Discovery and Annotation of *Microbacterium foliorum* phage Juicer and *Gordonia rubripertincta* phage GiKK.

Brahim Azeni, Merry Bilbo, Robyn Cashins, Cristina Gonzalez, Nick Migneault, Mariah Panza, Serjio Trevino and Amy B. Sprenkle, PhD Salem State University, Salem, MA, 01970 USA asprenkle@salemstate.edu

Abstract

Salem State was fortunate to be able to run the Phage Discovery semester of SEA-PHAGES in person in the Fall of 2020 under COVID-19 compliant protocols. Two actinobacterial hosts were used; *Microbacterium foliorum* and *Gordonia rubripertincta*. Student enriched soil samples yielded a number of phages on both hosts. We were able to amplify and purify two unique phages on each host, and finally send both DNA samples for full genome sequencing at the University of Pittsburgh. *Microbacterium* phage Juicer is a lytic *siphoviridae* in cluster EA6 at 41099 base pairs in length and displays typical genome organization. Genome annotation is accomplished by comparison to several close relatives. *Gordonia* phage GiKK is a lytic *siphoviridae* in cluster CT at 47537 base pairs and also has typical synteny and close relatives to compare for annotation during the Bioinformatics semester of SEA-PHAGES at Salem State University.

Introduction

The '20/'21 academic year was a year like no other, and at Salem State we dove into face-to face phage discovery labs in the fall and a remote bioinformatics semester in the spring. The physical distancing required in our lab spaces made for an excellent de-densified learning environment where the student/faculty/TA ratio was ideal. The twice weekly meeting time recommended for phage discovery allowed us to divide the students into two cohorts that would occupy the same bench space on different days, and share results remotely, via an electronic OneNote laboratory notebook. The instructor could also keep a master lab notebook data and protocol repository to accommodate absence due to isolation/quarantine. Salem State (Cohort 11) has so far annotated 6 phages, two from the genome exchange during the inaugural semester of bioinformatics, and 4 phages isolated by students in the program, three from two different Actinobacterial genera, *Microbacterium foliorum*, and *Gordonia rubripertincta*. *M. foliorum* SEA B-24224 was isolated from grass in Germany and is one of more than 90 species that are in the genus *Microbacterium*, which are small Gram-positive rods, giving them their diminutive name. They are found in some cheese rinds, lending to the distinctive flavor of the cheese. *G.rubripertincta* is also a Gram-positive rod-shaped soil bacterium, but is more acid-fast in nature and more closely related to *Mycobacterium spp.*. Gordonia species are attractive for use in bioremediation and biotechnology and are also known to have caused opportunistic infection in humans. We continue to accumulate data on phage diversity in the phylum Actinobacteria in hopes of being able to apply this to future understanding of phage biology and for use in treating antibiotic resistant actinobacterial infections through phage therapy[1].

Methods

The SEA-PHAGES program has OER in the form of the Phage Discovery and Phage Genomics Guide[2]. All phages resulting from the fall semester discovery were from enriched cultures which were then purified, amplified and characterized at the DNA level to insure that they were unique phages. TEM samples were prepared by negative uranyl acetate staining and grids sent to UMassMedical EM facility[3], and DNA samples were sent to Pitt for sequencing. Analysis of the genomes returned via FASTA file from phages.db[4] begins with auto-annotation of coding sequences and collection of BLAST data. Phamerator[5] is a data driven graphical representation of the sequence data from PhagesDB. It provides comparative genomics analysis of annotated phage genomes. Finally, PECAAN (Phage Evidence Collection And Annotation Network)[6] is a web-based platform that draws together multiple analysis tools for genome analysis in an web-based format.

Results

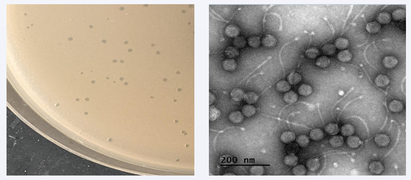

Figure 1: *Gordonia* actinobacteriophage **GiKK** plaque morphology (left) and TEM image (right).

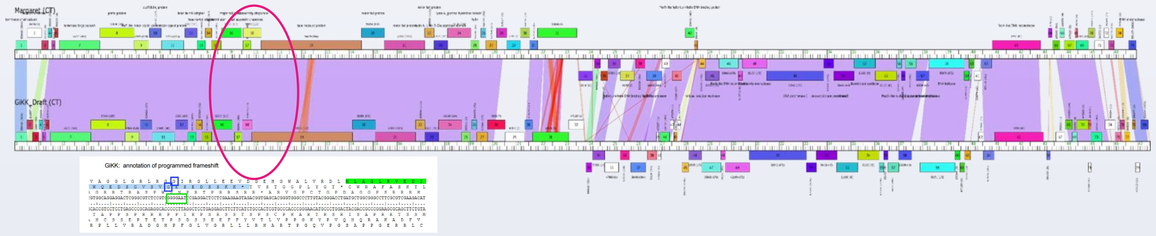

Figure 2: Phamerator map of **GiKK** (47,537 bp) and its closest CT cluster relative, Margaret. Annotation of the programmed translational frameshift of the tail assembly chaperone below the red oval.

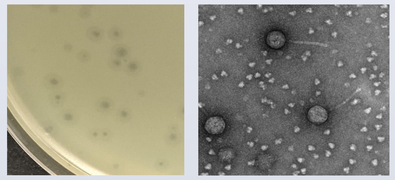

Figure 3: *Microbacterium* actinobacteriophage **Juicer** plaque morphology (left) and TEM image (right).

Figure 4: Phamerator map of **Juicer** (41,099 bp) and its closest EA cluster relative, Chepli. Annotation of the programmed translational frameshift of the tail assembly chaperone below the red oval.

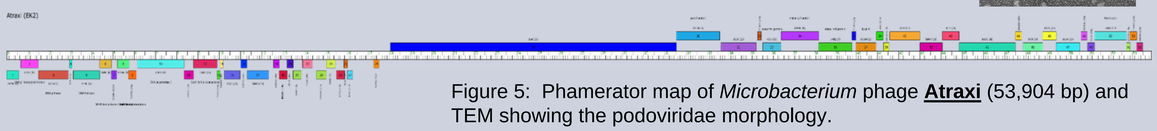

Figure 5: Phamerator map of *Microbacterium* phage **Atraxi** (53,904 bp) and TEM showing the podoviridae morphology.

Discussion

Salem State added a new host to our phage hunt this year, and discovered the lytic siphoviridae with a long tail (240 nm) on *Gordonia*. **GiKK** (CT, 47537 bp) showed the typical organization with the structural genes in the left arm and the control genes in the right. An exception to synteny in cluster CT phages is that the lysin B occurs in the right arm, rather than in a lysis cassette with lysin A and holin.

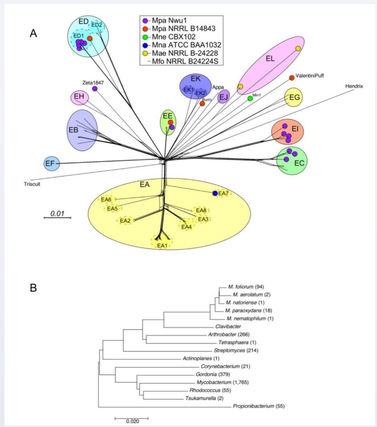

Figure 6: Figure from Jacobs-Sera et. al[7] showing phylogeny of the Microbacterium phages and their relationships to other Actinobacteria.

Juicer (EA6) was discovered on *Microbacterium*, and is a lytic siphoviridae with a shorter tail than GiKK, at approximately 175 nm on average. The sequence of Juicer displayed the typical synteny and tail assembly chaperone programmed translational frameshift. During AY 19/20, we isolated another actinobacteriophage, **Atraxi** (EK2) shown in Figure 5. Atraxi was unusual because it was a podoviridae, which lacks the long flexible tail of the siphoviridae. Atraxi also demonstrates unusual genome structure, with a large gene of unknown function in the forward direction in the center of the genome. The structural genes are in the right arm, rather than the left, and the large gene is not tape measure. There were no obvious genes indicating a temperate life cycle, but the cluster life cycle is listed as unknown in phagesdb. Atraxi had slow-forming, cloudy plaques and an area of future study is to continue to explore the life cycle of this unusual phage.

References

1. Dedrick, RM et. al. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant *Mycobacterium abscessus*. Nature Medicine 2019 Vol 25(5):730-733. doi: 10.1038/s41591-019-0437-z
2. https://seaphagesbioinformatics.helpdocsonline.com/home
3. https://www.umassmed.edu/cemf/
4. https://phagesdb.org/
5. https://phamerator.org/
6. PECAAN https://discover.kbrinsgd.org
7. Jacobs-Sera D, et.al. Genomic diversity of bacteriophages infecting Microbacterium spp. PLoS One. 2020 Jun 18;15(6):e0234636. doi: 10.1371/journal.pone.0234636. PMID: 32555720; PMCID: PMC7302621.

Acknowledgements

Thank you to:

- Graham Hatfull and the HHMI SEA for valuable mentoring and assistance.
- the members of the Fall 20 BIO 132 Phage Discovery Lab: JJ Castillo, Arlyn Cosme, Nomar Fernandez, Israa Kadri, Shanta Khatun, Gennadiy Kolesnichenko, Madelaine Kutscheke, Carolyn Lamontagne, Rosa Martinez, Jay Monroe, Emerald Moretti, Marita Nevins, Julieris Payero Nunez, Spencer Wightman
- Alyssa Palladino, BS BIO '21, Teaching Assistant