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Amplification and Cloning of Mycobacteriophage Pixie and ThetaBob Genes

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Bacteriophages are gaining increasing recognition in the health community for their potential to combat antibiotic-resistant bacteria. As resistance to traditional antibiotics grows, phages present a promising alternative due to their specificity and diversity. However, the field of bacteriophage research is still in its early stages, and the functions of many phage-encoded proteins remain unknown. Through the SEA-GENES program, our team worked to characterize gene functions in two bacteriophages, Pixie and ThetaBob, to assess their potential roles in antimicrobial strategies. High titer lysate DNA served as the PCR template, and products were validated through gel electrophoresis. Based on band patterns, DNA fragments were purified using column purification or gel extraction. The resulting DNA was inserted into plasmids via isothermal assembly, transformed into *E.coli* 5-alpha F’Iq cells, extracted, sequenced, and electroporated into *M.smegmatis* for expression. For Pixie, we amplified and cloned 99 of 100 genes into pExTra for phenotypic assays. Of these genes, 95 were sequenced and verified. Preliminary phenotypic assays using Pixie encoded genes identified 38 potentially cytotoxic gene products, which were grouped into functional clusters for further analysis. We amplified by PCR 102 out of 106 genes from ThetaBob, with 46 genes sequence-verified to date. Out of 58 genes tested for cytotoxicity, 46 genes are possibly toxic. These findings expand the growing database of known phage gene functions and reinforce the value of phages in developing future treatments for drug-resistant infections. Ongoing efforts aim to complete ThetaBob sequence verification and functional screening of both phages.