Isolation of a Novel Lytic and *Siphoviridae* Mycobacteriophage Using *Mycobacterium smegmatis* mc^{2}155

Michael Dempsey, Aaron Levy
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Abstract:
Usually, antibiotics are used as treatment for bacterial infections, but some bacteria are resistant due to antibiotic overuse. Phages are able to infect and destroy bacteria, meaning they can be used as alternative treatments. Phage treatment in the United States is conducted under compassionate use. While there have been plenty of phages that have been put in a database, many remain unidentified. Through enrichment, purification, and the formation of a high titer lysate, we were able to successfully isolate a novel species of phage that was located in the soil at Williams Village Campus in Boulder, Colorado (39°59'54.5"N 105°14'53.4"W). Enrichment produced 0.5-1 mm plaques that were barely visible. The purification plate contained clear, well-defined plaques that were 10-15 mm in diameter. Our phage had a head that was about 63 nm in diameter and a tail that was about 150 nm. Additionally, the resulting 10 mm plaques were clearly defined, indicating that it was lytic. More phages will be isolated in the future, growing the ever-expanding database. Hopefully, our phage will one day be considered a candidate to treat antibiotic resistant bacterial infections.

Background:
Phages, which are viruses, are found within soil and bodies of water and are known to infect and kill bacteria, with each species of phage being specific to one type of bacteria that it is able to kill. A typical phage consists of 3 distinct structures, a head which contains its viral DNA, a tail that allows it to attach itself to a host bacteria, and tail fibers which allow the phage to inject its DNA through the host bacteria's cell wall to infect it. There are two major classifications of phages, lytic or the temperate, which undergo two distinctive life cycles: lytic or lysogenic.

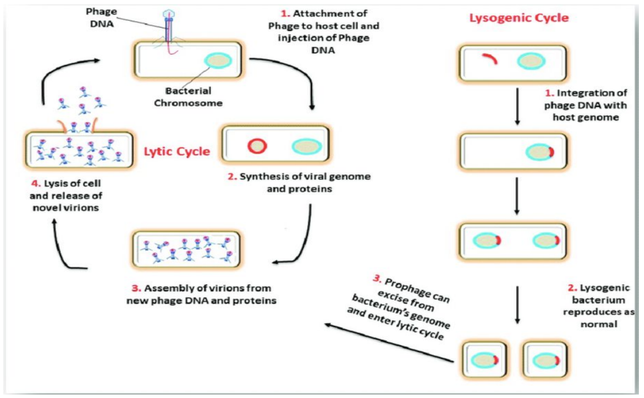

Figure: Lytic Versus Lysogenic Phage Life Cycle:
The rise of antibiotic resistant bacteria, and the inability of antibiotics to treat these bacteria, have increased the relevancy of phage research as phages are able to kill these antibiotic resistant bacteria when antibiotics fail through a process known as phage therapy.

Results:

Figure 1: Plaques 0.5-1 mm in Diameter Formed After Enrichment of Soil Sample:
A soil sample, LB media, and M. smeg bacteria solution was incubated and filtered using a 0.2µm filter. The solution was then mixed with M. smeg bacteria and top agar on an L-agar plate. This is the resulting plate after enrichment. Little (0.5-1mm), barely visible plaques were spread across the plate. Small white dots appeared in the centers of some of the plaques.

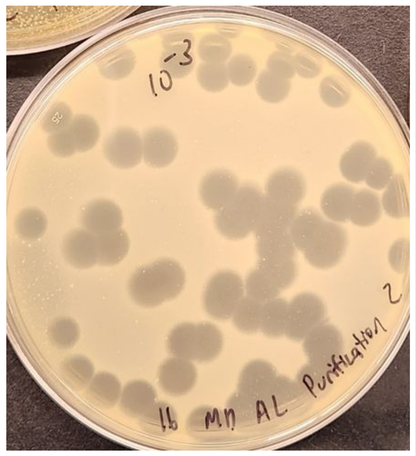

Figure 2: 10-15 mm Uniform Plaques Were Formed After Second Round of Purification:
A sample was collected from the spot test plate for the purification process. For the first round of purification, four 1:10 serial dilutions were performed. Then, a sample from the most dilute plate that had plaques was taken, and four more 1:10 serial dilutions were performed. This was the resulting 10^{-3} plate. The plaques appeared uniform and well-defined. There were about 55 plaques on the 10^{-3} plate. Some plaques overlapped, so getting an exact amount was difficult. The plaques were approximately 10-15 mm in diameter.

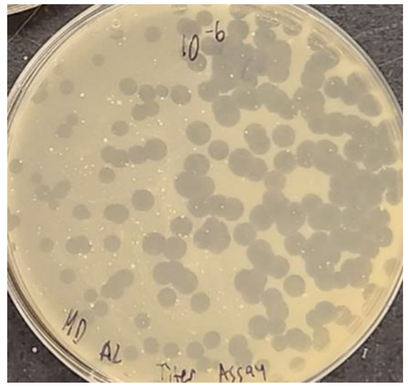

Figure 3: ~160 10 mm Plaques Formed On 10^{-6} Plate While Calculating the Titer:
Nine 1:10 serial dilutions were performed on the high titer lysate, and the 10^{-6} dilution was plated. The plate had approximately 160 non-cloudy plaques, which were all about 10 mm in diameter. This yielded a titer of $1.6*10^{10}$ pfu/mL. There was some variation in the size of the plaques, and there were more plaques on one side of the plate.

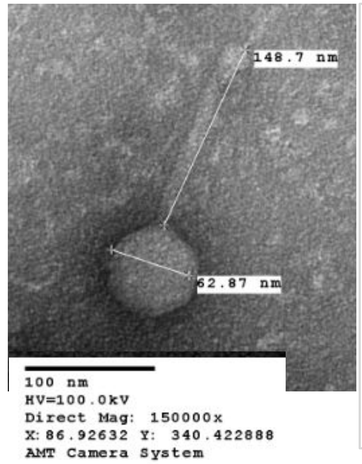

Figure 4: Phage Head Size Approximately 63 nm and Tail Length 150 nm
10µL of the high titer lysate was absorbed onto a fresh grid, which was then washed twice by adding 10µL of sterile water. 10µL of 1.0% uranyl acetate was added to the grid. This is the resulting image of the phage. The head size is about 63 nm in diameter, and the length of the tail is about 150 nm.

Conclusions:
- Phage was observed to be a lytic phage due to the clear, defined nature of the plaques that it formed
  - lytic phages form clear plaques while temperate phages form cloudy plaques
- Phage identified as a *Siphoviridae* as EM imaging revealed its head to be approximately 63 nm and a long, flexible tail with a length of approximately 150 nm
  - *Siphoviridae* are known for having longer and more flexible tails than *Myoviridae* phage
- The titer of this phage was $1.6*10^{10}$ pfu/mL as 160 plaques formed on HTL plaque assay dilution plate 10^{-6}
- By isolating this bacteriophage, we have added to the ever-expanding database, adding another potential treatment for bacterial infections

Future Directions:
In the future, more tests could be performed on our isolated phage, including performing a restriction digest and PCR. Cytotoxic genes could be identified, helping us gain a better understanding of how much a phage's genome contributes to the lysing of bacterial cells. Additionally, the phage cluster could be identified, and the close relatives of our phage could be analyzed. We could then look at the characteristics of the close relatives and test to see if our phage has similar characteristics. This could then be used to formulate ideas regarding the evolution of phages.

References
Cisek, A. A., Dąbrowska, I., Gregorczyk, K. P., & Wyżewski, Z. (2017). Phage Therapy in Bacterial Infections Treatment: One Hundred Years After the Discovery of Bacteriophages. Current microbiology, 74(2), 277–283. https://doi.org/10.1007/s00284-016-1166-x
Liu, Steven (2014) "Characterizing the response of multidrug-resistant Klebsiella pneumoniae species to the application of a phage cocktail," Symposium: Vol. 1: Iss. 1, Article 2. https://doi.org/10.15368/symp.2014v1n1.1
Stone, Edel & Campbell, Katrina & Grant, Irene & Mcauliffe, Olivia. (2019). Understanding and Exploiting Phage–Host Interactions. Viruses. 11. 567. 10.3390/v11060567.

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