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Functional analysis of conserved hypothetical genes from cluster K mycobacteriophages Hammy and Waterfoul

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Bacteriophages that infect mycobacteria attract a lot of recent interest as therapeutic agents for treating infections caused by antibiotic-resistant *Mycobacterium tuberculosis*. To date, over 1500 phages that infect *M. smegmantis* and *M. tuberculosis* were discovered and characterized through genome sequencing and bioinformatics analysis. Most of these phages belong to the family Siphoviridae and are grouped into clusters based on genetic similarity. In addition to well-characterized genes that encode structural, regulatory, DNA metabolism, and lytic proteins mycobacteriophages carry numerous conserved hypothetical genes. Such genes are identified via cross-genome comparisons, but their function is currently unknown. In this study, we employed a combination of high-fidelity PCR and Gibson assembly to clone 70 hypothetical genes from the cluster K bacteriophage Hammy. The genes were cloned into the shuttle vector pSMEG and introduced into *M. smegmatis*, the natural host of Hammy. We then treated the plasmid-carrying clones with the inducer anhydrotetracycline and demonstrated that several hypothetical genes exhibit cytotoxicity and kill the bacterial host. Some of the newly-characterized cytotoxic Hammy genes have homologs in bacteriophage Waterfoul and are variably present in other clusters of mycobacteriophages. The ongoing work involves the identification of *M. smegmatis* targets of the cytotoxic proteins using a bacterial two-hybrid system. Results of this study will help to elucidate the role of poorly characterized viral genes in the biology of phages that infect *M. smegmatis*, *M. tuberculosis*, and closely related bacteria.