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9th Annual SEA-PHAGES Symposium Abstract

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BEARS in the SEA Unearth Arthrobacter Phage Diversity Along Waco’s Brazos River

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Bacteriophage are prolific throughout our biosphere, yet little is known about their genome. *Arthrobacter* phage infect *Arthrobacter*, a genus of common soil bacteria known to metabolize a variety of organic substrates. An increased interest in *Arthrobacter* phage has resulted in over 150 sequenced genomes since 2012. These phages were all isolated on *Arthrobacter* sp. 21022 and are classified in 12 clusters based on nucleotide similarity. As this area of phage genomics grows, a more efficient method of clustering phages that does not require whole genome sequencing would facilitate further studies in this field. Our research adds the annotations of four *Arthrobacter* phage genomes from four different clusters to the phagesdb.org database: Lore (AN), Shrooms (AL), Caterpillar (AU), and Nubia (AK) and proposes a new method for cluster identification using phage lysates. Phages were isolated from soil in various locations in Waco, TX, and purified on host *Arthrobacter* sp. 21022. DNA was purified and sequenced using Illumina Sequencing and annotated using DNAMaster. Genes and their putative functions were predicted using Glimmer, Genemark, NCBI BLASTp, and HHpred. Furthermore, comparative analysis using Phamerator and Starterator supported gene annotations. Overall annotations reveal that *Arthrobacter* phage Lore is similar to the other ~15,550 base pair phages of the AN cluster. Interestingly a function not previously called in AL phages was discovered for Shrooms gene 51 through an HHpred hit that matched a MazG nucleotide pyrophosphohydrolase. Caterpillar was unique in that its genome contained four large intergenic gaps. Nubia, like other AK phages, contained a frameshift within the tail assembly chaperone gene. Promoter and terminase analysis was conducted in each cluster using DNAMaster, BPROM, and ARNold. Sigma 70 like promoters were identified in the gaps between the forward and the reverse genes of Lore, Nubia, and Shrooms. Probable rho-independent transcription terminase sequences were also identified in each of the four phages. Tape measure protein (TMP) sequences from each cluster were compared using Gepard dotplots and Mega7 was used to produce a phylogenetic tree. The results indicate that TMP single-gene clustering is identical to whole genome clustering. Therefore, we used the variable regions of the TMP genes from each cluster to design 12 unique primer sets. In the future, these primers will be tested against a panel of known *Arthrobacter* phage. *Arthrobacter* phage diversity is predicted to be high, similar to Mycobacteriophage diversity, based on the large number of clusters already recognized. As in Mycobacteriophage research, TMP analysis has the potential to provide an efficient way to cluster *Arthrobacter* phage in the laboratory without sequencing the entire genome.