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Isolation of phage that infect Gordonia rubripertincta and annotation of Nithya and Vine

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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 (~ 2,220 with 548 sequenced, according to The Actinobacteriophage Database), students used *Gordonia rubripertincta* NRRL B-16540 as the host in the Fall 2020 Virus Hunting course. A total of five phage were identified, two from direct isolation (BigEd, DoubleDipper), and three from enriched isolation (Nithya, ObLaDi and Vine). As judged by their morphology viewed by transmission electron microscopy, BigEd appears to be a myoviridae (average tail length of 147 nm) while the other four are siphoviridae (average tail lengths ranged from 220 to 314 nm). Phages were added to PhagesDB.org and lysates were archived. Genomic DNA of phages Nithya (61,092 bp, 51.3% GC; Cluster DJ) and Vine (48,092 bp, 60.4% GC; Cluster CT) was sequenced by Illumina sequencing by the Pittsburgh Bacteriophage Institute. Previously sequenced UNL phage from 2018 and 2019 also were from Clusters DJ and CT, which are categorized as lytic phage. Auto-annotation using DNA Master predicts 89 and 73 protein coding genes, in Nithya and Vine, respectively. An obvious difference between the phage genomes, based on the auto-annotation, is the presence of 23 reverse ORFs in Vine clustered largely in the right arm of its genome, while Nithya has a small single reverse (and dubious) ORF in the middle of its genome. Close relatives of Nithya, phages Duffington and AlainaMarie, identified by BLAST, were isolated using *Gordonia terrae* 3612 as the host, which raised the interesting questions of whether they can infect *G. rubripertincta* and if Nithya can infect G. terrae. Preliminary plaque assay spot tests using s high titer lysate of Nithya showed it capable of infecting *G. terrae*. Phamerator analyses showed that Nithya and Vine shared no Phams although both had the requisite known proteins of tailed phages including terminase, HNH endonuclease, portal protein, capsid maturation protease, scaffolding protein, major and minor capsid proteins, endolysins, major and minor tail proteins, and tape measure protein. Annotation of the genomes by the class is in progress this Spring semester.