An Investigation of a Cluster S Mycobacteriophage Genome, Corazon, Genes 4-16: Location and Function

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An Investigation of A Cluster S Mycobacteriophage Genome, Corazon, Genes 4-16: Location and Function

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Abstract

The purpose of this investigation is to establish the presence, location, and function of genes within the genome of a novel bacteriophage, Corazon and add to the Actinobacteriophages Database. The Corazon genome was analyzed with the program DNA Master as well as NCBI BLAST, HHPred, and Phamerator to determine the location and function of genes within the auto-annotated range of genes 4-16. Only one of these genes was assigned a function, and the investigation of significant gaps in the genome resulted in an additional gene being added.

Introduction

Mycobacteriophages are a type of virus that specifically attack mycobacteria. This attribute can be exploited to fight antibiotic resistance mycobacteria. As of February 2019, only 14 Cluster S types (a specific group of mycobacteriophage) have been completely sequenced and published in the Actinobacteriophage Database. The Corazon genome was analyzed to determine the accurate locations and functions of 13 auto-annotated genes and one additional manually discovered gene. The main goal of this investigation was to prepare a high-quality annotation of the phage genome. The published work will be used for future investigations.

Contribution of the global understanding of bacteriophages is of interest since the phage-bacteria model has expanded scientists’ capabilities of studying evolution and exploring novel medical applications. Publishing these annotations will allow generations of researchers to compare their results to this member of Cluster S and potentially identify a new candidate for phage-mediated transduction, phage therapy, or other applications.

Materials and Methods

<table>
<thead>
<tr>
<th>Procuring A Phage</th>
<th>Analyzing the Phage</th>
<th>Analyzing Phage Genomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A dirt sample was obtained.</td>
<td>The phage was viewed through electron microscopy.</td>
<td>Using several programs' locations and functions were determined.</td>
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<tr>
<td>A phage was isolated and a high centrifugation lysate was made.</td>
<td>DNA analysis was done through Restriction Digest.</td>
<td>Using several programs' locations and functions were determined.</td>
</tr>
</tbody>
</table>

Remote Analysis:
- Auto Annotating the phage genome using the program DNA Master.
- Confirming gene start site locations and gene functions using
  - GeneMark, Startator, Phamerator Maps, NCBI BLAST, PhagesDB BLAST, HHPred, and Ribosomal Binding Sites scores

Figure 6 shows the program to evaluate the genes. The Green text shows each gene numerically and its base pair position. The bottom right band corner shows the notes we found from researching the gene.

Procuring a Phage

Small, circular plaques (1-3 mm diameter) are associated with lytic phage.

Results

Procuring a Phage

Fig. 1 Antibiotic Plate of Phages from Environmental Extraction
Fig. 2 Beolyzob (col) Plate with Purified Plague Morphology
Fig. 3 Actinobacteriophage Plate Spread with an Mycobacteria and lysate (purified plaque sample)
Fig. 4 LittleLafl Electron Microscopy Image
Fig. 5 VasuNzinga Electron Microscopy Image
Fig. 6 Denotes how the start sites of genes were modified or added during annotation.
Fig. 7 Summarizes the function calls of the 14 total genes annotated

Conclusions and Future Directions

Including m.smegavianti bacteria on the initial extraction plates and subsequent attempts to plate an isolated plaque morphology (Figures 1 and 2) ensure that the bacteriophage present are mycobacteria. The presence of small, circular plaques observed on the plates (Figures 1 and 2) suggest that the phage particles quickly lyse their host bacteria, which is characteristic of a lytic life cycle. The electron microscopy images (Figures 3-5) were used to characterize their respective phages as members of the Siphoviridae family. Siphoviridae have long, non-contractile tails and geometric, round capsids. Cluster S is a family of phage genomes that encompasses Siphoviridae mycobacteria with a lytic life cycle. The wet-lab portion of this investigation successfully isolated a phage type from an environmental sample and characterized it to be a member of Cluster S.

Subsequent DNA sequencing allowed the genome of a Cluster S member, Corazon, to be obtained. Investigating Genes 4-16 of Corazon with bioinformatics tools revealed the phage was highly similar to the phages Beelzebub, LittleLafl, and MosMoris. The similarity colored boxes correspond to similar genes. The purple regions show strong correlations between genomes.

Fig. 7 shows the BLAST results show genes from other phages that are very similar to the selected gene. The BLAST results are analyzed for similarity to determine the function and startsite of an unknown gene based on this information from known, similar genes. The other similar genes are shown in the top left corner, and various scores describing similarity are shown on the bottom right.

The Phamerator map (Figure 5) compares the genome of Corazon with several similar phages, including Beelzebub, MosMoris, LittleLafl, and MosMoris. The similar colored boxes correspond to similar genes. The purple regions show strong correlations between genomes.

The initial hypothesis was rejected since the annotated Corazon genes had no known function (Figure 7). A future investigation could involve protein analysis on bacteriophage proteins to potentially characterize their functions, discover what type of interactions they perform within phage, and relate the activity of phage to its expression level.

Bioinformatics

Fig. 4 LittleLafl Electron Microscopy Image
Fig. 5 VasuNzinga Electron Microscopy Image
LietzkeMehling, LittleLafl, and VasuNzinga are all Siphoviridae phages. They are characterized by having long, non-contractile tails and geometric, rounded capsids (heads).

References and Acknowledgements

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Notes:
- The genomes were obtained from the Actinobacteriophage Database by Kimberly Mac Menage at Lafayette College and run through the Phamerator bacteriophage Institute. Gene annotation was performed at Purdue University with quality from Dr. Kari Clase, Bonnie Gleadthorpe, and Emily Jamie. Procedures for vaccines and diagnostics were provided by the Howard Hughes Medical Institute Science Education Alliance. Also recognized are the Bioinformatics and Regulatory Science Center at Purdue University, Bradley Bioscience Center at Purdue University, Polytechnic Institute, and the Department of Agricultural and Biological Engineering at Purdue University.