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Isolation of phage that infect Gordonia rubripertincta and annotation of Fulcrum

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The *Gordonia* genus includes species capable of transforming and degrading hydrocarbons, making them candidates for environmental and industrial biotechnology. Phages of *Gordonia* are potential genetic tools that can be used to study the genes coding for the metabolic enzymes of the bacteria. To bolster the numbers of identified phage that infect the *Gordonia* genus (2,790 with 689 sequenced, according to The Actinobacteriophage Database as of Mar. 20, 2023), students used *Gordonia rubripertincta* NRRL B-16540 as the host in the Fall 2022 Virus Hunting course. Two phage were identified from local soil samples using enriched isolation protocols: Tabbat and LittleBother and one from direct isolation, Fulcrum. Viewed by transmission electron microscopy, LittleBother appears to be a myoviridae with an average tail length of 209 nm while Tabbat and Fulcrum are siphoviridae with average tail lengths of 279 and 476 nm, respectively. Phages were added to PhagesDB.org and lysates were archived. Genomic DNA of phage Fulcrum (67,607 bp, 58% GC; Cluster DV) was sequenced by Illumina sequencing by the Pittsburgh Bacteriophage Institute. Within Cluster DV, 25 of the 26 phage were isolated on *G. rubripertincta*; phage Zany was isolated on host *G. terrae*. Using the auto-annotation feature of DNA Master, the Fulcrum genome is predicted to have 92 forward and 4 reverse open reading frames (ORFs), and no tRNAs. Phamerator predicts the same number of forward genes and identifies one less reverse ORF. The reverse genes all are located within different regions of forward genes, making them less credible ORFs. Annotation analyses using Phamerator, HHPred and BLAST predict a total of 28 protein functions (30% of the ORFs), including terminase, portal protein, major and minor capsid proteins, major and minor tail proteins, lysins A and B, major and minor tail proteins, and tape measure protein. Four ORFs predicted to be membrane proteins may include a holin protein. The capsid maturation protease and scaffolding proteins have not been identified in Cluster D phage. Fulcrum produced clear plaques with diameters of approximately 1 mm consistent with the absence of ORFs with predicted protein functions such as an immunity repressor or integrase expected of temperate phage.