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Analysis of Additional Arthrobacter Phage with Small Genomes (Cluster D) and the Singleton Kitkat

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We identified six new bacteriophage that infect Arthrobacter sp. All of these phage have small genomes of just over 15kbp and are closely related to each other and the Cluster D Arthrobacter phage listed in Phamerator. Two of the phage, Yank and Decurro, were isolated from the same soil sample based on different plaque morphologies. Upon sequencing it was determined that there is a single base change at position 11,243, which changes a serine to isoleucine in gp15. The predicted amino acid sequence of gp15 has no strong similarity to any proteins in Genbank, which makes the different plaque morphologies very interesting. The genomes of the other four phage show high levels of similarity to each other and to the genome of Sandman, which we isolated last year. The major differences between these genomes are near the beginning, where some of the genomes either lack “gene 2” (Toulous) or have one of two variants of this gene (pham 892 or pham 972). The version of gene 2 found in Sandman (pham 671) is different from the other Cluster D phage and represents an orpham. There is a single reverse gene that was not identified by DNA Master in all of these genomes. Even though all of the genomes appear to contain this reverse gene, which encodes a protein with predicted helix-turn-helix structure, small sequences deletions near the start of this orf determined whether it was called or not. This reverse gene is likely the repressor, since all of these phage appear to be temperate and upon lysogen testing are homoimmune to each other. We note that some aspect of our phage hunting technique enhances the isolation of phage in this cluster and changes in various conditions will be tested to see if they foster discovery of phage with more diverse genomes. In addition to the analysis of the Cluster D Arthrobacter phage, we analyzed the genome of Kitkat, an Arthrobacter phage discovered last year. The genome of Kitkat is 58560bp in length and appears to be a new singleton. All of the 100 orfs encoded by Kitkat are in the forward orientation. Kitkat is predicted to be lytic since it produces clear, small plaques and lysogens could not be isolated. This corresponds with the lack of an orf that encodes a putative repressor protein. Of the 100 proteins that Kitkat encodes, many appear to have putative enzymatic functions involved in DNA replication, recombination and repair. Some of these contain intriguing homologies to proteins involved in the CRISPR/Cas system, which have been identified in other phage and could provide a counterattack to the host defense system.