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Using Cytotoxicity and Bacterial-2-Hybrid Assays to Investigate Phage Sheen Gene Functions

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A bacteriophage is a virus that infects and kills their bacterial hosts. Bacteriophages have increasingly been used for medicinal therapies and for maintaining environmental bacterial populations. Bacteriophage are the most abundant microbe on the planet; however, most bacteriophage genomes and functions are not known. This research focuses on further characterizing the genes within Mycobacterium phage Sheen. Characterization required the use of standard amplification and assembly techniques such as PCR and Isothermal Assembly for each gene. To date, 22 of the 86 genes have been amplified and assembled into a plasmid backbone. These genes were analyzed using a cytotoxicity assay with ten of these genes characterized as toxic to the host. Sheen gene ten is a toxic gene that is known to code for lysin A. A Bacterial-2-Hybrid assay is being conducted with lysin A to identify the possible protein-protein interactions with the proteome of its primary host, Mycobacterium smegmatis. These findings contribute to the understanding of phage gene function.