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2022 SEA Symposium Abstract

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Elucidating Mycobacteriophage Taptic Gene Functions with the SEA-GENES Research Project at Lehigh University

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Taptic is a Cluster W mycobacteriophage that was isolated from a soil sample by a Lehigh University undergraduate student in 2015. Its genome was completely sequenced by the Pittsburgh Bacteriophage Institute that same year and then fully annotated at Lehigh. The Taptic genome is 60,973 base pairs in length and consists of 92 protein-coding genes and one tRNA-encoding gene. Taptic is an interesting bacteriophage for several different reasons. The Taptic genome lacks the integrase and immunity repressor genes and has an incomplete set of chromosomal partitioning genes (the Taptic *gene 74* encodes a ParB protein but there is no annotated ParA). As such, Taptic has been characterized as a lytic bacteriophage. Interestingly though, Taptic is able to form a lysogen (though relatively unstable), but the mechanism by which it accomplishes this is currently unknown. We hypothesize that it may do so via extrachromosomal prophages, as revealed from sequencing mc2155(Taptic). One Taptic gene (Taptic *29*) encodes a protein that has a significant degree of homology with a bacterial small subunit ribosomal protein, raising the intriguing possibility that this protein might alter host cell translation. We chose Taptic as the target bacteriophage for the SEA-GENES Research Project for the spring 2022 semester. To date, we have successfully PCR amplified about two-thirds of Taptic’s protein-coding genes, and most of these have been cloned into the pExTra vector so that their expression can be regulated by the tet-inducible pTet promoter. Many genes have been tested to determine if overexpression is toxic to Taptic’s host, *Mycobacterium smegmatis*. So far, we have identified one gene, Taptic *58*, which is cytotoxic when overexpressed in *M. smegmatis*. Taptic *58* gene homologs are found only in other Cluster W mycobacteriophages. However, HHpred analysis of this protein does not provide us with a clear indication of how Taptic gp58 overexpression kills the host cell. The spring 2022 SEA-GENES Research Project is ongoing, and by the end of the semester we plan to perform cytotoxicity tests on all cloned genes, together with defense assays to determine if Taptic gene overexpression protects *M. smegmatis* from infection by Taptic and other bacteriophages.