CONSIDER FOR TALK

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Proteomic and Genomic Analysis of Novel Cluster A, B and P Mycobacteriophages

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Mycobacteriophages TNguyen7, Brusacoram, and Hetaeria were isolated and characterized using proteomic and genomic tools as part of the SEA-PHAGES program at The College of St. Scholastica in Duluth, MN. All phages were isolated from soil through enrichment using Mycobacterium smegmatis as host. Genome sequencing placed TNguyen7 in cluster A3, Brusacoram in P and Hetaeria in B1. A total of 93, 78, and 100 protein coding genes were predicted in TNguyen7, Brusacoram, and Hetaeria, respectively. 6 putative promoters were identified in TNguyen7 (-35 TTGACG and -10 TAT\_CT) and Brusacoram (-35 TTGA\_T and -10 TATAAT) involved with the transcription of these genes. Multiple stoperator sequences responsible for immunity repressor binding were identified in all three phages. In addition, sequences with predicted integrase function were identified in both TNguyen7 and Brusacoram. Interestingly, MEGA 6 phylogenetic tree analysis of integrase sequences indicated that the integrase in Brusacoram is more closely related to the integrases in cluster I than other cluster P integrases. Although TNguyen7 produced large turbid plaques typical of A3 phages, both Hetaeria and Brusacoram produced medium/small clear plaques. These plaque morphologies in Hetaeria and Brusacoram are inconsistent with the presence of multiple stoperator sequences in both phages, and the presence of in silico predicted gene functions associated with the maintenance of lysogeny in Brusacoram (e.g. integrase, immunity repressor). Thus, it appears that both Hetaeria and Brusacoram either have a preference for host cell lysis or an inability to enter or maintain the lysogenic cycle despite genome traits that suggest otherwise. In support of this, we have been unable to isolate lysogens with Hetaeria. The high degree of host cell lysis in Hetaeria and Brusacoram (as opposed to lysogeny and prophage formation) made both of these phages candidates for proteomic analysis of expressed proteins during active infection. To achieve these aims, LC-MS/MS was used identify phage proteins expressed 4 hours post infection. Proteomic analysis of Hetaeria resulted in the detection of nearly 70% of in silico predicted proteins, and the verification of 26% of called translational start sites. Amino acid modifications were present in over 20% of identified proteins. Gene functions were predicted for 33% of identified proteins, with functions encompassing phage structure, genome replication and assembly/release. 66% of identified proteins expressed by Hetaeria have no known function. LC-MS/MS analysis of expressed Brusacoram proteins identified approximately 50% of in silico predicted proteins. Identified proteins occur throughout the genome and are responsible for a variety of functions associated with phage structure and replication.