Thermostable Phage at Gonzaga University

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INTRODUCTION

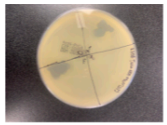
Bacteriophages are viruses that infect bacteria. There is an incredible amount of bacteriophages that inhabit the Earth. It is estimated that there are 10^{31} types of phage. While phages have been around for a very long time, they were only formally discovered about 100 years ago by Frederick Twort and Felix D'Herrelle (Travis, 2003). It was D'Herelle who was the first person to characterize the nature of bacteriophages by experimentation using plaque assays (Poxleitner et al., 2017). Researchers still use D'Herelle's techniques today in bacteriophage research. Learning more about bacteriophages is important because they can be the key to unlocking new medicine. Discovering a bacteriophage that can kill bacteria harmful to humans or flora could be revolutionary. Phage therapy has become an increasingly popular treatment method for various diseases as an alternative to using antibiotics. Phages also help scientists learn more about gene structure, function, and mutation due to their simple structure.With only about 2×10^3 bacteriophages discovered, there is so much potential that has yet to be reached (Poxleitner et al., 2017). The phage that we are researching comes from 47.66837° N, 117.40328° W in Spokane, Washington. The environment had wet soil and the temperature was 34°F. We are growing our phage in the bacterial host *Microbacterium foliorum.*

OBJECTIVE

To isolate a unique phage that has not been discovered and learn about how it behaves in various environments.

METHODS

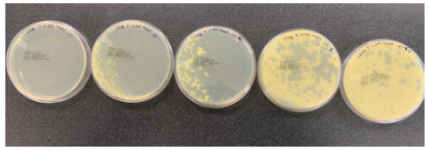
Collection of Phage: We collected three environmental samples in hopes to collect a phage. We collected samples from the following locations. Sample 1: Collected from 47.66837° N, 117.40328° W, wet



In sections 1, and 3 the *M. foliorum* was killed, indicating the presence of phage. They looked different than the control section which was full of *M. foliorum.* Sections 1 and 3 had medium-sized translucent spots that with no *M. foliorum.* The edges were curved and have an irregular shape. Sample 3 was hazier than Sample 1. Because of that, we decided to continue with the phage present Sample 1.

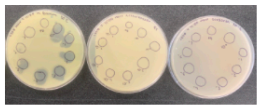
Isolation of Phage:

We performed serial dilutions to isolate our phage. Serial dilutions were a helpful tool for phage isolation because the end result was a diluted plate with separated plaques. Individual plaques indicate a single type of phage present in the host *M. foliorum*. We did this by preparing liquid phage samples of decreasing concentrations and plating them on to plates with the host *M. foliorum*.



In this image, each of the plates decreased in the amount of spots and plaques. The plaques were small, round and had defined edges. The plaques present on the 10^{-3} and 10^{-4} plates had the same morphology, which means that we isolated a single type of phage.

Testing for New Characteristics:



We tested to see if our phage would grow in the alternate hosts *Gordonia rubripertincta* or *Arthobacter globiformis*, as well as testing if it would grow at 30 degrees C. We did this by creating plates with the new hosts as well as storing the a plate with M. foliorum as the host in the 30 degrees C condition. Our plates are shown in the image.

RESULTS

Our Plaque Morphology

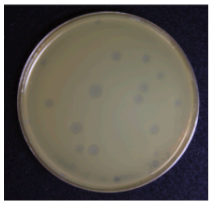


Figure 1: In its control state and at the concentration 10^{-1}, our phage can clear a full plate of the host *M. foliorum.* When at the concentration 10^{-2}, it forms a webbed plate. At 10^{-3} and 10^{-4}, individual plaques start occurring. Our phage form small, round plaques that have defined edges. We never observed any haziness in the plaques or unusually large plaques throughout our research process.

Our Phage is Thermostable

M. foliorum RT *M. foliorum* 30C *A. globiformis* RT *G. rubripertincta* RT

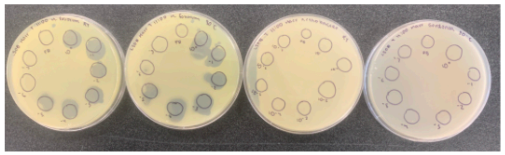


Figure 2: Our phage thrived on the *M. foliorum* plates. On the *M. foliorum* plate that was kept at RT, the phage had spots all the way out to 10^{-5}. On the plate kept at 30 degrees C, there was a plaque in the 10^{-4} spot. There was no growth on the Gordonia rubripertincta or Arthobacter globiformis plates. It was predicted that our phage would not be able to occupy new hosts. However, our phage was able to grow in 30 degree C conditions, which we did not originally expect. In fact, our phage grew slightly better in 30 degrees C. This meant that our phage likely prefers hotter temperatures than room temperature. Because there was no growth on the Gordonia rubripertincta or Arthobacter globiformis plates, this indicated that our phage is not able to occupy those hosts. It is possible that there are other hosts our phage could occupy, just not these ones.

Gel Electrophoresis Reveals EE Family

Ladder 3 (plaque pick)

1000 bp -
820 bp -
500 bp -

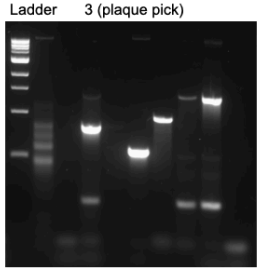


Figure 3: The image above is a picture of our Gel electrophoresis. The bar on the left is the ladder, and the lowest ring is 500 bp. Our plaque pick is the third lane in, and is highly amplified at 820bp. The EE control did not work because there was a preparation error, but it would have been amplified at 820 bp. Even though the EE control did not show up it would have been amplified at 820 bp. Our plaque pick sample was amplified at 820 bp, meaning that it is a part of the EE family of phage. In the lanes to the left of our plaque pick sample, there are other controls including EG, EK1, and EB which are not the family types of our phage.

CONCLUSIONS

In this study, we wanted to discover and characterize a phage found on Gonzaga University's campus. To do this, we isolated and amplified our phage so that we could study it knowing it was of a single phage type. We plated our phage and took a high resolution photo so that we could carefully examine the morphology. Our phage creates small, round plaques with clean edges. We also calculated a titer for our phage to see how many viable phage particles are in our lysate. Our titer test was calculated as 1.7×10^8. After studying our phage, we also wanted to perform additional experiments to see if it had unique characteristics about what hosts and termatures it could survive in. We set set up four different plates with different host bacteria and for different incubation temperatures: two with *M. foliorum* (room temperature and 30°C), one with *Gordonia rubripertincta* (30°C), one with *Arthrobacter globitormus* (room temperature). What we found is that our phage grew extremely well in both of the *M. foliorum* temperature environments. This indicated to us that our phage is thermostable. We categorized our phage as being part of family EE by interpreting our gel electrophoresis results. The control EE failed to be amplified, but our phage was amplified at 820 bp which is what the EE would have amplified at. We were pleased with our accumulation of results this semester, but more research could be conducted to look at what other temperatures our phage is able to survive at in the bacterial host *M. foliorum.*

LITERATURE CITED

Poxleitner M, Pope W, Jacobs-Sera D, Sivanathan V, Hatfull G. 2017. Phage Discovery Guide, Adapted for use with *Microbacterium foliorum*. Howard Hughes Medical Institute.

Travis J. 2003. All the world's a phage: Viruses that eat bacteria abound – and surprise. *Science News* 164, 26–27.

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