CONSIDER FOR TALK

7th Annual SEA-PHAGES Symposium Abstract

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Matchsticks, Spaghettis, and Podos, Oh MY!

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Interesting and varied morphologies of phages have been an exciting part of Bacillus phage hunting at JMU this year. We used the strain, *Bacillus pumilus*, F036B, as the host for isolation. Three different classes isolated 51 phages, of which we have obtained 25 TEM images. These are the first reported phages isolated on *B. pumilus* F036B. A mixture of morphotypes in virtually every sample led us to hypothesize that a prophage might be emerging as a result of infection of our host bacterium with environmental phages. We observed a “matchstick” myovirus frequently, in association with small podoviruses, more typical myoviruses, or siphoviruses with very long tails. Some samples had so many of the long-tailed, large head siphoviruses, that these images gave the impression of spaghetti and meatballs.

Our challenge in the discovery semester was the “case of the disappearing nucleic acid”, which plagued most students in every section. Tests for RNA, dsDNA and ssDNA indicated that at least some samples contained ssDNA genomes, which was confirmed by tests at our sequencing center. Ten samples appeared stable enough for sequencing, and we obtained raw data for 5 of these (Angel, Boney, Chester, Hogan, and VRKOLAKAS).

Assembling contigs from raw data was a challenge our genomic sections took on, and we utilized different size datasets to experiment with how best to find authentic phage genomes. Because we expected to find a small prophage genome, we related genome size to phage head size using previous data. Based on the expected genome size for the “matchstick” head, we could not find an appropriate size contig match. We also looked for our contigs in the genome sequence of a closely related *B. pumilus* strain, but were not able to find it. It is likely that the genome of strain F036B differs in prophage content from the sequenced strains.

The five phage genomes we analyzed were all unique, meaning that no matches were found in GenBank. However, three of these had some similarity to each other. Annotation was completed for Angel (238,553bp) and Hogan (130,213bp). These have a G+C content of about 33%, similar to the Bacillus genomic G+C content. Like previous Bacillus phages we have studied, these have terminal direct repeats (17,040 and 1,762bp, respectively) that can easily be identified using the assembly view in Consed. The genome arrangement shows predicted structural genes clustered at one end, with enzymes for DNA metabolism and entry and exit from the bacterium at the other end. Hogan is predicted to be a temperate phage due to recombinase and integrase genes. We were able to assign functions to 33/330 predicted genes in Angel and 24/236 predicted genes in Hogan.

In future studies, we would like to obtain the chromosome sequence of our host bacterium, F036B, to help figure out the prophage question, and we would like to follow up on the presence of ssDNA in our genomic preparations.