The use of MST can allow for corrective action to be taken to help prevent further contamination and potential transmission of pathogenic microbes, reducing the incidence of illness. Our project aims to alter RNA-based methods and investigational protocols and techniques to detect the β-glucuronidase gene sequence, along with the usage of propidium monoazide treatment to detect viable Escherichia coli and to quantify and trace the identity of the microbes found to the precise source.

GP 13: Reverse Genetics Analysis of Radial Spoke Protein 1 (RSP1) in *Chlamydomonas reinhardtii*

Marcus Ross, Anne Gaillard
Sam Houston State University, Huntsville, USA

Abstract

The radial spoke is a conserved component of `9+2' cilia and plays an essential role in regulating dynein activity. This T-shaped structure relays signals from the central pair microtubules to the dynein arms. The Chlamydomonas reinhardtii radial spoke complex is comprised of 23 proteins including 13 identified stalk proteins and 5 head proteins. Radial Spoke Protein 1 (RSP1) is a head protein highly conserved across taxa. In mammalian models, deletion RSP1 has led to a decrease in ciliary motility. However, there is limited understanding of the role RSP1 plays within Chlamydomonas reinhardtii ciliary motility. Here, we employ a reverse genetics approach to understand the effect on cilia motility when RSP1 is absent within the radial spoke head. RSP1 mutants display an increase in loss of motility and palmelloid formation. Analysis with dark-field microscopy exhibited a decrease in general motility, and alterations in swim speed, and waveform.

GP 14: Source Tracking of Fecal Indicator Bacteria in Texas Coastal Waters

Kristin Sefcik[1,2], Janice Speshock[2], Jesse Meik[2], Stephanie Brady[3], Jeff Brady[1]
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Abstract

Detection of fecal indicator bacteria in Texas typically involves culture-based technology. While the culture tests are simple, they take 24 hours to produce results and offer no indication of the source of the pollution. The United States Environmental Protection Agency (EPA) has validated DNA-based tests for fecal indicator bacteria (FIB). In this study, EPA method 1696 will be conducted on water samples from Texas coastal waters for the detection of human fecal pollution by HF183/BacR287 quantitative polymerase chain reaction (qPCR) assays. While the DNA-based detection methods provide more rapid quantification of FIB, the source of the biological pollution is critical when assessing human risk from exposure. Microbial source tracking (MST) will be used to detect the source of FIB from the water samples using multiple qPCR assays. In addition to human sources, the MST qPCR assays used in this study will track FIB from avian, equine, bovine, swine, and canine hosts. Initial results gathered from the qPCR assays performed showed all water samples tested positive for *Bacteroides* with 55% of the samples testing positive for avian fecal contamination. Each of the qPCR assays conducted in this study provided rapid determination of pollution sources which allows for a more refined assessment of health risks in recreational waters.

GP 15: Agent-Based Modeling to Assess Optimal Conditions for Reducing Pathogenic Air Transmission

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Abstract

It has become painfully apparent in the last two years the efficiency with which viruses can spread through aerosols. While this is not new information, we did not yet have the tools to create effective safety measures. Our team is working on a method of measuring and modeling how aerosols containing viral particles suspend and move throughout the air. We have previously used a nebulizer to simulate the spread of aerosols filled with a detectable bacteriophage and were able to observe how viable particles spread and factors such as humidity that can affect this. We will expand on previous work by using a new air sampler to quantify DNA. We will also be introducing more variables such as airflow and movement of the source to see how these changes may affect how much the aerosols spread. Using an agent-based model we will further explain the phenomenon of aerosol travel and potentially use this as a future guideline for social distancing or other safety protocols. An issue with current models is that little experimental data is collected and many of the outcomes are hypothetical. With experimental data used to create the baseline for our model, we anticipate that it will be more accurate and applicable to those trying to create safety protocols while keeping aerosols in mind.

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Eppinger, Mark	South Texas Center for Emerging Infectious Diseases (STCEID), San Antonio, USA. Department of Molecular Microbiology & Immunology, University of Texas at San Antonio, San Antonio, USA.	UP 24, UP 30
Estrada, Diana	Tarleton State University, Dublin, USA. Tarleton State University, Stephenville, USA	UP 13, UP 19
farsi, soroush	Paul L Foster School of Medicine, El Paso, USA	GP 04
Fernandez Robles, Rebeca	Tarleton State University, Stephenville, USA	UP 13
Fernandez-Robles, Rebecca	Tarleton State University, Stephenville, USA	UP 15
Feroz, Tadeen	Department of Biology and Kinesiology, Letourneau University, Longview, USA	UP 41
Fitzgerald, Katelyn	Tarleton State University, Stephenville, USA	UP 13
Foree, Hannah L.	Department of Biology & Kinesiology, LeTourneau University, Longview, USA	UP 16
Frost, Macy	Abilene Christian University, Abilene, USA	UP 06
Gaillard, Anne	Sam Houston State University, Huntsville, USA	GP 13
Gallegos, Kyle	Texas Christian University, Fort Worth, USA	GO 3
Garcia, Isabel	University of Dallas, Irving, USA	UP 44
Gardner, Joshua	Texas Tech University HSC El Paso, El Paso, USA	GP 05
Garrett, Charley	Del Mar College, Corpus Christi, USA. Texas A&M University, Corpus Christi, USA	UP 07
Geddie, Mary	University of Dallas, Irving, USA	UP 25
George, Isaiah K.	Texas Tech University, Lubbock, USA	UP 27

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Gold, Daniel A.	St. Edward's University, Austin, USA.	UO 2, UP 28
Goppert, Marlee	Tarleton State University, Stephenville, USA.	UP 12, UP 13, UP 15, UP 19, UP 20, UP 22
Graupmann, Dylan	Midwestern State University, Wichita Falls, USA	UP 08
Greene, Mariah	Texas Christian University, Fort Worth, USA	GO 3
Griffin, Jen	Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, El Paso, TX, USA	GP 01
Groth, Austin	Midwestern State University, Wichita Falls, USA	UP 11
Gu, Christina	University of Texas at Dallas, Richardson, USA	UP 43
Guan, Ziqiang	Duke University Medical Center, Durham, USA	GP 07
Gutierrez, Eduardo	Sam Houston State University, Huntsville Tx, USA	GP 06
Haines, Ian	Tarleton State University, Stephenville, USA	UP 13
Hall, Sydney	University of Texas at Dallas, Richardson, USA	GP 07
Hamilton, Luke	Texas Christian University, Fort Worth, USA	UP 29
Hamood, Abdul	Texas Tech University Health Sciences Center, Lubbock, USA	UP 35
Harbison, Zoe J.	Department of Biology & Kinesiology, LeTourneau University, Longview, TX, USA	UP 18
Hauser, Charles	St. Edward's University, Austin, USA.	UO 2, UP 28
Hennigan, Jennifer	Abilene Christian University, Abilene, USA	UO 3
Hernandez, Amber	Del Mar College, Corpus Christi, USA	UP 14
Higgins, Ashley	Department of Molecular Microbiology & Immunology, University of Texas at San Antonio, San Antonio, USA.	UP 24, UP 30

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Hogins, Jacob	University of Texas at Dallas, Richardson, USA.	GO 4, UP 40, UP 43
Hona, Salina	Texas Christian University, Fort Worth, USA.	GP 08, UP 36
Hosseini, Mehrdad	Texas Tech University HSC El Paso, El Paso, USA	GP 05
Huddleston, Jennifer	Abilene Christian University, Abilene, USA.	ES 4, UP 03, UP 06
Hughes, Lee E.	University of North Texas, Denton, USA	GP 09
Hughes, Rachel	University of North Texas, Denton, USA	GP 09
Hulyalkar, Neha	University of Texas at Dallas, Richardson, USA	UP 34
Hung, Chiung-Yu	The University of Texas at San Antonio, San Antonio, USA	GO 5
Hwang, Jisoo S.	University of Dallas, Irving, USA	UP 44
Jeffers, Shelby	Tarleton State University, Stephenville, USA.	UP 13, UP 15
Jefferys, Sean	The University of Texas at San Antonio, San Antonio, USA	GO 5
Jenkins, Emily	Stephenville Research and Extension Center, Stephenville, USA	UP 09
Jett, Adrienne M.	Department of Biology & Kinesiology, LeTourneau University, Longview, USA	UP 16
Johnson, Dustin	Texas Christian University, Fort Worth, USA	GO 2
Jones, Bryce	St. Edward's University, Austin, USA	UP 45
Jones, Laura	MolecuLight, LLC, Toronto, Canada	UP 35, UP 26
Jorge, Cervantes	Paul L Foster School of Medicine, El Paso, USA	GP 04
Kalalah, Anwar	South Texas Center for Emerging Infectious Diseases (STCEID), San Antonio, USA. Department of Molecular Microbiology & Immunology, University of Texas at San Antonio, San Antonio, USA.	UP 24, UP 30
Kasriel, Gregory	Texas A&M University-San Antonio, San Antonio, USA	UP 05

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Kaur, Gurvinder	Texas Tech University Health Science Center, Lubbock, USA	ES 1
Keim, Klara C.	Texas Tech University, Lubbock, USA	UP 27
Kelly, Taylor	Texas Christian University, Fort Worth, USA	UP 29
Khan, Ahmed	Paul L Foster School of Medicine, El Paso, USA	GP 04
Khan, Nasiha	Midwestern State University, Wichita Falls, USA	UP 46
Klose, Karl	The University of Texas at San Antonio, San Antonio, USA	GO 5
Konig, Sara S.K.	South Texas Center for Emerging Infectious Diseases (STCEID), San Antonio, USA. Department of Molecular Microbiology & Immunology, University of Texas at San Antonio, San Antonio, USA.	UP 24, UP 30
Kruczek, Cassandra	Texas Tech University Health Science Center, Lubbock, USA	ES 1
Kuchar, Alexa	Delmar College, Corpus Christi, USA	UP 17
Kumar, Niraj	Department of Botany, Biotechnology, and Botany, Patna University, Patna, India	GP 10
Labry, Faith	University of North Texas, Denton, USA	GP 09
Landrye Reynolds, Landrye	Texas Tech University, Lubbock, USA	UP 27
Lawson, Shelby	Stephenville Research and Extension Center, Stephenville, USA	UP 09
Leija, Caroly	Texas A&M AgriLife Research, Stephenville, USA	UP 09
Leung, TimYee	Tarleton State University, Stephenville, USA	UP 12
Leung, Timyee	Tarleton State University, Stephenville, USA	UP 13
Levent, Gizem	Texas Tech University, Lubbock, USA. Texas Tech University School of Veterinary Medicine, Amarillo, USA	GO 1, GP 03
Lima, Isabela	University of Dallas, Irving, USA	UP 44

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Macario, Jordan	St. Edward's University, Austin, USA	UP 45
Machunis-Masuoka, Elizabeth	Midwestern State University, Wichita Falls, USA.	UP 04, UP 08, UP 11
Malmquist, Jacob	Texas Christian University, Fort Worth, USA	GO 3
Manceras, Julio	Texas Christian University, Fort Worth, USA	GO 3
Maneekul, Jindanuch	University of North Texas, Denton, USA	GP 09
Mares, Chris	Texas A&M University-San Antonio, San Antonio, USA	UP 31
Martin, Cody	Texas A&M University, College Station, USA	GP 02
Martinez, Clarissa A.	Department of Biology & Kinesiology, LeTourneau University, Longview, TX, USA	UP 18
Masuoka, James	Midwestern State University, Wichita Falls, USA.	UP 04, UP 37, UP 46, UP 49
Mathews, Kaitlyn E.	Department of Biology & Kinesiology, LeTourneau University, Longview, USA	UP 48
McCutchen, Bill	Texas A&M AgriLife Research, Stephenville, USA	UP 09
McGehee, Rebecca	Texas A&M University-San Antonio, San Antonio, USA	UP 31
McGillivray, Shauna	Texas Christian University, Fort Worth, USA.	GO 2, GO 3, GP 08, UP 29
McGillivray, Shauna M.	Texas Christian University, Fort Worth, USA.	UO 5, UP 36, UP 38
McLean, Robert	Texas State University, San Marcos, USA	GP 11
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Meik, Jesse	Tarleton State University, Stephenville, USA	GP 14
Miller, Kathleen M.	University of Dallas, Irving, USA	UP 44
Munoz, Jasmine	Tarleton State University, Dublin, USA.	UP 13, UP 19
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Navarro, Stephany	Texas Tech University Health Sciences Center, Lubbock, USA	UP 35
Negron, Austin	The University of Texas at San Antonio, San Antonio, USA	GO 5
Negron, Elizabeth	The University of Texas at San Antonio, San Antonio, USA	UP 32
Neugent, Dr. Michael	University of Texas at Dallas, Richardson, USA	UP 34
Nicolay, Jacqueline	Del Mar College, Corpus Christi, USA. Texas A&M University, Corpus Christi, USA	UP 07
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Ogwo, Morgan	Texas Tech University HSC El Paso, El Paso, USA	GP 05
Okolie, Star	UT Southwestern Medical Center, Dallas, USA. University of Texas at Dallas, Richardson, USA	UP 34
Olivo, Benjamin	Texas Tech University Health Science Center, Lubbock, USA	ES 1
Olivos, Ashley	Tarleton State University, Stephenville, USA.	UP 13, UP 20
Palmer, Kelli	University of Texas at Dallas, Richardson, USA	GP 07
Papp, Sara	The University of Texas at San Antonio, San Antonio, USA	GO 5
Parekh, Trusha	University of Texas at Dallas, Richardson, USA	UO 4

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Pascal, Francine	Midwestern State University, Wichita Falls, USA	UP 49
Pham, Emily	Department of Biological Sciences, Texas Tech University, Lubbock, USA	UP 26, UP 27, UP 35
Pitman, Jacob I.	Department of Biology and Kinesiology, Letourneau University, Longview, USA	UP 41
Pledger, Taryn M.	Department of Biology & Kinesiology, LeTourneau University, Longview, TX, USA	UP 18
Powers, Nicole	Texas A&M University, Corpus Christi, USA	UP 07
Prasad, Birendra	Department of Botany, Biotechnology, and Botany, Patna University, Patna, India	GP 10
Ramirez, Anayeli	Tarleton State University, Stephenville, USA	UP 13, UP 20
Ramos, Abigail	Texas A&M, Corpus Christi, USA. University of Dallas, Irving, USA	UP 44
Ramsey, Jolene	Texas A&M University, College Station, USA	GP 02, ES 2
Ranasinghe, Weerakkody	Department of Biological Sciences, Sam Houston State University, Huntsville, USA	GP 10
Ranson, Taylor	Texas State University, San Marcos, USA	GP 11
Reese, Tiffany	UT Southwestern, Dallas, USA	UP 50
Reitzer, Larry	The University of Texas at Dallas, Richardson, USA.	GO 4, UP 40, UP 43
Reynolds, Landrye	Texas Tech University, Lubbock, USA	UP 35
Richeson, John T.	West Texas A&M University Department of Agricultural Sciences, Canyon, USA	GP 03
Robles, Ariel	Texas A&M University - San Antonio, San Antonio, USA	GP 12
Rodriguez, Jesus	Sam Houston State University, Huntsville Tx, USA	GP 06
Romo, Jesús	The University of Texas at San Antonio, San Antonio, USA	UP 32
Ross, Marcus	Sam Houston State University, Huntsville, USA	GP 13
Rouseau, Zach	Texas Christian University, Fort Worth, USA	UP 29
Saito, Joshua	University of North Texas, Denton, USA	GP 09

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Samuelson, Kendall L.	West Texas A&M University Department of Agricultural Sciences, Canyon, USA	GP 03
Sanchez, Leah	Abilene Christian University, Abilene, USA	UO 3
Sanchez, Mia	St. Edward's University, Austin, USA	UP 28, UO 2
Sandford, Nicholas	Texas Tech University, Lubbock, USA	UP 27
Sawant, Namrata	University of Texas at Dallas, Richardson, USA	UP 34
Schlimme, Sarah G.	Department of Biology and Kinesiology, Letourneau University, Longview, USA	UP 41
Schneid, Kasi	West Texas A&M University Department of Agricultural Sciences, Canyon, USA	GP 03
Scott, H. Morgan	Texas A&M University, College Station, USA	GO 1
scullion, kaylee	Del Mar, corpus christi, USA	UP 21
Sefcik, Kristin	Tarleton State University, Stephenville, USA. Texas A&M AgriLife Research, Stephenville, USA	GP 14
Shariat, Nikki	University of Georgia, Athens, USA	GO 1
Siceloff, Amy	University of Georgia, Athens, USA	GO 1
Smith, Allie	Texas Tech University, Lubbock, USA.	UP 27, UP 35
Smith, Allie Clinton	Honors College, Texas Tech University, Lubbock, USA	UP 26
Smith, Braden	Abilene Christian University, Abilene, USA	UP 03
Smith, Caressa	Tarleton State University, Stephenville, USA	UP 13
Smyth, Davida	Texas A&M University-San Antonio, San Antonio, USA.	GP 15, UP 05, UP 31
Snowden, Jason	Texas A&M University, College Station, USA	GP 02
Soto, Carolina	University of Dallas, Irving, USA	UP 44
Spere, Justin	Tarleton State University, Stephenville, USA.	UP 13, UP 22
Speshock, Janice	Tarleton State University, Stephenville, USA	GP 14
Spiro, Stephen	University of Texas at Dallas, Richardson, USA	UO 4

Author name	Affiliation name	Program Codes*
Stacy, Lyndsy	Texas A&M University-San Antonio, San Antonio, USA.	GP 15, UP 05
Stenesen, Drew	University of Dallas, Irving, USA	UP 25
Strzhemechny, Yuri	Texas Christian University, Fort Worth, USA	GO 2
Sullivan, Tes	Texas A&M University, College Station, USA	GP 02
Tan, Michael J.	Department of Biology & Kinesiology, LeTourneau University, Longview, USA	UP 41
Tasmin, Sarah	Texas A&M University, College Station, USA	UP 09
Tavarez, Idaleth	Tarleton State University, Stephenville, USA	UP 13
Taylor, Andre	Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center El Paso, El Paso, TX, USA	GP 01
Teufel, Ashley	Texas A&M University San Antonio, San Antonio, USA	GP 15
Tiemann, Kami	Del Mar College, Corpus Christi, USA	UP 23
Toalson, Claire	Stephenville Research and Extension Center, Stephenville, USA	UP 09
Tomaszewski, Adam	Texas A&M University, College Station, USA	GP 02
Tompkins, Megan	The University of Texas at San Antonio, San Antonio, USA	GO 5
Tran, Steven	Texas A&M University, College Station, USA	GP 02
Tsai, Marcus	University of Texas at Dallas, Richardson, USA	UO 4
Turnbow, Jacob	Midwestern State University, Wichita Falls, USA	UP 11
Turner, Jeffrey	Texas A&M University, Corpus Christi, USA	UP 07
Vazquez, Sabrina	Department of Biological Sciences, Sam Houston State University, Huntsville, USA	GP 10
Verchot, Jeanmarie	Texas A&M AgriLife Research, College Station, USA	UP 09
Wagle, Sophia	Abilene Christian, Abilene, USA	UP 06
Waite, Kelsey	Texas Christian University, Fort Worth, USA.	GP 08, UP 36

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Wood, Bethany	Stephenville Research and Extension Center, Stephenville, USA	UP 09
Wood, Isabella	Tarleton State University, Stephenville, USA	UP 13
Wright, Justin	University of Texas at Tyler, Tyler, USA	UO 6
Ylostalo, Joni H.	University of Mary Hardin-Baylor, Temple, USA	ES 3
Young, Ryland	Texas A&M University, College Station, USA	GP 02
Yu, Jieh-Juen	The University of Texas at San Antonio, San Antonio, USA	GO 5
Zavala, Ethan	Tarleton State University, Stephenville, USA	UP 13, UP 22
Zeng, Alexander	Brown University, Plano, USA	UP 50
Zhang, Daiyuan	Del Mar College, Corpus Christi, USA	UP 07, UP 14, UP 21, UP 23
Zimmern, Philippe	The University of Texas Southwestern, Dallas, USA.	GO 4, UP 34, UP 40
Zogaj, Xhavit	The University of Texas at San Antonio, San Antonio, USA	GO 5
Zucchi, Paola	The University of Texas at San Antonio, San Antonio, USA	UP 32

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IMAGE: LEFT
“Fiesta Flamenca” by Mireya Duran from Texas Health Presbyterian Hospital in Dallas, Texas won third place in the Traditional (Professional) Category in ASM’s 2021 Agar Art Contest. “Her skirt is tastefully painted with *Klebsiella pneumoniae* and *Enterococcus faecalis*. Her ivory white skin is made from a blend of *Staphylococcus aureus* and *Candida albicans*. The pink flowers in her hair and her gold hoop earrings are thanks to *Staphylococcus saprophyticus* and *Micrococcus luteus*, respectively. Her black curls, elegantly gathered in a bun, are composed of *Salmonella enterica*.” - https://asm.org/Events/ASM-Agar-Art-Contest/Previous-Winners/2021

IMAGE: CENTER
"My Living Portrait," by Korey Abram, a Digital Media Arts undergraduate student at Prairie View University, Texas, won first place in the Maker category in ASM’s 2019 Agar Art Contest. “His piece is a bacterial self-portrait, utilizing both *Serratia* (red) and *Micrococcus* (yellow) bacteria.” - https://asm.org/Press-Releases/2019/November-1/ASM-s-5th-Agar-Art-Contest-Showcases-the-Beauty-of

IMAGE: RIGHT
"Twelve Years of Yuck," Laura Bryan, Sara Lawhon, Sara V. Little, Texas A&M University, College Station, TX, won third place in ASM’s 2016 Agar Art Contest. "Microbial pathogens were painted with *Salmonella enterica* serovar Typhimurium and *Escherichia coli* MG1655 on Hektoen enteric agar to yield black and yellow colonies, respectively. *Salmonella* spp. produce hydrogen sulfide which precipitates thiosulfate and ferric ammonium citrate in plates. *E. coli* ferments sugars and acidifies the agar, causing the yellow color change.” - https://asm.org/Events/ASM-Agar-Art-Contest/Previous-Winners/2016