Isolation and Genomic Analysis of Mycobacterium smegmatis and Rhodobacter capsulatus bacteriophages
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Introduction
The 2015-16 Illinois Wesleyan SEA-PHAGES class isolated a total of 16 novel phages that infect the host organism Mycobacterium smegmatis MC2 155 (Ms), and 6 novel phages which infect the host Rhodobacter capsulatus (Rc), strain YW1. Unlike Mc—an organism found in a wide variety of environments—Rc is primarily found in aquatic environments, and has rarely been used to study bacteriophages. Whereas the extensively-studied Ms phages are present in over 1,000 isolates, only six phages that infect Rc had been isolated before our work. All phages were characterized and categorized based on their plaque morphology, ability to form lysogens, susceptibility of these lysogens to infection by other phages, and, for the Rc phages, their ability to infect related host strains. Through such testing, three Ms phages (Daffodil, Erdmann and Yucca) and three Rc phages (Dormio, McDreamy, and Tiptonus) were sequenced and studied further. The genomes of each of these phages was then annotated using bioinformatics resources, and their relatedness was then determined using the sequencing results.

Immunity Testing:
For each phage, 3 attempts were made to isolate an infection-resistant lysogenic bacterial strain. These strains were then challenged with each of the other captured phages to reveal possible relatedness. An example is depicted below. The ability of eight different phage isolates and the phage used to create the possible lysogen (center spot) to infect a candidate lysogen is compared to the original host used for the isolation.

Ms Phage Sequencing Results:
All three sequenced Ms phages were identified as cluster C1 phages. Table 3 (right) contains a brief comparison of the genomes of our isolated phages with that of the average C1 phage, the host Ms MC2 155, and the two C1 phages isolated by IWU in 2011.

Mx Phage isolation sites:
Below: Map of Ms C1 phages found at IWU.

Rc Phage Sequencing Results:
After sequencing the three Rc phages Dormio, McDreamy and Tiptonus, several things were discovered:
1. Dormio is very similar to Oceanus, and thus allowed the creation of the third cluster, Cluster C.
2. McDreamy currently has the second largest Rc phage genome, and though it contains many orphans, it also demonstrates mosaicism as it shares genes with phages from all clusters (see the image below).

Immunity Testing:
Using Immunity Testing procedures for the Rc phage Tiptonus, a lysogen resistant to Tiptonus was created and then spot-tested against the phages listed in the "Phages Key" below. Of those phages, all save for Belator and Titan formed a plaque on the Tiptonus resistant lysogen.

Future Directions Ms phages:
1. Determine functions of the many genes with no known function.
2. The remaining 13 phages that were not sequenced could be sequenced in order to determine better understand them.

Future Directions Rc phages:
1. The three phages that were found and not sequenced could be sequenced, once purified DNA is obtained.
2. Determine how related Tiptonus is to Belator and Titan.
3. Determine why Dormio is unable to infect the Rc strain B10, whether this lies in the Tape Measure Protein or in the post-repressor Gene.

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