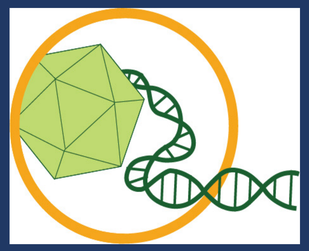
Cloning and Overexpression of Phayonce Genes to Assess Cytotoxicity in *Mycobacterium Smegmatis*

Abbey Montoya, Olivia Sundman , Dr. Daniel Williams, Dr. Michael Pierce
Department of Biology

Abstract:

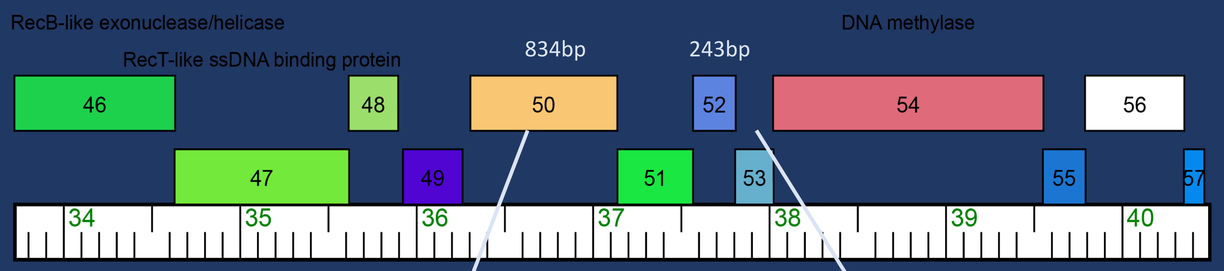
Bacteriophages, viruses that infect bacteria, possess the potential to be utilized for therapeutic purposes. Despite being the most abundant biological entity, the biological function of many phage genes has not been established. Our lab is analyzing each gene of the phage Phayonce, which infects *Mycobacterium smegmatis*. First, individual genes are inserted into an inducible expression vector. Then, these plasmids were used to transform *M. smegmatis* and determine if individual genes have toxic effects on host cells. Because functions of gene number 50 and 52 of Phayonce cannot be inferred by sequence comparison, they were selected for analysis. These genes were cloned into the pExTra inducible expression vector and used to transform M. smegmatis. We assessed cytotoxicity of these gene products by assaying host cell growth rates on media that induces expression of the phage genes. Our results will establish a biological role of these bacteriophage proteins that could be developed into therapeutic strategies to combat bacterial diseases.

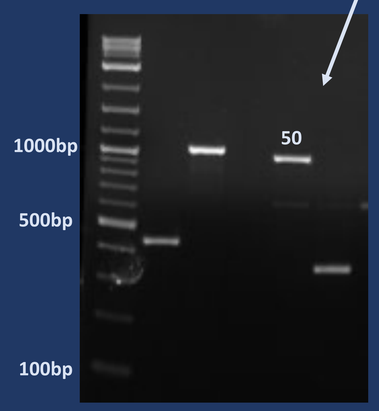


Bacteriophages account for a large majority of all organisms in the biosphere. A phage will only infect one, or a few, specific species of bacteria. The number of individual phages that are isolated are substantial and have increased in recent years, but the functions of individual genes remain unknown.

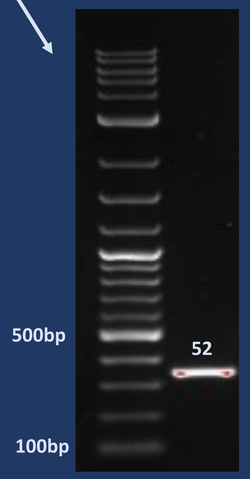
The SEA-GENES project aims to analyze phage genomes in order to identify cytotoxic genes

Coastal Carolina University's SEA-GENES lab selected mycobacteriophage Phayonce, whose genome is comprised of 77 genes. Using PCR, genes 50 and 52 were amplified.

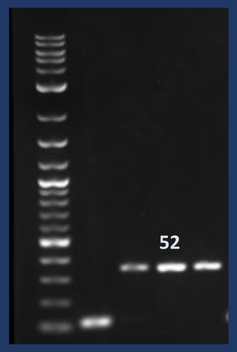


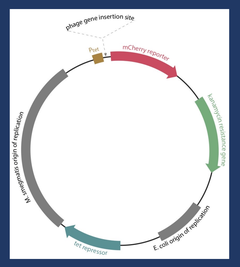


1. Gene isolation and amplification using gene specific primers

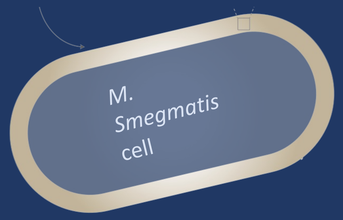


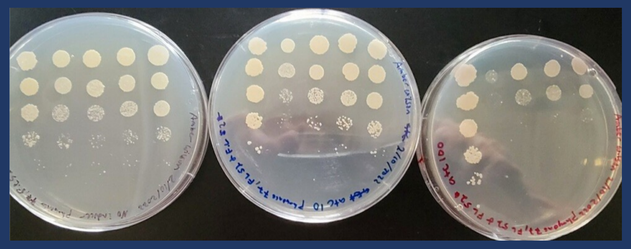
2. Ligation into pExTra plasmid by isothermal assembly





3. Electroporation and cytotoxicity assay



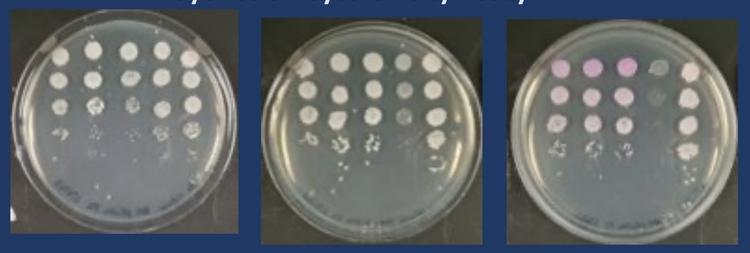


Phayonce 77 (Cytotoxic)

Results and Conclusion:

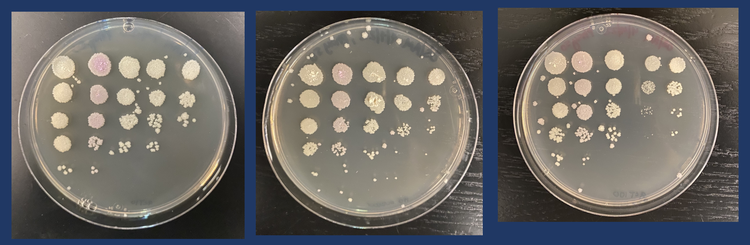
Gene 50 and 52 were determined to be non-cytotoxic. We can infer that the gene products are not detrimental to the host bacterial cell because gene 50 and 52 did not affect the fitness and growth of *M. smegmatis.* It is possible that gene 50 and 52 can be involved in the assembly of viral particles. If there are no particles to assemble, there will be no effect on the growth of bacteria. It is also likely that they could be involved in the packaging of DNA into viral particles. In both instances, the lack of DNA or particles does not affect cytotoxicity.

Phayonce 52 Cytotoxicity Assay



No Inducer aTc10 aTc100

Phayonce 50 Cytotoxicity Assay



Further Research:

We will continue to assess other genes within the Phayonce genome within our lab and in conjunction with the SEA-GENES project. Gene 35 has been isolated and will be electroporated into M. smegmatis in order to perform a cytotoxicity assay to assess effects. In the event of cytotoxicity, genes will be analyzed to determine what biological function they have in the event of an infection.

References:
Hatfull GF, Hendrix RW. Bacteriophages and their genomes. *Curr Opin Virol*. 2011;1(4):298-303. doi:10.1016/j.coviro.2011.06.009

Deighan, P., Gainey, M., Ali, I., Mohamed, A., Amaya, I., Heller, D., & Sivanathan, V. (2019). Home. Retrieved April 8, 2022, from https://seagenes.helpdocsonline.com/home

Wilson, Amber. Overexpression and Functional Analysis of Phayonce Gene 77. February 2, 2022.