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2023 SEA Symposium Abstract

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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. As part of the SEA-GENES network over six semesters, we have amplified and cloned all 100 genes from Mycobacteriophage Pixie for study in cytotoxicity assays. Genes were amplified from Pixie high titer lysate by PCR amplification, and the products were purified and ligated into a pExTra plasmid by isothermal assembly. Plasmids were confirmed by sequencing and 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 the production of gene inserts. Cytotoxicity was determined by spotting serially diluted transformed *M. smegmatis* growth versus controls. Our study revealed 34 potentially cytotoxic genes: a putative major capsid protein, a holin protein downstream of the lysin A/B proteins, portal protein, putative tyrosine integrase, and a series of uncharacterized cytotoxic genes adjacent to the immunity repressor. We are currently assessing bacteriophage-bacteria protein-protein interactions of cytotoxic genes using bacterial 2-hybrid assays. This information broadens our understanding of bacteriophage-host interactions and the potential clinical use of cytotoxic genes to treat bacterial infections.