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Isolation and Genomic Analysis of New B3 and C1 Mycobacteriophages

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We are reporting on the isolation and genome annotation of two new mycobacteriophages – Halfpint (B3) and Asterius (C1). Halfpint was isolated from a North Commerce Lake, Michigan water sample by direct plating at 34°C, and Asterius was isolated from a Worthington, Ohio backyard garden soil sample following enrichment, also performed at 34°C. Both phages are from well-represented (sub)clusters but still presented interesting genomic features. The Halfpint genome is 68,449 bp in length and contains approximately 103 protein-coding genes and no tRNA genes. BlastN analysis of Halfpint against the Actinobacteriophage database showed it most similar to B3 mycobacteriophages Kamiyu, isolated 15 years ago from sample collected in central Pennsylvania, and GlenHope, isolated from a soil sample collected at Hope College 12 years ago. That high degree of nucleotide identity was supported in an analysis of gene content – Halfpint in comparisons to Kamiyu and GlenHope share more than 90% of phams. Like Kamiyu and GlenHope, Halfpint also lacks an intein in its terminase gene. However, Halfpint is missing a small gene sequence found as gene 2 in both Kamiyu and GlenHope. And Halfpint, like other B3 mycobacteriophages, contains an extensive collection of a conserved sequences, seen most readily in the right end of aligned Phamerator genome maps. Asterius was isolated via a modified enrichment protocol designed to inactivate fast-reproducing phages and increase the probability of isolating slower-reproducing phages. The Asterius genome is 154,871 bp in length and contains approximately 230 protein encoding genes, 33 tRNA genes, and a single tmRNA gene. Asterius is most similar by BlastN analysis to C1 mycobacteriophage Erdmann, which was isolated in Bloomington, Illinois 8 years ago, and expectedly, they share more than 90% of their phams. Although an aligned Phamerator genome map between Asterius and Erdmann confirms their high degree of sequence identity, there are patches of differences. One region of interest across C1 mycobacteriophage genomes is in the sequence for a predicted Holliday junction resolvase (Asterius draft gp97). Clustal Omega alignments of nucleotide and amino acid sequences show strongest conservation at the C-terminal coding active domain position. Asterius is also in two minority subgroups of C1 mycobacteriophages, one that lacks the sequence corresponding to Erdmann\_24 and a second defined by having Asterius\_68; both gene are of unknown function. And similar to the B3 mycobacteriophages, Asterius in alignment with other C1 phages also shows a collection of conserved repeat sequences, but more predominantly in the left end of the genome. These and other genomic features of the new mycobacteriophages Halfpint and Asterius are being explored.