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2023 SEA Symposium Abstract

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Identification of 34 genes from phage Lebron that are toxic upon overexpression in Mycobacterium smegmatis

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Bacteriophages engage in complex dynamic interactions with their bacterial hosts and their genomes display high genetic diversity. Although bioinformatics has helped assign function to many of the genes encoded by bacteriophages, most of the genes cannot be assigned a predicted function. Gene overexpression is a powerful strategy for studying gene function, linking genes to observable phenotypes. To this end, the GENES class at La Sierra University studied Mycobacteriophage Lebron. Lebron, which infects *Mycobacterium smegmatis*, is a temperate phage with a 73,453 bp genome and contains 123 putative protein-coding genes, 65 of which can be assigned a function via bioinformatics, and 9 tRNAs. To begin to elucidate the gene functions of the other genes, the last 57 genes of the genome were amplified by PCR and cloned into plasmid pExtra-01. After verifying by PCR and gel electrophoresis that each plasmid had the right insert, the plasmids were introduced into *Mycobacterium smegmatis* via electroporation. Using cellular toxicity of phage gene overexpression as an assay, the expression of 22 genes showed partial to full cytotoxicity. Some of the genes encode for transcription factors, proteins involved in DNA synthesis, synthesis of nucleotides, and about a dozen genes with no known function. Altogether, 34 out of the 123 genes displayed different levels of cytotoxicity, including 20 with no known 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.