Phage Discovery of Phages LestyG, Tamron, and Kbm Phage

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Introduction

Phages are viruses that infect bacterial cells [Kasman *et al.*, 2019]. They depend on bacterial cells for replication, utilizing the cells' machinery to replicate intracellularly until they are finally released from the host by cell lysis [Carson et al., 2010]. Bacteriophages were independently discovered 100 years ago by F. W. Twort [Twort, 1915] and Felix d'Hérelle [d'Hérelle, 1917]. Discovery of bacteriophages have had a huge impact in the treatment of bacterial infections [Morozova et al., 2018]. Bacteriophages became an important field of study because the use of bacteriophages in medicine in the 1900s—to treat dysentery patients. Ever since, phages have not been used very often in medicine. The decline in the interest of bacteriophages could be due to the negative perceptions about viruses, the cost, and the unpredictable nature of viruses. However, the surge in antibacterial resistant bacteria has made bacteriophages a stronger alternative and potential treatment for pathogenic bacterial infectious diseases such as gonorrhea, pneumonia, tuberculosis, leprosy, and wound infections; this idea is called Phage Therapy. Phage Therapy is the use of bacteriophages therapeutically, as antibiotics, to treat pathogenic bacterial infections. Recently, bacteriophages are used in meat treatment to reduce salmonella in poultry products [Higgins *et al.,* 2005]. It is estimated that there are 10^{31} phages on earth, and only 18,000 have been discovered [LaFee & Buschman, 2017]. Discovering bacteriophages could also help better scientific knowledge: to help discover more bacteriophages, to make bacteriophages available for other scientists, to know the phylogenetic relationships bacteriophages share, and the evolution of viruses.

Mycobacterium smegmatis is a common non-pathogenic environmental bacterium. Due to this, *M. smegmatis* is usually used in research projects concerning the *Mycobacterium* species. Therefore, the bacteriophages that are discovered using *M. smegmatis* could potentially be used for phage therapy. The objective of this research was to discover bacteriophages from environmental samples using *Mycobacterium smegmatis* as the host bacteria and characterize the newly discovered bacteriophages.

Methods and Materials

Soil samples were collected from various locations in a 50ml conical tubes. The information such as the GPS and characteristics of the samples were recorded. Soil samples were isolated through the enriched isolation method to amplify bacteriophages in the soil samples. Bacteriophages were detected using the Plaque Assay method by creating a lawn of *M. smegmatis* on agar and infecting the lawn with the soil samples.

The set-ups were incubated in 30 degrees and 37 degrees Celsius for 1-3 days: depending on the rate at which plaques were formed or developed fully. Phages were picked from plaques on the lawn and isolated in phage buffer for further assessment. A spot test was used on each plaque picked to confirm the presence of bacteriophages. 10-fold serial dilutions were performed to purify the bacteriophages and find the dilution that can create a webbed-plate for collection of phage lysates. The full plate titer was calculated for every phage to determine the concentration of phage particles in a lysate—plaques per milliliters of a phage lysate(pfu/ml). After determining the titer of the phages, webbed plates were created for each phage and collected for future use.

Collected phage samples were archived for long-term storage. Newly discovered phages were entered into the Actinobacteriophage Database to make the phages available to other scientists.

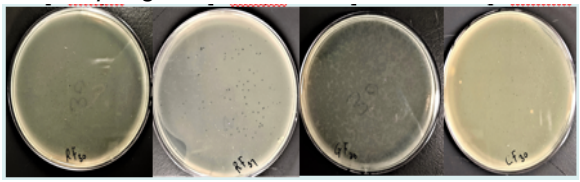
50 microliters of each phage lysate was submitted to Mary Washington University. The DNA samples of each phage were extracted to isolate the genomic DNA from the phages and submitted to the University of Pittsburgh for sequencing.

Results

Plaque assay

Plaques were found from the isolated soil sample. Three different bacteriophages were discovered from three separate soil samples. Phage LestyG was discovered under the Aztec grass in front of the Rhetoric Studio of Hampden-Sydney College. Phage LestyG developed in 37 degrees Celsius (takes less than 24 hours to fully develop) and 30 degrees Celsius (takes 1 to 2 days to fully): forms medium sized plaques. Phage Tamron was discovered under the Northern Red Oak tree in front of Gilmer, the main science building of Hampden-Sydney College. Phage Tamron was developed in 30 degrees Celsius incubation: takes less than 18 hours to fully develop and forms large plaques. Phage Kbmars was discovered under the Privet plant in the Presbyterian church's cemetery in Hampden-Sydney College. Phage Kbmars only developed in 30 degrees Celsius incubation: takes 2 to 3 days to fully developed and forms small plaques.

Figure 1: Plaques of newly discovered bacteriophages



Phage LestyG Phage LestyG Phage Tamron Phage Kbmars

Serial Dilutions

Figure 2: Serial Dilution of Phage LestyG

10^{-1} 10^{-2} 10^{-3} 10^{-4}

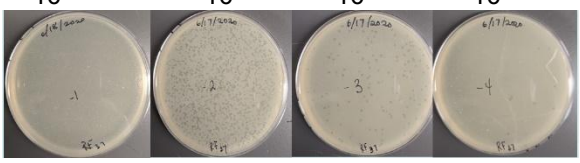


Figure 3: Serial Dilution of Phage Tamron

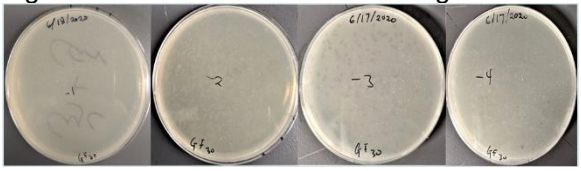
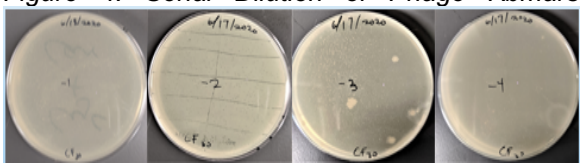


Figure 4: Serial Dilution of Phage Kbmars



Serial dilutions were made on Phage LestyG, Phage Tamron, and Phage Kbmars respectively. The dilution shows the decrease in the number of plaques when the dilution increases. Phage LestyG in 37 degrees Celsius incubation had a titer value of $3.2 * 10^{10}$ pfu/ml whiles 30 degrees Celsius incubation had a titer value of $1.9* 10^{10}$ pfu/ml. Phage Tamron had a titer value of $1.11*10^{10}$ pfu/ml and Phage Kbmars had a titer value of $7.0* 10^{9}$ pfu/ml.

Electron Microscopy Images

Figure 5: Electron microscopy of newly discovered bacteriophages

Phage Kbmars Phage Tamron Phage LestyG

The images show the distinct structure of a bacteriophage: an icosahedral head and a tail.

Discussion

The expectation for this research was to discover bacteriophages from the from the environment. This research looks to expand scientific knowledge through discovering (for database collection), characterizing, and analyzing bacteriophages. Figure 1 is the first step to determine the presence of bacteriophages (plaque assay). Each black spot represents a plaque, and each plaque denotes the presence of bacteriophages. The variations in location of soil samples, growth times, growth temperatures, and titer values indicate the discovery of distinct bacteriophages.

Serial Dilutions determines the dilution needed to create a webbed plate and the titer of the phage lysate. The serial dilution shows the decrease in the number of plaques when the dilution increases; this confirms the presence of bacteriophages.

The growth time and the titer of Phage LestyG in 37 degrees Celsius incubation (less than 24 hours and $3.2 * 10^{10}$ pfu/ml) compared

that of 30 degrees Celsius incubation (1-2 days and 1.9* 10^{10} pfu/ml) indicate that the ideal temperature for Phage LestyG is 37 degrees Celsius. With the idea of phage therapy, Phage LestyG will be a suitable phage to be used in a human host; due to the average body temperature being 37 degrees Celsius. Because Phage Tamron forms large plaques, takes a shorter period to develop and easily clears a plate, Phage Tamron is highly invasive. Also, because bacteriophages were discovered from locations with similar ecosystems, the phages might have a close Phylogenetic relationship.

Conclusion

Three distinct bacteriophages were discovered at the end of the research. All three phages were entered into the Actinobacteriophage Database. Further research on the characterization and host range assay of the phages will be conducted.

References

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