**Isolation and Characterization of Phages infecting Bacillus**


Students, Teaching Assistants and Instructor Fall 2013/Spring 2014 BINFO 251/252: Phage Discovery Laboratory I/II

**Introduction:** The Bacillus "ACT" group include B. anthracis, B. cereus, and B. thuringiensis. These bacteria are closely related by sequence, but present a variety of phenotypes and pathogen profiles. Our class has found phage discovery to be a challenge with B. cereus, but is relatively easy with B. thuringiensis. For example, after 57 attempts at finding phages using B. cereus as a host, we obtained only four positive tests (7%). A recent paper indicated 2 plaques were revealed after testing 40 soil samples (5%, Lee et al., 2012, 52:456-464, Letters in App Micro). In contrast, all students found a phage on their first attempt with B. thuringiensis. Our work in progress is presented here.

**Methods:** Soil samples were collected around the Richmond, VA region. Students used enrichment cultures (30°C, overnight, with B. cereus or B. thuringiensis) platting to detect phage plaques. All plates and cultures were grown at 30°C for 24h in TSB media. Samples were purified by multiple rounds of plaque picking, serial dilution into phage buffer, infection into aliquots of bacteria, and plating with top agar. Purification was performed until a consistent plaque morphology was observed. Alternatively phages infecting B. cereus required 'plaque enrichment' prior to infection and plating, to amplify the phages present in the sample. Phage particles were spotted onto formvar coated grids, stained with 1% uranyl acetate, and visualized by transmission electron microscopy. DNA was purified from high titer lysate and sequenced by 454 FLX next-generation sequencing technology. Nigalana was annotated using DNA Master (Glimmer, GenBank to identify ORFs and Aragorn for tRNA prediction) and blastp, HHpred and Phamer for functional annotation. The dotplot was created with Gepard, using a 10bp word size, and the phylogenetic trees were created by ClustalW2.

We sequenced the genomes of four phages that were isolated using B. thuringiensis as a host bacteria.

### Table 1. Genome characteristics of four sequenced Bacillus phages.

<table>
<thead>
<tr>
<th>Phage</th>
<th>Genome Length</th>
<th>Terminal repeat</th>
<th>% GC</th>
<th># predicted genes</th>
<th># tRNAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigalana</td>
<td>163041</td>
<td>2867</td>
<td>38.8</td>
<td>305</td>
<td>0</td>
</tr>
<tr>
<td>NotTheCreek</td>
<td>159336</td>
<td>2585</td>
<td>38.2</td>
<td>296</td>
<td>0</td>
</tr>
<tr>
<td>SageFayge</td>
<td>159547</td>
<td>2812</td>
<td>38.1</td>
<td>299</td>
<td>0</td>
</tr>
<tr>
<td>Vinny</td>
<td>162865</td>
<td>2358</td>
<td>36.4</td>
<td>296</td>
<td>7</td>
</tr>
</tbody>
</table>

Three of our four sequenced Bacillus phages are similar in genome characteristics. Vinny is unique, with a lower %GC, 7 tRNAs, and lower sequence similarity.

**Results:**

We annotated Nigalana, NotTheCreek and Vinny. 31 genes with functional predictions were shared by all three.

* Each gene had >20 genes with non-phase best blast hits or ‘no significant similarity’

**Methods**

- **Plaque Enrichment method** to be able to see B. cereus phages on plates

We explored gene transcription and translation by examining genome features related to these processes. Regulation of expression is complex, with promoters, ribosomal binding sites and transcription terminators in close proximity, often overlapping. We wrote a python program to analyze the Shine-Dalgaro sequence to initiate translation.

**Phage genome map:**

- **Exhibiting structure:** Key structural proteins were identified, and mapped. We found gene order maintained while sequence may change, and we were able to use this knowledge to identify a potential tail fiber in PhAgeTE.

Rho-independent transcription terminator

- We used two tools, Arnold and mfold, to predict 57 transcription terminators. Based on four characteristics, 23/57 were selected as putative terminators. These examples are shown relative to the right, and the middle hairpin is the only one predicted to be a transcription terminator.

**Predicted** 3’- Promoter Consensus Nigalana α30 (early) Promoter Consensus

- We used a python program to grab promoter regions from fasta file.

**Generating consensus sequence using multiple sequence alignment and weblogo**

- The toolbox would be predicted to allow transcription of early phage genes.

**Grouping by host bacteria**

Results in grouping by host bacteria, with promoters, ribosome binding sites and transcription terminators in close proximity, often overlapping. One example is show below.

**Can we create a better consensus SD sequence?**

- Wrote python program to grab 22 nucleotides before each predicted gene start.

- Generated consensus sequence using multiple sequence alignment and weblogo.

- We identified the central GGAGG is well conserved, working on what to do with the outside 5 nucleotides, and to compare auto vs. curated annotation.

**Discussion:**

- The first 16s rRNA recognizes the Shine-Dalgaro sequence to initiate translation.

**DNAMasters uses a 10 nucleotide consensus to generate the SD score.**

- The 3’-end of the 16s rRNA recognizes the Shine-Dalgaro sequence to initiate translation.

**Exploring gene expression:**

- Key structural proteins were identified, and mapped. We found gene order maintained while sequence may change, and we were able to use this knowledge to identify a potential tail fiber in PhAGETE.