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TOWARD A BETTER UNDERSTANDING OF...

Genomic Analysis of Mycobacteriophage, Kradal: Evolutionary Insights within the Recently Discovered BM Cluster

Aja Drain, Lauren Kang, Tuka Mousa, and Jerry Sun

Mentor: Chris Shaffer

The goal of this research was to isolate and characterize a novel bacteriophage and deduce the position and functionality of its genes. Phage KRADAL was isolated through direct isolation with host bacteria Streptomyces griseofuscus, purified through five rounds of purification, amplified to create a high-titer lysate, and characterized through transmission electron microscopy. Phage DNA extracted from the high titer lysate was also characterized through gel electrophoresis of various restriction enzyme digests and subsequently sequenced. This process of phage isolation, purification, and characterization revealed that KRADAL forms very small (< 2 mm in diameter), circular, and clear plaques. Transmission electron microscopy revealed that it has a flexible tail and a rod-shaped head, in contrast with the more common icosahedral head. The phage genome is 184,673 base pairs long with a 1053 base-pair terminal repeat, and genomic analysis has placed KRADAL in phage cluster BM. The phage shares distinct Gene product similarities with two phages: JustBecause and Satis. For example, gel electrophoresis of KRADAL's DNA and that of other rod-shaped phage revealed similar restriction enzyme digest patterns. We performed positional and functional annotation on the middle section of the phage genome (from base 60,409 to 149,338) utilizing computational tools including PECAAN, DNA Master, GBrowse, and NCBI/PhagesDB BLASTX searches. In total, the middle section of KRADAL has 168 protein-coding genes. Functional annotation will be performed on these genes in order to discern any possible functions of these proteins. This research adds to the limited body of knowledge currently available about the genomes of rod-shaped phages in general, and more specifically, the BM cluster. This research can reveal differences in protein function that differentiate rod-shaped phages from icosahedral phages.