Apr 8th, 2:00 PM - 3:00 PM

The Bioinformatic Analysis of a Cluster S Mycobacterium Smegmatis Bacteriophage Tesla

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Fitzgerald, Janna; Laughlin, Zoephia; Page, Colin; Reichert, Bailey; Alvey, Faculty Advisor, Richard; and Bollivar, Faculty Advisor, David, "The Bioinformatic Analysis of a Cluster S Mycobacterium Smegmatis Bacteriophage Tesla" (2017). *John Wesley Powell Student Research Conference*. 6.  
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THE BIOINFORMATIC ANALYSIS OF A CLUSTER S MYCOBACTERIUM SMEGMAVIS BACTERIOPHAGE TESLA

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Introduction

The SEA-PHAGES program expands the knowledge of its participants while increasing the diversity of the collection of bacteriophages. The bacteriophage Tesla was collected in Owego, Illinois by Colin Page, isolated at Illinois Wesleyan University, sequenced at the University of Pittsburgh and annotated at Illinois Wesleyan University. The objective of this project was to interpret the genome of Tesla and then compare the evolutionary relationship existing between the bacteriophage Tesla and the other seven bacteriophages within the S Cluster. A cluster of bacteriophages is a group that shares at least 50 percent of recognizable nucleotide similarities. Using the genomic information of other S cluster phages found on the program Phamerator, it was found that many of Tesla’s functions were shared with the other phages. In comparison with Marvin, the original member of the S Cluster, Tesla has 106 genes that are the same and 1 that are different from other annotated phages. In the Tesla genome, 70 are hypothetical proteins and 42 were found to have their functions identified. Based on this information, we have learned that members of the S Cluster are highly conserved but have some differences. This supports the mosaic nature of bacteriophage genomes.

Materials and Methods

- Blastp and HHpred were used to compare gene functions with other phages.
- Phamaterator was used to compare genomic similarity to other phages in S cluster
- Starterator was used to compare start sites for genes with start sites of homologous genes in other phages
- GeneMark and Glimmer were used to help determine start sites for genes based on coding potential
- DNA Master was used to annotate Tesla’s genome

Genome Results

- Genes 3-13 are evidence of the mosaic nature of bacteriophage genomes since these genes differed from the other bacteriophages in the S cluster. These genes shared more similarities with genes from bacteriophages in other clusters than those in the S cluster, showing how Tesla is a unique phage.
- There is a translational frameshift that affects genes 45 and 46. Both are tail assembly chaperones.
- Tesla, like other phages in the S cluster, contains genes that function as minor tail proteins that are very close together in the genome.

Annotation Results

- Initial results: Tesla is a bacteriophage in the S cluster that contains 64,625 base pairs and 113 genes. Of these genes, 23, 31, 95-107, and 113 were reverse reading genes and the remaining genes were forward reading. This information was provided by the University of Pittsburgh.
- Gene 73 was deleted from the genome because there was not sufficient coding potential and the gap between genes 72 and 74 only included one base pair, lowering the gene total to 112.

Gene Function Chart

- This figure shows how large the S cluster is compared to other bacteriophages.

Gene Function Table

<table>
<thead>
<tr>
<th>Gene function</th>
<th>Placement</th>
<th>Length</th>
<th>Clusters with Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysin A</td>
<td>Middle</td>
<td>1380 bp</td>
<td>B4, B7, K2, S, &amp; X Cluster</td>
</tr>
<tr>
<td>HNH Homing Endonuclease</td>
<td>End</td>
<td>327 bp</td>
<td>S Cluster</td>
</tr>
<tr>
<td>Minor Tail Protein</td>
<td>End</td>
<td>2142 bp</td>
<td>S &amp; V Clusters</td>
</tr>
</tbody>
</table>

Acknowledgements

- TA’s were Ellen Stumph, Brooke Koebele, and Niyant Vora
- Lab manuals used were the DNA Master guide and the Phages Discovery Guide (provided by the SEA phages program)
- Splitree diagram was provided by Dr. Bollivar
- Cluster tree provided by elifesciences.org in their article “Whole genome comparison of a large collection of mycobacteriophages reveals a continuum of phage genetic diversity”