CONSIDER FOR TALK

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Temperate or Virulent? The Search for Lysogens of Cousin Phages, Belinda and Smudge, Newly Discovered from Two Continents

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Bacteriophages, Belinda and Smudge, are members of the Myoviridae family, isolated on Bacillus thuringiensis (Bt) DSM-350. Bt is a common soil microbe that acts as a natural predator for soil nematodes, but does not cause human disease. Belinda was discovered in 2015 in soil from George Town, Grand Cayman; whereas, Smudge was derived from soil on the James Madison University campus.

Standard SEA-PHAGES procedures were used the plaque purify and characterize these two phages, which were shown by transmission electron microscopy to be Myoviridae, observing contractile tails. When tested on 6 closely and distantly related Bacillus species, Smudge and Belinda were found to infect B. thuringiensis Kurstaki, but Belinda also infected B. subtilis, while Smudge did not infect at detectable levels. Because these phages are about 90% identical at the nucleotide level, the source of host range differences could potentially be identified in the dissimilar regions.

The highly related genomes consisted of just over 162,000 bases, with a GC-content of ~3%, and had a direct terminal repeats of nearly 3000 bp, based on the occurrence of the double coverage region in the assembled contigs. The terminal repeats include approximately 1500 bases of non-coding sequence and two predicted hypothetical proteins. Approximately 40 genes encoding functional proteins were putatively identified in these genomes. Of these, 11 structural proteins were identified, including tail fiber proteins, baseplate proteins, tail lysins, a prohead protease, and a major capsid protein. Lysis genes included three hydrolases and a holin. DNA binding proteins with potential to affect gene expression were identified (helix-turn-helix proteins and sigma factors). Eight DNA replication proteins were identified, including helicase, primase, and polymerase. The DNA polymerase is intact in contrast to several reported Bacillus phages where these genes are interrupted by introns or frame shifts. Ten proteins utilized in nucleic acid metabolism were identified, such as ribonucleotide-diphosphate reductase, adenylate kinase, thymidylate synthase, and nicotinamide phophribosyl transferase.

Recombinase A and Holliday junction resolvase were also predicted, suggesting that Smudge and Belinda might be temperate phages and form lysogens. We isolated putative lysogens by picking within plaques and culturing; several bacterial cultures further tested appeared resistant to infection. However, a test for these potential lysogens releasing phages was negative. The chromosome lacked any putative repressor genes, the presence of which would be expected in a temperate bacteriophage. Because other C1 phages are reported to be virulent, it is likely that these are virulent phages, and the presence of predicted recombination proteins remains a conundrum.