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Isolation and Genomic Analysis of the new F1 Mycobacteriophage Stap

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Fifteen new mycobacteriophages were isolated from soil samples collected around the campus of Hope College and other locations in Michigan. All phages were capable of infecting Mycobacterium smegmatis and were isolated through either enrichment at 37°C, enrichment at 34°C, or direct plating at 34-35°C. A variety of plaque morphologies were produced based on size, shape, and clarity; both lytic and temperate phages appear represented in this collection. The mycobacteriophage Stap was chosen as one of two phages for complete genome sequencing and comparative genomic analyses. The predominant plaque produced by Stap after 24 hours at 34-35°C is a clear plaque about 1 mm in size. After one week at 4°C, the plaque will gain a crescent comet tail about 3mm in diameter. The complete genome sequence for Stap showed it was similar to mycobacteriophages of cluster F, subcluster F1, which now contains over 200 sequenced members. Comparative genomic analyses show it most similar to the F1 phage Ramsey isolated in 2005 in White Bear, Minnesota, and least similar to phage Bipolar isolated in 2013 in Caldwell, Indiana. Stap has a genome size of 54,395 bp and contains about 100 protein-encoding genes but no tRNA or tmRNA genes. This genome size is at the small end of all F1 phages, which range from 52,141 bp to 61,164 bp. Like other F1 mycobacteriophages, Stap shows a high degree of genomic sequence and structural variability making annotation of this genome an interesting and exciting challenge. For example, there were multiple initial auto-annotation predictions for genes being encoded in overlapping segments on both strands of the double-stranded DNA genome (e.g., genes 34-35, 45-46, and 99-100) – an unusual organization of genetic information even for phage genomes. Stap\_Draft gene 59 is a novel single gene arrangement of coding sequences found as two adjacent genes in other related F1 phages (e.g., Ramsey, Beakin). The genetic cause is a single base insertion in the Stap\_Draft gene 59 sequence. Another genomic region of great interest is gene 80; it is bounded by direct repeat sequences and codes for a small 50-residue protein containing two CXXC segments bioinformatically recognized as part of zinc-binding protein domains. Stap and some other F1 phages also show a high degree of variability in sequence, sequence space, and gene content in the region centered around Stap genome coordinate 43,000 bp. These and other genomic features are the focus of our continuing exploration of mycobacteriophage Stap.