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Systematic Analysis of Bacteriophage Phayonce Gene Toxicity on Host Mycobacterium smegmatis

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Because they are toxic to bacterial hosts, bacteriophages are emerging as potential avenues in treating significant bacterial diseases. Systematic analysis of individual gene function on the host allows for an increased understanding of phage biology and possible therapeutic use. This work aims to generate a gene library of the mycobacteriophage Phayonce in an inducible expression vector, and analyze possible cytotoxic effects caused by overexpression of each gene in host cells. Each of Phayonce’s 77 genes were amplified by PCR using gene specific primers and assembled into the pExTra plasmid. The pExTra plasmid contains a tetracycline inducible promoter for controlled expression of the phage gene. Each plasmid was verified by colony PCR using universal pimers. Subsequent sequencing of the recombinant pExTra plasmid established that they are error-free. After verification, each pExTra clone was transformed into *Mycobacterium smegmatis*, Phayonce’s host, for phenotypic analysis to determine cytotoxic impacts of each gene on host cells. We identified a number of genes that reduce cell growth in a manner that ranges from a slight reduction in colony size to complete elimination of colony formation. One interesting cytotoxic gene is Phayonce 77. Phayonce 77 has similarity to T4 polynucleotide kinase, which is involved in the phosphorylation of nucleic acids. Future work is aimed toward determining interactions between Phayonce 77 and *M. smegmatis* proteins using a bacterial two-hybrid assay. These tentative protein protein interactions will provide insight into the mechanism behind this gene’s toxic impact on the host, *Mycobacterium smegmatis*.