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Graduate Oral Presentations

GO1 Bacteriophage N4 lysis inhibition – ancient conundrum meets novel culprit

Michael Awuah, Jolene Ramsey

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Abstract

Bacteriophage (phage), viruses that infect and kill bacteria, are reemerging as an important player in the fight against bacterial antimicrobial-resistant (AMR) infections. To treat AMR infections with phage, we need reproducible large-scale production. This is a manufacturing challenge blocking cost-effective phage therapy. However, some phage naturally overcome this challenge. Phage infections typically terminate in lysis, an explosive release of viral progeny that results in cell death. Double-stranded DNA phages of Gram-negative bacteria regulate a class of lysis proteins called the holins to delay the onset of lysis, allowing the phage to maximize the cell's resources to produce more virions through lysis inhibition (LIN). Bacteriophage N4 exhibits the LIN phenotype. N4 phage infections yield 3000 phage/cell compared to 25 ~ 100 phage/cell in other phages. To deconstruct N4 LIN for future applications, this study focuses on understanding the molecular basis of LIN by N4 using RNA Sequencing. Samples of wildtype N4 phage and a rapid lyser variant that does not exhibit LIN were collected to capture the important stages of the N4 lysis program. Transcriptomic data show the expression of an unannotated open reading frame we named gp75 which has nonsense mutations in all sequenced rapid lyser lines but not the wildtype, implying a strong association of the rapid lysis phenotype with this genomic region in LIN. Future studies seek to delineate the function of gp75 whilst also examining the role of differentially expressed host & N4 genes.

GO2 Impact of land development on soil microdiversity

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Abstract

The impact of land development on soil microbial diversity and antibiotic-producing properties is a critical area of investigation due to the escalating expansion of industrialization and scarcity of effective antibiotics. Low Impact Development (LID) used by development companies aims to preserve ecosystems within their communities, however, the efficacy of LID in maintaining microbial diversity remains to be determined. The central question driving our research is how land development influences microbial diversity and their capacity to produce antibiotics. To address this, we are conducting field sampling across nine sites, including three undisturbed, three disturbed, and three Greenway sites, representing LID efforts at a local residential development area and the Texas A&M University-San Antonio (TAMUSA) campus. Bacteria collected from these sites are isolated, screened against four different bacterial strains for antibiotic production, and will be genetically characterized to identify bacterial compositions in each site. Preliminary findings indicate that undisturbed sites harbor more bacteria with antibiotic-producing properties than Greenway and disturbed sites. This suggests a potential negative impact of land development on microbial communities and questions the efficiency of LID in preserving these soil bacteria.

GO3 Investigating altered metabolism during KSHV lytic infection

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Abstract

Kaposi's Sarcoma Herpes Virus (KSHV) is the etiological agent of Kaposi's Sarcoma (KS) and a leading cause of cancer in AIDS patients. With limited treatment available for KS, it is essential to understand the underlying mechanics behind KSHV infection. KSHV undergoes latent and lytic viral phases in the host cell. Previous metabolomic analysis showed that KSHV latently-infected endothelial cells modulate central carbon pathways (glycolysis, fatty acid synthesis (FAS), and amino-acid metabolism). Nonetheless, global metabolic changes during lytic KSHV infection are still unknown. We hypothesize that global host cell metabolism is modulated to support maximal lytic infection and viral infection. Drug inhibition of FAS using TOFA resulted in reduced survival of latently infected cells and a significant reduction in virion production during the lytic phase. Despite that, it is important to quantify FAS enzymes in lytic infection to better understand the cellular mechanism. ACC, ACLY, and FASN are the major enzymes of FAS. We examined FAS gene expression via qPCR in a KSHV inducible cell line during lytic replication and found that ACC, ACLY, and FASN are not significantly altered during the lytic infection compared to latent infection. We are currently preparing samples for global metabolomics during KSHV lytic infection to analyze all the changes in central carbon metabolism. We expect to see significant metabolite changes in central carbon metabolic pathways and potentially reveal novel pathways modulated by KSHV lytic infection.

GO4 Alignment-free distance-based method to identify prions and prion-like proteins.

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Abstract

The functional role of prion proteins is exhibited in yeast cells. Yeast cells modulate cell functioning to counterbalance extreme environmental conditions with the help of one or more than one prion protein as a prion system. Whereas in the context of mammalian cells, prions are not functional. There are mammalian proteins which share fewer characteristics of prion proteins. For example, proteins involved in human neurodegenerative diseases form aggregates and exhibit infectious characteristics through cytoplasmic transfer. In general, they are termed amyloid-like proteins. Prion proteins share very diminished sequence similarity and hence sequence similarity-based alignment methods fail to identify prion proteins. Sequence composition-based methods are developed to discover prion proteins. Here in the study, an alignment-free distance-based method is proposed to find prion-like protein sequences whose physico-chemical context is like that of prion proteins and may exhibit prion-like characteristics. Protein sequences are converted to series form based on their physicochemical properties and the Dynamic Time Warping method is applied in window-based fashion to identify sequence level similarity in terms of DTW distances. Alignment is performed in a one-to-one fashion between query and template which is known prion protein, and results are analyzed for the best match. Study design will help us to leverage the advantages of alignment-free distance-based method in prion research and will pave a path to identify and screen prion-like proteins across various kingdoms of life.

GO5 Microbiome composition of soil Sediments at polluted locations of River Ganges, India

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Abstract

River Ganges is one of the major rivers in the world and the largest freshwater river system in the Indian sub-continent. However, Ganges has been strongly affected by pollution due to various pollutants, including untreated sewage from industries and cities, pesticides from agriculture, medical waste and religious activities. To understand the impact of the pollution on the microbiome composition, we analyzed the soil sediments from six different locations along the Ganges where unique anthropogenic activities take place. DNA was extracted from 18 soil sediment samples collected from Rishikesh, Haridwar, Kanpur, Varanasi, Patna, and Kolkata. The V3-V4 region of the 16S rRNA gene was amplified using polymerase chain reaction, and the DNA was sequenced by Illumina Mi-Seq. The raw sequences were then processed and analyzed using *mothur v. 1.48.0* miseq SOP in Galaxy. Results reveal that all the locations share several dominant genera including *Clostridium*, *Lachnospiracea*, *Roseburia*, *Faecalibacterium*, *Ruminococcus*, *Rhizobiales*, and *Acidobacteria,* but differ in the presence of several rare genera. The alpha (α) diversity was estimated using Shannon and Simpson index. Varanasi exhibits the highest alpha diversity compared to other locations indicating the unique anthropogenic activities at Varanasi could lead to a higher microbial diversity. Beta (β) diversity was estimated using Bray Curtis Dissimilarity Index. Patna shows the highest dissimilarity compared to other sampling locations whereas Kanpur and Kolkata show the most similarity. The understanding of the microbiome community structure-function will further provide insights in essential nutrient cycles, including decomposition of organic compounds and primary production in aquatic food chains.

Undergraduate Oral Presentations

UO1 Prevalence of bacterial pathogens in seafood harvested from Galveston Bay

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Abstract

Blue crabs (*Callinectes sapidus*) and Gafftopsail catfish (*Bagre marinus*) are frequently harvested commercially and recreationally from Galveston Bay and handling these animals often results in wounds that can lead to fatal infections; however, few studies have determined the prevalence of bacterial pathogens associated with catfish and crabs. To address this, catfish and crabs that had been harvested from Galveston Bay were purchased at seafood markets in Kemah, TX. The catfish were purchased in September 2023 and January 2024. The crabs were purchased from February 2023 to January 2024. A library of readily-culturable, aerobic heterotrophic bacteria was generated from the spines of catfish and the hemolymph and exoskeleton of blue crabs and isolates were identified with a matrix-assisted laser desorption ionization – time of flight mass spec (MALDI-TOF MS). The library generated from catfish was dominated by two opportunistic pathogens - *Kurthia gibsonii* (7/23) and *Macrococcus caseolyticus* (3/23). Cluster analysis of mass spectra generated by MALDI-TOF MS suggested that crabs host different communities on their exoskeleton and in their hemolymph. The library generated from the exoskeletons of crabs was dominated by *Vibrio* spp., including *V. pomeroyi* and *V. anguillarum* (18/130), and *Psychrobacter* spp. (14/130). The library generated from the hemolymph of crabs was dominated by *Proteus hauseri* (7/35) and the pathogen *Aeromonas veronii* (3/35). This suggests that the exoskeletons of crabs are a hotspot for vibrios but Gafftopsail catfish should also be handled with caution because of pathogens associated with their spines.

UO2 Inhibiting the growth of a human pathogenic fungus *Cryptococcus neoformans* via genetic alteration

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Abstract

Cryptococcus neoformans is a yeast fungus found throughout the globe. *C. neoformans* poses little threat to healthy individuals, however, it is an opportunistic pathogen for immunocompromised people. The immunocompromised population in underdeveloped countries is highly susceptible to cryptococcosis infections. Calcineurin, an enzyme, plays an essential role in thermo-tolerance/virulence, and it negatively regulates expression of the *BYC1* and *BYC4* genes. Our goal was to elucidate how the *BYC1* and *BYC4* genes of calcineurin are involved in the growth of *C. neoformans* at body temperature. We hypothesized that the yeast would be hypersensitive to the overexpression of these genes and show low growth at 37^{o}C, mimicking the thermos-sensitivity caused by calcineurin inhibitors. Methods included insertions of *BYC1* and *BYC4* constructs into the wildtype strain (H99) via electroporation. Resulting strains were screened to visualize growth at 30^{o}C and 37^{o}C. A mutant expressing thermos-sensitivity was then tested in an intranasal-infected murine study to determine survival and CFUs in the brain and lungs. In conclusion, *BYC1* transformed strains (n=4) exhibited a reduced growth at 37^{o}C compared to the wildtype. A successful mutant was then used for a murine study with the wildtype. The mice infected with the mutant had a significant increase in survival compared to the wildtype. There was a significant decrease in the CFUs of mice lungs infected with the mutant compared to those infected with the wildtype strain. This project can lead to the research of potential antifungal treatments overexpressing the *BYC1* gene, negatively impacting *C. neoformans* cells and not patients.

UO3 Repurposing a serotonin receptor antagonist as a potential novel antibiotic

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Abstract

With the surge of multidrug resistant bacteria and increasing antibiotic resistance, there is a critical need for the development of new drug therapies. A new antimicrobial technique revolves around targeting virulence factors, which enable the bacterial pathogen to evade host immune defenses. Inhibitors that target pathogenicity hinder the capacity of the bacterium to cause an infection, thus allowing the host immune system to better clear the infection. In this study, we aim to inhibit the ClpXP protease, a highly conserved intracellular protease involved in virulence in different bacterial pathogens. Previous studies have shown that inhibition of ClpX completely attenuates virulence in *Bacillus anthracis*, rendering the pathogen more susceptible to cell envelope targeting antibiotics such as penicillin, daptomycin and LL-37. Computational modeling was performed and ten commercially available inhibitors with predicted activity against ClpX were identified, with ritanserin showing the most promise. In this study we explore the antimicrobial effects of ritanserin, a previously identified serotonin 2A receptor antagonist that underwent clinical trials as a potential treatment for schizophrenia and substance dependence. We hypothesized that if ritanserin inhibits ClpX in *B. anthracis* Sterne it should mimic the phenotype of the knockout clpX mutant, *ΔclpX*. We found that ritanserin increased WT *Bacillus anthracis*susceptibility to the cell envelope targeting antibiotics penicillin and daptomycin. Future studies will look at interactions host defenses such as antimicrobial peptides including LL-37. This demonstrates that ritanserin could be potentially repurposed as an antibacterial drug with the potential to be used by itself or in combination with antibiotics.

UO4 Choline degradation in *Paracoccus denitrificans*: identifying sources of formaldehyde

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Abstract

Paracoccus denitrificans is a non-obligate methylotroph that can grow on the C1 compounds methanol and methylamine as sole sources of carbon and energy. Both are oxidized to formaldehyde then to formate. Thus, growth on C1 substrates induces the expression of genes encoding enzymes required for the oxidation of formaldehyde. FlhSR, a two-component regulatory system, is believed to activate transcription of the operons encoding the methanol, methylamine and formaldehyde dehydrogenases. Deletion mutants of this system are known to be unable to grow on C1 compounds. Under aerobic conditions, choline is degraded in the cytoplasm through betaine aldehyde, glycine betaine, dimethylglycine and sarcosine to glycine. Catabolism of this organic substrate has the potential to generate 0-3 molecules of formaldehyde, though the sources of formaldehyde made during this process in P. denitrificans are not known. By exploring FlhSR mutant growth phenotypes and the activities of a promoter and enzyme known to be up-regulated by formaldehyde, we identify two steps of the choline oxidation process that generate formaldehyde: the oxidations of glycine betaine and dimethylglycine. Formaldehyde, which is toxic and must be removed, is then oxidized to formate by a cytoplasmic glutathione (GSH)-dependent formaldehyde dehydrogenase, which was assessed. Our work sheds further light on metabolic pathways that are likely important in a variety of environmental contexts.

Graduate Poster Presentations

Molecular and Environmental Microbiology

GP1 Iron-dependent regulation of membrane iron transporters by the nonsense-mediated mRNA decay pathway in *Saccharomyces cerevisiae*

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Abstract

The nonsense-mediated mRNA decay (NMD) pathway is highly conserved within eukaryotes and known to serve a surveillance role in degrading mRNAs containing premature termination codons (PTCs). Additionally, NMD functions as a regulatory pathway in targeting naturally occurring mRNA transcripts for degradation. Certain features of mRNA transcripts, such as atypically long 3'UTRs, have been shown to render these transcripts sensitive to NMD-mediated degradation. It has also been shown that NMD may differentially regulate transcripts in different cellular contexts, such as varying levels of extracellular iron. Dysregulation of iron homeostasis has implications in human health in various cancers, neurodegenerative conditions, and anemias. Prior and preliminary research shows that NMD is implicated in maintaining iron homeostasis, though the extent is unknown. To explore the relationship between NMD and iron homeostasis as it affects the iron transcriptome, RNAseq analysis of wildtype and NMD mutant *Saccharomyces cerevisiae* was done. Differential gene expression in the low-iron transcriptional activators *AFT1* and *AFT2* were observed in the wildtype and NMD mutants between iron sufficient and iron deficient conditions. The downstream targets of *AFT1* and *AFT2* include ferric cupric reductases (FREs) responsible for iron uptake, which were also explored and shown to be differentially regulated by NMD in iron sufficient and iron deficient conditions.

GP2 Integrating multi-omics data and advanced machine learning models to characterize antimicrobial resistant genes

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Abstract

Antimicrobial resistance (AMR) is a global health crisis and remains a challenging problem for clinicians and researchers alike. As traditional methods for AMR detection are often time-consuming and costly, we leverage state-of-the-art computational methodology to characterize drug-resistant pathogens. The development of next-generation sequencing technologies, along with the rapidly-evolving field of deep learning has innovated the manner in which researchers may investigate pathogenic data to further elucidate AMR. We employ machine learning models, including deep neural networks to demonstrate their powerful performance in predicting and classifying antibiotic resistance gene susceptibility/resistance to various clinically relevant antibiotics.

GP3 The fate of survivors: the impact of released phage peptides on neighboring host bacteria

Michael Debrah, Jolene Ramsey

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Abstract

Bacteria are a key element of cellular life found everywhere on Earth. Another major component of this community is bacteriophages, which modulate the turnover of these bacteria. Phages infect bacteria, replicate their genetic material inside their host and eventually lyse the host to release their progeny, leaving bacteria debris in the environment. The released progeny continues this cycle by infecting bystander host cells. Bacteria sense debris as a danger signal. Exposure to these cues and changes in the microenvironment influence the composition of host cell surfaces, which sometimes leads to resistance to subsequent phage infections. Our lab recently reported that phiKT phage uses the gp28 protein to disrupt the *E. coli* outer membrane. We hypothesize that gp28 released in the environment after cycles of phiKT infection will sensitize bystander cells making them resistant to subsequent infections. To test this hypothesis, we sought to determine whether the host cells detect gp28 as a stress cue. We used a luciferase gene transcriptionally fused to the promoter of an outer membrane stress response gene, to measure the response to gp28 at different times. Interestingly, the host cells primed with gp28 compared were more susceptible to phage challenge than controls and responded in a concentration-dependent fashion. This data suggests gp28 binds to host cells. To determine host factors responsible for gp28 binding, a complete collection of single gene knock-out host cells is being screened. Understanding how phage peptides affect host cells will inform our models of phage modulating bacterial community composition.

GP4 Envelope stress responses functionally coordinate to maintain cell homeostasis in *Escherichia coli*

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Abstract

The envelope stress responses (ESRs) are essential pathways that maintain cell integrity and protect bacteria from antibiotics. Two critical ESRs that monitor outer membrane integrity in *Escherichia coli* are the Regulator of Capsule Synthesis (Rcs) and the extracytoplasmic σE response. Rcs and σE survey lipopolysaccharide (LPS) and outer membrane protein (OMP) biogenesis and are often induced under the same conditions. My project investigates potential regulatory feedback between them. Using σE-dependent transcriptional fusions, I showed that Rcs stimulates σE activity. Next, I focused on the mechanism underlying this stimulation. σE activity is regulated post-translationally by the anti-σ factor RseA, which is degraded in response to OMP defects, allowing free σE to regulate gene expression. My genetic epistasis analysis demonstrated that not only Rcs acts independently of RseA but can also act synergistically with the RseA degradation pathway to stimulate σE activity. σE - encoding gene, *rpoE*, forms an operon with *rseA*, and their expression is highly regulated. Previous studies implicated RcsB in controlling one of the minor *rpoE* promoters. I generated several transcriptional reporter fusions and showed that when Rcs is strongly activated, it indeed upregulates the expression of the *rpoE-rseA* operon. We hypothesize that by doing so, Rcs increases the amount of the σE protein that is made, while the RseA degradation pathway increases the fraction that is active, enabling a synergistic response. As LPS and OMPs are the major components of the outer membrane, this Rcs/σE regulatory feedback may help coordinate outer membrane homeostasis as a whole.

GP5 Impacts of land development on chemical and physical properties and its effect on microbial diversity

Robert Garcia

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Abstract

The impact of land development on soil microbial diversity and antibiotic-producing properties is a critical area of investigation due to the escalating expansion of industrialization and subsequent disturbances to soil micro diversity. This research aims to investigate the effects that land development has on beneficial bacteria, which play a crucial role in the production of antibiotics. Given the current scarcity of effective antibiotics, understanding these effects is important for developing strategies to preserve and protect these essential microorganisms. An example is Low Impact Development (LID), a strategy implemented by VIDA, a developmental company that is adopting LID strategies to preserve ecosystems within their communities. However, the efficacy of LID in maintaining microbial diversity and the bacteria's antibiotic-producing properties remains uncertain. The central question driving our research is how land development influences microbial diversity and their capacity to produce antibiotics. Soil samples were collected from nine field sites over a year three disturbed, and three Greenway sites within the VIDA development operating under LID guidelines and three undisturbed sites were collected outside of the LID area all sites located on Texas A&M University-San Antonio campus compared and evaluated. Isolated bacteria were screened for antibiotic production, cataloged and preserved for later genomic sequencing. Preliminary findings indicate that undisturbed sites harbor higher diversity of bacteria with antibiotic-producing properties compared to Greenway and disturbed sites. Future research will utilize soil analysis to investigate physical and chemical properties of sample sites to provide an understanding of allopatric populations.

GP6 Impact of simulated microgravity on gene expression in *Acidovorax* sp. 1608163

Eduardo Gutierrez, Hyuk Cho, Madhusudan Choudhary

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Abstract

Previous studies reported that a number of bacterial species under microgravity stress undergo differential gene expression leading to diverse 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 bacterium capable of infecting a variety of crops including corn, rice, oats, and wheat. The hypothesis that “Acidovorax grown under microgravity alter the gene expression patterns”, will be tested by comparing transcriptomic data grown under normal and microgravity conditions. Bacterial samples were collected during the log phase of growth (48 and 72 hours), RNA was extracted and reverse transcribed, and then the DNA was sequenced using Illumina Mi-Seq. Results revealed that growth rates were significantly higher under microgravity conditions compared to the normal gravity conditions. Two different comparisons (normal gravity vs microgravity at the log phase and stationary phase) were made. A total of 846 differentially expressed genes (18.76% of the total genome), 296 (34.98%) and 534 (63.12%) genes were up- and down-regulated, respectively, whereas at the stationary phase, of the total 246 differentially expressed genes, 54 (21.95%) and 192 (78.04%) genes were up- and down-regulated, respectively. Differentially expressed gene analysis indicated that genes coding for several COG, KEGG, and GO functions, such as metabolism, signal-transduction, transcription, translation, chemotaxis, and cell motility are differentially expressed to adapt and survive microgravity.

GP8 Differences in growth, PCA1 and CRS5 mRNA expression in response to cadmium in different *Saccharomyces cerevisiae* genetic backgrounds

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Abstract

Environmental toxins like cadmium influence mRNA-level gene expression, impacting cell viability. The Nonsense-mediated mRNA (NMD) decay pathway, known for degrading abnormal mRNAs containing premature termination codons (PTCs), also regulates natural mRNAs crucial for metal ion homeostasis and detoxification. Our study investigates NMD's control over these genes and their resulting physiological effects. We observed strain-specific and condition-dependent regulation of PCA1 mRNA by NMD. RM11-1a yeast strain has a functional PCA1 allele compared to common laboratory strains like W303a and BY4741. In wild-type BY4741 yeast, PCA1 mRNA levels were unexpectedly higher than in NMD mutants under both normal and cadmium conditions, contrasting with findings in other strains. Deletion of PCA1 led to improved growth in cadmium-exposed yeast, suggesting compensatory mechanisms by other genes. To explore this further, we focused on CRS5, a copper metallothionein potentially involved in cadmium binding. Indeed, CRS5 mRNA levels increased in NMD mutants relative to wild-type strains under cadmium exposure. Additionally, we observed biphasic CRS5 mRNA decay rates by NMD in complete minimal media, indicating complex regulatory dynamics. These findings shed light on the intricate interplay between NMD and metal detoxification genes, highlighting the importance of considering strain variations and environmental conditions in studying cellular responses to toxic metal exposures.

GP9 Deciphering the role of FtsA structural states in *Escherichia coli* divisome activation and assembly

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Abstract

Bacterial cytokinesis, essential for cell proliferation, involves a complex interplay of numerous proteins within a dynamic nanomachine (divisome), which is organized as a ring-like structure at the mid-cell by the tubulin-like FtsZ protein. Actin-like FtsA plays a crucial role during early FtsZ-ring formation by anchoring FtsZ to the inner cell membrane and helping to recruit other essential divisome proteins. Previous studies have shown *in vitro*, FtsA assembles into three distinct oligomeric states: a closed mini-ring that may inhibit premature divisome assembly, an open arc conformation that is hypothesized to initiate divisome assembly, and a double-stranded (DS) antiparallel filament conformation proposed to be necessary for full activation of the divisome and synthesis of septal peptidoglycan. Moreover, genetic variants of FtsA, $FtsA_{R286W}$ and $FtsA_{G50E}$, can bypass one or more of the early stages and mimic the arc and double-stranded oligomeric states, respectively. Although these distinct forms of FtsA have been identified, it remains unproven which distinct conformation is responsible for assembling and activating the divisome. To address this, we used an *in vivo* crosslinking assay for FtsA DS filaments along with a dominant negative mutant $FtsA_{M96E\ R153D}$ to investigate the roles of FtsA oligomeric states in assembly and activation. We show that $FtsA_{M96E\ R153D}$, which cannot form DS filaments *in vivo*, is not functional for cell division but can be rescued by ectopically hyperactivating the divisome. Through these specific FtsA variants, we aim to conclusively determine the role of FtsA's structural states in the divisome's assembly and activation.

GP10 Polymicrobial biofilm formation and influence on corrosion during spaceflight

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Abstract

Biofilms are commonly found throughout nature as surface-adherent polymicrobial communities. These are problematic due to their high tolerance to most antibiotics and disinfectants, and their association with fouling and corrosion. In human spaceflight, one concern is biofilm formation within the water recovery system (WRS) on the International Space Station (ISS). Within the WRS, bacteria-containing liquids, including urine, are treated through physical (filtration and distillation) and chemical (iodine or silver fluoride disinfection) methods to be recycled as potable water. Since incoming liquids are not sterile, biofilm formation in the WRS has resulted in clogging and potential corrosion of key components. To study polymicrobial biofilm formation and potential control by AgF, *Escherichia coli* F11-mCherry and *Pseudomonas aeruginosa* PAO1-gfp were cultured during spaceflight as well as in ground control experiments in an artificial urine medium. In the absence of AgF, biofilm and planktonic bacteria were observed, whereas in the presence of AgF, only biofilm bacteria were detected. Ongoing experimentation includes the use of scanning electron microscopy and X-ray analysis (SEM-EDX) to measure microbial corrosion; transcriptome analysis to assess gene expression; and genomic analysis to evaluate mutations due to spaceflight.

GP11 Functional metagenomics reveals diverse anti-phage defenses from human and environmental microbiomes

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Abstract

Bacteria are under constant attack by an arsenal of bacteriophages (phages), leading to the development of anti-phage defense systems. Simultaneously, phages have evolved counter-defenses, leading to a competitive coevolution between bacteria and phages. Many defense systems have been identified using a ‘guilt-by-association’ approach, which finds new defense genes based on their tendency to co-occur with known defense systems in discrete genomic loci called ‘defense islands’. However, this approach can only find defense systems where others exist. Thus, sequence-independent means of discovering anti-phage defenses should reveal many new defense genes with fundamental impacts on Earth's longest-standing and most abundant arms race. Here, we performed a novel metagenomic functional selection screening in *E. coli* to discover new bacterial defense systems. Specifically, nine metagenomic libraries were selected for resistance against a panel of seven diverse phages. Excitingly, the 63 functional selections yielded 203 metagenomic DNA inserts with putative phage defense activity. Of these 203 inserts, 73 were chosen for individual validation experiments, with 64 exhibiting strong phage defense phenotypes. More than 90% of these 64 validated inserts conferred protection to at least two phages when re-tested against the full panel. These 64 sequences include 24 without any known phage defense gene, indicating that we have identified many novel anti-phage defenses. The discovery of new defense systems holds promise for improving our ability to predict the outcomes of phage infection, uncovering new tools for biotechnology, and revealing the evolutionary origins of some eukaryotic immune factors.

GP12 Elucidating the functional effect of KSHV latent-host protein-protein interactions in endothelial cells

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Abstract

Kaposi's Sarcoma-Associated Herpesvirus (KSHV) is an oncogenic virus that causes Kaposi's Sarcoma (KS). KSHV latent and lytic phases contribute to its pathogenesis. Viral-host protein interactions (PPIs) may remodel the host cell machinery for successful virus replication. However, the molecular mechanisms behind these interactions are still understudied. We hypothesize that KSHV latent-host PPIs are crucial for the regulation of cellular processes that contribute to KSHV infection. Therefore, we aim to determine the functional implications of KSHV latent-host PPIs. In this study, we evaluate the role of latent KSHV-host PPIs in either maintaining latency or supporting viral replication in a doxycycline (DOX)-inducible KSHV reporter cell line (iSLK.219) through qPCR. Previous studies have reported the interaction of the latency-associated nuclear antigen, LANA, with the host death domain-associated protein (DAXX), and vCyclin, another latency-associated KSHV protein, interaction with the host Cyclin-dependent kinases (CDK2 and CDK5). Our preliminary data shows increased mRNA expression levels of early (ORF45) and late (K8.1) lytic genes after DAXX-knockdown in DOX viral-induced iSLK.219 cells. Conversely, CDK2-knockdown caused a decreased expression of lytic genes, while CDK5-knockdown shows no differential expression. Our results suggest that DAXX is necessary for KSHV latency maintenance, while CDK2 is required for lytic reactivation in iSLK.219 cells. Further studies are underway to understand the role of other KSHV latent (LANA, vCyclin, and vFLIP)-host PPIs in iSLK and endothelial cells. Understanding the mechanistic significance of latent KSHV-host PPIs in modulating the host endothelial cell response during infection is critical for the development of potential treatment strategies.

GP13 Elucidating the role of fatty acid binding proteins during lytic KSHV infection

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Abstract

Kaposi's Sarcoma Herpes Virus (KSHV), a double-stranded enveloped DNA virus, is the etiological agent of Kaposi's Sarcoma (KS); an endothelial cell-based tumor, and the most common cancer in HIV-infected individuals. KSHV undergoes both latent and lytic viral phases. KSHV infection of endothelial cells results in morphological, metabolic, lifespan, and gene expression changes to facilitate virion production. In a previous study, the fatty acid binding protein (FABP) genes were observed to be differentially regulated by KSHV in Burkitt Lymphoma cells (BJAB). Fatty acid binding proteins are cytosolic proteins that play a central role as lipid chaperones, trafficking fatty acids to specific cellular compartments. Nevertheless, the role of FABPs in lytic KSHV infected endothelial cells has not been explored. Our preliminary data in iSLK.219 cells, a KSHV reporter cell line and model of latent and lytic infection, show increased FABP2 and FABP4 mRNA expression levels post lytic induction. We hypothesize that the differential expression of FABPs in endothelial cells during lytic KSHV infection leads to altered lipid metabolism. To test this hypothesis, mRNA expression levels of key enzymes involved in TAG synthesis, lipolysis, and □-oxidation will be investigated during lytic replication and post FABP knockdown. We expect FABPs to play a significant role in lipid metabolism in lytic KSHV infected endothelial cells to facilitate maximal virion production and expose metabolic vulnerabilities for future targeting.

GP14 Challenges in predicting zonal trends using a model parametrized with national-level COVID-19 data

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Abstract

Infectious disease models play an important role in public health decision making, but their effectiveness depends on the quality of the data (and the underlying assumptions). During the COVID-19 outbreak, some affected regions had sparse epidemiological data due to various factors. To address this issue, often data from different regions are aggregated and country-wide trends are considered as a proxy for regional trends. This is needed for an expedient intervention, particularly for regions that lack data for modeling and prediction. However, studies assessing these aspects are yet lacking. We, therefore, focused on studying country-wide and zonal COVID-19 trends in India. We first fitted a Susceptible-Exposed-Infected-Recovered-Deceased (SEIRD) model to the COVID-19 data from India and then assessed the predictability of this model across six zones of India. We observed that the country-wide model was not able to predict zonal trends in several instances. To understand the reason for the discordance, we fitted the SEIRD model to the COVID-19 data from each zone and then compared the parameters estimated for each zonal model to those of the national model. Remarkable differences in the values of several model parameters between the national model and zonal model were observed. Based on our findings, we conclude that caution should be exercised in the use of a country-wide model for planning appropriate measures and actions to mitigate the effects of an epidemic in different regions, and where possible, the interventions should be based on region-specific models.

GP15 Comprehensive characterization of *Nakaseomyces glabrata* oral clinical isolates

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Abstract

Nakaseomyces glabrata (formerly *Candida glabrata*) is a fungal pathobiont commonlyfound in the human gastrointestinal (GI) tract. The World Health Organization (WHO) classified *N. glabrata* as a high priority fungal pathogen due to resistance to antifungals and high mortality. *N. glabrata* colonization of the GI tract is poorly characterized. We conducted phenotypic characterization studies of two oral isolates of *N. glabrata* AE2 and D1 from two distinct patients and compared them to two reference strains (BG2 and CBS138). We hypothesized these isolates had adapted to growth in the alimentary tract and these adaptations impact fungal biology and virulence. We characterized growth patterns, biofilm formation, antifungal susceptibility, impact of environmental stressors, virulence in a *Galleria mellonella* model, and GI colonization capabilities and preference in an antibiotic-treated murine model. We show all strains display similar growth patterns in YPD and RPMI. Media-specific differences in antifungal susceptibility were observed for all strains. While all strains showed comparable sensitivities to amphotericin B and fluconazole, AE2 displays decreased susceptibility to caspofungin. AE2 also displayed the highest level of biofilm formation. The strains displayed distinct sensitivities to environmental stressors. AE2 was highly sensitive to SDS and caffeine. Employing the *G. mellonella* model to characterize virulence, AE2 was avirulent, while D1 displayed highest virulence. Colonization capability was assessed in our murine model by measuring CFU/gm in the GI tract. AE2 and D1 showed comparable colonization capabilities in the small intestine and colon. Characterizing clinical isolates will allow us to better understand *N. glabrata* biology and develop novel therapeutics.

GP16 Inhibition of ANO1 suppresses Kaposi sarcoma through inactivating apoptotic signaling pathway

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Abstract

Kaposi's Sarcoma-Associated Herpesvirus (KSHV) is an oncogenic virus that causes Kaposi Sarcoma (KS). KSHV exists in lytic and latent replicative phases and is involved in host cell transcription modulation for disease progression. Viral G Protein-Coupled Receptor (vGPCR), expressed in the lytic stage, promotes cell transformation and angiogenesis to drive KS oncogenesis. Previous studies have focused on apoptosis during KSHV infection, yet the role of vGPCR in apoptosis and cell death is still unknown. Whole-cell transcriptomic analysis on vGPCR overexpressing (OE) HMEC-1 cells revealed changes in many transcripts, including the upregulation of anoctamin 1 (ANO1, Ca-activated Cl channel). ANO1 is overexpressed in several cancer types where it plays a role in tumor growth, invasion, and metastasis. Additionally, published studies show that activation of ANO1 inhibits apoptosis in cancer cells. We thus hypothesize that vGPCR-mediated inhibition of host cell death requires ANO1 upregulation, under apoptotic conditions. Our preliminary data on vGPCR OE and ANO1 siRNA knocked down endothelial cells show an increase in cell death when exposed to apoptotic stimulus. My ongoing research includes ANO1 inhibiting drugs to study apoptosis using fluorescence assays and show that its anticancer effect is mediated through PI3K/AKT apoptotic pathway. Furthermore, we intend to expand our research using the cell model of KSHV infection to study the role of latent and lytic genes involved in the maintenance and reactivation of infection. As such, novel insights into molecular pathways involving vGPCR and ANO1 are needed to develop novel therapeutics aimed at the prevention and treatment of KS.

GP17 Mathematical modeling of co-infection dynamics of influenza and COVID-19

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Abstract

Infectious diseases pose a significant challenge to global health. The co-occurrence of two or more infectious diseases further overburdens the healthcare systems and brings a new set of challenges in addressing the co-infections. While numerous studies have used mathematical concepts to understand the dynamics of individual infectious diseases, co-infection remains understudied. Based upon the established compartmental models, namely, Susceptible-Vaccinated- Infected-Recovered (SVIR) for influenza and Susceptible-Vaccinated-Exposed-(Asymptomatic/Symptomatic) Infected-Hospitalized-Recovered (SVEA/IHR) for COVID-19, we constructed a novel framework (SVIR-SVEA/IHR) to model the co-infection dynamics of the simultaneous occurrence of influenza and COVID-19 in a single population. Unlike prior works focusing primarily on secondary infections, our approach incorporates both- the secondary infections and the co-infections. Using parameters estimated from the US population and epidemiological data, we estimated the effective reproduction number with vaccination as a preventive measure and deduced that nearly 54% of the population should be vaccinated with the influenza vaccine (with vaccine efficacy of 64%) and 74% of the population should be vaccinated with the COVID-19 vaccine (with 95% efficacy) in order to control the influenza and COVD-19 co-infection. These threshold values can be brought further down if additional control measures are incorporated along with vaccinations. Our model, even though focused only on the influenza and COVID-19 co-infection, presents a general framework that is applicable to various co-circulating diseases, including a co-infection involving two strains causing the same disease. Our model thus facilitates further understanding of co-infections and can be leveraged to address co-circulating infectious diseases in a population.

GP18 Deciphering microbial shifts in the gut and lung microbiomes of COVID-19 patients

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Abstract

COVID-19, caused by SARS-CoV-2, results in respiratory and cardiopulmonary infections. There is an urgent need to understand not just the pathogenic mechanisms of this disease but also its impact on the physiology of different organs and the microbiome. Multiple studies have reported the effects of COVID-19 on gastrointestinal microbiota, such as in promoting dysbiosis (imbalances in the microbiome) following the disease progression. Deconstructing the dynamic changes in microbiome composition and their impact on genetic mechanisms and functional pathways at the systems level remains a challenge. Motivated by this problem, we implemented a biomarker discovery pipeline to identify candidate microbes specific to COVID-19. This involved meta-analysis of large-scale COVID-19 metagenomic data to decipher the impact of COVID-19 on the human gut and lung microbiotas. Metagenomic studies of COVID-19 patients revealed differentially abundant microbes and shed light on complex effects of this disease on human gut and lung microbiome's functional components. Several functional targets were identified from a microbial co-occurrence network. Additionally, by utilizing machine learning models (LASSO and XGBoost), we demonstrated that the power of microbial features in discriminating COVID-19 samples from metagenomic samples representing other respiratory diseases. Overall, our study provides insights into microbiome shifts occurring in COVID-19 patients, shining light on the compositional and functional changes.

GP19 Role of ClpX and MsrB on cell envelope antibiotic tolerance of *Bacillus anthracis* Sterne

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Texas Christian University, Fort Worth, USA

Abstract

The prevalence of antimicrobial-resistant bacteria is a rapidly growing public health crisis. This, combined with a decline in the development of novel antimicrobial therapies, makes the search for unique drug targets essential. Previous work from our lab has identified a promising antimicrobial drug target within *Bacillus anthracis*, the regulatory ATPase, ClpX. ClpX is essential for virulence in *B. anthracis* and critical for resistance to a host of cell envelope-targeting antimicrobials. ClpX works with ClpP to form a global protease that regulates a wide range of proteins, including transcriptional regulators. Previously, we conducted a microarray of a Δ*clpX* mutant and found 119 genes with altered expression. One such gene, *msrB*, has been studied for reactive oxygen species tolerance in other pathogens. This gene encodes for methionine sulfoxide reductase, an antioxidant enzyme that restores functionality to oxidized methionine residues. Increased *msrB* expression was seen with oxacillin exposure in *S. aureus,* indicating a potential connection between MsrB and cell wall-targeting antimicrobials. In *B. anthracis* Sterne, loss of *msrB* induces sensitivity to penicillin, but unlike Δ*clpX,* this phenotype is not seen with daptomycin or LL-37. This suggests that the role of *msrB* in antimicrobial tolerance may be limited to cell wall active antibiotics. Further experiments will include testing the Δ*msrB* mutant with additional cell wall-specific antimicrobials (e.g., bacitracin and vancomycin). Our research provides additional information regarding the role of MsrB in the bacterial cell and its potential suitability as a pharmacological target to increase susceptibility to antibiotics.

GP20 Digesting the alphabet soup: combining resources to promote a conceptual understanding of immunology

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Abstract

Introduction

At the Texas Tech University Health Sciences Center School of Medicine (TTUHSC SOM), first year students are taught Immunology during the spring of their first year. A needs assessment administered in spring of 2023 to year one medical students (MS1, Class of 2026) revealed that out of 49 survey responses, 63% reported facing challenges in grasping mechanisms in which innate immunity and adaptive immunity work together, and 51% reported difficulties in comprehending B and T cell development. It is our goal that a combination of supplemental resources enhances student learning and application of these specific topics.

Methods

To promote a conceptual understanding of B and T cell development and the integration of innate immunity and adaptive immunity, three detailed note guides, three fillable concept maps, and two practice online quizzes were provided to a subsequent class of MS1 students (Class of 2027). A student satisfaction survey has been released following the recent conclusion of the Immunology unit.

Results/Conclusions

Results indicate 96% of 47 survey respondents expressing that at least one of the provided resources promoted active learning and a conceptual understanding of specific Immunology topics. Similarly, 96% of respondents expressed that using one or more of the provided resources helped with retention and/or recall while taking a summative assessment. Overall, students report high levels of satisfaction in utilizing the provided supplemental resources, and further results regarding effects of practice quiz utilization on student exam performance is currently being assessed.

Undergraduate Poster Presentations

Antimicrobial Microbiology

UP1 The analysis of *Punica granatum* and *Terminalia chebula* for antimicrobial activity

Dalila Aranday, Patricia J. Baynham

St. Edward's University, Austin, USA

Abstract

In the U.S., over 2.8 million people acquire and 35,000 people die from antimicrobial-resistant (AMR) infections. 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. Extracts were tested for antimicrobial activity by using the Kirby-Bauer Disk Diffusion (KBDD) assay against Escherichia coli lptD4213-, a strain that contains a mutation that causes a permeable lipopolysaccharide layer. The average zone of inhibition (ZOI) for the pomegranate extract was 10.75 mm and for the black myrobalan extract this was 7.75 mm. In order to determine if these extracts could be used to target AMR bacteria, they were tested against Staphylococcus aureus 269, a strain that is resistant to methicillin and vancomycin, using KBDD. In these assays, the average ZOI of black myrobalan was 15 mm and of pomegranate was 20 mm. To quantify the antimicrobial activity, a minimum inhibitory concentration (MIC) assay indicated that pomegranate extract had an MIC of 1000 ug/ml and black myrobalan 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.

UP3 Digging Deeper: Antibiotic resistance genes in aqueous environment

Baruti Prince Oredy, Gabriela Escobar-Verdezoto, Megan Romeo

Dallas College, Dallas, USA

Abstract

This study aimed to elucidate microbial diversity and the occurrence of *tetM* and *tetB* genes in soil samples obtained from distinct geographical sites. The primary focus was to compare the prevalence of resistance genes within clayey soil adjacent to an aqueous environment (White Rock Lake) with three other locations (Irving, Kirbyville, Valley Creek) characterized by diverse ecosystems. This strategic approach sought to investigate potential correlations between environmental moisture levels and microbial dynamics, considering the influence of water content [1] and clay-rich properties [2] on microbial community structures and interactions. Hence, the confirmation of *tetB* or *tetM* genes in clayey soils aimed to support the relationship between water retention, drainage patterns, and nutrient availability with the presence of resistance genes in the soil. Furthermore, the study expanded to the isolation of some bacteria from White Rock soil sample to assess their resistance to common antibiotics and attempting to isolate the resistant bacteria. This project aimed to offer valuable insights into whether specific area properties and geographical locations, concerning water content and soil composition, might indeed increase the chance of detecting tetracycline and antibiotic resistance genes in the soil of the investigated region.

UP4 Hurt dirt, antibiotic resistances

Angel Quinones, Brandon Esterhuizen, Megan Romeo

Dallas College, Dallas, USA

Abstract

Microbes are a major part of all ecosystems. Soil is a large ecosystem for microbes, but because there is a wide range of types of soils from different areas common microbes are exposed to different chemicals. This factor influences the rise of specific resistances to microbe killing chemicals known as antibiotics. Antibiotics are created organically by other microbes, that kill or inhibit the growth of certain microbes. But due to the exposure of microbes to wide range of chemicals found in the soil samples resistances have developed through mutation and horizontal gene transfer. Gene transfer can happen through transformation (the picking up of DNA from the environment), transduction (the absorption of DNA through infection), and conjugation (uses extracellular to exchange DNA). This research investigates these resistances of common microbes gathered from different locations in Texas. Through the collection, dilution/separation of microbes from soil samples, and isolation of selected microbes and their colony growth, then finally exposing similar selected microbes to the same chosen antibiotics and recording the differences in antibiotic resistances.

UP5 Inhibitory effects of oxymel combined with ampicillin on the growth of *Aeromonas hydrophila*

Tris Flores, Victoria Haynes, Payton Reinke, Selasi Sarfo, Jennifer Huddleston

Abilene Christian University, Abilene, USA

Abstract

This study explores the inhibitory effects of oxymel, a traditional blend of honey and vinegar, in conjunction with the antibiotic, ampicillin, against Aeromonas hydrophila. A. hydrophila, a gram-negative pathogen, poses significant health risks, bringing the need for innovative therapeutic approaches. Through a series of in vitro experiments, we assess the combined impact of oxymel and ampicillin on the growth and viability of A. hydrophila. Minimum Inhibitory Concentration (MIC) assays revealed that 6% or more oxymel effectively inhibited bacterial growth, while ampicillin alone demonstrated resistance. The measured pH decreased with higher oxymel concentrations. Combining oxymel and ampicillin resulted in growth inhibition, confirmed by spectroscopy, introducing a potential synergistic therapeutic strategy against A. hydrophila. This research contributes valuable insights into the potential therapeutic effects of oxymel in combination with ampicillin against A. hydrophila infections. By shedding light on these interactions, our study aims to advance the understanding of novel therapeutic interventions, exploring the promising avenue of combining traditional remedies with conventional antibiotics to address challenges associated with antibiotic resistance in bacterial infections.

UP7 Determining the antimicrobial effects of *Eysenhardtia polystachya* and *Pinus maritima* extracts to combat antimicrobial resistance

Nicholas Purcell, Nichole Abrego, Patricia Baynham

St. Edward's University, Austin, USA

Abstract

Over 2.8 million people in the United States acquired antimicrobial-resistant (AMR) infections, with over 35,000 deaths attributed to AMR infections in 2019. The global rise in AMR infections and lack of effective treatments is an imminent threat and must be addressed. The overuse of antibiotics is one of the leading causes of AMR. *Eysenhardtia polystachya* (Palo Azul) and *Pinus maritima* (Pine Bark) were investigated for antimicrobial activity. Palo Azul and Pine bark extracts were made by extracting powdered bark with water, filtering, condensing, and resuspending in deionized water to a concentration of 100 mg/mL. They were then evaluated for antimicrobial activity using Kirby-Bauer disk diffusion assay against *Escherichia coli lptd4213*-, which contains a mutation that results in increased permeability. Palo Azul and Pine Bark had average ZOIs of 7mm and 10mm, respectively. To quantify antimicrobial activity, a minimum inhibitory concentration (MIC) assay was performed and indicated MICs of 1,280 µg/mL for Palo Azul and 640 µg/mL for Pine Bark. Bacterial cytological profiling (BCP) was then conducted to identify the mechanism of action (MOA) of the extracts. Cells treated with Palo Azul extract appeared elongated, while cells treated with Pine Bark extract appeared to show both elongation and DNA condensation when compared to untreated cells. By identifying and characterizing antibacterial properties of plants, this will open avenues of development of new therapies for AMR infections. Personal and global health depend on the identification and development of novel treatments to combat the rise in resistant bacteria.

UP8 Evaluation of essential oils for the anti-protozoal activity against trichomonad pathogens

Rima Shaaban, Vishal Mundodi, Robert Corbett, Ashwini Kucknoor

Lamar University, Beaumont, USA

Abstract

Due to the rise in metronidazole-resistant trichomonads, there is a need to search for an effective treatment option for trichomoniasis, a prevalent sexually transmitted disease in humans and a cause of reproductive issues in cattle. In this study, various essential oils (EO's) were evaluated for their antimicrobial potential against the human pathogen: *Trichomonas vaginalis* (TV) and the cattle pathogen: *Tritrichomonas foetus* (TF). Concurrently, the oils were assessed for their impact on HeLa cells to ensure cellular safety of the mammalian host. Following a screening process, five essential oils exhibiting antimicrobial activity against TV and TF while being non-toxic to HeLa cells were identified. Subsequently, the selected oils were subjected to testing across three distinct concentrations: 0.5%, 1%, and 1.5%. Among the oils tested, rose essential oil emerged as the most efficacious in eradicating the protozoan cells, notably at the lowest concentration. This efficacy can be attributed to the presence of terpenes, prominent constituents known for their antimicrobial properties. These findings underscore the potential of rose essential oil as a promising candidate for the topical treatment of trichomoniasis. Their selective action against pathogenic protozoa, coupled with the preservation of HeLa cell integrity, highlights their suitability for further exploration as alternative therapeutic agents against trichomoniasis.

UP9 Antibiotic resistance reversion in *E. coli*

Luis Tamez, James Masuoka

Midwestern State University, Wichita Falls, USA

Abstract

A proposed approach to addressing antibiotic resistance is to alternate drugs. Previous studies suggested that long-term exposure of a tetracycline-susceptible, erythromycin-resistant strain of *E.coli* to subinhibitory concentrations (0.5x minimum inhibitory concentration, MIC) of tetracycline increased susceptibility of the strain to erythromycin. The aim of this project was to answer two questions: 1) was the originally observed effect due to long-term culturing alone? 2) would the effect be seen sooner if a higher concentration of drug was used? The tetracycline exposure experiment (0.5x MIC) was repeated in parallel with cultures with no added tetracycline or tetracycline added at 0.9x MIC. Samples were taken every 24 hours, and tested for tetracycline and erythromycin susceptibility. We saw no change in susceptibility to tetracycline. Overall, there was an apparent upward trend in erythromycin susceptibility. The observed change at day 10 was comparable to previous results, but inhibition zone diameter fluctuated throughout the culture period. There wasn't a consistent, constant increase in inhibition over time. Furthermore, after day 10, the susceptibility to erythromycin seemed to return to baseline. Subsequent replica plating of selected timepoints suggested that all cells were equally affected by culture conditions. Current efforts are trying to determine the differences between the isolates with increased erythromycin susceptibility and the original strain. The results of these studies will help us further understand mechanisms by which treatment with one antibiotic affects resistance to another.

UP10 Testing and improving antibiotic resistance in bacteria found in environmental soil samples

Jesus Torres, Domenica Gaibor Verdezoto, Megan Romeo

Dallas College, Dallas, USA

Abstract

Irving Towne Lake is a popular park that attracts a variety of humans, animals, and bacteria; the area presents a unique opportunity to assess bacteria and their response to antibiotics. Although the bacteria is sensitive to the antibiotic used, not all bacteria respond in the same manner; natural resistance can occur in nature. In the lab, bacteria that are normally sensitive to antibiotics can be challenged against this antibiotic, and over time, a more resistant strain can appear. This study manipulates variables such as antibiotic concentration for a specific strain of bacteria and analyses the effect on its antibiotic sensitivity. Methods like Kirby-Bauer and minimum inhibitory concentrations were used to assess developing antibiotic resistance of the sample. Resistance mechanism to counteract antibiotics does not happen as quickly as popular belief suggests, lending credence to the hypothesis that varying amounts of antibiotic exposure can impact the rate of bacterial resistance development.

UP11 Exploring phenotypic growth characteristics of environmental nontuberculous mycobacteria under climate change scenarios

Layla Behrens[1], Kade Brackin[1], Rachel Wilsey[2], Jennifer Honda[2], Todd Primm[1]

[1]Sam Houston State University, Huntsville, USA. [2]UT Tyler Health Science Center, Tyler, USA

Abstract

There are over 90 species in the genus *Mycobacterium*, with the most infamous being the obligate human pathogens, *M. tuberculosis* and *M. leprae*. However, most of the species are environmental in soil and water, and opportunistic pathogens in humans and other animals. These are often called NTM, nontuberculous mycobacteria, which are responsible for increasing rates of respiratory infections in the population. Humans have constant exposure to NTM, which vary widely in virulence, and the exact nature and factors that underly the virulence are poorly understood. These infections in humans tend to be under-diagnosed yet difficult-to-treat. In addition, we hypothesize that environmental changes associated with warming and more humid climates select for more pathogenic NTM strains. A panel of ten NTM strains is being examined for phenotypic characteristics that may correlate with virulence, including growth and colony morphology under increasing heat and acidic conditions. The goal is to discover characteristics that are exhibited or amplified in more pathogenic strains as the basis to begin to determine mechanisms of virulence.

UP12 The role of the nonsense-mediated mRNA secay pathway in *S. cerevisiae* response to low iron conditions

Jana Chao, Jacqueline Carroll, Bessie Kebaara

Baylor University, Waco, USA

Abstract

The Nonsense-Mediated mRNA Decay (NMD) pathway, a highly conserved pathway in eukaryotic organisms, degrades mRNAs with premature termination codons and regulates the expression of certain fully functional natural mRNAs. The NMD pathway has been shown to regulate mRNAs involved in iron homeostasis. Iron homeostasis is critical to all eukaryotes because while iron is an essential micronutrient involved in crucial metabolic pathways, high levels of iron are toxic to the cell. Iron deficiency is a physiologically significant issue that has pathological implications in humans. When Saccharomyces cerevisiae experiences iron deficiency, transcription factors Aft1 and Aft2, activate the iron regulon, a group of genes involved in the cell's response to low iron conditions. Previous studies have demonstrated NMD regulation of mRNAs in the iron regulon such as the ferric cupric reductase, FRE2. Our research focus is to investigate whether the NMD pathway regulates the ARN family of genes in low iron conditions in S. cerevisiae. The ARN family (ARN1-4) is a group of iron-xenosiderophore-specific transporters belonging to the yeast iron regulon in the non-reductive iron import machinery. Northern Blotting was conducted to evaluate mRNA expression in low iron conditions using a wild type and NMD mutant S. cerevisiae. We found that ARN1 gene expression was NMD-dependent and induced by low iron conditions. ARN1's base level expression appears to be regulated by the NMD pathway.

UP13 Ribozyme-mediated gene-fragment complementation for non-destructive reporting of DNA transfer within soil

Andy Corliss, Malyn Selinidis, James Chappell, Jonathan Silberg

Rice University, Houston, TX, USA

Abstract

Bacteria can share genes through a variety of methods, collectively known as horizontal gene transfer (HGT). This process impacts bacterial evolution and metabolism and can increase the spread of antibiotic resistance genes. One of the principal environments where HGT occurs is soil, but in situ soil studies have been hindered by a lack of suitable tools. To address the challenges of soil environments, we have developed a robust, real-time reporting tool based on the enzyme methyl halide transferase (MHT). MHT produces methyl bromide gas, which can escape soil and be detected as a signal. To achieve the tight control necessary to monitor an event as rare as HGT, the MHT transcript is split into two RNA halves. One half is produced by the recipient strain, and the other half is coded on a conjugation plasmid in the donor strain. When the recipient strain and the donor strain are present in the same environment, the donor will give its plasmid to the recipient (an HGT event), and the two halves of the system will combine via a self-splicing ribozyme mechanism to produce MHT, and in turn, methyl bromide gas. We have implemented this tool in E. coli in real soil matrices, exploring the optimizations needed to apply synthetic biology tools to realistic environments. Preliminary results with P. putida suggest that this tool can also monitor interspecies HGT. Moving forward, we plan to use our tool to investigate how different soil properties affect HGT rates.

UP14 Microbial communities associated with microplastics and detritus in Galveston Bay

Drew Eisenbach[1], Joel Flores[1], Allison Forrest[2], Andrew Patterson[2], Sasha Stephenson[2], Alicia Rios[2], Michael LaMontagne[1]

[1]University of Houston - Clear Lake, Houston, USA. [2]Clear Creek High School, League City, USA

Abstract

Microplastics (μPs) are a contaminant of concern along coastlines of Texas. These pollution particles adversely effect the health of marine animals and biofilms on μPs could facilitate the transfer of antibiotic-resistance genes and lead to the emergence of virulent strains of bacteria. Detritus is also dispersed along Texas coastlines, but few studies have compared the microbial communities between these two types of debris. To address this, we collected μPs and detritus from two beaches along Galveston Bay. Bacteria were isolated from these particles on marine agar and R2A and identified with a matrix-assisted laser desorption – time of flight mass spectrometry (MALDI-TOF MS) system. Cluster analysis of mass spectra generated from 66 isolates (37 from μPs and 29 from detritus), identified 38 MALDI-TOF taxonomic units (MTUs). Many MTUs were common to μPs and detritus; however, the library generated from detritus included strains of *Exiguobacterium* species and *Priestia megaterium*. These opportunistic pathogens were not observed on μPs. The library generated from μPs did not contain any MTUs that were identifiable with the MALDI-TOF MS system and included MTUs primarily associated with μPs. This suggests that detritus and μPs could both support potentially virulent bacterial strains.

UP15 Inhibitory effects of phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN) on quorum sensing in *C. violaceum*

Sarah Hudson, Brianna Garrett, Jennifer Hennigan

Abilene Christian University, Abilene, USA

Abstract

Quorum sensing (QS) systems allow bacteria to modulate gene expression in response to chemical signals, such as N-acylhomoserine lactones (AHLs), that accumulate in dense bacterial populations. Common pathways that are under QS control include biofilm formation, bioluminescence, and toxin and pigmentation production. As with traditional antibiotics, naturally competing species of bacteria may be a good source of novel quorum sensing inhibitors (QSIs). QSIs can interrupt communication in pathogenic bacteria. Our previous work isolated bacterial strains that secrete QSIs from Sorcerer's Cave in Texas. Of these isolates, several were identified through 16S PCR as subspecies of Pseudomonas chlororaphis, which are known to secrete various phenazines, such as phenazine-1-carboxylic acid (PCA) and phenazine-1-carboxamide (PCN). We hypothesize that purified PCA and PCN will disrupt QS in C. violaceum. We tested the impact of the cave isolates on violacein production using a C. violaceum mutant (CV026) deficient in AHL synthesis and supplemented with exogenous N-hexanoyl-L-homoserine lactone (C6-HSL) and N-octanoyl-L-homoserine lactone (C8-HSL). Loss of pigmentation was evident around isolates. In a soft agar assay, we also plated CV026 supplemented exogenous C6- and C8-HSLs and added purified PCA and PCN to wells. Loss of pigmentation was also observed around the wells. Our results suggest that PCA and PCN lead to inhibition of QS pathways mediated by C6 and C8-HSLs.

UP16 Characterization of mitochondrial ferric-cupric reductase, *FRE5*, as regulated by the nonsense-mediated mRNA decay pathway

Elizabeth John, Jacqueline Carroll, Bessie Kebaara

Baylor University, Waco, USA

Abstract

The nonsense-mediated mRNA decay (NMD) pathway is a conserved pathway in eukaryotes known to degrade mRNAs with premature termination codons, as well as natural mRNAs. Natural mRNAs regulated by NMD have unique features such as atypically long 3' untranslated regions (3'UTR). NMD is also known to be affected by environmental stimuli, such as varying concentrations of extracellular iron. Iron homeostasis is paramount to energy production and oxygen transport in mammals and is relatively underexplored in relation to the NMD pathway. *Saccharomyces cerevisiae* bears extensive gene homology to humans as well as similar NMD mechanisms and iron homeostatic processes. In *S. cerevisiae*, the genes involved in maintaining iron homeostasis are referred to as the Iron Regulon. In iron-deficient conditions, transcription factors *AFT1* and *AFT2* activate the Iron Regulon, which includes *FRE5*, a ferric-cupric reductase located in the mitochondrial membrane. Prior research showed that NMD regulates a related cellular membrane homolog, *FRE2*. Due to the connection between mitochondrial and cytosolic iron homeostasis, it is hypothesized that *FRE5* also experiences NMD-mediated regulation. Preliminary data has since been collected indicating that *FRE5* is sensitive to NMD degradation and is predicted to have an atypically long 3'UTR. Further clarification of the relationship between NMD and iron homeostasis within S. cerevisiae will improve our understanding in humans and elucidate potential therapies for iron-related diseases, such as iron-deficiency anemia, Friedrich's ataxia, myopathies, and respiratory complex deficiencies.

UP17 Exploring the impact of varying temperatures on bacterial growth

Desiree Molinar

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Abstract

Microbial growth is greatly affected by temperature. Each bacterial species exhibits diverse survival mechanisms in response to temperature changes. The optimal growth of most microbes occurs within a specific temperature range determined by the functionality of proteins within their cells. Typically, microbial growth is slower at lower temperatures. However, bacteria that can survive drastic conditions such as cold temperatures are better suited to their environment. This research explores competitive coexistence under low temperatures among eight common BS-L1 bacteria found in the environment. In this study, eight bacteria (Escherichia Coli, Staphylococcus epidermidis, Bacillus subtilis, Micrococcus luteus, Serratia marscences, Enterobacter aerogenes, Citrobacter freundii, and Enterococcus faecalis) were pitted against each other using the NCCA March Madness-bracket style elimination process. The ultimate victor in this bacterial competition is Enterobacter aerogenes (E.a.), due to its rapid growth rate, ability to assimilate various carbon sources, low nutritional requirements, and the presence of a capsid. Furthermore, E.a. can transition between different metabolic pathways, allowing it to outcompete bacteria with limited metabolic capabilities. E.a. is also able to form biofilms that protect against harsh conditions, like UV radiation, extreme temperatures, and antibiotics. In conclusion, Enterobacter aerogenes employs a suite of highly effective survival strategies that contribute to its exceptional ability to outgrow other bacteria in competitive environments.

UP18 Estimating the limit of detection of a handheld bacterial imaging device *in vitro*

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Abstract

Chronic wounds are injuries which fail to progress through the normal stages of repair within a typical timeframe, and are a common cause of morbidity and mortality in a significant patient population. To detect bacterial bioburden within the wound bed, a handheld bacterial imaging device has been developed that can detect the autofluorescence properties of most clinically relevant species of bacteria in real time. While the limit of detection of the device has already been estimated *in vivo*, this study seeks to determine the limit of detection *in vitro* independent of background signal from the wound bed. To do this, we conducted a series of time course experiments to determine the inflection point where bacterial colonies move from signal negative to positive via visible fluorescence, and then determined the colony forming units before and after signal conversion to estimate the minimum bacterial concentration necessary for detection with the handheld bacterial imaging device. These findings are useful to understand the environmental conditions required for bacterial detection with the device, and can inform use and results interpretation when the device is used in the clinical setting.

UP19 Challenges in genomic DNA extraction from environmental *E. coli* isolates

Zaniya Medlin, James Masuoka

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Abstract

Antimicrobial resistance is a major global public health threat. Genetic transfer between cells can spread antimicrobial resistance and other virulence factors, resulting in difficult-to-treat multidrug-resistant strains. Migratory birds can act as bacteria carriers, introducing new strains into their seasonal habitats. We hypothesized that gulls arriving in Wichita Falls each winter play such a role and that the virulence factors present would change each time the gulls arrive. Fecal samples were collected from resident Canada geese (Branta canadensis) for a year, and bacterial isolates, presumptively identified as Escherichia coli, were selected. The presence of virulence factors was screened by multiplex PCR. While initial results suggested that virulence factors were present in some isolates, absence of an expected amplicon for the uidA gene suggested that these bands may have been artifacts. We tested each parameter separately using a single primer set (16S rDNA). Extracted DNA served as a control for PCR template. A Staphylococcus aureus strain was used to determine if problems were constrained to gram-negative organisms. Results suggested that the cell lysis procedure worked well with S. aureus but wasn't releasing DNA from E. coli. Once we can consistently get amplification of the 16S rRNA gene, we will use these preparations for multiplex PCR. The pattern of gene sequences present will allow us to group the isolates by pathotype and to determine pattern changes over time. Understanding how genes associated with virulence are introduced to our local environment will help inform decisions related to public health and antibiotic stewardship.

UP20 Protocol development for identification of *Staphylococcus aureus* from wastewater

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Abstract

Wastewater can be a source of human-associated microbes which can tell us about the health of a community. This study aims to develop a protocol to isolate MRSA (methicillin-resistant Staphylococcus aureus) from wastewater samples and to identify the types of MRSA found. We receive weekly wastewater samples from different treatment plants around the greater San Antonio region and we are using them to design our protocol which is in the early stages of development. We begin by preparing Baird Parker Broth and mixing it with our wastewater sample. The broth and wastewater mixture is incubated in a shaker overnight and then streaked onto Baird Parker agar, which is selective for Staphylococcus, and incubated overnight. Of the colonies that form, eight colonies are selected and patched onto a mannitol salt agar (MSA) plate. After overnight incubation, we identify all the colonies that grow and ferment (presumptive *S. aureus*) on the MSA plates. Our next step is to make glycerol stocks and prepare simple boil preps for PCR and eventual sequencing. We are currently optimizing our PCR methods to identify not only the type of Staphylococcus species present but also to determine the strain of MRSA present. So far we have isolated 62 fermenting colonies and in the coming weeks, we will send our samples for sequencing. Our protocol will be developed further over the remainder of the Spring semester.

UP21 Determining the penetrating power of a handheld bacterial imaging device

Jacob Seidl, Eleanna Carris, Maya Panday, Laura Jones, Allie Smith

Texas Tech University, Texas, USA

Abstract

Chronic wound infections are a significant cause of morbidity and mortality in a significant patient population annually and are a major clinical concern. A handheld bacterial imaging device has been developed to aid in the diagnosis of wound infections in real-time by detecting the auto fluorescent properties of most clinically relevant bacteria. While there have been numerous efforts describing the ability of the device to detect bacterial bioburden, this project seeks to understand the penetrating power of the device. To do this, we used a series of culture media and varying depths to understand how the thickness of media affects the ability of the device to detect bacterial fluorescence and quantitatively assess the penetrating power of the device. This work contributes to understanding the capabilities of the handheld bacterial imaging device and can inform use and results interpretation when the device is used in the clinical setting.

UP22 *Pseudomonas aeruginosa* and hydrocarbon degradation in jet fuel

Angelina Viola, Mary Aumen, Carolina Soto, William Cody

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Abstract

Jet fuel spills threaten the well-being of the environment and the health of anyone who comes into contact with it due to the presence of hydrocarbons, which accumulate within the cells of living organisms, leading to dysregulation and death. It is estimated that 50 tons of jet fuel is spilled at airports and military bases across the globe each year. Currently, there is no effective way to degrade hydrocarbons contaminating the environment after one of these spills. Here, we demonstrate that *Pseudomonas aeruginosa* is commonly found in soil adjacent to runways, and investigate its potential use in biostimulation, the use of microorganisms already present in the environment to neutralize contamination. *P. aeruginosa* strain 33988 degrades hydrocarbons using Alkb1 and Alkb2, monooxygenases that catalyze the insertion of oxygen into the hydrocarbon chain, but the regulation of this mechanism is unknown. In an effort to elucidate the regulatory pathway, in this study we investigate genes previously linked to hydrocarbon degradation by shotgun RNA hybridization screen. Insertional inactivation mutants were created by the electroporation of genomic fragments from *P. aeruginosa* strain PAO1 transposon mutants into strain 33988, isolating recombinants on selective media, and verification by gel electrophoresis. The resulting insertional inactivation mutants were analyzed by growth curve. This study represents the first steps in understanding the regulation of hydrocarbon degradation in *P. aeruginosa,* which is necessary for its use as an effective bioremediation agent.

UP23 Pathogenesis of pulmonary injury in *Streptococcus pneumoniae* infection and inflammation: role of pneumolysin

Dami Aiyejuto[1], Joshua Tadegegn[1], Sofia Tamayo[1], Andrey Komissarov[2], Ali Azghani[1]

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Abstract

Streptococcus pneumoniae is an opportunistic pathogen known to cause severe pulmonary infections and empyema in individuals with underlying diseases. S. pneumoniae's multifaceted approach for pathogenesis includes adherence to mucosal surfaces, evasion of the host immune system, and production of several virulence factors including Pneumolysin (PLY), a pore-forming protein released by autolysis. We sought to investigate the hypothesis that the PLY in physiologic concentrations disrupts the integrity of the lung's epithelial barrier function. We cloned the PLY gene and used non-cytotoxic concentrations of purified PLY, determined by LDH assay. Confluent monolayers of human airway Calu-3 cell line cultured on porous membrane inserts were used to study the role of PLY in epithelial paracellular permeability and inflammatory responses. We used Transepithelial electrical resistance (TEER) to assess the integrity of the epithelial junctional complexes. The TEER data showed a significant drop in resistance after treating the cells with various concentrations of rPLY (5,15, and 30 µg/mL) for a period of up to 50 minutes for the lowest dose. Images from fluorescence microscopy studies indicated subtle changes in the intensity and localization of tight junction proteins ZO-1 and Occludin. In conclusion, S. pneumoniae disrupts the integrity of the epithelial barrier in a Calu-3 cell culture model. Our findings aim to contribute to understanding the mechanisms underlying lung injury during pneumococcal infections, potentially informing the development of preventative and therapeutic strategies.

UP24 Estimating the bacterial bioburden of chronic wound infections using a handheld bacterial imaging device

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Abstract

Chronic wound infections often lead to high rates of morbidity and mortality, and are a common clinical concern. A handheld bacterial imaging device was developed that is able to detect most clinically relevant species of bacteria within the wound bed in real time. The device is able to detect the autofluorescent properties of bacteria, allowing them to be visualized as red fluorescent with the device. We seek to investigate the inflection point where bacterial bioburden converts from signal negative to positive via visible fluorescence, and investigate the impact of device settings on detection of the bacteria. To test this, a series of time course experiments were conducted using the common wound pathogen Staphylococcus aureus. The overall goal of this project is to develop a system that ‘grades’ wounds based on their potential bacterial bioburden according to estimates made using the handheld bacterial imaging device. This could be used as an alternative to time-consuming culture-dependent methods to detecting bacterial infection in a wound bed, allowing physicians to treat chronic wounds in real-time and therefore improve patient outcomes.

UP25 The identification of novel genes related to iron acquisition in *Bacillus Anthracis* Sterne

Jessica Guilhas, Kyle Gallegos, Julio Manceras, Mariah Green, Jacob Malmquist, Shauna McGillivray

Texas Christian University, Fort Worth, USA

Abstract

Bacillus anthracis, the causative agent of anthrax, is a spore-forming, gram-positive bacterium. Its virulence mechanisms are of interest due to its potential use as a biological weapon and high lethality. For *B. anthracis* to survive and reproduce in a host, it must evade the host's immune response and acquire nutrients. One important nutrient *B. anthracis* must acquire is iron. Iron is a limiting nutrient in the host because it is usually found sequestered to hemoglobin or bound to host proteins such as transferrin. To acquire iron, pathogens must strip it from the host proteins. To find genes important for iron acquisition from hemoglobin, we screened genetic mutants created through transposon mutagenesis. Media was chelated to remove all divalent cations, including iron, and then hemoglobin was added as the sole iron source. The mutants that were unable to grow were chosen to be tested in a larger volume hemoglobin assay. We confirmed the phenotype of several mutants using this larger volume assay and we are working to confirm the site of transposon disruption via PCR. The mutants thus identified include a mutation in a dUTPase gene and an L-aspartate oxidase gene, neither of which has been previously linked to iron acquisition from hemoglobin. Future directions include making independent mutations and/or complement the disrupted genes to confirm the gene disruption is linked to loss of iron acquisition from hemoglobin. This study allows for a further understanding of how *B. anthracis* acquires iron and sheds new light on potentially novel virulence mechanisms.

UP26 Effects of triglycerides on the mouse gut microbiome

Madison Wolfrom[1], Samirah Jackson[2], Carol Kumamoto[3], Jesus Romo[4], Todd Primm[1]

[1]Sam Houston State University, Huntsville, USA. [2]Houston Christian University, Houston, USA. [3]Tufts University, Boston, USA. [4]UT San Antonio, San Antonio, USA

Abstract

Clostridioides difficile is a serious gut infection in humans, often brought on by antibiotic treatment. Previous work (Romo, 2020) showed that a microbiome protective against a *C. diff* infection has higher levels of certain lipids as well. Mice fed olive oil as a lipid source had enhanced survival vs *C. diff* compared to controls. In this study, groups of C57BL/6 mice were dosed with PBS control or triglycerides. The gut microbiome composition was examined using 16S gene fragment sequencing. Triglyceride diet increased the relative abundance of *Peptostreptococcaceae* and decreased that of *Akkermansia*. The dominant genus in all groups was *Bacteroides*. How these composition changes related to protection against *C. diff* remains to be determined.

UP27 Investigating the role of KSHV viral G-Protein Coupled receptor in innate immunity

George Jose, Dr. Erica Sanchez

University of Texas at Dallas, Richardson, USA

Abstract

Kaposi's Sarcoma Herpes Virus (KSHV), an enveloped double-stranded DNA virus, is the leading cause of cancer in HIV/AIDS patients. KSHV is a temperate virus with two phases, latent and lytic. Viral G-protein coupled receptor (vGPCR, ORF-74) is a prominent oncogene in KSHV infection and is exclusively expressed during the lytic phase of the viral life cycle. KSHV-infected cells expressing vGPCR show activation of the complement system, a major component of innate immunity and a first line of immune defense. However, the specific role vGPCR plays in innate immunity through activation of the complement system and other host immune responses, including Interferon Stimulating Genes (ISGs) responses, is still to be explored. I hypothesize that cells expressing vGPCR activate the complement system by C5B9 deposition through autocrine signaling as well as on the bystander cells through paracrine signaling. Our preliminary data shows the visible C5B9 deposition on HeLa cells that stably express vGPCR. We also visualized C5B9 deposition on HeLa wild-type cells that have been treated with vGPCR-expressing HeLa cell media. Additionally, HeLa cells transfected with vGPCR showed an increase in certain ISGs. Current work is focused on understanding why specific ISGs are upregulated in response to vGPCR expression and their mechanism of action. In the future, we will work to uncover key vGPCR-regulated host factor(s) along with their mechanism of action. Understanding the fundamentals and key player(s) of the innate immune response to vGPCR-mediated signaling may help further our knowledge of KSHV pathogenesis.

UP28 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 10^6 to 10^7 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.

UP29 A Survey of awareness of antimicrobial resistance among the university community on Lamar campus

Sergio Mendez, Helena Bautista, Estrella Balderas, Jordan Nelson, Ashwini Kucknoor

Lamar University, Beaumont, USA

Abstract

Antimicrobial resistance (AMR) is a hidden problem that affects individuals of all groups. Our study focused on understanding the student body's knowledge of AMR on campus and to increase awareness of this problem. A survey was conducted amongst the student body and faculty at Lamar University with 100 participants in total. The survey used was adapted from the World Health Organization's "Antibiotic Resistance: Multi-Country Public Awareness" . The modified survey consisted of five sections. The first section was over demographic information which included gender, age, where you live, and level of higher education. The results were analyzed in tables and charts, and conclusions were drawn. Most responses were neutral, when asked whether antibiotic resistance was a leading problem in the world. Many participants had a neutral response on important topics regarding AMR with 49% of participants (n=46) believing medical experts will solve antibiotic resistance. Additionally, 69% (n=65) of those surveyed believe that the flu can be treated with antibiotics. Although most of the data reflects neutrality, the surprising number of students who misuse antibiotics prescribed both off the counter and by a physician has nearly doubled in comparing it to other categories expressed throughout the survey. The results varied due to the unfamiliarity of AMR amongst the participants on campus, but the questionnaire aided in the recognition that improvements should be made for campus-wide efforts to increase knowledge of antimicrobial resistance among individuals. This emphasizes the need for action and antimicrobial stewardship from all parties involved to combat this grave problem.

UP30 Screening for novel virulence factors using H_2O_2 in *Bacillus anthracis* Sterne

Abi Plylar, Luke Hamilton, Shauna McGillivray

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Abstract

Bacillus anthracis is a gram-positive, spore-forming bacterial pathogen and the causative agent of the deadly disease, anthrax. This pathogen produces a lethal infection due to the potency of its virulence factors in inflicting harm upon and defending against their host. While anthrax toxin and capsule encoded in the *B. anthracis* plasmids are well-studied, there is minimal research into the over 5,000 chromosomal genes. To identify potential chromosomal virulence factors, a *B. anthracis* Sterne strain transposon mutant library containing thousands of randomly disrupted genomes was created and previously used to successfully screen for loss of virulence-associated phenotypes. In our current screen, we examined attenuation of mutants exposed to oxidative stress in the form of H_2O_2. ROS are released by innate immune response cells and destroy invading pathogens lacking adequate defense mechanisms. While there are some known antioxidant-encoding genes in *B. anthracis*, like the catalase gene, we predict there are others that may influence the bacteria's susceptibility to ROS. To search for additional genes, we screened over 1,300 transposon mutants using H_2O_2 and selected mutants with growth attenuation compared to wild-type *B. anthracis* Sterne. Mutants with increased H_2O_2 susceptibility were further tested to confirm *in-vitro* phenotypes. Ultimately, we want to screen selected mutants in the *G. mellonella* invertebrate infection models to prioritize mutants with both *in-vitro* and *in-vivo* phenotypes. Our goal is to discover novel virulence factors while also developing validated methods and procedures to study *B. anthracis* pathogenesis.

UP31 Coevolution of *Pseudomonas aeruginosa* and *Drosophila melanogaster* in a chronic infection model and the Red Queen hypothesis

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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 seventh 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.

UP32 Resistance profiles of multi-drug resistant Shiga toxin-producing *Escherichia coli* (MDR-STEC) of diverse serotypes

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Abstract

Background: The production of phage-borne Shiga toxin (Stx) is a virulence hallmark of Stx-producing *Escherichia coli* (STEC). Among STEC, the emergence of antibiotic-resistant poses significant challenges in both medical and agricultural settings, especially with *E. coli*, being one of the most prevalent Gram-negative pathogens in humans. In this study, we determined the Minimum Inhibitory Concentration (MIC) for a panel of ten Multi-Drug Resistant STEC of diverse serogroups. Genome sequencing and resistance profiling detected a total of eleven resistance loci of different antibiotic classes (Amoxicillin/Clavulanic Acid, Azithromycin, Cefoxitin, Ceftriaxone, Streptomycin, and Tetracycline) present in different combinations in these strains.

Methods and Materials: Bacteria growth in LB and in response to the addition of serial dilations of the relevant 11 antibiotics were recorded with the starting concentration informed by Biolog Microbial Sensitivity Assay. The Optical Density at 600 nm was measured in a 48-well Micro Arrayplate for 12 hours with 10-minute read intervals.

Result: The recorded phenotypes confirmed the cataloged antibiotic resistance profiles of the tested STEC strains. We determined MICs for each strain's individual set of resistance genes. Overall, our data provide evidence of the plasticity in MDR-profiles and conferred resistance levels that can be found in clinical STEC lineage.

UP33 Refining methods for isolating, purifying, and characterizing bacteriophages

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Abstract

Bacteriophages, the most abundant biological entities on Earth, specifically infect bacteria. These viruses initiate the lytic cycle, hijacking the cellular machinery of their bacterial hosts to replicate, which ultimately leads to the host's destruction. Phage therapy has shown promising results in treating antibiotic-resistant infections, though clinical trials are ongoing to fully establish its safety and efficacy. Identifying suitable phages is crucial in developing successful therapy due to the specificity of bacteriophage-host interactions. Our study refined methods for isolating and studying bacteriophages against *Enterobacter aerogenes*, a critical ESKAPE pathogen contributing to antibiotic resistance. We evaluated two isolation techniques: the overnight enrichment assay and direct isolation via the whole plate spotting assay. Our comparison found an advantage of the direct isolation method—it not only matched the efficacy of the overnight enrichment but surpassed it by offering accelerated results and minimizing resource utilization. A key refinement for purification was the incorporation of calcium chloride into the soft agar, which markedly enhanced plaque clarity and visibility. Moreover, our exploration of DNA extraction techniques revealed the superiority of zinc chloride precipitation over commercial kits, with the former delivering higher DNA yield and purity. We isolated three phages, K-1, BB-1, and M-1, effective against *E. aerogenes*. Noteworthy, phage BB-1 exhibited a rapid lytic cycle, clearing plates in under 10 hours. Future research will focus on examining their infectivity across *Enterobacter strains*, lysis of host cells, and absorption rates. We will also analyze their genome sequences to determine their novelty and potential for addressing antibiotic resistance.

UP34 Isolation and characterization of Jackal: a *Mycobacterium* phage

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Abstract

Bacteriophages are bacterial viruses, also called phages. Phage isolation and characterization is important for multiple reasons: phages have served as model organisms in genetics and molecular biology, are used to treat antibiotic-resistant bacterial infections, and have been used as vectors for genetic engineering. Our team isolated *Mycobacterium* Phage Jackal during Fall 2023 from a soil sample obtained at 32.46324° N, 94.72627° W at LeTourneau University, in Longview, Texas. The enriched isolation method was used, using *Mycobacterium smegmatis* mc^2 155 as the bacterial host. The sample was tested for phages with a spot test, with the bacterial lawn divided into three sections which were inoculated with 5 µl, 10 µl, and 100 µl of our 0.22 µm-filtered enriched sample. Plaques were seen in all three inoculated spots. Purification, amplification, DNA extraction, and TEM followed the SEA-PHAGES Phage Discovery Guide. After aseptically picking a plaque from the spot plate, Jackal went through 4 rounds of purification, each time picking a plaque with was at least 1.59 cm away from its nearest neighbor. Jackal had clear, circular plaques with average diameter 0.89 mm (range 0.5 to 1.2 mm; n = 14). Jackal's titer was 6.7×10^{10} PFU/mL, and TEM showed that it has siphovirus morphotype. Jackal's genomic DNA and a sample of its lysate are archived at the University of Pittsburg. Jackal's genome awaits sequencing. Its genomic and morphological information may be useful in future research, therapeutical applications, and genetical engineering.

UP35 Comparative analysis of bacteriophage tape measure and minor tail protein phamilies

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Abstract

Bacteriophages are extremely host specific and usually only infect a single bacterial species. During infection, bacteriophage DNA goes through the tail into the host cell cytoplasm. The tape measure protein (TMP) determines phage tail length. By controlling tail length, TMPs ensure that phages can effectively recognize and infect suitable hosts. Bacteriophage minor tail proteins (MTP) assist in phage tail assembly, adsorption, host cell wall penetration, and DNA ejection. Therefore, bacteriophage MTPs and TMPs could function as reliable tools for bacterial pathogen identification and host range determination. To further knowledge of host range and host infection dynamics, it is necessary to investigate the sequence and structural diversity of MTPs and TMPs. Five phages were analyzed per subcluster (A1, A2, A3, A4), together with phage Chargerpower, the sole non-subclustered cluster A member. The TMP and one MTP were analyzed per phage, giving 21 total TMPs and 21 total MTPs analyzed. MEGA11 was used to construct maximum likelihood cladograms for the TMPs and MTPs. For both TMPs and MTPs, phages in subclusters A1, A2, A3, and A4 grouped in their own clades, while cluster A fell between A2 and A4 with TMPs and between A2 and A3 with MTPs. Using the SWISS-MODEL, three-dimensional protein folding models were constructed. Structural differences were observed among the MTPs and TMPs. Further studies are warranted to investigate MTP and TMP diversity and structural conservation across all A phamily members in relation to host range, bacterial identification, and phylogenetic analysis.

UP36 Isolation and discovery of the Sue2 phage

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Baylor University, Waco, USA

Abstract

In an era of antibiotic resistance, bacteriophage research has emerged to combat this emerging crisis. Bacteriophages are viruses that infect and replicate in their specific host bacteria. Through the Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, a study was conducted to characterize a newly discovered Arthrobacter bacteriophage. The bacteriophage, Sue2, was discovered in soil in Elmhurst, Illinois. It was isolated through soil washing and was purified by performing plaque assays with the lysate to confirm the presence of the phage on the host Arthrobacter atrocyaneus. Amplification and characterization were performed through procedures of calculating titer, phage precipitation, DNA purification, polymerase chain reaction, gel electrophoresis, and transmission electron microscopy (TEM). These procedures resulted in a bacteriophage with a high titer of 3.0 x 1010 pfu/mL. Cluster determination was unsuccessful using available cluster-specific primers for the tape measure protein. TEM indicated a Siphoviridae morphology with an average head diameter of 48 nm and a tail length of 108 nm. Sue2 was archived into the SEA-PHAGES database, and its phage genome was sequenced at the University of Pittsburgh to compare it to other known bacteriophages. Using bioinformatics tools such as BLAST, HHPred, Glimmer, and GeneMark, annotations of each of the 67 genes from Sue2 were completed. Genome sequencing revealed that Sue2 is a member of the AZ1 cluster. The knowledge gained from isolating and characterizing Sue2 can be applied to new scientific discoveries and medicinal applications, such as phage therapy.

UP37 Isolation of five novel *Serratia* bacteriophages from Central Texas

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Abstract

Serratia marcescens is a facultative anaerobic bacterium commonly found in aqueous environments such as mud, ponds, lakes and sewage water. It is known to cause infections in neonates and immunocompromised humans and is an emerging antibiotic-resistant pathogen. For this reason scientists are interested in studying its bacteriophages. We wanted to research more into phage to see if it can be helpful in the treatment of *S. marcescens* for phage therapy. Here we present a phage hunt from eight distinct Central Texas locations, five of which proved positive results from areas near Hearne, Navasota and College Station. These areas yielded five novel phages that kill the host bacteria *S. marcescens*. After processing these samples and filtering out all other contaminants we isolated and amplified the five different types of phage plaques. The morphologies of the phage varied in size and shape and the TEM pictures revealed most of them are likely podophages. Then we processed the genomic DNA for short-read Illumina whole-genome sequencing. Analyses of the sequencing data are ongoing. Our results demonstrate that diverse *Serratia* phages are present within the Texas environment and can be readily sequenced and studied. We anticipate our research to aid in further studies, including phage therapy for bacterial infections.

UP38 Perceived student difficulties and confidence while annotating their first phage genome: before, during, and after

Abraham Gonzalez Pimentel, Fred Baliraine, Josh McLoud

LeTourneau, Longview, USA

Abstract

The past two decades brought a data revolution in life sciences, especially in bioinformatic analysis of microbial genomes. Therefore, it is crucial to teach bioinformatics at the undergraduate level. However, there are few investigations on students' perceived difficulty or confidence levels before, during, or after the training. Fortunately, our Biology Program collaborates with the SEA-PHAGES Program to help us learn these essential bioinformatics skills with well-defined tools and protocols during the training. We surveyed a class of 14 students after they annotated a phage genome and received 11 responses. The survey focused on student's perceived difficulty or confidence level before, during, and after the training. The annotation training included 3 separate sections. The end of the survey had open-ended questions about which section they found to be most difficult and which one they found to be the easiest, whether they improved from before and after, and their overall thoughts on the entire training. Results from the survey showed a pattern; the student's perceived confidence increased, while difficulty decreased over time. In parallel, the data included incremental increases in confidence before, during, and after the training. When asked which section was easiest, students had the most confidence with section 3. When asked which section was difficult, there were 4 responses for both sections 1 and 3; although, comments on section 3 included: 2 on software, 1 on data interpretation, and 1 on interpretation due to missing training. Overall, 82% of respondents reported improvement in their confidence in phage genome annotation.

UP39 Isolation of EarlyBird from a *Microbacterium foliorum* culture

Timothy Hester, Alyssa Reza, Kelci Hill, Cole Moore, Dustin Edwards

Tarleton State University, Stephenville, USA

Abstract

The study of bacteriophages increases our understanding of bacteria and virus interaction and evolution over time. Microbacteriophage EarlyBird was isolated from a soil sample collected in the backyard of a house after rainfall in Stephenville, TX (GPS coordinates 32.20844, -98.2227077). Soil samples were washed with peptone-yeast extract-calcium (PYCa) media and filtered with a 0.22-μm filter to directly isolate bacteriophages. The filtered supernatant was inoculated with 250 μL of host bacteria Microbacterium foliorum, placed in a top agar overlay, and incubated at 29°C for 48 hours. EarlyBird formed small clear plaques, which were present on all plates up to 1x104 dilution. Bacteriophage EarlyBird was isolated through two rounds of serial dilution. The 1 x103 dilution was used to create a webbed plate for the high titer. A high-titer lysate at 2.9 x109 pfu/mL was used to prepare transmission electron microscopy (TEM) grids by staining with 1% uranyl acetate. TEM imaging showed that the microbacteriophage EarlyBird has siphovirus morphology, the approximate capsid diameter was 58-nm, and the tail length was 125-nm. DNA extraction was performed using a modified zinc chloride method. DNA genome length was approximated to be between 10,000 and 23,000 bp by a gel electrophoresis comparison with λ-HindIII. EarlyBird was archived at Tarleton State University and Pittsburgh Bacteriophage Institute.

UP40 Isolation and characterization of phage Pygmy

Benjamin Hughes, Josh McLoud, Frederick Baliraine

LeTourneau University, Longview, USA

Abstract

Bacteriophage discovery and characterization is increasingly becoming an important undertaking, to add to the arsenal of tools to combat antibiotic-resistant infections which claim about 5 million lives globally every year. Phage Pygmy was isolated from a soil sample collected at 32.464167 N, 94.726111 W at Letourneau University on August 22, 2023. Combining the sample with Middlebrook 7H9 Complete medium, we utilized a spot test and 10-fold serial dilutions for three 48-hour rounds of plating at 37°C to isolate Pygmy, named for its small plaque sizes. Eight webbed plates were prepared and flooded with 5 ml of phage buffer, which was then filtered through 0.22 µm pore size filters. This process yielded a titer of 2.9×10^{10} PFU. Transmission electron microscopy at the University of Arkansas for Medical Sciences revealed Pygmy to have a siphovirus morphotype. After extracting Pygmy's genomic DNA from the lysate using the Promega Wizard DNA cleanup Kit and determining its DNA quality by 1% agarose gel electrophoresis, Pygmy's DNA was sent off for Illumina sequencing at the University of Pittsburg. We annotated the genome using various bioinformatic software and databases, including DNA Master, HHPred, Phamerator, Starterator, BLASTp in NCBI GenBank and PhagesDB, DeepTMHMM, and searched for tRNA using Aragorn and tRNAscan-SE but found none. Genome sequence analysis placed Pygmy in the P1 Subcluster, with a 48612 bp genome length, 66.9% GC content, and a temperate life cycle. Pygmy adds to our inventory of phages as an asset in the potentially life-saving field of bacteriophage research.

UP42 Isolation of microbacteriophage Tiland

Chaney Kelly, Luke Reynolds, Melody Hunter, Lauren Bower, Cole Moore, Dustin Edwards

Tarleton State University, Stephenville, USA

Abstract

Bacteriophages are viruses that infect specific host bacteria. There is a renewed interest in the therapeutic application of bacteriophages as a treatment for antibiotic-resistant bacteria. The SEA-PHAGES program at Tarleton State University, through the Howard Hughes Research Institute, is working to build a library of characterized bacteriophages. Microbacteriophage Tiland was isolated from a soil sample collected from an ant hill outside of the West End Cemetery in Stephenville, Texas (GPS Coordinates 32.213125, -98.216560). The soil sample was flooded with peptone-yeast extract-calcium (PYCa) liquid media, filtered through a 0.22-μm filter, and then incubated with Microbacterium foliorum NRRL-24224 SEA for 48 hours at 29°C. Two rounds of serial dilutions and plaque assays were performed, and lytic plaques were observed. Negative-staining transmission electron microscopy showed Tiland to have siphovirus morphology with a 58-nm icosahedral head and 164-nm noncontractile tail. DNA was extracted from Tiland by a zinc chloride precipitation method and then treated with HaeIII, MseI, NspI, SacII, and SalI restriction enzymes. Gel electrophoresis revealed that none of the enzymes successfully digested the DNA. Tiland was archived at Pittsburgh Bacteriophage Institute and Tarleton State University.

UP43 Discovery, isolation and annotation of cluster BD2 bacteriophage Puginator on *Streptomyces baarnensis*

Dalya Kinsizer, Kathelyn Herrera, Ben Bishop, Pedro Rodriguez, Hunter Brown, Joanna Roy, Aaliyah Khan, Maharjan Abhyarthana, Sreemoye Nath, Lee Hughes, Ahmad Sulaiman

University of North Texas, Denton, USA

Abstract

Bacteriophages are viruses that infect bacterial host cells. Bacteriophage Puginator was discovered from a soil sample collected in Denton, Texas. It was enriched with the host bacterium Streptomyces baarnensis. Puginator was isolated, purified, amplified to produce a lysate with a titer of 6x108 pfu/ml. DNA was extracted using this lysate, with a DNA concentration of 122.3 ng/μL and a total yield of 15.3 μg. Upon sequencing, Puginator was determined to be a member of Cluster BD2. The genome has 51731 base pairs including an 11bp-3' overhang, a GC content of 67.6%, 87 annotated protein-coding genes, and no tRNAs.

UP44 Analyzing the unique presence of DNA methyltransferases in the subcluster A4 bacteriophages

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Abstract

DNA methyltransferases (MTases) catalyze the transfer of a methyl group to DNA, often resulting in silenced gene expression. In bacteria, DNA methylation protects against the cells' own restriction enzymes that recognize specific foreign, unmethylated nucleotide bases in infecting bacteriophages, and "cut" into their DNA, thereby killing or preventing infection by the invader. Bacteriophages that have MTases can outsmart the host bacteria defenses by methylating their bases, thereby evading recognition by the host's restriction endonucleases. While annotating the A4 subcluster phage Lunsford, we noted that this virus has two consecutive MTase genes in the right flank toward the end of the genome, downstream the Cas4 exonuclease and immunity repressor. Further analysis in a total of 608 phages across cluster A and subclusters A1-A5 in phagesDB and phamerator revealed that 99% of subcluster A4 phages have these two consecutive MTase genes in this region. However, no MTases were identified in this region among the A, A3, and A5 phages, while 40% A1 and 95% A2 phages had only one MTase, with ~1% of the A1 and A2 phages having two MTases. Additionally, 100% of the A and A4 phages had the Cas4 exonuclease, which was present in 96-99% of the A1, A2, A3 and A5 phages. The immunity repressor was present in 95–100% of the analyzed phages. The presence of two DNA methyltransferases in addition to the immunity repressor indicates that subcluster A4 members could have a wider host range than other cluster A bacteriophages. This observation warrants further investigation.

UP45 Isolation and characterization of phage FreddyB

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Abstract

Bacteriophages (phages) are important in phage therapy, to combat antibiotic-resistant infections. Using the enriched method with Middlebrook 7H9 medium and Mycobacterium smegmatis mc2155 as the host, Mycobacterium phage FreddyB was isolated from a soil sample collected at LeTourneau University in Longview, TX (32.463333 N, 94.7275 W), in August 2023. The appearance of plaques on the spot test confirmed phage presence. Phage purification was done through three rounds of ten-fold serial dilutions and plating on Middlebrook 7H9 top agar, followed by webbed-plate preparation and flooding with phage buffer. This yielded a high titer lysate of 3.2 x 1010 PFU/ml. Phage FreddyB produced small, turbid plaques (average diameter 1.3 mm, range 0.7 – 2.2 mm; n = 10) after 48 h of incubation at 37°C. Negative-stain TEM showed FreddyB to be a siphovirus with an isometric capsid measuring ~43 nm (range 41 - 45 nm; n = 5) in diameter and a long, flexible, non-contractile tail measuring ~156 nm (range 137 - 178 nm; n = 5) in length. Following DNA extraction using the Promega Wizard® DNA Clean-Up Kit, Illumina Genome sequencing was done at the University of Pittsburgh. Annotation was done using DNA Master, Starterator, PhagesDB, NCBI, HHPred, Phamerator, Aragorn, tRNAscan-SE and DeepTMHMM. FreddyB is a temperate phage, and its sequence data show that it belongs to sub-cluster F1 and is the first F cluster phage isolated in Longview. FreddyB has a 10 bp 3’ sticky overhang (CGGACGGCGC) and a genome length of 58318 bp, with 61.7% GC content.

UP46 The isolation and characterization of novel bacteriophage Woodbury

Sierra Martinez, Daiyuan Zhang

Del Mar College, Corpus Christi, USA

Abstract

Bacteriophages are viruses that target and selectively kill bacteria. They are more abundant than every other organism on earth and have proven useful in developing treatments for antibiotic-resistant bacteria, as well as having other applications in medicine and gene therapy. The purpose of this study was to isolate and characterize a novel bacteriophageusing *Mycobacterium smegmatis* as a host. To achieve this, a variety of procedures were performed including soilenrichment with *M.smegmatis,* spottest confirmation, streak test purification, and harvesting of the high titer lysate (HTL). The phages gDNA was isolated from HTL and used for restriction digest. Woodbury was imaged using uranyl acetate staining and Transmission Electron Microscopy. Lysogeny was then isolated and confirmed using a spot test and a patch test. Finally,an efficiency test was performed to investigate virusefficiency. Bacteriophage Woodbury was observed to be lytic after isolation and TEM image shows acapsid of 80 nm with a tail of ~250 nm. The restriction digestperformed with enzymes *BamHI, EcoR, ClaI, HaeIII* and *HindIII* shows only recognition sites for the last two and remainsmainly uncutfor the rest.The low efficiency rate of 36.7% suggests that the phage is less than ideal for treatment of bacteria like *M.smegmatis*.Woodbury is published on Phages.DB and future study may include DNA sequencing.

UP47 Isolation of microbacteriophage Ashaug

Ashlynn Nowlin, Augur Buchholz, Cole Moore, Dustin Edwards

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Abstract

Bacteriophages, being the predominant entities on Earth, are a central focus in the field of genomics, driving continuous efforts aimed at discovering novel viruses. Microbacteriophage Ashaug was extracted from a soil sample taken under a tree frequented by animals in Stephenville, Texas [Global Positioning System (GPS) Coordinates: 32.229704 N, 98.201283 E]. The phage was isolated by incubating the soil with peptone yeast calcium (PYCa) liquid media while shaking at 225 rpm for 1 hour at 29°C. The supernatant was passed through a 0.22 µm filter and incubated with host Microbacterium foliorum at 29°C for 24 hours in a soft agar overlay. A well-isolated plaque was picked, and then two rounds of 10-fold serial dilutions in phage buffer were performed and incubated with the host bacterium as before. The plate with a “webbed” appearance was selected, flooded with phage buffer for 6 hours, and a high-titer lysate of 7.1×10^{6} pfu/mL was collected. A negative-staining transmission electron microscopy was prepared, showing a siphovirus morphology with approximately 15,500 bp, an estimated tail length of 105 nm, and a capsid diameter of approximately 45 nm. Genomic DNA was extracted using a modified zinc chloride precipitation method and was treated with HaeIII, MseI, NspI, SacII, and SalI restriction enzymes in electrophoresis. DNA analysis using a spectrometer showed a DNA concentration of 2176.6 ng/µL. The genomic DNA sample was archived at Tarleton State University and sent to the Pittsburgh Bacteriophage Institute.

UP48 Teuta: discovering a *Streptomyces*-specific bacteriophage

Anyla Paschall

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Abstract

Bacteriophages are viruses that exclusively infect bacteria. They are typically specific to very few species of a particular genus of bacteria. Teuta, a recently discovered phage, infects species of the genus *Streptomyces*. Teuta was discovered from a soil sample which was enriched with the host bacterium *Streptomyces baarnensis* and then isolated and amplified. DNA was extracted from the phage lysate, allowing for genetic analysis of the phage. The final lysate titer was $1.6x10^9$ pfu/mL. The DNA concentration was 556.4 ng/µL, the 260/280 ratio was 1.98, and the yield was 41.62 µg. The phage was able to infect several other species of *Streptomyces*, including *S. azureus, S. xanthochromogenes,* and *S. bicolor*. The gel electrophoresis results for the phage were most similar to those seen in the BI1 subcluster, however there were some variations, so this analysis was not conclusive.

UP49 Isolation and characterization of the *S. baarnensis* bacteriophage Eviev

Sydney Porter, Ahmad Sulaiman, Jindanuch Maneekul, Lee Hughes

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Abstract

Here, we describe the isolation and characterization of Eviev, a phage taken from a soil sample collected in Denton, Texas using the bacterial host *Streptomyces baarnensis* NRRL B-2842. *Streptomyces* is a genus of bacteria with a fungi-like appearance commonly found in soil samples. Eviev was isolated from an enriched sample that then underwent several rounds of purification and amplification obtaining a high titer lysate and extracting DNA. Eviev has plaques that are clear and circular with an average diameter of 0.75mm. The final high titer lysate was 1.0 x 10^8pfu/mL. The extracted DNA had a concentration of 780 ng/µL with a DNA yield of 42.9µg. Restriction analysis was performed and used to estimate a genome size of 24.43kbp. The restriction pattern showed no exact matches to already sequenced *Streptomyces* bacteriophages, with the closest similarity being cluster BK2. In host range testing, Eviev was able to infect 6/10 of the tested bacterial strains.

UP50 Isolation of microbateriophage Kudou

Mia Prahl, Dustin Edwards, Cole Moore

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Abstract

Bacteriophages are the most abundant biological entities on Earth, outnumbering all other organisms. Bacteriophages are present in every environment where bacteria exist, from the oceans and soil to the living organisms around us. Bacteriophages play a crucial role in microbial ecosystems. Microbacteriophage Kudou was collected from a soil sample near a clearing by a forest in Fort Worth, Texas (32.612 N, -97.289 W). The soil sample was flooded with peptone-yeast extract-calcium (PYCa) liquid media, placed in a shaking incubator at 250 rpm for 1 hour, and filtered through a 0.22-µL syringe. The supernatant was inoculated with the host bacteria, Microbacterium foliorum, and then plated in a soft agar overlay for 48 hours at 29°C. Approximately one hundred single 2-mm diameter lytic plaques formed. Additional plaque assays with two rounds of 10-fold serially diluted supernatant in a phage buffer produced well-isolated lytic plaques. One plaque was selected, diluted 1:1000, and incubated again with the host to produce webbed plates. A webbed plate was flooded with PYCa liquid media to obtain a high-volume lysate of 4.2x108 pfu/mL. DNA was extracted using a modified zinc chloride precipitation method. Microbacteriophage Kudou was archived at Tarleton State University and the Pittsburgh Bacteriophage Institute.

UP51 Looking at the HNH endonuclease: classification and analysis of Lakshmi gp 60, Wildwest gp 64, and Wildwest gp 65

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Abstract

HNH endonucleases are free-standing enzymes that cleave DNA at the HNH motif. These enzymes play a necessary role in bacteriophage terminase activity and are largely conserved in both sequence and position. Analysis of HNH endonucleases can indicate important details about bacteriophage evolution and cluster relationships. These endonucleases often exist as one of three main groups: Intron 1 coded proteins, Intron group 2 proteins, and self-splicing inteins. This study investigated three HNH endonucleases across two isolated Arthrobacter bacteriophages- Lakshmi and Wildwest. Specific gene proteins were visualized through SWISS-MODEL and tested against templates for similarity using FATCAT pairwise alignment. Genetic relationships and evolutionary origins were also determined through multiple sequence alignment and phylogenetic modeling. FATCAT analysis suggests that Wildwest gp 65 and Lakshmi gp 60 should be classified as Intron group 1 coded proteins, with pairwise alignment scores of 185.39 and 186.90 respectively. Analysis of Wildwest gp 64 showed similarity to a PacI restriction endonuclease compared to classic HNH enzymes. Phylogenetic analysis revealed evolutionary relationships between Wildwest gp 64 and an arthrobacter protein, as well as conservation of Wildwest gp 65 and Lakshmi gp 60. These conclusions indicate the presence of horizontal gene transfer in the phage Wildwest and the presence of Intron 1 coded HNH endonucleases in the novel phages Wildwest and Lakshmi. Further examination of these endonucleases can be done to understand the importance of specific proteins, such as Wildwest gp 64, in the context of phage-phage and phage-host interactions.

UP52 Isolation and characterization of microbacteriophage Godfather

Augur Buchholz, Joshua Hutchings, Anayah Copeland, Cole Moore, Dustin Edwards

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Abstract

Studying new bacteriophages contributes to ongoing efforts to advance our understanding of phage genomics. Bacteriophage Godfather was directly isolated by incubating moist soil from a flowerbed near an anthill in Stephenville, Texas, with peptone-yeast-calcium (PYCa) liquid media, passing the supernatant through a 0.22 μm filter and incubating with a host culture of Microbacterium foliorum. Following two rounds of serial dilutions and plaque assays on PYCa agar plates to isolate the bacteriophage, Godfather formed small, lytic plaques. Negative-staining transmission electron microscopy showed Godfather has siphovirus morphology with an approximate tail length of 116 nm and capsid diameter of 51.5 nm. Bacteriophage DNA was extracted, and libraries were sequenced by Illumina MiSeq at the Pittsburgh Bacteriophage Institute to generate single-end reads of 150-base read length, resulting in a single bacteriophage contig with 38× coverage. Godfather has a double-stranded DNA genome of 17,452 base pairs containing a 9-base sticky overhang. Whole-genome sequence analysis using Starterator, GeneMark, and Phamerator software revealed Godfather has 68.7% G+C content and 27 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. Godfather is a cluster EE bacteriophage and is most closely related to microbacteriophages Scamander and Aries55.

UP53 The discovery and characterization of the bacteriophage ElGato

Nicole Reyes, Ahmad Sulaiman, Jindanuch Maneekul, Lee Hughes

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Abstract

Bacteriophages, also known as phages, are viruses that infect bacteria. Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) is a program that works with a phage to isolate, purify, and amplify from a soil sample so it can get characterized. *Streptomyces baarnensis* NRRL B-2842 was the host bacterium used in this study. Streptomyces phage ElGato was isolated from soil collected at Community gardening in Denton, TX. It was enriched, purified, and amplified until it achieved a high titer lysate of 1 x 10^8 pfu/mL. After DNA extraction, the phage yielded a DNA concentration of 189.9 ng/µL, with a 260/280 result of 1.96, and a total DNA yield of 28.9 µg. Restriction enzyme was performed to find the potential DNA cluster. Moreso, a host range test was performed that showed that ElGato could infect almost all other *Streptomyces* species that were tested. The phage lysate was archived, and ElGato's information was uploaded to PhagesDB database. Following sequencing, ElGato was identified as part of Cluster BD3, with a genome length of 48922 base pairs. Currently, the genome is undergoing annotation.

UP54 The isolation and characterization of the bacteriophage Erock

Elizabeth Rocklage, Daisy Zhang

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Abstract

Bacteriophages are a type of virus that infects bacteria. Bacteriophages have multiple uses in research and medical fields such as vectors for moving genes and alternative treatment for antibiotic resistant bacteria. In this project, a novel bacteriophage Erock was isolated from a soil sample by enrichment with *Mycobacterium smegmatis*. The gDNA was extracted from the high titer lysate, followed by restriction enzyme digest. The morphology of Erock was studied with uranyl acetate staining and transmission electron microscopy. The lysogen of Erock was isolated with an extended spot test and confirmed with a patch test. Plates were set up to measure virus efficiency on its host by seeding different amounts of bacteria on phage-coated plates. The phage morphology was observed to be more lytic. The restriction digest analysis shows that Erock contains multiple sites for the enzymes *BamH I*, *EcoR I*, *Cla I*, and *Hae III*. The TEM image indicates the capsid is 86nm in diameter and the tail 400nm long. The virus efficiency is estimated to be 20%-30%. Due to the high survival rate of the host, Erock would not make a suitable candidate for antibiotic replacement. However, Erock could be a tool to study gene transfer.

UP55 Isolation and characterization of microbacteriophage Ganandorf

Elijah Rodriguez, Dustin Edwards, Katelyn Kaase, Cole Moore

Tarleton State University, Stephenville, USA

Abstract

Microbacteriophage Ganandorf contains a 52,941-bp double-stranded genome. Ganandorf was isolated from an anthill soil sample collected in Stephenville, TX (GPS coordinates 32.25634 N, 98.21932 W). Soil samples were submerged and washed with a peptone-yeast extract-calcium (PYCa) liquid medium and incubated at 250 rpm and 29°C for 1 hour. The supernatant was filtered through a 0.22-μm syringe filter, inoculated in molten agar with the bacterial host Microbacterium foliorum, plated on a PYCa agar plate, and incubated at 29°C for 48 hours. Bacteriophage replication produced small, lytic plaques on the soft agar overlay. Ganandorf was isolated by two rounds of 10-fold serial dilutions. High-titer lysates of 1.5 x 1010 pfu/mL were produced by flooding webbed plates with phage buffer at room temperature for 4 hours. Negative-staining transmission electron microscopy showed that Ganandorf displayed siphovirus morphology with a tail length of 150-nm and an isometric capsid 77-nm in diameter. Bacteriophage DNA was extracted and sequenced through Illumina Sequencing at Pittsburgh Bacteriophage Institute to generate single-end reads of 150-base length, which resulted in an approximate coverage of 24. Whole-genome sequence analysis using Phamerator, GeneMark, and Starterator determined that Ganandorf has 68.9% G+C content and 89 protein-coding genes. Putative functions for genes encoding structural proteins, an endolysin, and nucleases, such as three HNH endonucleases, a VRR-Nuc domain protein, and a Cas4 exonuclease, were identified using HHPred and BLASTp databases. Ganandorf is a cluster EC bacteriophage most closely related to Busephilis and CrazyRich.

UP56 The isolation and characteristics of the bacteriophage Tsan1019

Teresa Sanchez, Daiyuan Zhang

Del Mar College, Corpus Christi, USA

Abstract

Bacteriophage, also referred to as “phage” is a virus that infects bacteria and archaea. Once a bacteriophage attaches and enter their host, they begin to replicate which then causes bacterial death. Bacteriophage are known as the most abundant biological agents with unique infectious characteristics. Due to their uniqueness, they share a clinically significant role by being used for gene therapy and antibiotic resistance studying. This study began with a soil sample that was enriched with the host bacteria, *Mycobacterium smegmatis*. After the virus named Tsan1019 was identified and purified, a high titer lysate was harvested and later used to isolate the genomic DNA followed by a restriction digest analysis. The phage sample was fixed on a copper grid with uranyl acetate staining and sent for transmission electron imaging. Finally, the lysogen of Tsan1019 was isolated by a spot test and later confirmed using a patch test. The plaque morphology of Tsan1019 indicated a lytic life cycle. The restriction digest suggests that the genome of Tsan1019 contains recognition sites for ClaI, EcoRI, and HindIII. TEM imaging shows the phage capsid is estimated to be about 60nm in diameter and the tail is estimated to be 280nm in length. The phage Tsan1019 was published on the Phages Database online at https://phagesdb.org/.

UP57 A novel discovery and annotation of *Arthrobacter* bacteriophage Kukla

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Abstract

Bacteriophages are the most abundant organisms in the biosphere and a unique type of virus, composed of unique genetic sequences. This study describes the discovery and genome annotation of a new bacteriophage, Kukla. Kukla was initially isolated from a soil sample collected in Richmond, Illinois. Initial isolation of Kukla was performed using the Gram-positive bacteria, Arthrobacter globiformis, as the host organism. Purification and characterization of the bacteriophage were performed through phage cluster PCR typing and transmission electron microscopy. The TEM image revealed Kukla to be a Siphoviridae with an average tail length of 175 nm and an average head diameter of 57 nm. DNA was purified and the genome sequence was determined at Pittsburgh Bacteriophage Institute. The length of Kukla's genome is 46,695 bp, with a GC content of 64.5%, and it is in the phage cluster FJ. The initial auto-annotation identified 87 genes. Currently, programs such as DNAMaster, HHPred, NCBI Blast, PECAAN, and Phamerator are being used to annotate Kukla. These tools will provide insights into the functions of Kukla's genes. Annotating bacteriophage Kukla may further inform fundamental molecular biology and explain host interactions, horizontal gene transfer, and evolution of phage genomes. This information on phage genomics may lead to new phage-based treatments. After annotations are complete for Kukla, it will help to improve what we already know about bacteriophages and phage therapy.

UP58 Amplification and cloning of mycobacteriophage Pixie genes

Grace Clements, Alexis Gastin, Morgan Karianen, Karerina Leone, Elias Maki, Pamela Meriscal-Arzola, Santos Munoz, Hayden Ordner, Megan Prindle, Parker Wilstead, Justin Spere, Selena Alvarado, Harold Rathburn, Dustin Edwards

Tarleton State University, Stephenville, USA

Abstract

Bacteriophages are rapidly becoming a topic of interest in the health community because of their use in combating antibiotic-resistant bacteria. The field of bacteriophage research is not fully developed yet, and only a small amount of knowledge has been gathered on the diverse bacteriophage population. A large portion of protein functions are still unknown to us, but this research helps us grow the library of known functions. Working through the Science Education Alliance Gene-function Exploration by a Network of Emerging Scientists (SEA-GENES) program hosted by the Howard Hughes Medical Institute, our group amplified, cloned, and sequence verified 71 of 100 genes. Continuing that work, we amplified 24 and cloned 18 genes for phenotypic assays to study their use against antibiotic-resistant bacteria. Using the Pixie high titer lysate DNA, we amplified the genes via PCR. Amplified products were then verified through gel electrophoresis. PCR products were then purified by either column purification or gel extraction, depending on the banding patterns of the gel. The purified products were then ligated into the pExTra01 plasmid with isothermal assembly. Ligated plasmids were transformed into 5-alpha F'Iq Escherichia coli and sequenced by Azenta. Extracted DNA was electroporated into Mycobacterium smegmatis. The 18 clone-verified genes will undergo phenotypic assays to determine effectiveness against antibiotic-resistant bacteria.

UP59 Discovery and identification of Lakshmi bacteriophage from Plano, Texas

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Abstract

Bacteriophages are an abundant type of virus with the ability to infect and kill bacteria while using them to replicate through lytic or lysogenic processes. Bacteriophages play a key role in the regulation of bacterial populations in various environments and are being explored as potential tools in phage therapy. The aim of this study was to isolate, characterize, and manually annotate the genome of the bacteriophage Lakshmi collected from the Arbor Hills Nature Preserve in Plano, Texas. Lakshmi was isolated from an enriched soil sample on *Arthrobacter* sp. ATCC 21022. Plaque assays, spot tests, and serial dilutions were used to purify the lysate; tape measure protein polymerase chain reaction (TMP PCR) of Lakshmi DNA and transmission electron microscopy (TEM) were performed to determine phage characteristics. Lakshmi DNA was sequenced at the Pittsburgh Bacteriophage Institute. Various bioinformatics tools, including PECAAN, GeneMarkS, Phamerator, BLASTP, HHPred, TMHMM, and SOSUI, were used to determine open reading frames and predict gene function. Lakshmi belongs to cluster AK and displays Siphoviridae morphology, with a head diameter of approximately 50 nm and a tail length of 100 nm. Its genome has 44018 base pairs, a GC content of 60.8%, and 60 confirmed genes. Twenty-nine of these genes code for proteins that have determined functions, including minor tail proteins, membrane proteins, and helix-turn-helix DNA binding domain. The annotation of the Lakshmi phage will advance the current understanding of bacteriophage diversity, and further bioinformatic analysis can be performed to explore the structure and function of the predicted protein.

UP60 Comparison of the A4 cluster mycobacteriophages: focus on phage Lunsford's serine integrases

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Abstract

Mycobacteriophages are viruses that infect bacteria of the family *Mycobacteriaceae*. Serine and tyrosine integrases are enzymes that facilitate recombination between a specific attachment site (attP) in the phage genome and the corresponding attachment site (attB) in the host bacterial genome. Whereas serine integrases do not need host cofactors, tyrosine integrases require the help of host cofactors. Serine integrases are therefore believed to be more efficient at site-specific recombination, and bear more promise than tyrosine integrases for use in synthetic biology, cell line manipulation, genetic engineering, biotechnology, and gene therapy via the introduction of att sites or sequences with partial homology to the att sites. HHPred and Phamerator analysis showed subcluster A4 phage Lunsford to have a serine integrase. Although there was significant homology among the upstream subcluster A4 minor tail proteins, lack of protein sequence homology was observed among the integrase genes. Notably, a large majority (78.1%) of the fully annotated A4 phages had the serine integrase, while only 16.8% of the A4 phages had the tyrosine integrase. Lunsford is a temperate phage, so its serine integrase facilitates a symbiotic relationship with its bacterial host. The high prevalence of the serine integrase in phage Lunsford and other A4 members implies that these phages have great potential to be used by scientists in genetic engineering and synthetic biology applications, including developing genetically engineered food crops to resist adverse environmental conditions, pests, and pathogens, and to increase plant and animal productivity to mitigate against world hunger, besides providing cures for genetic diseases.

UP61 of microbacteriophage BoneCarver

Rylee Widger, Isabel Gonzaba, Cole Moore, Dustin Edwards

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Abstract

Microbacteriophage BoneCarver was collected in the backyard of a house in Stephenville, TX (GPS coordinates: 32.21326 °N, 98.22464 °W). BoneCarver was isolated using the direct isolation method by adding the soil sample to peptone-yeast extract-calcium (PYCa) liquid media and passed through a 0.22μm filter before being incubated with host Microbacterium foliorum. After incubation at 29°C for 48 hours in a soft agar overlay, microbacteriophage BoneCarver formed clear isolated plaques. A well-separated plaque was picked for replication and added to 100μL of phage buffer; two ten-fold serial dilutions were performed to isolate the phage and produce a set of webbed plates by incubating with the host bacteria as before. Flooding the webbed plates with phage buffer and filtering the supernatants produced a high-volume lysate of 2.7 x 108 pfu/mL. The high-volume lysate was stained with uranyl acetate for transmission electron microscopy, which showed that microbacteriophage BoneCarver has siphovirus morphology. Phage DNA was extracted using a modified zinc chloride precipitation method, incubated with restriction enzymes, and resolved in a 2% agarose gel. Restriction enzymes HaeIII, NspI, SalI, and SacII were unable to digest the bacteriophage DNA. Microbacteriophage BoneCarver was archived at Tarleton State University and Pittsburgh Bacteriophage Institute.

UP62 Beneath the surface: The genomic exploration of bacteriophage Ozwaldo

Catherine Wright, Daisy Zhang

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Abstract

Bacteriophage, commonly known as “phage,” is a virus that infects bacteria and archaea. After infection, phage replicate within the bacteria, commonly resulting in bacterial death and phage multiplication to continue their life cycle. With a population estimating around 1031 phage particles on the planet, phage are the most abundant biological entities. Due to their parasitic nature to bacteria, phage have a clinical significance that can be used for gene therapy and strengthening the fight against infection from antibiotic resistant bacteria. Beginning with an enriched soil sample with the host bacteria, Mycobacterium smegmatis, a high titer lysate was harvested and used to isolate the phage genomic DNA, followed by a restriction digest analysis. A phage sample was fixed on a copper grid with uranyl acetate staining and sent for transmission electron microscope imaging. Lastly, the lysogen of Ozwald’ was isolated by spot test and confirmed by patch test. The phage’s efficiency on its host was measured by plating different amounts of host bacteria on virus-coated plates. The plaque morphology of Ozwaldo indicates a lytic life cycle. According to restriction digest gel results, Ozwaldo’s genome contains multiple recognition sites for enzymesBamHI, ClaI, EcoRI, and HindIII. Indicated by TEM imaging, the phage capsid is about 60 nm in diameter and the tail is 240 nm in length. The phage efficiency was calculated to 64.9% and the host survival rate was 35.1%. These results suggest that Ozwaldo may be a good candidate to study gene therapy and may aid in alternative antibiotic resistance studies. The phage Ozwaldo was published on the Phages Database online at https://phagesdb.org/.

Postdoctoral Poster Presentations

PP1 Lysis of phage Mu: the release of its endolysin

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Abstract

Recently, bacteriophages or phages have received renewed attention due their potential use as clinical treatment for multi-drug resistant “super bugs”. Phages are viruses that infect and kill bacterial populations by lytic infection. During their lytic infection, phages adsorb to bacteria, inject their DNA for genome replication, assemble virions, and subsequently lyse bacteria to release virions. The lysis process in Gram-negative bacterial hosts by phages requires disrupting the cell envelope. Canonically, this involves a holin that forms micron-scale holes in the inner membrane, an endolysin that degrades peptidoglycan, and spanins for disruption of the outer membrane.
The spanins and endolysin of phage Mu use mechanisms common to other phages. However, phage Mu lacks a holin. Instead, the membrane protein gp25, with a conserved domain of unknown function, is required for gp22 release. The principal aim of this study is to test two models for gp25 activity in host lysis. In the first model, gp25 works as a protease to cleave the amino terminal region of gp22 for its release. In the second model, gp25 forms a channel for gp22 release. We are purifying both proteins for biochemical and structural characterization. From another angle, we are investigating a nonessential neighboring gene with preliminary lysis regulation phenotypes. Using CRISPR-Cas13a we deleted the gp24 gene to confirm its role in lysis timing. The characterization of proteins involved in the lysis process will aid in their biotechnological or therapeutic use.