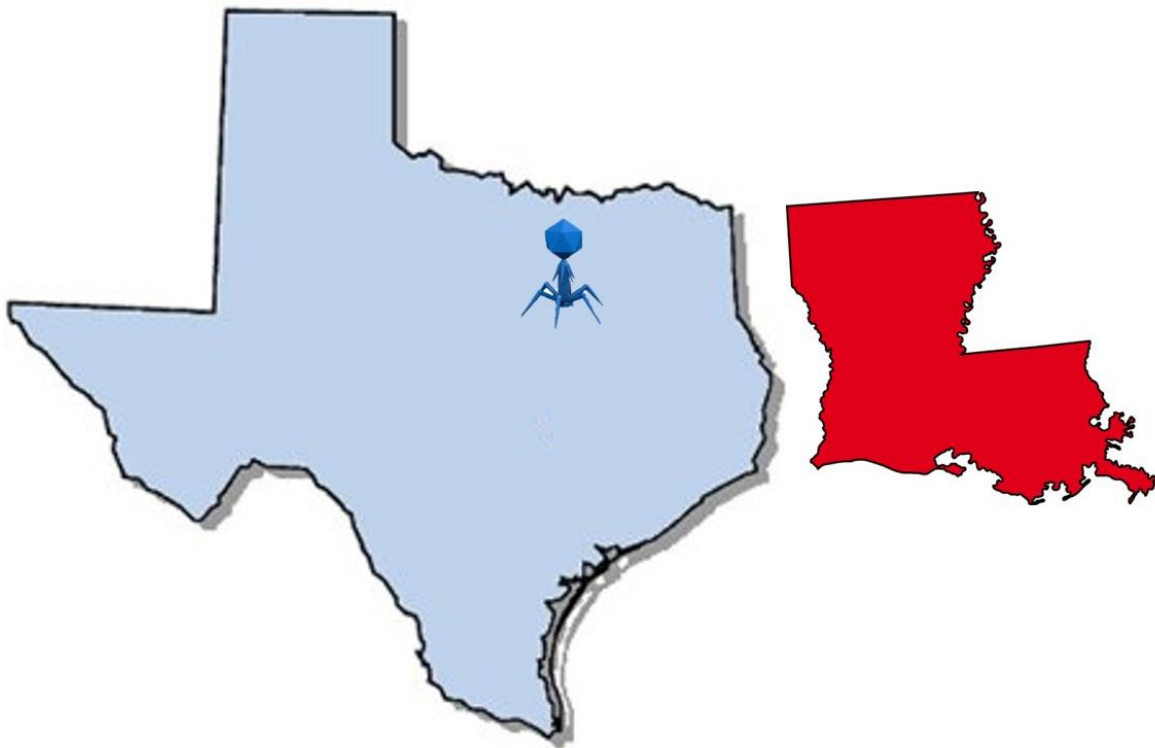


**The Third Annual  
South-Central U.S.A.  
SEA-PHAGES Research  
Symposium**

**April 12-13, 2019**



**Hosted by:  
University of North Texas,  
Denton, Texas**

# Symposium Schedule

**Friday, April 12, 2019**

Note: All meeting activities will be held in the UNT University Union (See room numbers on schedule below. Campus map found at back of program)

## ***Optional Pre-meeting Event***

3:00pm      Fossils, Genes, and the Origin of Limbs by Dr. Neil Shubin (author of the book, *Your Inner Fish*)  
Scientific seminar, UNT Union Lyceum.

## ***Symposium Events***

4:30pm      Symposium Check-in Table opens  
Union 333

5:00pm      Introduction of meeting organizers and Official Welcome from Dr. John Quintanilla, Associate Dean for Academics, UNT College of Science  
Union 333

5:15pm      Program and Research updates by SEA-PHAGES Student Representatives  
Union 333

**Isolating and Annotating *Streptomyces* Phages at UNT.**  
Brendon Williams, UNT

**SEA PHAGES at Collin College, two different perspectives.**  
Danielle Davis and Huy Nguyen, Collin College

**Tips and Tricks for Increased Arthrobacterphage Discovery.**  
Lathan Lucas, Baylor University

**Specialized Genes in Lysogenic Phages and Implications Involving Phage Therapy.**  
Sara M. Henry\*, Gregory D. Frederick, and Frederick N. Baliraine,  
LeTourneau University

**Possible Cas4 Gene Encoded by Subcluster A1 Bacteriophages.**

Tiffany Lujan\*, Faith Cox, Camille Trautman, Trey Carter, Nikki Cody, Jackson Royce, Harold Rathburn, and Dustin Edwards, Tarleton State University

6:15pm      Dinner and a Movie  
Union 332

7:15pm      Practical Matters: Presentations by Faculty, Alumni, and Students  
Union 333

**SEA-PHAGES: Inspiring Change and Refocusing our Vision for Undergraduate Biology Education.**

Dr. Jonathan Lawson, Collin College

**Exploring Phage Holin and Endolysin Structure Through Bioinformatics**

Gabriel Andino, Baylor University

**Life After SEA-PHAGES**

Lydia Flores, Collin College/UNT, Nic Mercado, UNT, and Willow Rock, UNT

8:00-9:00pm      Symposium Group Activities  
Union 333

**Saturday, April 13, 2019**

8:00am      Poster Setup  
Union 333

Light snacks  
Union 332

8:30am      Student Poster Session A (Even-numbered posters)  
Union 333

9:20am      Group Photo  
Outside Union 333

- 9:30am      Break
- 9:40am      Student Poster Session B (Odd-numbered posters)  
Union 333
- 10:30am      Short Break (please remove posters at this time)
- 10:45am      **Keynote Presentation: “A Brief History of Phage”**  
Dr. Welkin Pope, University of Pittsburgh  
Union 332
- 11:45am      Wrap up and Closing  
Union 332
- 12:00pm      Safe travels home!

Special “Thank You” to the UNT American Society for Microbiology Student Chapter for sponsoring and helping to organize this symposium!

# Abstracts

## Oral Student Presentations

### 1. Isolating and Annotating *Streptomyces* Phages at UNT. Brendon Williams

An overview of phage hunting on *Streptomyces* hosts at the University of North Texas

### 2. SEA PHAGES at Collin College, two different perspectives. Danielle Davis and Huy Nguyen, Collin College

Collin offers SEA PHAGES discovery both in Intro to Biotech I and Microbiology and the annotation in Intro to Biotech II and as a part of a project in Center for advanced studies in math and natural sciences (CASMNS). This presentation will provide different perspectives regarding the courses taken and research opportunities.

### 3. Tips and Tricks for Increased Arthrobacterphage Discovery. Lathan Lucas, Baylor University

### 4. Specialized Genes in Lysogenic Phages and Implications Involving Phage Therapy. Sara M. Henry\*, Gregory D. Frederick, and Frederick N. Baliraine, LeTourneau University

Bacteriophages go through one of two life cycles, lytic or lysogenic. Because of the way each life cycle occurs, it can be expected that viruses would require different genes in order to either effectively integrate into a host's DNA or destroy the host cell. In phages with the lysogenic cycle, the mutualistic lysogenic phage-bacterial host relationship may increase the host's fitness with a display of superinfection immunity which curtails the effectiveness of phage therapy. In some cases, lysogenic genes may even trigger the bacteria to release virulence factors, including toxins. If the genes and their products that cause these effects could be removed, this process could then make the lysogenic phages more viable to be used in therapy. In order to observe the genomes of lysogenic phages, bioinformatics software, such as Phamerator, HHPred, and phagesdb.org were used. In this study, investigations were performed into known proteins that differ between the two cycles. Some of the functions appearing only in lysogenic phages that could be considered for removal will be discussed in this study.

### 5. Possible Cas4 Gene Encoded by Subcluster A1 Bacteriophages. Tiffany Lujan\*, Faith Cox, Camille Trautman, Trey Carter, Nikki Cody, Jackson Royce, Harold Rathburn, and Dustin Edwards, Tarleton State University

Bacteriophages are viruses that are able to infect and lyse bacteria. Bacteria have evolved anti-viral defense mechanisms, such as clustered regularly interspaced short palindromic repeats (CRISPR) to combat bacteriophage invasion. We hypothesize phages may have acquired a counter-invasion mechanism by uptaking a CRISPR-Cas-associated gene. Subcluster A1 mycobacteriophage Arlo was directly isolated from host *Mycobacterium smegmatis* mc<sup>2</sup>155, and its genome (Genbank MH576971) was predicted to have 96 protein coding genes with gene 67

being annotated as a Rec-B like exonuclease. However, through further characterization using bioinformatics software HHpred and databases Pfam, SCOPe, PDB, BLASTp and NCBI, it showed a 99% identity to a CRISPR-Cas4-associated protein. CRISPR-Cas4 proteins are characterized as containing a Rec-B like exonuclease domain and are relatively uncharacterized as stand-alone genes. Using the software MEGA X to create a phylogenetic tree based on bootstrap statistical method provided high evidence of gene 67 being more closely related to other Cas4 type I-A than Rec-B proteins. MEGA X MUSCLE analysis showed a QXXXY amino acid motif that is found in all CRISPR-Cas4-associated proteins was present in gene 67, but not in RecB proteins. A secondary MUSCLE analysis showed the motif DYK to be highly-conserved in RecB protein sequences at the C terminus, but not present in gene 67 or other cas4 proteins. All proteins were found to contain highly-conserved cysteine residues at the same area on the C-terminus that are thought to be responsible for enzymatic activity. An in silico model of gene 67 using PHYRE2 software was used for comparative analysis to Cas4 which resulted in 99.8% identity. Current directions include doing a comparative analysis against all tertiary structures analyzed in the phylogenetic tree and characterization of the evolutionary origin of gene 67.

## Poster Presentations

**6. Siphoviridae Bacteriophage McGalleon Gene Annotation.** Kirk Niekamp\*, Huy Nguyen\*, Danielle Davis\*, Elizabeth Hampton\*, Kaitlyn Riley, Carole Twichell, Jonathan Lawson, Bridgette Kirkpatrick. Collin College

Here we will highlight the annotation of the siphoviridae bacteriophage McGalleon which was discovered from a soil sample in Forney, TX. The phage was isolated using the host *Microbacterium foliorum*. McGalleon is a member of cluster EA and subcluster EA1, and has a genome length of 42562 base pairs (bp). McGalleon has a G-C percentage of 63.7%, the 11th highest percentage within the subcluster. The bioinformatics tools DNAMaster, Glimmer, Genemark, PhagesDB Blast, NCBI Blast, HHpred, and Phamerator were used in this study. Genome-wide and gene-focused analysis was done to identify gene coordinates and potential functions of encoded proteins. Out of the 66 original genes identified, four were non-coding DNA sequences with minimal evidence of coding potential suggesting possible functional RNAs. Two additional genes were not found within McGalleon that have been identified in closely related bacteriophage. Additionally, there are 6 genomic regions within McGalleon's genome that appear to be unique within this subcluster. 26 of the 62 protein-coding genes had putative functions based on our analysis and included many of the common structural genes. The remainder 36 coding sequences included proteins that had no known function using the bioinformatics tools used in this study. Interesting features of McGalleon that will require further analysis are the unique gene 2 and the potential genes 41-43 of McGalleon within this cluster. Additionally, 3 other genomic regions that appear unique to McGalleon will be further investigated.

**7. *Agrobacterium tumefaciens* Bacteriophage Classification Using a Superinfection Immunity Based Lysogen Library.** Similoluwa Ogundare\*, Cassidy Horton, and Allison Wiedemeier. University of Louisiana at Monroe

Bacteriophages can carry out lytic or lysogenic life cycles. Lytic cycles are defined by progeny production preceding cell lysis, while lysogenic cycles are defined by phage genome insertion (prophage) within the bacterial genome. When bacterial cultures possess resident prophages, they are known as lysogens. The choice to carry out lytic or lysogenic cycles depends on the presence of repressors which prevent the transcription of lytic genes, favoring the lysogenic cycle. Superinfection immunity of lysogens is defined as immunity to infections by phages genetically related to the resident prophage. Using the principle of superinfection immunity, this study aimed to classify novel *Agrobacterium tumefaciens* (now known as *Rhizobium radiobacter*) bacteriophages into genetically related clusters. Supporting information was also obtained via the analysis and comparison of restriction digestion patterns of bacteriophage DNA samples. Three previously isolated bacteriophages were used to infect *Agrobacterium tumefaciens* cultures. Candidate lysogens were chosen from colonies on infection plates and were screened for being true lysogens via patch and supernatant spot tests. Positive results were indicated by clearings on *Rhizobium radiobacter* lawns. Immunity spot tests and infection assays were carried out on 3 true lysogens using 11 bacteriophage lysates. Plaque morphologies resulting from the infection of candidate lysogens and *Agrobacterium tumefaciens* were analyzed and compared. Percent reductions in the number of plaques on lysogen infections vs. *Agrobacterium tumefaciens* infections were also calculated, and subject bacteriophages were placed into bins based on these values. Preliminary results do not suggest genetic relatedness between all 3 bacteriophages used to create the lysogens tested in this study. However, trends that suggest the presence of genetic similarities between other bacteriophages have been observed in results from both the binning and restriction digest analysis. Future plans include annotating the now sequenced bacteriophage genomes, followed by PCR assays to confirm results of lysogen production. Immunity infections will also be repeated to confirm results. The lysogen library created from these results will serve as a classification tool to determine which bacteriophages belong to the same taxonomic group, prior to annotating their genomes.

**8. Annotating *Streptomyces* phage Araceli.** Joelyn Hanson\*, Cameron Theiss, Subhayu Nayek, and Lee E. Hughes. University of North Texas

Phages are a very large population with an estimated  $10^{31}$  phages in the biosphere. However, only a small portion are known to the scientific world. One of the largest collections, the actinobacteriophages found through the SEA-PHAGES program, are still fewer than 3,000 isolates. What is so interesting about bacteriophages is they are viruses that infect a bacterial host because of their inability to replicate on their own, making them extremely successful in destroying bacteria. These bacteriophages could eventually have the reach to be the counter to antibiotic-resistant bacteria, possibly making bacteriophages the future of medicine. The specific host that I used during my research was *Streptomyces griseus*. The project will focus on *Streptomyces* phage Araceli with a total of 84 genes. While annotating Araceli, my partner and I found that it had very few identifiable functions with a total of 13. We were able to determine these function by looking for a proper E-value with a low number, preferably that of 0, and the query to and from numbers which show the protein alignment. Some functions that were found

range to terminase, portal protein, helicase, and DNA polymerase. Araceli compared to the other phages in the same cluster, lined up with other phages and typically had few moments when it strayed from the similarities. Overall this process has brought a new light about the smaller world beneath our feet and we feel our hard work with these phages will someday account for something very important.

**9. Annotation and Analysis of Streptomyces phage Tribute.** Rebecca Lamb\*, Heather Bullock, Samantha Julsaint, Brandy Mason, Jonah Pfeiffer, Subhayu Nayek, and Lee E. Hughes. University of North Texas

Streptomyces phage Tribute was discovered in Frisco, TX in 2016 by UNT PHAGES student Meera Patel. This phage was isolated on the host bacterium *Streptomyces griseus* using the HHMI Phage Discovery Guide protocols. Tribute is a member of cluster BE, subcluster BE1. This phage appears to be lytic, has a 50% GC content, and was sequenced at the Pittsburgh Bacteriophage Institute. Our team adopted Tribute for bioinformatics analysis in our lab and performed annotations by utilizing comparative tools through PECAAN, following the SEA-PHAGES Bioinformatics Guide. Our annotations show the function of most genes in Tribute is currently unknown. The functions we have been able to assign include HNH-endonuclease, tail assembly chaperones, minor tail proteins, tape measure proteins, rIIA-like protein, rIIB-like protein, and exonuclease. Annotating and analyzing this phage will further the understanding of basic phage biology. Understanding phage biology could also further developments in using phages as molecular biology tools or in phage therapy for humans with bacterial infections.

**10. Annotation of Cluster A1 Bacteriophages Tripl3t and Zeuska.** Haze Murphy\*, Rheaven Sandoval, Faith Cox, Matthew Bristerpostma, Leah Dowell, Tiffany Lujan, and Dustin Edwards. Tarleton State University

Bacteriophages Tripl3t and Zeuska were isolated from enriched soil samples incubated with *Mycobacterium smegmatis* mc<sup>2</sup>155 in 2013. Tripl3t was collected in Washington, DC by students at Howard University and sequenced at Virginia Commonwealth University Nucleic Acids Research Facilities, while Zeuska was collected in Providence, RI by students at Brown University and sequenced at Pittsburgh Bacteriophage Institute. Both bacteriophages have Siphoviridae morphology and contain a linear double-stranded DNA genome of approximately 53,500 base pairs with 63.7% G+C content and a 10 base 3' sticky overhang of 5'CGGATGGTAA3'. Annotation was performed by students at rural high schools in Tolar and Bluff Dale, TX as part of a science outreach activity with Tarleton State University. Bacteriophage Tripl3t and Zeuska genomes contains 91 and 93 predicted protein-coding genes, respectively, including for HNH endonuclease, terminase, lysin A, lysin B, integrase, DNA polymerase I, RNA polymerase sigma, phosphodiesterase, DNA primase, DNA methyltransferase, endonuclease VII, NrdH-like glutaredoxin, Cas4 family exonuclease, immunity repressor proteins.

**11. Annotation of Streptomyces Bacteriophage Animus.** Kevin Volkmann\*, Noah Cone, Subhayu Nayek, and Lee E. Hughes. University of North Texas

The bacteriophage Animus was isolated using *Streptomyces griseus* by Tony Du in 2018 at the University of North Texas. Animus was found in a soil sample from Carrollton, TX at 33.001746 North, 96.864174 West. On the day of collection, the soil was crumbly, dry, and dark-brown. The bacteriophage's plaques were between 0.5 and 1 millimeter and comet-shaped. It is uncertain whether Animus is a temperate phage, more testing is required. Upon performing an electron microscopy, the bacteriophage appeared to belong to the family Siphoviridae. Once isolated, Animus was sequenced at the University of North Texas. The sequencing of the bacteriophage was completed on 15 January 2019 using Illumina Sequencing. The bacteriophage was found to have a genome length of 50793 base pairs and a 67% GC content. The bacteriophage was classified under sub-cluster BD2. The genomic annotation of Animus began on 28 January, 2019. Of the 78 genes identified, 45 are forward and 33 are reversed. Forty-two of Animus's genes have no known function. The other 36 genes include functions such as: small subunit terminase, head-to-tail connector complex protein, single-strand-DNA binding protein, and helix-turn-helix DNA binding domain.

**12. Assembly, Finishing, and Annotation of Agrobacterium tumefaciens Bacteriophages Genomes.** Aaron Nguyen\* and Allison Wiedemeier. University of Louisiana at Monroe

With today's technology, DNA can quickly and accurately be sequenced through automated processes. However, the sequencer must read many short sequences of DNA simultaneously resulting in millions of randomly organized short reads. Due to each DNA strand's uniqueness and size, a single automated process cannot create an accurate complete sequence for every genome. As a result, human-mediated bioinformatics work must be done for each sequence in order to ensure a quality genome is produced and annotated. Using Newbler, Consed, AceUtil, DNA Master, BLAST, and HHPRED, a researcher can compile millions of short sequences and assemble a complete genome, check for quality, and mark it for possible protein calls. Of interest for this process are bacteriophage genomes. We have not even sequenced 1% of all bacteriophage genomes, so bacteriophages contain untapped genetic power. They also are relatively short, contain no large noncoding regions, contain many proteins with unknown function, evolve quickly, and are easy to isolate. In this study, *Agrobacterium tumefaciens* bacteriophages have been selected since only two phages have been sequenced with both having completely different attributes. Using sequencing reads from novel bacteriophages, this paper shall demonstrate and propose a protocol to effectively use the above programs to create a quality novel genome and call possible proteins.

**13. Bacteriophage Educational Laboratory Exercise Utilizing Raspberry Pi.** Rheaven Sandoval\*, Faith Cox, Tiffany Lujan, Dustin Edwards, and Keith Emmert. Tarleton State University

Incorporating meaningful real-world experiences into classrooms is a persistent challenge. Bacteriophages are an ideal tool for creating a hands-on collaborative learning environment for high school and undergraduate biology students. As bacteriophages lyse their host, they begin to form cleared areas of host cells called plaques. Bacteriophage plaque assays

are a common technique used in virology to visualize and quantitate replication, as well as measure lytic virulence. We have designed a laboratory exercise for high school and lower-level undergraduate courses that combines inexpensive computing devices and open source programming to visualize and quantitate actual or virtual bacteriophage plaque assays. Students assemble Raspberry Pi 3 Model B+ computing device, Raspberry Pi 7 inch Touch Display, and camera module V2 components and program with Python language to acquire time lapse images over a time course of 72 hours. Bacteriophage plaque assays are incubated above a backlit LED light board panel allowing plaque formation to be imaged in a field-of-view camera. A virtual plaque assay can also be performed in classes without laboratory access by using a variable size hole punch on paper to simulate plaque formation. Students are then able analyze the time lapse images of the plaque assay to quantitate plaque forming units and to determine bacteriophage virulence.

**14. Characterization of Streptomyces phage TomSawyer and the use of Bioinformatics in Genomic.** Brenda Banda\*, Morgan Marshall, Maxine Nguyen, Kevin Mariena, Adam Tesso, Michael Tran, Subhayu Nayek, and Lee E. Hughes. University of North Texas

In this laboratory, we isolate and analyze bacteriophages that infect *Streptomyces griseus*. For this study, Streptomyces phage TomSawyer was isolated by enrichment in Harrisonburg, VA in 2017 by Sawyer Kearns. This phage was found to be in the BE cluster and BE2 subcluster. TomSawyer was sent to the Pittsburgh Bacteriophage Institute, where it underwent DNA sequencing. It has a GC content of 49.3%. This phage also seems to be lytic. The genome contains 133961 bp with terminal repeats of 12182. We adopted the phage for genome annotation in our laboratory. We used bioinformatic software including Glimmer and GeneMark which predicted 262 genes. Each gene then had to be individually examined for the correct start and probable functions that it could have. In total we had two rounds of annotations, one primary annotation and a secondary review by another peer. The software we used was primarily PECAAN because it was a collection of most of the bioinformatic software we needed.

**15. Can CA Cluster *Rhodococcus erythropolis* Phages Infect *Gordonia terrae*?** Mallory Crawford, Anne Marie Hancock, Emily Bryant, ULM SEA PHAGES Program Students, Allison M.D. Wiedemeier, Christopher Gissendanner, and Ann M. Findley. University of Louisiana at Monroe

During the 2014-2017 academic years, ULM SEA PHAGES Program students isolated numerous CA cluster *Rhodococcus erythropolis* phages and a limited number of CB and Singleton phages from the same host system. Although the CA cluster of *Rhodococcus* phages share a high degree of sequence homology, there are some differences in the Phams to which many of the open reading frames (ORFs) belong. In an attempt to determine the possible broad host-range infectivity of these isolates, we have conducted spot tests of all *Rhodococcus erythropolis* phages (each with approximately the same phage titer) isolated by ULM phage students against the *Gordonia terrae* host system. Of the *Rhodococcus* phages tested, only four CA cluster phages (Angry Orchard, Bobby Dazzler, Dinger, Partridge) and a single unsequenced phage (Dangloo) produce a hazy area of clearing. Attempts to produce true infections of the *Gordonia terrae* host with these five phages have proven unsuccessful. A comparative Phamerator analysis of the CA cluster isolates is provided in an attempt to identify possible

reasons for the observed spot test results. (Funding was provided by the NIH-LBRN Administrative Supplement Award to ULM and the HHMI SEA PHAGES Program.)

**16. Comparison of *Gordonia terrae* CU Cluster Phages with *Gordonia terrae* Singleton Phage Catfish.** AnneMarie Hancock, Aaron Nguyen, Lauren Nguyen, ULM SEA PHAGES Program Students, Allison M.D. Wiedemeier, Christopher Gissendanner and Ann M. Findley. University of Louisiana at Monroe

During the 2017-18 academic year, ULM freshmen SEA PHAGES Program students isolated 24 novel bacteriophages using the *Gordonia terrae* host system. *Gordonia* phage Catfish was sequenced and determined to be a Singleton with no clear homology to any previously sequenced *Gordonia* phage. In functionally annotating the Catfish genome, highest BLAST hits for the entire genome and many of its functional open reading frames (ORFs) corresponded to members of the CU cluster of *Gordonia terrae* phages. We have since adopted *Gordonia* phage Dardanus for annotation (isolated by Florida Gulf Coast University). Dardanus is a CU3 cluster temperate phage which shares many of its functional ORFs with members of the CU1 cluster and Catfish. We present a comparative functional genome analysis of CU1 phages, the CU3 phage Dardanus, and the Singleton Catfish. Using DotPlot and Phamerator maps, we provide a picture of the relative genomic similarities for the entire genomes and individual ORFs of these phages. Moreover, using the Phage Enzyme Tool (PET), we show that sole reliance on the standard series of restriction enzymes to digest the DNA isolated from these phages (Bam H1, ClaI, EcoR1, HaeIII, HindIII) is of limited use in predicting subcluster designations within the CU group of *Gordonia terrae* phages. (Funding was provided by the NIH-LBRN Administrative Supplement Award to ULM and the HHMI SEA PHAGES Program.)

**17. Defense or Disguise: Implications of DNA Methylase Homologies in Phages and Soil-Dwelling Bacterial Species.** Christina E Spencer\*, Frederick N Baliraine, and Gregory D Frederick. LeTourneau University

Genome annotation of Phage Phalm (P1) revealed two genes with homology to methylase/methyltransferase (MTases) genes in other bacteriophage and soil-dwelling bacteria. MTase function in bacteria is known to be important to cell survival and other aspects of nucleic acid stability. MTases are enzymes which methylate specific bases within nucleic acid sequences of the host organism. MTases also function in restriction-modification systems by helping defend the host genome from its own restriction enzymes. While the functions of MTases are relatively well known in bacteria, the exact functions of MTases within bacteriophage genomes remain unelucidated. The location of the MTase genes suggests a hypothesis for the function in the phage genome. In Phage Phalm and other members of the P1 subcluster, such as Brusacoram and Shipwreck, MTases genes are located directly next to proteins of unknown function. However, a gene encoding an endonuclease or a helix-turn-helix DNA binding domain protein always exist within a few genes on either side of the MTase genes. Due to being located in a region of the genome related to genome maintenance, MTase's function in temperate bacteriophage is most likely related to protection of the phage genome inside of susceptible hosts. Specifically, in a phage, MTases most likely disguise the phage genome from the host's restriction enzymes, protecting the phage genome. Previous investigations indicate that methyltransferase is necessary for stable lysogeny. Additionally, characterization of phage-encoded MTase could have

relevance in host-range determination. NCBI BlastP analysis of genes 53 and 55 in Phage Phalm both align with multiple soil-dwelling organisms. These include *Mycobacterium* sp. UM\_RHS, *Mycobacteroides abscessus*, *Mycobacteroides salmoniphilum*, *Rhodococcus*, *Mycobacteroides chelonae*, and *Mycolicibacterium fortuitum*. The most prevalent alignment for both genes 53 and 55 is to MTase genes in *Mycobacteroides abscessus*. The results for gene 53 and 55 vary slightly in the organisms to which they are aligned with. The presence of the two MTases that have slightly variant bacterial homologues implies that Phage Phalm frequently inhabits bacterial species possessing more than one restriction system. Thus, acquired MTase genes are conserved within its genome, protecting future generations. This study describes the application of various bioinformatics tools, such as NCBI BlastP, Phylogeny.fr, and others, to elucidate plausible significance and function of MTase genes within bacteriophage genomes.

**18. Discovery and gene function analysis of Arthrobacter Phage NapoleonB.** Kathryn Adkins\*, Gabriel Andino\*, Sriram Avirneni\*, Emily Balint\*, Henry Burns, Lucy Fisher, Lauren Foley, Emily Gaw, Lily Goodman, Soo-Un Jeong, Cooper Johnson, Melissa Leon Norena, Michael Lum, Rachel Melone, Nathan Newton, Aman Patel\*, Sabin Patel, Lucy Potts, Brandon Reider, Shepard Saabye, Sona Subramanian, Joseph Yu, Justin Yu, Lathan Lucas, Leo Rule, and Aadil Sheikh. Baylor University

*Arthrobacter* is a type of bacteria commonly found in soil and sewage that can be utilized in bioremediation and in the degradation of pesticides. *Arthrobacter* sp. ATCC KY3901 was used as a host to isolate *Arthrobacter* phage. Bacteriophage are a genetically diverse important tool in biotechnology. This study was conducted to explore trends between soil composition and phage presence and to discover and sequence a bacteriophage. The isolation of 8 phage was accomplished by screening over 60 soil lysates using plaque assays and spot tests. Isolated phage were imaged using TEM and the DNA was extracted. PCR was used to determine the potential phage cluster. For one AM cluster phage, NapoleonB, the DNA was sequenced using Illumina sequencing. NapoleonB was discovered from soil near a Chinkapin Oak on the Brazos River on Baylor University. Annotations were manually curated using tools such as DNA Master, NCBI databases, PhagesDB, HHpred, and Phamerator. NapoleonB exhibits siphoviridae morphology and produces two distinct sizes of clear plaques with average diameters of 1.5 mm or 0.1 mm. The genome size of 57,846 base pairs. Annotations indicate 100 potential genes, 75 with no known function and 25 with predicted functions. Among these genes, both structural and regulator genes were predicted. The annotations for this genome will serve to further the understanding of bacteriophage diversity. Further bioinformatic analysis can be done to explore the intergenic regions and the structure and function of the predicted proteins.

**19. Functional Annotation and Comparative Analysis of Three *Gordonia terrae* Phages: Sombbrero (CS2 cluster), Catfish (singleton), and Dogfish (DT cluster).** Katherine E Feroben, Ivan A Alvarez, Misisipi Bhandari, Abby D Byford, Joe R Deselle, Quoc-Nghia N Duong, Ashlyn F Dupree, Minnisa E Garrison, Jeanelle L Higginbotham, Cade W Hunter, Beth A Knight, Jaden A Lee, Isabel C Lewis, Elizabeth L Long, Lauren Q Nguyen, Arohan Rimal, Connor C Roan, Salena Sinnasone, Jamuna Tandukar, Carl E Willis, Aaron V Nguyen, AnneMarie Hancock, Austin P Dicus, Grant E Gallien, Paul D Wiedemeier, Allison M Wiedemeier, Christopher R Gissendanner, Ann M Findley. University of Louisiana at Monroe

We have successfully isolated twenty-four phages that infect the *Gordonia terrae* host, three of which have been sequenced. *Gordonia* phage Sombrero is similar to other CS2 cluster members, has 76,485 bp with a direct terminal repeat of 201 bases, 110 open reading frames, one tRNA, and a GC content of 59.0%. Dogfish constitutes one of two members of the DT cluster of *Gordonia* phages (with Nyceirae). It has 41,907 bp with a 3' sticky overhang of nine bases, 56 open reading frames, and a GC content of 67.5%. Catfish is presently classified as a Singleton and shares a limited degree of sequence homology with the CU1 subcluster of *Gordonia terrae* phages. Catfish has 46,888 bp with a 3' sticky overhang of eleven bases, 79 open reading frames, and a GC content of 65%. We provide functional annotations of these phage genomes and explore their relationship to other *Gordonia* phage clusters using the SplitsTree, Gepard DotPlot, and Phamerator visualization tools. Such analyses provide insight not only into the relationship between the *Gordonia terrae* phages but can point to extended comparisons between other Actinobacter phage group isolates.

**20. Gene Annotations of the unique Phage McGalleon from Subcluster EA1 in host *Microbacterium foliorum*.** Danielle J. Davis\*, Elizabeth S. Hampton, Thanh-Huy Nguyen\*, Kirk S. Niekamp\*, Kaitlyn M. Riley\*, Carole M. Twichell, Jonathan N. Lawson, Bridgette L. Kirkpatrick. Collin College

Here we highlight the annotation of the siphoviridae bacteriophage McGalleon which was discovered in a soil sample from Forney, TX. The phage was isolated using the host *Microbacterium foliorum*. McGalleon is a member of cluster EA, subcluster EA1, with a genome length of 42,562 base pairs, the longest of the subcluster. McGalleon has a 63.7% G-C content, the 11th highest percentage within the subcluster. We used the bioinformatics tools DNAMaster, Glimmer, Genemark, PhagesDB Blast, NCBI Blast, HHPred, and Phamerator in this study. Of the 66 auto-annotated genes identified through auto-annotation, four genes were deleted due to lack of coding potential. Additionally, 26 of the 62 annotated genes had putative functions, with multiple regions that differ when compared to the 42 non-draft genomes within the subcluster. McGalleon does not contain a gene for a phosphoesterase which was present in all other members of subcluster EA1. Multiple features will require further analysis: Gene Product 2, an orpham with homology to only two other phages; two regions where phosphoesterase is expected based on its position in closely related phage but have no homology. Our annotation work on phage McGalleon highlights the detailed work that goes into genome analysis in order to advance potential phage therapy. We are excited to enter our annotation into public database so that it may be used for future reference and research.

**21. Isolation and Annotation of Streptomyces phage Bordeaux.** Brendon Williams\*, Subhayu Nayek, Hannah Klug, Lee E. Hughes. University of North Texas

Bacteriophages are the most abundant organisms on the planet, with estimations placing their numbers higher than all other organisms (including bacteria) combined, despite their relatively simple structures. Although there is an astoundingly high number of bacteriophages in the world, our knowledge on bacteriophages as a whole is relatively limited. Bacteriophages replicate by attaching to a compatible bacterial host cell and inserting the genetic material stored in their capsid. From here, it will initiate one of two life cycles, depending on the phage. The new genetic material will either integrate into the genome and be passed down to future

generations of the cell in the lysogenic life cycle, or immediately hijack the cell's protein synthesizing machinery and start producing and assembling phages until the cell bursts, releasing a number of new phages. This being the lytic life cycle. Once further research is done on bacteriophages, they may be considered as a form of treatment for bacterial infections. The SEA-PHAGES program is an initiative that studies bacteriophages, providing undergraduate students the opportunity to get experience in the field of research whilst helping fill out the roster of known bacteriophages. This poster will focus on the bacteriophage Bordeaux, isolated and annotated by the University of North Texas SEA-PHAGES program. The main bacterial host during the process of isolation was *Streptomyces griseus*, however a host range experiment showed the potential of the phage to infect a handful of other *Streptomyces* species. The phage created relatively large, clear plaques in the bacterial lawns used during the course of isolation, meaning it likely utilizes the lytic life cycle. In the later stages of isolation, an experiment was run to break down the protective capsid to extract viral DNA, which met the requirements to be sent to the main lab at the University of Pittsburgh to be sequenced. The sequencing process was successful and the genome was sent back to begin being annotated back at the University of North Texas. At the time of the creation of this poster, the genome (consisting of 250 possible coding genes and 45 tRNA genes) has gone through a primary and secondary annotation in the PECAAN program, and is awaiting further review. Approximately 53 of the genes showed enough evidence to make a call on the probable function of the gene, or what protein it likely codes for according to a host of computer algorithms.

**22. Isolation of Cluster EA2 Bacteriophage Finny.** Faith Cox\*, Stacy Luka, Tiffany Lee, Michaela Aguirre, Shey Andrews, Kayla Bahr, Abigail Ballard, Matthew Bristerpostma, Leah Dowell, David Kiker, Tiffany Lujan, Haze Murphy, Abigail Ramirez, Rheaven Sandoval, Kenneth Underhill, and Dustin Edwards. Tarleton State University

Bacteriophage Finny was purified from *Microbacterium foliorum* at 29°C in PYCa media by direct isolation method from a soil sample collected from a chicken coop in New Braunfels, Texas. Following initial isolation, two rounds of serial dilutions and plaque assays were performed for bacteriophage isolation and purification. Bacteriophage Finny plaque morphology consists of small-to-medium-sized lytic plaques with turbid halo rings. High titer lysate was stained with uranyl acetate to visualize bacteriophage Finny by transmission electron microscopy, which showed the virus has Siphoviridae morphology with an icosahedral capsid. DNA was extracted by a zinc chloride method and the whole genome sequenced at the Pittsburgh Bacteriophage Institute. Whole genome sequence comparison determined that bacteriophage Finny is a Cluster EA2 cluster with a circularly permuted genome 40,313 bp in length with 62.1% G+C content. Bacteriophage Finny genome contains 63 predicted protein-coding genes, including lysin A, holin, RecA-like DNA recombinase, AAA-ATPase, MazG-like nucleotide pyrophosphohydrolase, thymidylate kinase, and ThyX thymidylate synthase.

**23. Parameter Estimation and Simulation of Bacteriophage Infection Model.** Abigail Ballard\*, David Kiker\*, Shey Andrews, Michaela Aguirre, Matthew Bristerpostma, Dustin Edwards, and Keith Emmert. Tarleton State University

Bacteriophages are an emerging focus in research due to their potential use in treating antibiotic resistant bacterial infections. Tarleton State University, as part of the international

HHMI SEA-PHAGES bacteriophage discovery program, is working on an interdepartmental project between the biology and mathematics departments to create a mathematical model for the complexity of bacteriophage infection of host cells. We constructed a Compartmental Diagram using  $3n+1$  differential equations to model interactions between bacterial cells and bacteriophage, in which  $n$  different types of phages are capable of infecting a cell. Susceptible cells are represented as Class S. Class  $C_j$  represents those bacteria which have become infected with the  $j$ th phage where the bacteriophages are circularizing. Bacteria in Class  $C_j$  will then move to either a lytic or lysogenic cycle. Class  $I_j$  represents the cells infected by the  $j$ th bacteriophage and are currently following a lytic cycle. Class  $R_j$  represents the cells infected by the  $j$ th bacteriophage which are currently following a lysogenic cycle and exhibiting bacteriophage homoimmunity. We assume it possible to move from Class  $R_j$  to Class  $I_j$  when suitable environmental stress occurs. The Classes  $C_j$ ,  $I_j$ , and  $R_j$  are considered immune to infection by a different virus. Finally, Class  $V_j$  represents  $j$ th virus in the environment produced from lysis of infected cells. A simple mass-action interaction term,  $\delta \square \times \frac{1}{4} j S V_j$ , controls the creation of newly infected susceptible bacteria and removes spent virus. A single, constant carrying capacity is used to control the growth of all classes of bacteria. Local asymptotic stability criteria for selected equilibria are then derived. Currently, we are designing experiments to evaluate and verify the model that has been created. *Microbacterium foliorum* will be used as a host for bacteriophages to establish parameters which that can be used to verify the model.

## 24. Phylogenetic and Evolutionary Relationships of Small Genome Bacteriophages.

Matthew Brister-Postma\*, Abigail Ballard, Shey Andrews, Michaela Aguirre, Tiffany Lee, David Kiker, Kayla Bahr, Hunter Underhill, Leah Dowell, Abbigail Ramirez, Stacy Luka, and Dustin Edwards. Tarleton State University

Bacteriophages, viruses that can infect bacteria, are a unique and diverse category of viruses that show wide variability, from host range to genome length, even within their respective clusters. The goal of this study was to characterize bacteriophages that contain genomes of less than 20,000 base pairs, which include Clusters AN, BO, CW, EE, FE, DM, AX, and FD. MEGA X phylogenetic software was used to construct an initial phylogenetic tree to group 83 small genome bacteriophages, using the neighbor-joining method with a Tamura-Nei distance model. Genes were analysed using Phamerator, HHPred, NCBI Basic Local Alignment Tool-protein (BLASTp), and NCBI Basic Local Alignment Tool-nucleotide (BLASTn) to investigate possible gene functions and evolutionary relationships. Candidate genes were input into Venny software, which determined that two cluster BO bacteriophages share a LysM-like endonuclease which is also found in the cluster BE bacteriophages, which infect plant pathogenic bacteria. This result suggests a common ancestor for these two clusters of bacteriophages, and we plan to further categorize this result with further phylogenetic analysis. Likewise, both cluster AN and FE bacteriophages, which primarily follow lytic cycles, have a unique MerR-like Helix-turn-Helix binding domain, similar to the more temperate cluster A and cluster F bacteriophages. Geospatial analysis is currently being conducted to determine the effect of geographic distribution on genetic variability by using an interactive map with data from the Actinobacteriophage Database. This study could provide insights in bacteriophage evolution and their environmental roles.

**25. Preliminary Annotation of the *Gordonia terrae* CU3 Cluster Phage Dardanus.** Breanna Gottschalck, Austin Perkins, ULM SEA PHAGES Program Students, Anne Marie Hancock, Mallory Crawford, Christopher Gissendanner, and Ann M. Findley. University of Louisiana at Monroe

We have adopted *Gordonia terrae* phage Dardanus (isolated by Florida Gulf Coast University) for annotation. Dardanus, the sole member of the CU3 cluster of currently-sequenced *Gordonia* phages, has 43,143 bp, 74 ORFs, no tRNA genes, and a GC content of 66%. Dardanus shares considerable sequence homology with CU1 cluster *Gordonia* phages and the *Gordonia* phage Catfish, presently classified as a Singleton. Catfish has 46,888 bp with a 3'™ sticky overhang of eleven bases, 79 open reading frames, and a GC content of 65%. We provide a functional annotation of Dardanus and explore its relationship to CU1 cluster *Gordonia* phages and the Catfish singleton using the SplitsTree, Gepard DotPlot, and Phamerator visualization tools. Such analyses provide insight not only into the relationship between the *Gordonia terrae* phages but can point to extended comparisons between other Actinobacter phage group isolates.

**26. Preliminary Results of Bacteriophage Infection on Static Biofilm Production in *Agrobacterium tumefaciens*.** Darrell Bailey Mabou\* and Allison Wiedemeier. University of Louisiana at Monroe

Biofilms are communities of single-celled organisms living together to provide nourishment and protection for the entire group. Cells lie within an extracellular polymeric matrix which is produced at least in part by members of the community. The matrix allows the cells to adhere to each other, anchor to surfaces, easily trap nutrients, and survive in otherwise harmful conditions. Cells growing in a biofilm are physiologically distinct from planktonic individuals. This phenotypic shift can be triggered by a variety of factors, including exposure to low levels of antibiotics. In the biofilm phenotype the organisms express different genes than planktonic cells would, which can destroy or inhibit targets of many classic antibiotics, thus increase their resistance. Bacteriophages, viruses that infect bacteria, can infect biofilm bacteria and compromise their defenses, thus lowering the total biofilm mass. *Agrobacterium tumefaciens* (now *Rhizobium radiobacter*), the causative agent of crown gall disease in many crops, is the bacteria used for this study. A growth curve of *A. tumefaciens* over 36 hours was measured. In addition, the amount of biofilm was measured by staining the biofilm with Gentian violet on a 96 well plate after 24, 48, 72, and 96 hours of growth as a baseline measurement. We then added specific phage lysates to the forming biofilm and analyzed the total biofilm mass. We expected lytic phages to reduce overall bacteria number in the biofilm, thus reducing biofilm mass reported by absorbance. Results are reported in this study.

**27. Isolation and Annotation Streptomyces phage Celia, First Member of Cluster BD6.** Julia Lopez\*, Katherine N. Ball\*, Subhayu Nayek, Swapn Bhuiyan, Austin Sivoravong, Lee E. Hughes. University of North Texas

In Fall of 2018 the phage Celia was isolated from a soil sample in Denton, TX, using *Streptomyces xanthochromogenes* as the host. We used a series of protocols to isolate the phage, and then proceeded to extract the DNA. After the DNA was extracted, we used Gel Electrophoresis of Restriction Enzyme Digests and through this protocol we were able to get a

successful gel. Using the gel, we were able to compare it to other phage clusters to figure out which one Celia belongs to. Celia's genome was then sequenced at the Pittsburgh Bacteriophage Institute. This phage's DNA was sequenced using the Illumina Sequencing method; then, we began the annotation process. Celia's genome is 50493 bp long, with 77 ORFs, and one tRNA. The phage was placed in the BD cluster, however, its genome was different enough to warrant its own subcluster, BD6. We used several bioinformatic tools to determine the functions of Celia's ORFs, and found that 37 ORFs had known functions. We determined based on the EM picture of Celia that it is a siphoviridae phage, which means it has a long non-contractile tail, and a non-enveloped head.

**28. The Discovery and Annotation of Streptomyces phage Limpid.** Kimberly Anderson\*, Kate Summerhill\*, Carson Nick, Taeyah Roberts, Maiya Webster, Brandt Smith, and Lee E. Hughes. University of North Texas

Bacterial phages are the most abundant and diverse biological entities that infect a variety of bacterial hosts throughout the biosphere. Their ability to disrupt and lyse specific bacterial host cells are one of the main reasons they are being researched as a potential solution to the increases in antibiotic-resistant bacteria. Streptomyces phage Limpid was isolated using the host bacterium *Streptomyces sanglieri* by following the HHMI Phage Discovery Guide protocols. The phage was isolated and showed that it appears to have a lytic life cycle. We obtained an HTL titer of  $4.8 \times 10^9$  pfu/ml and found that Limpid was able to infect 6 different bacterial host. After isolation, Limpid was sent for further analysis and sequenced by Pittsburgh Bacteriophage Institute. The sequence showed that Limpid is a member of Cluster BK, subcluster BK1, and has a GC content of 47.6%. Whole genome comparison shows Blueyedbeauty and Annadreamy as two similar phages, with Annadreamy being the closest in relation. Annotation of the whole genome confirmed this similarity with most of the genes matching these other two phages as well as Sparkle Goddess and Comrade. Most of these confirmed genes had proteins with an unknown function; however, analysis of confirmed functions showed matches to proteins with unknown functions. Some genes show matches to functions related to the phages structure, various enzymes for replication, and binding proteins. After careful isolation and analysis of the bacteriophage Limpid, it found that this virus is a unique member of Cluster BK1.

