Morphology Comparison and Temperature Dependent Growth Experiment of Bacteriophage Mysteria

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INTRODUCTION

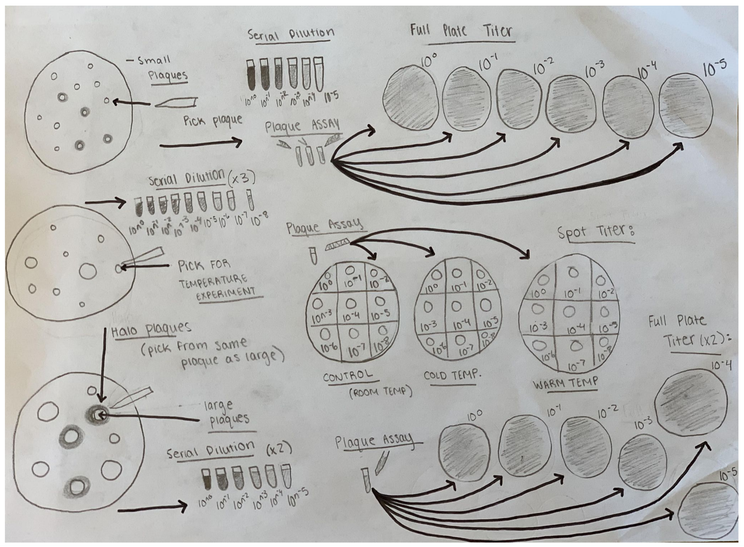
Bacteriophages, often referred to as phages, are a type of virus that infects bacterial cells. There are thought to be over 10^31 phages, therefore, they are recognized as the most abundant biological agent in the biosphere (Poxleitner et al., 2017). Every phage is unique, with a variety of different sizes and morphologies, and are often specific to only one type of bacterial host. While phages cannot infect and replicate in human cells, they are an integral part of the human microbiome and can help protect us from pathogenic bacteria (Kasman, 2021). The research for this course, BIOL 105 at Gonzaga University, focuses on phages that are hosted by *Microbacterium foliorum*. Phage were filtered from soil samples collected around Gonzaga University's campus, specifically, in front of Herak Hall (Lat 47.667009, Long -117.401969). The course research is part of a national research program, SEA-PHAGES, which has tasked themselves with exploring the bacteriophage population and discovering the evolutionary mechanisms that produce the wide variety of phages. Phage research, like the work done by SEA-PHAGES, is beneficial to the science community as a better understanding of phage would allow for the expansion of phage therapy.

OBJECTIVE: Using the sample collected near Herak Hall (Sample D), a spot test was performed to determine the presence of phage and then the sample was purified and the phage was amplified through a series of serial dilutions and plaque assay. The end result was a lysate that would be the basis of our research. There were two questions guiding our research: 1) what was the morphology of the phage present in sample D, and 2) how does incubation temperature affect plaque growth?

METHODS

In order to test temperature incubation effects on phage growth we completed a spot titer of our previously successful lysate. In order to do so, we picked from an isolated plaque with a sterile p200 micropipette and followed a traditional serial dilution series, from dilution level 10^0 all the way through 10^-8. We carried out plaque essays for each dilution, and ultimately did this procedure three times, giving us a total of three plates to test: one for the warmer temperatures, one for colder temperatures, and one as our control for room temperature.

For testing the possibility of different morphologies in our phage, Mysteria, many more plates were required. Once again we followed a traditional dilutions series, starting with picking from two different plaques. We picked from one small plaque, one large plaque, but also picking a third time at the halo of the same large plaque. We were sure to once again pick from isolated plaques and executed three serial dilutions that went from 10^0 to 10^-5. We performed full plate titers, only after completing plaque essays for each dilution. A full plate titer was used to clearly see and attempt to define a potential unity within each level to conclude whether or not there were different phage populations.



RESULTS

Morphological difference between Mysteria and Gufstrom

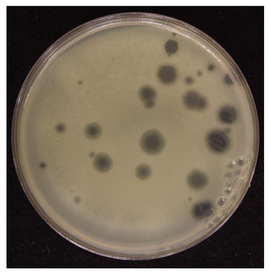


Figure 1

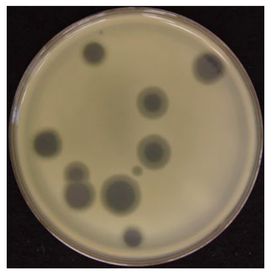


Figure 2

Figure 1 shows the morphology of the Mysteria phage. The plaques range from 1 mm to 5 mm in diameter. The plaques are clear, as opposed to turbid, with a small halo around them. Halos are no larger than 1.5 mm. Figure 2 shows the morphology of the Gufstrom phage, isolated by Brooke and Julian. Gufstroms' plaques are larger than those of Mysteria, ranging from 3 mm to 7 mm in diameter. All plaques have a clear center and a halo which is between 1 mm to 3 mm, which is larger than the halos around Mysteria plaques.

Additional data of Mysteria vs. Gufstrom phage

Figure 3

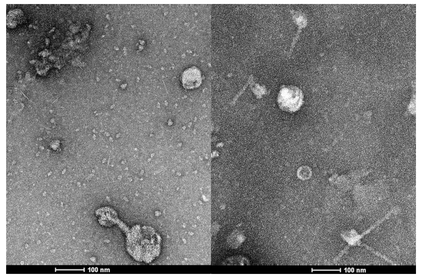


Figure 4

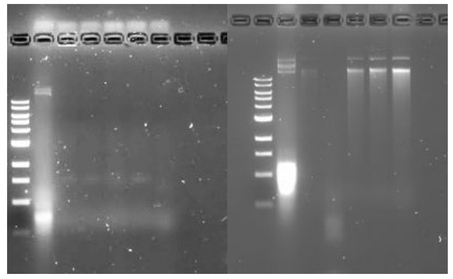


Figure 3 is a TEM (transmission electron microscope) image taken at Washington State University. Mysteria is pictured in the bottom right of the left image. The phage's capsid is approximately 116 nm in diameter and the tail is 109 nm long. Mysteria's tail fibers are visible at the end of the tail and are approximately 48 nm long. On the right, Gufstrom is pictured. The phage's capsid is approximately 48 nm in diameter and the tail is 98 nm long. Figure 4 is an image of two agarose gel electrophoresis done 4/13, on the left is Mysteria, on the right is Gufstrom. The first column is the ladder and the whitest widest row marks 3 bp, going up, each line increases by one and going down each line decreases by one. The second column is our uncut lysate DNA. On both gels there is a thick line near 1 bp which fades out in both directions and a thinner line above 8 bp (Gufstrom has several thinner lines above 8 bp). The third column was treated with the HaeIII enzyme, the fourth column with NspI, the fifth column was SacII, and the sixth column was Sal I. Even with contrast turned up, the results for Mysteria are difficult to read. Gufstrom had much clearer results.

Warmer temps during incubation resulted in little difference in plaque development

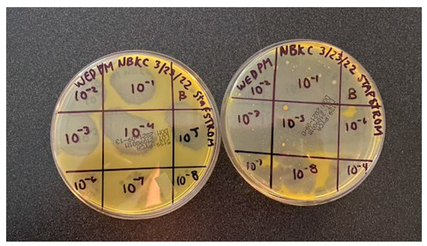


Figure 5

Figure 5. Two spot tests were made using lysate. The "warm" plates were incubated at 30 degrees celsius for 48 hours whereas the "regular" plates were incubated at room temperature, about 20 degrees C, for 48 hours. In figure 5, the normal plate (right) is almost completely cleared and the warm plate (left) has large spots through 10^-6 and then 10 plaques (2mm) on 10^-7 and 3 plaque (2mm) on the 10^-8 plate.

CONCLUSIONS

Phage Morphology:

- There is much variability in the size of Mysteria plaques which can range from 3 mm to 7 mm in diameter. Plaques are clear with small halos.
- Mysteria and Gufstrom are likely different species as Gufstrom plaques are larger in diameter and have larger halos.
- Further evidence of Mysteria and Gufstrom being different species lies in their difference in individual size. Mysteria's capsid is approximately 2 times larger than that of Gufstrom. The two species have similar length tails, but no tail fibers are visible in the image of Gufstrom.
- The gel electrophoresis of Mysteria showed little results, but the uncut lysate DNA was visible. When compared with Gufstrom, Gufstrom has longer strands of DNA as there are three lines above 8 bp compared to just one line for Mysteria.

Temperature Dependent Growth:

We concluded that temperature has some effect on the growth of Mysteria plaques. The spot test incubated at room temperature was almost completely cleared whereas the test incubated at 30 degrees celsius still had bacterial lawn remaining in every square. Because there were more plaques on the room temperature plate, we can deduce that Mysteria grows faster in temperate incubation climates compared to warm climates. These results make sense as Mysteria was collected from and thrives in soil, which is closer to room temperature than 30 degrees celsius. While there was a difference between the two plates, it was not incredibly drastic as both plates have a similar titer value (Warm: 1.33 x 10^11 and Room: 3.33 x 10^11). Thus, we could not conclude that incubation temperature has a serious effect on plaque growth. A future investigation on this topic would be necessary to test how cooler incubation compares to room temperature incubation.

LITERATURE CITED

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