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New Phage Phindings: Characterization of two new Bacteriophages Diversify the CS4 Subcluster

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Bacteriophages are the most abundant biological entity on Earth, and their ability to  
kill bacteria with specificity is continually being explored for environmental and  
medicinal applications. Expanding the diversity of bacteriophage characterization is  
important for both basic biology and for finding bacteriophage for a specific  
application. In the Fall of 2024, students from Durham Technical Community College discovered seven phages: Colssa, Honeymoon, Logzilla, Mikronejon, Obeleaja, Vitaenoii, and Wolfwood. Out of the seven, Vitaenoii was selected for sequencing and annotation. Vitaenoii is a lytic phage assigned to the CS4 subcluster. Philon9, another CS4 phage discovered by DurhamTech in 2021, was found to have 95.8% gene content similarity (GCS) to Vitaenoii. Both phages are uniquely dissimilar to the other ten members of the CS4 subcluster (<70% GCS). Hierarchical clustering analysis provides potential evidence that these phages may form their own subcluster. For annotation, programs including Phamerator, Starterator, Phages DB, HHpred, and NCBI BLAST were used within PECAAN (Phage Evidence Collection And Annotation Network). When the programs had conflicting information on start sites, we manually weighed the evidence to find consensus. Gene functions were determined by synteny and by using the above mentioned comparative genomics tools. Thirty-five of the ninety-six genes in Vitaenoii were assigned a functional call. These functions include the tail assembly chaperones, minor tail protein, lysin A, holin, and membrane proteins. Additionally, genome sequencing was performed through the DOGEMS (Deconvolution of Genomes En Masse Sequencing) approach, enabling the identification of individual phages from a mixed pool of the remaining six discovered phages. Five unique contig sequences were returned. Contig-specific primers were designed, and PCR was used to match contig to phage DNA within the pool. Four out of six phages were successfully matched to clusters. Mikronejon was matched to cluster DQ, and Colossa formed a new cluster with singleton VanLee. Our efforts resulted in the cluster identification of four additional phages, as well as the annotation of phages Vitaenoii and Philon9. These findings contribute valuable data to PhagesDB and underscore the evolutionary significance of CS4 phages.