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Tracking Down Tracker and Sephiroth: Using DOGEMS to Identify Gordonia terrae Bacteriophages

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Since the 1920s antibiotics have been used as part of modern medicine to fight bacterial infection. It is an effective method, but it has presented with one unfortunate drawback: bacterial resistance. Multidrug-resistant organisms plague hospitals, yet antibiotics are the preferred (and at times, the only available) therapy used to help patients. Research and some exciting applications have shown promise for the use of phage therapy as a viable alternative to antibiotics. In fall 2019, 20 phages were isolated by enriched isolation at Durham Tech using the host *Gordonia terrae* 3612. 12 phages were combined and sequenced using the DOGEMS (Deconvolution of Genomes after En Masse Sequencing) approach. Following sequencing and assembling, six complete genome sequences from different clusters were returned. NCBI Primer-BLAST was used to create primers that were specific to each of the six contig sequences so that phage identity could be matched with genome sequence. Using PCR, phages Tracker and Sephiroth were matched with their clusters and subsequently annotated. Both phages are in the Siphoviridae family, Tracker is in subcluster CR2 that includes 16 additional phages, while Sephiroth is part of the DU cluster that currently has seven members. Tracker is a lytic phage with a genome length of 66,607 base pairs (bp). It has 93 genes and 65.9% GC content, quite similar to its host *Gordonia terrae* (67.8%). In the process of annotating the Tracker genome, a new gene was annotated (gene 17) based on coding potential and on comparison with other subcluster members. Additionally, the head to tail connector proteins were further explored and categorized as head-to-tail adaptor, head-to-tail stopper, and tail terminator based on alignment to phage SPP1 gp15 – 17. No tRNAs were annotated in Tracker. Sephiroth has 76,058 bps, 137 genes and a GC content of 58.6 %. Translational frameshifts were annotated in the tail assembly chaperone genes of Tracker and Sephiroth. The DOGEMS approach yielded full genome sequences for six *Gordonia terrae* phages from different clusters and is a useful method for isolating and sequencing bacteriophages when diverse phages are present in the phage DNA pool. The continued annotation and isolation of bacteriophages is important because it contributes to the field’s growing knowledge of phage evolution and interactions with their host bacteria.