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9th Annual SEA-PHAGES Symposium Abstract

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Establishing Mycobacteriophage Cluster AA – Genomic Analyses of Settecandela and Phrappuccino

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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 32°C or 37°C. A variety of plaque morphologies were produced based on size, shape, and clarity; both lytic and temperate phages appear represented in this collection. Three phages, Opia (Cluster B2), KashFlow (Cluster J) and Settecandela (Cluster AA), were selected for complete genome sequencing and comparative genomic analyses. We focus here on comparative analyses of Settecandela. The predominant plaque produced by Settecandela at 37°C was 1 mm in diameter, and took 48 hours to appear. The complete genome sequence for Settecandela revealed a relationship to the singleton, Phrappuccino, discovered by the Hope College phage class in 2016. The genome of Settecandela is 145.2 Kb, 67.4% GC, and contains 224 genes. The close relationship with Phrappuccino leads to the formation of a new cluster of mycobacteriophages, Cluster AA. The two genomes are identical over much of their length, with the exception of an approximately 9000 bp region in Settecandela containing 26 genes. The Settecandela-unique region begins near the 5’ end of gp184; the first 75 nucleotides of this gene are identical with gp183 of Phrappuccino at which point the sequences diverge. Settecandela gp184 encodes a protein of unknown function that is found in *Mycobacterium*, *Rhodococcus* and *Gordonia* bacterial species. The highest BLAST hit (full length, e-value 6 x 10-108) is to a protein with no known function from *Mycobacterium koreense*, a slow growing mycobacterium isolated from a patient with pulmonary dysfunction. Three genes were assigned functions in the unique region; 16 of the genes are currently orphams. The remaining 9 genes are members of phams found in phages from multiple mycobacteriophage clusters. At the end of the unique region, the Settecandela genome contains a full-length homologue to Phrappuccino gp183 (the start of the unique region), with sequence variation occurring in the first 62 nucleotides of the gene. Plaque morphologies of the two phages differ, with Settecandela displaying a comet morphology for some plaques. There is strong evidence at the morphological (Myoviridae) and genomic levels for a relationship of Cluster AA phages to Cluster C phages. Despite this relationship, Cluster AA genomes do not carry any tRNA genes. Additionally, Cluster AA genomes contain homologues to *Rhodococcus* phage E3 and to multiple actinobacterial genera, suggesting the potential for a broadened host range.