The Isolation and Characterization of Novel Mycobacteriophages in San Diego

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June 21, 2009
BIMM 171 Class

Environmental Sample  →  Plaque Assay  →  Electron Microscopy

Annotation  ←  Restriction Analysis
Isolation Overview

• 491 direct plating
• 33 enrichments
• 21 samples yielded plaques from direct plating (4%)
• 16 samples yielded plaques from enrichment (48%)
• 19 novel phage isolated
## Isolation Overview

<table>
<thead>
<tr>
<th>#</th>
<th>PHAGE</th>
<th>OWNER(S)</th>
<th>#</th>
<th>PHAGE</th>
<th>OWNER(S)</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>Auxin</td>
<td>Andrew A.S. Ang</td>
<td>12.</td>
<td>Fun Box</td>
<td>Jason Ho &amp; Theresa Wong</td>
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<tr>
<td>2.</td>
<td>Yo-yo</td>
<td>Kayla Busby &amp; Simmi Deo</td>
<td>13.</td>
<td>Snuggles</td>
<td>Diana T. Li</td>
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<tr>
<td>3.</td>
<td>Grass Blade</td>
<td>Sahrish Ekram</td>
<td>14.</td>
<td>ET08</td>
<td>Tina Lu</td>
</tr>
<tr>
<td>4.</td>
<td>Sunny Delight (previously isolated as Orange Drop)</td>
<td>Crystal Estrada</td>
<td>15.</td>
<td>Helianthus</td>
<td>Emilie Nguyen</td>
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<tr>
<td>5.</td>
<td>Sun God Bones</td>
<td>Shannon Fox</td>
<td>16.</td>
<td>Anina</td>
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<tr>
<td>7.</td>
<td>Lemon Drop (previously isolated)</td>
<td>Bridget Guiza</td>
<td>18.</td>
<td>Dora</td>
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<td>9.</td>
<td>RingO</td>
<td>Leila Haghighat</td>
<td>20.</td>
<td>Tree Hollow</td>
<td>Tin-Yun Tang</td>
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<td>10.</td>
<td>Freckles</td>
<td>Kevin He</td>
<td>21.</td>
<td>Tin</td>
<td>Jon Tsay</td>
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<td>11.</td>
<td>Wilmo</td>
<td></td>
<td>22.</td>
<td>Cherry</td>
<td>Danielle Wang</td>
</tr>
</tbody>
</table>
Phage Phinding Trial 1

[Map of San Diego area with marked locations]

[Images of natural environments with Phage]

[Image of a culvert with water]
Phage Phinding Trial

- All but one positive site was from compost
- No compost samples were plaque negative
Plaque Purification
Plaque Morphologies

Clear

Lytic

Turbid

Lysogenic

Bulls-Eye

???
Electron Microscopy

- Icosahedral capsid
- Long flexible tail

Siphoviridae
ET08: Myoviridae

**Plaque Morphology**
- small
- dot-like
- clear

**Electron Microscopy**
- contractile tail
- capsid diameter ~80 nm
- tail length ~80 nm
DNA Restriction Analysis

~72 kb

~155,445 base pairs

Cla I
Annotation of ET08

- Glimmer and Genemark in Apollo identified ORFs
- Manually evaluated Shine Delgarno Sites and BLASTp matches
- Member of Cluster C1 contractile tailed mycobacteriophage family
- Phamerator showed 1 ET08 gene product to be novel phamily
- tBLASTn showed that this gene was not actually novel genetic information
- Extensive genetic similarity to other Cluster C1 phage
Novel Elements of ET08 Genome

- 4 inteins
  - 2 with conserved binding sites
  - 2 with novel binding sites
- 33 putative tRNAs
- 1 tmRNA
Conclusion

1. Isolation of environmental samples and plaque purification

2. Characterization through
   - Plaque assays
   - Electron microscopy
   - DNA restriction analysis

3. Annotation and identification of novel inteins and tRNA
Marcella Erb, Dr. Joseph Pogliano, Dr. Kit Pogliano, Dr. Madeline Butler, Dr. Lorlina Almazan, Howard Hughes Medical Institute, UCSD Department of Biology, VA San Diego Medical Center Electron Microscopy Facilities, Andrew A.S. Ang, Kayla Busby, Simmi Deo, Sahrish Ekram, Crystal Estrada, Shannon Fox, Ethan Fram, Bridget Guiza, Auroni Gupta, Leila Haghighat, Kevin He, Jason Ho, Diana T. Li, Tina Lu, Emilie Nguyen, Katrina Nguyen, J. Bradley Segal, Tin-Yun Tang, Jonathan Tsay, Danielle Wang, Theresa Wong, Phillip Wu, Yun jeong Yang