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8th Annual SEA-PHAGES Symposium Abstract

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Mycobacteriophages Krueger (K6) and Phrappuccino (Singleton) Harbor Unique Examples of Mosaic Architecture Linking Actinobacteriophages from Different Hosts

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Eighteen new mycobacteriophages were isolated from soil samples collected around the state of Michigan and parts of the United States. All phages were capable of infecting *Mycobacterium smegmatis* and were isolated through either enrichment or direct plating at 25°C. Two mycobacteriophages, Krueger and Phrappuccino, were chosen for genome sequencing and comparative genomic analyses. The predominant plaque produced by Krueger at 32°C was circular and 2 mm in diameter. The predominant plaque produced by Phrappuccino at 32°C was 1 mm in diameter, and took 48 hours to appear. The genome sequence for Krueger revealed relationships to members of the novel Subcluster K6. The genome of Krueger is 60.3 Kb, 66.5% GC, and contains 101 genes, including 1 tRNA(Lys-TTT) gene. Subcluster K6 comprises 5 phages, 2 of which were isolated at Hope College in successive years. Genome arrangement within the subcluster is similar through the length of the genomes; differences are apparent at the level of nucleotide identity and pham membership. While there are 27 phams unique to Subcluster K6, Krueger is a member of just 4 of these phams, revealing mosaicism within the subcluster. Functions were assigned to 39 protein coding genes, representing 38.6% of called genes. The genome sequence of Phrappuccino reveals a truly novel phage that expands the diversity of known Mycobacteriophages in several interesting ways. The genome of Phrappuccino is 136.3 Kb, 67.4% GC, and contains 200 genes, reminiscent of the largest Mycobacteriophage genomes found in Cluster C. Electron microscopy shows that Phrappuccino displays a myoviridae structure (capsid, 86 nm; tail, 85 nm). The genome of Phrappuccino strongly resembles the basic genetic architecture of Cluster C phages and further suggests a link between *Rhodococcus* phage E3 and mycobacteriophages with myoviridae structure. Known insertion points for tRNA cassettes in Cluster C phages are conserved in both Phrappuccino and E3, although Phrappuccino and E3 do not carry tRNA genes. They contain a small number of mostly orpham genes in place of the tRNA cassettes. Phrappuccino shares 42 and 39 phamilies with C1 and C2 phages, respectively. The highest number of shared phams with non-myoviridae mycobacteriophages is 5, including F1, M and J phages. In contrast, 32 phamilies are shared with *Rhodococcus* E3, a singleton with high GC content, similar genome size and a myoviridae structure. Other potential myoviridae *Rhodococcus* phages share a single pham with Phrappuccino. There are 25 phams exclusive to Phrappuccino, Cluster C, and E3; many of these genes encode functions necessary for the typical phage life cycle. Consistent with the Singleton classification, 125 orphams are located throughout the genome; thirty-eight (19%) protein coding genes were assigned functions. These observations reveal a fundamental relationship between Phrappuccino, the distinctive mycobacteriophage Cluster C, and emerging *Rhodococcus* phages.