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Isolation of phage that infect Gordonia rubripertincta and annotation of KappaFarmDelta

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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 (2,515 with 634 sequenced, according to The Actinobacteriophage Database as of Mar. 14, 2022), students used *Gordonia rubripertincta* NRRL B-16540 as the host in the Fall 2021 Virus Hunting course. Three phage were identified from local soil samples using enriched isolation protocols: KappaFarmDelta, Octavian and Toddson. Viewed by transmission electron microscopy, Octavian and Toddson appear to be myoviridae (average tail lengths of 136 and 146 nm, respectively) while KappaFarmDelta is a siphoviridae (average tail length 274 nm). Phages were added to PhagesDB.org and lysates were archived. Genomic DNA of phage KappaFarmDelta (49,606 bp, 67.3% GC; Cluster CV) was sequenced by Illumina sequencing by the Pittsburgh Bacteriophage Institute. Poor yields of Octavian and Toddson genomic DNA precluded their sequencing. Using the auto-annotation feature of DNA Master, the KappaFarmDelta sequence predicts the genome to have 74 forward and 12 reverse open reading frames (ORFs) and no tRNAs. Phamerator predicts the same number of forward genes and identifies one less reverse ORF. Three of the reverse genes are orphams and located within different regions of forward genes, making them less credible ORFs. Known gene functions of mycobacteriophages are predicted by Phamerator and Blast analyses by DNA Master, including terminase large and small subunits, holin, portal protein, capsid maturation protease, scaffolding protein, major and minor capsid proteins, lysins A and B, major and minor tail proteins, and tape measure protein. Consistent with the predicted temperate nature of CV Cluster phage, proteins belonging to the serine integrase and immunity repressor Phams are present. Most of the Cluster CV phage (34 out of 41 total members) were isolated using the host *G. terrae* 3612, including PhrostedPhlake, which BLAST shows to be a very close relative of KappaFarmDelta. We are participating in the collaborative Gordonia Host Range project and have begun to test whether KappaFarmDelta and other phage isolated using *G. rubripertincta* by students in the course are able to infect G. terrae and other *Gordonia* bacteria. The data from these studies may help narrow down which phage genes, in addition to those encoding phage tail proteins, are likely to contribute to host specificity.