Purification and Analysis of the Mycobacteriophage Moses
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ABSTRACT
The purpose of this research is to expand the base of information related to mycobacteriophages. Mycobacteriophages can be found anywhere that there is a high presence of bacteria, especially in the top level of soil. One soil sample was collected in Denton, Texas, and an enrichment process was completed to help isolate a mycobacteriophage. A spot test was completed to ensure that a phage existed within the solution. After four steps of purification, a single phage was identified as present. We then prepared a high titer lysate. The sample was prepared for DNA analysis and a grid was stained to be used for electron microscopy. A restriction digest was then performed to provide insight about what type of nucleotide sequences were present in the sample. The DNA concentration was of a high enough quantity, yet it was a bit lower than the average amount of DNA present in other comparable cases. This is probably due to a smaller genome than many others, or because some of the DNA was not of good quality. Moses has a hexagonal head, which is a common feature of many mycobacteriophages. Through the careful studying of the behavior and DNA sequence of different mycobacteriophages, researchers may be able to come to conclusions that will aid them in developing cures for tuberculosis and leprosy.

PURIFICATION OF THE PHAGE
The sample was organic, dark, and moist. The soil was taken from underneath a bush, and the plant was large and had healthy looking vegetation, indicating that the soil was of good quality. The soil was found in an air temperature of 105 degrees Fahrenheit, and the ground temperature was not recorded. Purification was done through an enrichment process. The plating of the enriched sample indicated that there is a possibility of a phage being present in the sample. The plate had several different sizes and varying clarity, indicating that the phage was not pure and there was many different bacterium and mycobacteriophages present in the solution (Figure 1).

A spot test was performed, which verified the presence of at least one phage. Because of the different sizes and clarity of the plaques on the plates, three phases of purification were performed to isolate a single phage for analysis. On the second round of purification, two different sizes of plaques presented themselves. The first was around .2 cm and the second around .1 cm. While the presence of two different sizes in plaques normally indicates that there are multiple different phages in solution, it is possible that a single phage can produce several different plaques. Additional purification was performed to ensure it was one phage that could develop two distinct morphologies (Figure 2).

DNA AND PHAGE ANALYSIS
Next, the phage was prepared to be seen under the electron microscope. Through the use of uranyl acetate and sterile water, the phage was placed onto a copper grid that was analyzed by the local EM facility. Electron microscopy is utilized to determine the size and morphology of the phage (Figure 4).

Next, DNA preparation was performed to determine the amount of DNA that there was in the phage solution. The total DNA that was obtained was 17.1492 ug. Next, a restriction digest was performed to estimate the genome size of the phage as well as provide some insight about its similarities to other phages that have been sequenced in the past. The genome size of Moses is about 47,000 bases long. This was calculated by comparing the DNA ladder in base pairs against the distance that each band traveled on the gel.

DISCUSSION
Since the phage population is estimated at being “10^12” particles and constantly changing, it is safe to assume that taking soil samples across the nation can provide some insight on the way the mycobacteriophage population works (Hatfull et al 387). This phage is very similar to other mycobacteriophages that have been analyzed across the nation, however, there are some qualities that make it especially interesting and more analysis should be done. These include:

- Differing Plaque morphology. Moses consistently was able to produce two different morphologies. The larger plaque size was around .2 centimeters and the smaller plaque was around .1 centimeter. The ratio between them was fifteen large plaques per each smaller plaque. The cause is unknown.
- Moses is a lytic phage - The plaque sizes were quite clear and very small.
- The size of the phage itself was similar to phages in the A cluster - The capsid is around 75 nm and the tail is around 250 nm. An average size capsid indicates that it has an average amount of genomic material. The longer tail indicates that it serves well its purpose of attaching onto bacteria and infecting it with its genomic data.

REFERENCES