Characterization and Classification of a Montana Mycobacteriophage

Undergraduate Research Project Proposal

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Hypothesis

Thorough characterization of a newly discovered mycobacteriophage will reveal unique properties compared to known bacteriophages.

Background

A bacteriophage, also called a phage, is defined as a virus that infects bacterial cells. Phages are the most numerous viruses on Earth. There are approximately 10^{31} bacteriophages on Earth (Wommack and Colwell, 2000).

Bacteriophages have three main structural features: a capsid, genetic material, and a tail (SEA-PHAGES, n.d.). Bacteriophages are smaller than the bacteria they infect; the capsid diameter of the phage depends on the size of its genome. The capsid is the head of the bacteriophage. The genetic material, typically double stranded DNA, is contained inside the capsid. The tail is the part of the bacteriophage that attaches to the bacterium. The tail is a hollow tube through which the genetic material travels to enter the bacterium.

Bacteriophages that infect bacteria in the phylum Actinobacteria are recorded in the Actinobacteriophage Database at www.phagesdb.org. Included in this website are mycobacteriophages. In 2001, Dr. Graham Hatfull and Dr. Roger Hendrix of the University of Pittsburgh allowed two high school students to participate in a study in which bacteriophages were isolated. The study was a success. Dr. Hatfull, Dr. Hendrix, and colleagues began a program involving students in discovering and isolating bacteriophages. The program has grown greatly since it recieved funding from the Howard Hughes Medical Institute. In the fall of 2015, there were 80 undergraduate institutions participating in the Science Education Alliance- Phage

Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program, encompassing approximately 3,000 undergraduate students annually. Additionally, there have been over 4,000 middle and high school students who have participated in the SEA-PHAGES program. As of April 17, 2016, there were 7,904 bacteriophages in the database ("The Actinobacteriophage Database," 2016). Of the 7,904 phages, 1,500 have been fully sequenced and classified into genomically similar clusters.

Dr. Marisa Pedulla conducted her doctorate research in Dr. Hatfull's laboratory at the University of Pittsburgh. She was involved in the phage programs early on at the university. Dr. Pedulla now is the head of an outreach program called "Bringing Research into the Classroom (BRIC)" in which she travels to Montana middle and high schools every Thursday. This program is an offshoot of the Pittsburgh program. Funding for BRIC was awarded to Dr. Pedulla as a Science Education Partnership Award (SEPA) from the National Institutes of Health (NIH), grant number 1R25OD016533-01. During the outreach program, students bring water or soil samples to school, and the presence of bacteriophages that infect *Mycobacterium smegmatis* is tested. If a bacteriophage is found in the sample, the student is allowed to name the phage. The newly found phage is isolated and purified, and is then able to be added to the Actinobacteriophage Database. Since January 2015, there were approximately 4,000 Montana high school students involved in the BRIC program, bringing the total number of students served by Montana Tech outreach phage discovery to more than 7,000.

Currently, Dr. Pedulla has dozens of uncharacterized bacteriophages in her collection. Initial purification and addition of bacteriophages to the Actinobacteriophage database is planned for the summer of 2016.

The proposed Undergraduate Research Project involves adopting, purifying, sequencing, and characterizing one of the bacteriophages in Dr. Pedulla's collection.

Methods

According to the www.phagesdb.org , there are seven major steps in the research experience. The first step is "isolation" of bacteriophages from the environment. The second step is "purification" of the phages through multiple rounds of plaque purification. The third step is "amplification" of the phage to generate high-titer stocks. The fourth step is "extraction" of the phage DNA. The fifth is "characterization" of the phage DNA through restriction digests. The sixth step is "sequencing" of the phage, which is performed in a specialized laboratory. The final step is "annotation" of the bacteriophage through comparison of the phage's genome to other known phage genomes and all sequences in GenBank ("The Actinobacteriophage Database," 2016).

Dozens of bacteriophages have been collected and isolated. For the adopted phage, the first step will be purification through multiple rounds of plaque purification. First, the bacteriophage will be plated, or used to infect *M. smegmatis* on Petri dishes, in order to determine the morphology of the resultant plaque.

Next, a high titer stock of bacteriophage will be amplified, and then its DNA will be extracted using phenol/chloroform. A restriction digest of the phage DNA will be performed in order to determine how the "cut" DNA compares to DNA of phages in the database. Next, the polymerase chain reaction (PCR) will be used for preliminary determination of cluster assignment. A phage cluster is a group of bacteriophages with similar genetic properties. Comparison of 627 bacteriophage genomes resulted in 28 different clusters (Pope et al., 2015).

Phage clusters can be determined by a set of primers used in PCR to determine genetic similarities to sequenced bacteriophages (Smith et al., 2013). When an electrophoresis gel is run after PCR has been completed, a band of DNA will be present if the phage matches the cluster. If no band is present, the phage does not match the phage cluster.

Three common structural morphologies of bacteriophages are Myoviridae, Podoviridae, and Siphoviridae. Determination of the bacteriophage's structural morphology will be done by viewing the phage under a transmission electron microscope at the University of Montana.

DNA of the adopted bacteriophage will be sent to a laboratory for the DNA sequence to be determined. Once sequenced, the DNA sequence will be annotated, meaning putative protein-coding and functional RNA-coding genes will be identified and described in relation to other known sequences (Russell, 2010), and submitted to a genomic database such as GenBank.

Findings will be presented in poster format in spring, 2017.

Preliminary Data

I am currently enrolled in Dr. Pedulla's General Genetics Laboratory. For one experiment, students collected environmental samples and attempted to isolate bacteriophages from the samples. No mycobacteriophages were isolated from the samples; however, Dr. Pedulla has dozens of bacteriophages available for adoption and classification.

Timeline

Table 1. Timeline for Proposed URP

Task Name	Duration	Start	Finish
Choose Phage	1 day	Mon 8/29/16	Mon 8/29/16
Purify Phage	20 days	Mon 8/29/16	Fri 9/23/16
Amplify Phage	15 days	Mon 9/26/16	Fri 10/14/16
Titer Lysate	3 days	Mon 10/17/16	Wed 10/19/16
Electron Microscopy	2 days	Thu 10/20/16	Fri 10/21/16
Isolate DNA	2 days	Mon 10/24/16	Tue 10/25/16
Restriction digests	2 days	Wed 10/26/16	Thu 10/27/16
PCR	2 days	Mon 10/31/16	Tue 11/1/16
Update Database	1 day	Wed 11/2/16	Wed 11/2/16
Isolate Lysogens	25 days	Thu 11/3/16	Wed 12/7/16
Sequence DNA	14 days	Thu 12/8/16	Tue 12/27/16
Annotate DNA Sequence	60 days	Mon 1/9/17	Fri 3/31/17
Test Immunity	35 days	Mon 1/9/17	Fri 2/24/17
Prepare Poster	14 days	Mon 2/27/17	Thu 3/16/17
Present Poster	1 day	Sat 4/1/17	Sat 4/1/17

The proposed timeline spans two academic semesters, Fall 2016 and Spring 2017. The duration for the events is measured in weekdays.

Budget

Table 2. Itemized Budget for Proposed URP

Laboratory Supplies	**Cost per Unit**
Petri Dishes	$134.92 / 500 plates
Promega GoTaq Hot Start Polymerase: Green Master Mix, 2X	$110.80
7H10 Agar	$178.80 / 500g
7H9 Broth	$162.10 / 500g

The estimated costs of the laboratory supplies were retrieved from https://www.fishersci.com/us/en/home.html.

The cost of travel to The University of Montana in Missoula, Montana to perform transmission electron microscopy is included below:

120 miles X 2 (round trip) X $\frac{\$0.39}{mile}$ = $93.60

Total Proposed Budget = $680.22

Any expenses exceeding $300 will be paid for with funds from Dr. Pedulla's indirect or research accounts.

Summary

I propose to adopt a bacteriophage isolated through Dr. Pedulla's BRIC program. I will adopt, purify, amplify, and characterize one of the bacteriophages in Dr. Pedulla's collection. If time allows, I will sequence, analyze, and annotate the bacteriophage DNA sequences and submit the bacteriophage to a genomic database.

References

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