Cloning and Overexpression of Phayonce Genes 12 and 77 in *M. Smegmatis*

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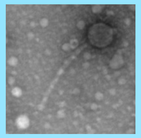
Abstract

Bacteriophage or phage are a diverse class of viruses that infect and reproduce in bacterial cells. Their diverse genomes represent an immense source of novel protein functions and a deeper understanding of phage genes will contribute to emerging treatments for antibiotic resistant bacterial infections. The goal of the research described here is to isolate and study individual genes from the bacteriophage Phayonce. Gene specific primers were used to PCR amplify Phayonce genes 12 and 77 (Phayonce 12 and Phayonce 77). The PCR products were then ligated into a plasmid vector by isothermal assembly. Following confirmation of the cloned gene by colony PCR, plasmids were transformed by electroporation into the Phayonce host bacterium, Mycobacterium smegmatis. To determine if the isolated phage genes interfered with cellular function, Phayonce genes were overexpressed from an inducible promoter in the host M. Smegmatis. Three isolated colonies overexpressing Phayonce 12 or Phayonce 77 were tested and comparted to toxic and non-toxic control strains of M. Smegmatis. Phayonce 12 overexpression did not result in a cytotoxic phenotype while the overexpression of Phayonce 77 did produce a cytotoxic phenotype. Future experiments will attempt to identify specific host proteins that interact with the proteins encoded by Phayonce genes 12 and 77.

Introduction & Background

The mycobacterium phage Phayonce was isolated from a soil sample at the University of Pittsburgh in 2014 as a part of the Phage Discovery class. The SEA-GENES group at Coastal Carolina began cloning and overexpressing all Phayonce genes in 2019 with the overall goal being to understand the function of all 77 Phayonce genes. I have cloned Phayonce genes 12 and 77 and have overexpressed the genes in the bacterial host *M. smegmatis* as part of my research project in the SEA-GENES.

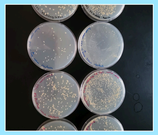


Research Questions:

- Are the Phayonce 12 and 77 genes cytotoxic to the host *M. smegmatis*?
- What are the functions of the Phayonce 12 and 77 genes.?
- If overexpression of the Phayonce gene makes the cells sick, what property of the encoded phage protein is responsible for the cytotoxicity?

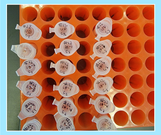
Experimental Methods

Step 1



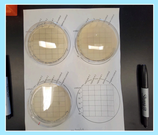
Colonies of transformed M. smegmatis. These cells carry a plasmid that will overexpress the cloned Phayonce genes in the presence of an inducer molecule called aTc.

Step 2



Dilution of M. smematis cells expressing either Phayonce 77 or a control strain.

Step 3



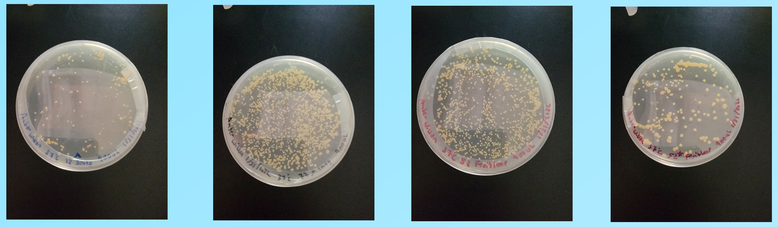
Five ul of each dilution was pipetted onto plates that contained 0, 10 ng/ml or 100 ng/ml of the aTc inducer. As the concentration of the inducer expression, the level of gene expression will also increase.

Step 4

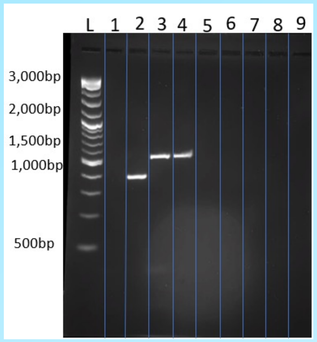
Plates were wrapped in aluminum foil to keep the aTc inducer from being inactivated by light exposure. They were placed in an incubator at 37ºC and grown for 5 days.

Results and Conclusion

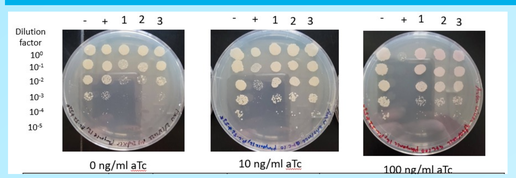
Serial dilutions of *M. Smegmatis* expressing the nontoxic control (-), the cytotoxic control (+) and 3 independent transformants expressing Phayonce 77 and 12 (1-3) were spotted onto plates containing increasing amounts of the inducer aTc. At the highest concentration of inducer, cells overexpressing Phayonce 77 exhibit a substantial growth defect compared to the nontoxic control whereas the overexpression of Phayonce 12 showed no cytotoxicity.



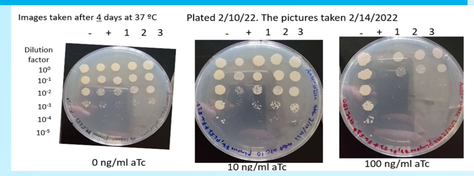
Verification of the successful cloning of Phayonce genes into the pEXTRA plasmid by PCR and gel electrophoresis analysis.



Cytotoxicity Plates for Phayonce Genes 12 and 77



Phayonce 12



Phayonce 77

Future Direction

- We will continue to test Phayonce genes for cytotoxicity until we have overexpressed each gene in the Phayonce genome.
- Further exploration of the mechanism of cytotoxicity resulting from Phayonce 77 overexpression will be investigated using a 2 hybrid screen to identify proteins that interact with Phayonce 77.
- We will also plan to co-express Phayonce 77 with the Phayonce 76 gene, a possible HNH endonuclease, to determine if there is increased cytotoxicity when both genes are overexpressed.

References

SEA-GENES Research Guide:
https://seagenes.helpdocsonline.com/

Acknowledgments

This research was made possible by the SEA-PHAGES, SEA-GENES programs, Dr. Graham Hatfull's group at the University of Pittsburgh and HHMI, and Coastal Carolina University's Department of Biological Sciences. Special thanks to Dr. Michael Pierce and Dr. Daniel Williams for the support and mentorship throughout this research endeavor.

https://www.hhmi.org/science-education/programs/science-education-alliance