Curation and Characterization of a Collection of Mycobacteriophages

Hannah E. Sparks  
_Montana Tech_

Marisa L. Pedulla  
_Montana Tech_

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Curation and Characterization of a Collection of Mycobacteriophages

Hannah E. Sparks & Dr. Marisa L. Pedulla

Bacteriophage Background

• Bacteriophages are the most abundant biological entity on earth, with an estimated number of $10^{31}$.
• Bacteriophages specifically infect and kill bacteria.
• Phages that infect *M. smegmatis* could potentially be used as an alternative to antibiotics.

Methods

• Isolation of single plaques from phages stored at various purification stages at Montana Tech.
• Creation of a high titer sample.
• DNA Extraction
  • Restriction Enzyme Digests & Gel Electrophoresis
  • Send sample for sequence analysis at the University of Montana
• Polymerase Chain Reaction (PCR) for cluster analysis
• Update Phage Information
  • Archive samples at the University of Pittsburgh
  • Upload information to phagesdb
  • Update spreadsheet of Montana Tech’s phages
• Transmission Electron Microscopy.

Conclusions

• Transmission electron microscopy indicated Siphoviridae morphology for all eight phages.
• Restriction enzyme digests indicated multiple cut sites for all phage genomes, giving each phage a unique fingerprint.
• Updating information of phages is important for future research.

Future Work

• Annotate genomic sequences when they are returned.
• Repeat methods for more phages stored at Montana Tech.
• Further discovery through bioinformatics or wet lab procedures.

Acknowledgements

• “This work was supported by Montana Tech’s Summer Undergraduate Research Fellowship (SURF). We thank…
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• University of Missoula & Dr. Jim Driver, EMTrix
• Dr. Joel Graff for resources utilized from his lab.
• HHMI, SEA- phages, University of Pittsburgh
• This research was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number P20GM103474. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.”

Results

<table>
<thead>
<tr>
<th>Phage Name</th>
<th>Concentration from DNA extraction (ng/µL)</th>
<th>Lysate Concentration (pfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemmarlo</td>
<td>14.2</td>
<td>$3.7 \times 10^{10}$</td>
</tr>
<tr>
<td>Moonbeam</td>
<td>55.8</td>
<td>$1.67 \times 10^{10}$</td>
</tr>
<tr>
<td>Prickles</td>
<td>34.3</td>
<td>$1.67 \times 10^{10}$</td>
</tr>
<tr>
<td>Solarflare</td>
<td>72.9</td>
<td>$6.67 \times 10^{9}$</td>
</tr>
<tr>
<td>Tomaszewsky</td>
<td>65.7</td>
<td>$2.33 \times 10^{10}$</td>
</tr>
<tr>
<td>T Rex</td>
<td>40.4</td>
<td>$1.67 \times 10^{10}$</td>
</tr>
<tr>
<td>Whatsapiecost</td>
<td>13.4</td>
<td>$6.0 \times 10^{10}$</td>
</tr>
</tbody>
</table>

PCR Cluster Analysis

<table>
<thead>
<tr>
<th>Phage Name</th>
<th>Putative Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whatsapiecost</td>
<td>A1, A2, A6, B1, F2, K</td>
</tr>
<tr>
<td>Prickles</td>
<td>D</td>
</tr>
<tr>
<td>Moonbeam</td>
<td>B1, B3, D, I1/II, A1, F2, I</td>
</tr>
<tr>
<td>Solarflare</td>
<td>A1, A2, B1, B3, D, E, F1, F2, G, I1/II, B4, C2</td>
</tr>
<tr>
<td>Paolini</td>
<td>A2</td>
</tr>
<tr>
<td>Tomaszewsky</td>
<td>A5, A6, B1, D, E, F1, F2, G, I1/II, B1, B2, B3, E, I</td>
</tr>
<tr>
<td>Gemmarlo</td>
<td>A2, A4, B3, E, I1/II, K, GGL, A1, B1, B2, F2, F1, G, H2, I</td>
</tr>
<tr>
<td>T Rex</td>
<td>A1, A2, A3, A4, A5E, F2, G, B3, C2</td>
</tr>
</tbody>
</table>