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Comparative genomic analysis of mycobacteriophages and the quest for new protein folds

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Mycobacteriophages are ubiquitous viruses that infect mycobacteria. They have potential uses in the field of biotechnology and medical science with applications ranging from disease diagnosis, through phage typing, phage vaccine and phage therapy. Meanwhile, only a meager number of mycobacteriophages have been identified and characterized out of the multitudes present in the biosphere. In addition, a far majority of the bacteriophage genes that are discovered have no known function or structure. In this study, thirty novel mycobacteriophages that infect Mycobacterium smegmatis were isolated, characterized and fourteen were annotated per the most recent guidelines using both PECAAN and DNA Master. One of the characterized mycobacteriophages, Ochi17, has a genome size of 58kbp and GC content of 61% and was classified into cluster F, and sub-cluster F1. The second phage, VasuNzinga, was identified for sequencing by Dr. Hatfull's research group at University of Pittsburgh during a screen of archived lysate inventory using DOGEMS. It is only the eighth Cluster S phage ever found. Both genomes contained expected virion structural genes, such as the tape measure protein and other genes involved in tail assembly. Capsid related genes were also annotated including capsid maturation protease, scaffolding protein, major capsid protein. Both phages also contained integrase and putative proteins that mediate the lytic cycle such as lysins A and B, and holin. Ochi17 contained three tRNAs and several defense related genes such as the exo- and endo- nucleases, the immunity repressor, and the antirepressor. Notably, VasuNzinga contained multiple putative transferase enzymes for post-translational modification. Several genes were annotated with no known function (NKF), 59 genes in Ochi17 and 68 genes in VasuNzinga, respectively. In order to investigate the role of the genes with no known function and to confirm the functions of the predicted genes, the course research experience was expanded to include collaborations with a structural biology research group. The first step toward trying to discover new protein folds hidden within these uncharacterized genes from bacteriophages isolated through the SEA-PHAGES program, was piloted with the first phage discovered at Purdue in 2010, called ‘MrGordo.’ MrGordo contains 92 genes, 31 with homology to known proteins of function and/or structure; 61 have unknown function/structure. Twenty genes from Mr. Gordo were cloned, expressed using recombinant expression methods, and purified using metal affinity chromatography. Twelve of the proteins were soluble or could be refolded and structural characterization was performed using SEC, CD analysis, and/or SEC-SAXS. Current efforts are to determine the crystal structures of these twelve proteins. The goal of this project is to gain insight into the 3D fold of each of these mysterious novel phage genes in search for new protein folds.