This project involved the isolation of a new strain of mycobacteriophage from soil collected in Denton, Texas near the University of North Texas campus using a Mycobacterium smegmatis host and an enrichment protocol.

Individual plaques were selected and purified through 4 successive rounds of isolation. A high titer lysoyme of 2x 10^6 pfu/mL was created and used to perform electron microscopy and DNA isolation. Transmission electron microscopy revealed the phage to have a diameter of 50 nm and a tail length of 150 nm. DNA restriction of the phage genome produced a unique gel electrophoresis pattern when compared to other known mycobacteriophage. This phage isolation and characterization was performed as part of the HHMI-sponsored National Genomics Research Initiative. The isolation and the one of 24 obtained from freshmen students in the program. Many mycobacteriophage also infect the related pathogenic Mycobacterium tuberculosis, and it is hoped that such phage may eventually be used to treat antibiotic resistant strains of this human pathogen.

**RESULTS**

The Homorl soil sample was collected from 35° 12'26"N, 97° 09'11.5"W underneath a tree. Bird feces was not collected in the vicinity of extraction. Soil was slightly damp. The bacteriophage was enriched to increase likelihood of obtaining bacteriophage. The phage was isolated from a plate grown on a bacterial lawn, serial dilutions were performed on sample from first filtration. The first phage titer assay was performed on the 10^4 plate that had 27 plaques and incubated at 37°C for 24 hours. The second titer assay was performed from the 10^6 plate and incubated at 37°C for 24 hours. The second attempted assay purification had no plaques on the plates. As the second assay was unsuccessful, the 2nd assay was performed again from the 10^4 plate and incubated at 37°C for 36 hours. The results from the successful third assay were as follows. The positive control contained four 3.5 centimeters plate, 10^4 plate contained about 500 plaques, 10^6 plate contained about 100 plaques, 10^5 plate contained 5 plaques, and 10^4 plate contained no visible plaques. 10^7 plate had too many plaques to count and was used for flooding. The 10^6 plate was flooding with phage buffer. The PB was aspirated from the plate and placed into conical tube. Phage lysate was performed from 10^4 to 10^7 with 10 minutes of M. smegmatis infection and left at 37°C for 24 hours of incubation. The phage titer assay was calculated utilizing the 10^4 plate. The plaque area was calculated as a x plate area (42.5 mm)^2 * (n, plaques/well) = 106.25 mm^2 / 106.25 mm^2. Titer lyase calculation: 1 plaque/μL = 10^6/1 μL/mL * 10^6/1 μL/mL = 1.0 x 10^12 plaque/mL. Necessary dilutions was calculated to be 10^3 plaque/mL. Serial dilution was then performed from 10^3 to 10^5, and infected with M. smegmatis. Samples then plated and incubated. The goal of the 10^3 phage plate infection and harvest was to obtain a higher titer phage lysate with high enough plaque concentration for progression to DNA isolation. The PB plates were flooded then harvested into the conical tube and stored at 4°C. Serial dilutions on lysate harvest were performed from 10^3 to 10^5 and plated from 10^3 to 10^5, then incubated. Results from the serial dilution plates were shown on 10^4 plate with about 140 plaques. The 10^3 lysate harvest was transferred to 50 mL. Oakridge tube and remaining lysate saved to 40 μL. Nucleic acid was added to tube, incubated and cooled 4°C. Enzyme precipitation solution was added and mixed by gentle inversion, then incubated at 37°C for 24 hours. Nuclease was added to 2 μL of plating grids and sent for electron microscopy. After DNA isolation and purification, 49 μL DNA solution had accumulated. The DNA concentration was 200.80 ng. 241 μL * 49.8 = 11890 ng of total recovered DNA. Electron microscopy was performed for various restriction enzymes.

**CONCLUSIONS**

Considering the two types of bacteriophages, the phage from this experiment was most likely lytic, considering the clear plaque appearance. With either type plaque, there are four steps to infection. Adsorption of phage to bacterial surface is done by tip of tail that adheres to receptor. Adsorption is irreversible. The second step is penetration of extension of adsorption minus specificity. Penetration of bacterial wall, the third step, occurs due to contraction of bacterial sheath forming something like a vacuum. Some phages produce enzyme which munice on the bacterial cell wall. The final step, nucelic acid injection from the phage head occurs after phage has made its way through the bacterial envelope. DNA is injected into the cell. With a lytic phage, after total DNA injection, the newly synthesized DNA can be used. The head and tail components are synthesized and assembled, the DNA is loaded into capsid, and both are attached to form whole infectious particles. After titer calculation, there was a high enough titer lysate for empirical data to be assembled, the DNA is loaded into capsid, and both are attached to form whole infectious particles. The purpose of the electron microscopy is to observe the individual phage.

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