Mycobacterium Phage Study

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Background

- Bacteriophages (phages) are viruses that parasitize a bacterium by infecting it and reproducing within it.
- *Mycobacterium smegmatis* (*M. smegmatis*) is the host bacterium for Montana Tech's PHAGES program.

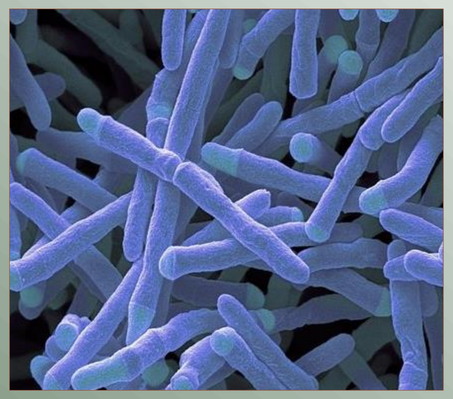

Figure 1 ***Mycobacterium smegmatis.*** A scanning electron micrograph of *M. smegmatis* is shown. [1]

- PhagesDB.org is where the information for thousands of Actinobacteriophages are stored. As of 7/28/21, there were 18,889 phages catalogued in phagesdb.org. [2]
- Phages with similar overall DNA sequence belong to the same cluster, which can be determined through cluster Polymerase Chain Reaction. [3]

References

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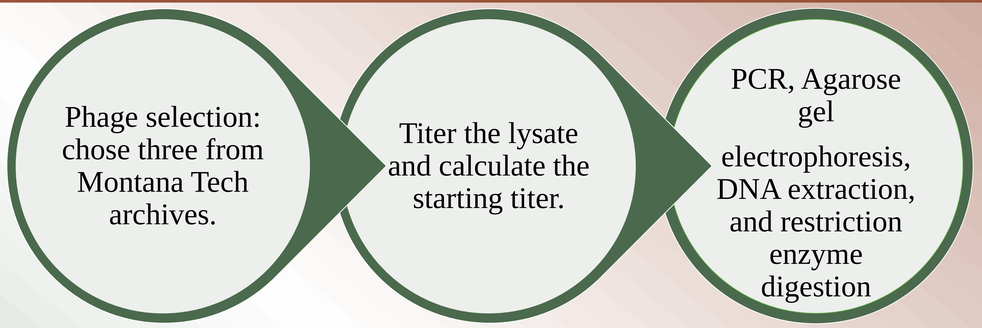
Methods



Serial dilutions were conducted to perform plaque assays. *M. smegmatis* was infected with serially diluted phage, plated, and incubated for 24 hours. Webbed plates were flooded and filtered to increase the titer of each phage. Titering procedures were repeated until the desired titer of 10^{10} PFU/mL was reached to permit DNA Extraction and Restriction Enzyme Digest (PFU: Plaque Forming Units). Cluster Polymerase Chain Reaction (PCR) was performed for LadyHelm and Farahbithia with 35 primer pairs, testing for 26 unique clusters.[4]

Results

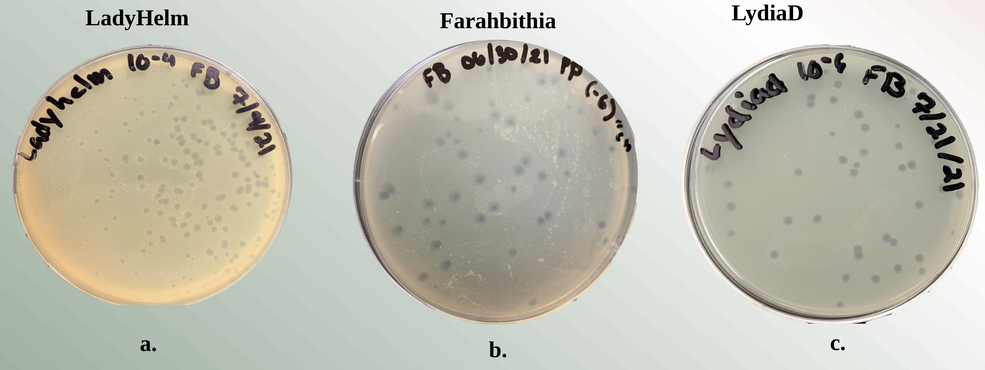
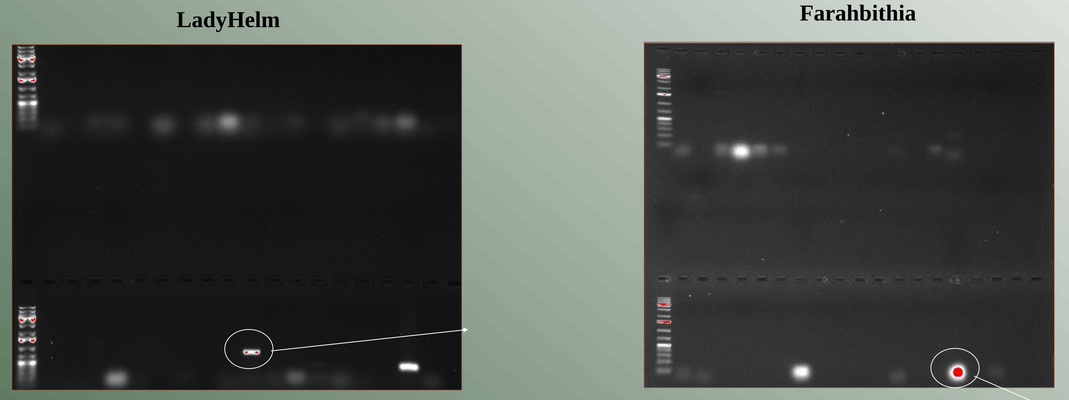


Figure 2. Plaque assays. a. LadyHelm resulted in a titer 2.3 x 10^8 PFU/ml. b. Farahbithia resulted titer 5.2 x 10^9 PFU/mL. c. LydiaD resulting titer 4.6 x 10^9 PFU/mL.

Titer Calculation

$$\frac{plaqes}{10uL} \text{X} \frac{1{,}000uL}{1mL} X\ 10^{inverse\ dilution} \text{PFU/mL}$$



Figue 3. Cluster PCR. a. LadyHelm has provisionally been identified as an A1 cluster phage. b. Farahbithia has been provisionally been identified as an I cluster phage. Positive bands are circled on each gel.

Conclusions

Phages	LadyHelm	Farahbithia	LydiaD
Calculated Titer (PFU/mL)	2.3 x 10^8	5.2 X 10^9	4.6 X 10^9
Provisional Cluster	A1/F1	I	A1
Archived	No	Sent 7/29/21	Previously

Future Work

- Amplify lysate titers to 10^{10} PFU/mL.
- DNA extraction.
- Restriction Enzyme Digests.
- Ship LadyHelm lysate and all three phages' DNA to University of Pittsburgh for archival and DNA sequence analysis.

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