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2021 SEA Symposium Abstract

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Fifteen genes from bacteriophage Amelie are toxic when overexpressed in Mycobacterium smegmatis

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Bacteriophages engage in complex dynamic interactions with their bacterial hosts and their genomes display high genetic diversity. Bioinformatics tools have been used to assign function to many of the genes but the vast majority of genes have unknown function. Determining the functions of phage genes will help elucidate the mechanisms of infection. To this end, the GENES class at La Sierra University studied Mycobacteriophage Amelie. Amelie, which infects *Mycobacterium smegmatis*, is a temperate phage with a 56,439 bp genome and contains 77 putative protein-coding genes, 38 of which had been previously assigned a function. In order to begin to elucidate the gene functions of other, 76 out of the 77 were amplified by PCR and cloned into plasmid pExtra-01. After verifying that each plasmid had the right insert, 59 of the plasmids were introduced into *Mycobacterium smegmatis* via electroporation. Using cellular toxicity of phage gene overexpression as an assay, the expression of fifteen genes resulted in inhibition of *M. smegmatis* growth either by reducing colony number and/or size. Out of the 15 genes, 8 of them have not been assigned a function. Combining the cytotoxicity data with future defense assays and phage-host interactions will provide further insight into the role of those genes in the phage life cycle and possibly identify novel phage therapies.