The Discovery and Analysis of Mycobacteriophage "Pembroke"

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Introduction

The Mycobacteriophage "Pembroke" was discovered from an enriched soil sample from Pembroke, Massachusetts. Using *Mycobacterium smegmatis* mc^{2}155, it was possible to isolate Pembroke and perform additional experiments to further characterize the phage. Pembroke was found to be an A3 cluster phage, meaning it has the potential to live a temperate lifestyle and could be used for phage therapy to treat infections caused by pathogenic Mycobacterium such as Mycobacterium tuberculosis and Mycobacterium abscessus. Analysis of Pembroke's genome was conducted to better understand the phage's unique qualities and how they can be utilized.

Figure 1: Purification
"Pembroke" phage plaques during purification procedures.

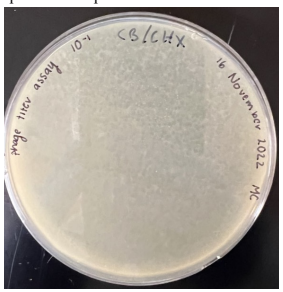
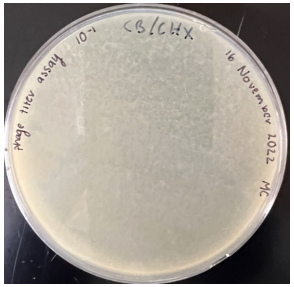


Figure 2: Formation of Lysate
The webbed plate that was utilized to form phage lysate.



Methods

The soil sample containing Pembroke was first enriched, filtrated, and plated in order to form phage plaques. Utilizing plaque-picking purification technique, the phage was isolated on a plate containing M. smegmatis mc^{2}155. Flooding the plate with phage buffer in order to form phage lysate and the newly formed phage lysate was used to then isolate phage DNA. Extraction procedures we carried out, utilizing a serries of incubations and centrifugations with materials including DNAase I, RNAse I, Proteinase K, EDTA, PCI, isopropanol, and sodium acetate pH 5, resulting in purified Pembroke DNA. To annotate the genome of Pembroke, a combination of bioinformatics software including DNA Master, Phamerator, and PECAAN were used. With this a G-Block was identified. The DNA was combined with primers that were unique to Pembroke, loaded onto a gel, and ran through electrophoresis. This process makes it possible to remove the G-Block that was identified in the genome of Pembroke, better preparing Pembroke for its potential use in genome therapy.

Figure 6: Immunity Assays Using Pembroke
Pembroke was tested in comparison A3 cluster phage LBerry and A5 Cluster Phage Zolita

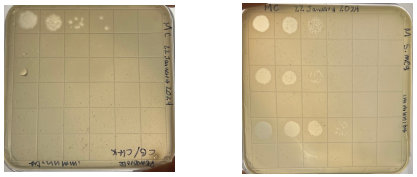


Figure 4: Example Annotation on DNA Master

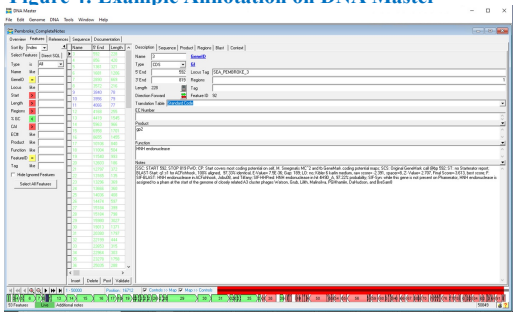
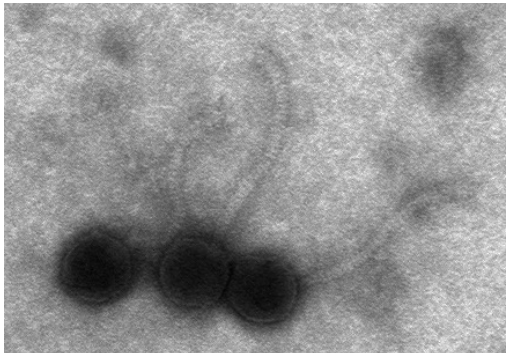


Figure 5: Electron Microscopy Photo of a Phage Particle



Results

Mycobacteriophage Pembroke was determined to be assigned to cluster A and subcluster A3 through the PCR analysis. Genome annotation determined that Pembroke's genomic diversity from other phages could be identified; containing 3 tRNAs and no orphams.

Figure 3: Complete Phamerator Map of Phage "Pembroke" and Similar Phages of the Same Subcluster