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Modulation of Bacterial Host Phenotypes by Mycobacteriophage Pixie Gene Products

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Bacteriophage genes are being studied for their potential clinical use in phage therapy for antibiotic-resistant infections. With the support of the Howard Hughes Medical Institute, as part of the Science Education Alliance Gene-function Exploration by a Network of Emerging Scientists (SEA-GENES), we have amplified 42 out of 100 genes from Mycobacteriophage Pixie and cloned 32 genes for study in cytotoxicity and superinfection assays. Genes were amplified from Pixie high titer lysate by PCR amplification, and the products purified and ligated into a pExTra plasmid by isothermal assembly. Plasmids were transformed into 5-alpha F’Iq *Escherichia coli*, and the extracted plasmid DNA was electroporated into *Mycobacterium smegmatis* mc2155. Phenotypic assays were conducted by plating transformed *M. smegmatis* on agar containing anhydrotetracycline to induce production of gene inserts. Cytotoxicity was determined by spotting serially diluted transformed *M. smegmatis* growth versus controls. Superinfection assays were conducted by inoculation of transformed *M. smegmatis* and control *M. smegmatis* lawns with serially diluted phages to determine the efficiency of plating. Our study revealed 4 potentially cytotoxic genes (a putative major capsid protein, uncharacterized gene downstream of the lysin A/B proteins, putative tyrosine integrase, and uncharacterized gene adjacent to the immunity repressor) and screened 18 genes for superimmunity. This information broadens the understanding of bacteriophage-bacteria interactions and can allow us to apply it to clinical use of bacteriophages.