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Comparative Genomic Analysis of Bacteriophages Chartreuse and Helmet from Subcluster A6

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Bacteriophages are critical tools for understanding microbial genetics and evolution. Here, we compare Chartreuse and Helmet, two temperate Siphoviridae Mycobacteriophages from Subcluster A6, isolated from soil samples obtained from Pennsylvania at the University of Pittsburgh. Chartreuse, discovered in 2020, has a 51,493 bp genome, while Helmet, discovered in 2023, has a genome size of 52,631 bp. Both phages were annotated using DNA Master, Phamerator, GeneMark, BLAST, HHPred, and Starterator, among other tools. Chartreuse has 97 ORFs, while Helmet contains 106 ORFs, with three tRNA genes in both genomes.  
Genome organization in the two phages is highly similar, with a comparable distribution of forward and reverse genes (37.1% forward, 62.8% reverse in Chartreuse; 36.5% forward, 63.5% reverse in Helmet). Notably, the tape measure protein is the longest gene (2,205 bp) in both phages. Functional annotation revealed that Chartreuse has 41 putative genes with known functions, whereas, Helmet has 38 functional genes. Both phages share key structural proteins, including the major capsid protein, terminase (large and small subunits), portal protein, and tape measure protein, which are crucial for virion assembly. They also encode functional proteins, such as DNA methyltransferase and deoxycytidylate deaminase, which play roles in DNA modification and repair. Additionally, the presence of an immunity repressor suggests regulatory mechanisms that enable these temperate phages to transition between lysogenic and lytic cycles.  
Gene content similarity (GCS) analysis shows Chartreuse shares 91.9 and 93.3% GCS with closely related phages VohminGhazi and EricB respectively, while Helmet exhibits higher similarity to Garak and Temprado (98.1%). Chartreuse and Helmet share 88 phams in common, supporting their close evolutionary relationship. In comparison to the other phages annotated previously by Unilag, Helmet exhibits more extensive similarity, sharing >22 phams with multiple phages (Kenmech, Peterson, and Big3). Chartreuse showed 24.2% relatedness with Kenmech. Despite differences in their genomes, Chartreuse and Helmet exhibit significant gene order conservation, thus, reinforcing the evolutionary relatedness of Subcluster A6 members.