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Phorget-Me-Not: Annotation of PotPie and Deconvolution of Gordonia Bacteriophages

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Bacteriophages are the most common biological entities on the planet, and their uses are becoming increasingly relevant. Their application as an alternative to antibiotics has led to a push for new phages to be discovered and sequenced. PotPie was discovered by Kalli Simmons and Messiah King at Durham Technical Community College during the fall of 2023. It was isolated by enriched isolation with *Gordonia rubripertincta* as the host. Through further characterization and sequencing, PotPie was determined to be a lytic phage of the CT cluster, with a genomic length of 48,182 base-pairs and with a GC content of 60.7%. PotPie was annotated and gene functions were identified using DNA Master and PECAAN in conjunction with other resources including NCBI BLAST, Starterator, Phamerator, Glimmer, Genemark, PhagesDB, HHPRED, CDD, DeepTMHMM, and synteny evaluation. Out of PotPie’s 73 genes, we identified functions for 34 genes. Potpie’s organization is similar to other CT phages, with the left arm encoding well-conserved structural proteins including terminase (small and large subunits), portal protein, capsid maturation protein, head-to-tail adaptor, tail terminator, tail assembly chaperones, and at least four minor tail proteins. Lysin A is split into two adjacent genes encoding a D-Glu peptidase domain and a glycosyl hydrolase domain, respectively. Two annotated genes of note are gene 48 encoding deoxycytidylate deaminase and gene 51 encoding thymidylate kinase. Both enzymes play a role in pyrimidine synthesis and may potentially work together in DNA synthesis. In addition to PotPie, seven other phages were discovered and sequenced using the Deconvolution of Genomic En Masse Sequencing (DOGEMS) approach, which is where individually isolated phage DNA samples are deliberately mixed before sequencing to reduce library preparation costs. DOGEMS resulted in 13 contiguous sequences (contigs), four of which had complete sequences. To match the four complete contigs to their respective phages, PCR primers specific to each of the contigs were designed using NCBI Primer-BLAST and all phage DNA samples were tested by PCR. We were able to successfully match all four contigs with their respective phages. Compostlurker and HippoPololi were identified as CT cluster phages, ModicumRichard was identified as a DZ cluster phage, and Avian was identified as a DV cluster phage. We will annotate one of the phages identified through DOGEMS and the other two will be available for adoption through the SEA-PHAGES Genome Exchange. Collectively, this work adds sequence for five new phages to the Actinobacteriophage Database and contributes to growing knowledge of phage diversity.