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University of South Florida

Tampa FL

Corresponding Faculty Member: Richard Pollenz (pollenz@usf.edu)



Sydni Schlosser

An Analysis of Possible Cytotoxicity and Defense Characteristics of Mycobacterium Phage Girr Gene 40

Sydni Schlosser

The substantial increase in Bacteriophage discovery and analysis of their properties has led to recent advances in the medicinal potential of bacteriophage therapy to treat bacterial infections. Two properties that could contribute to the potential for a phage to be used for phage therapy are cytotoxicity and immunity properties. Mycobacterium phage Girr is a Siphoviridae, F1 cluster bacteriophage that infects M. smegmatis and contains 102 genes in its genome. Girr appears to have a lytic life cycle, and creates medium sized, clear plaques. Gene 40, a small 153 bp gene, was originally annotated in 2012 as having no known function. Further analysis using HHPRED reveals no new information about a known function or conserved domains. The purpose of this project was to identify whether or not the expression of gene 40 has an effect on the growth of M. smegmatis or potentially protects M. smegmatis from infection by other phages, to potentially reveal a function for gene 40. PCR of gene 40 was performed in order to prepare for isothermal assembly of gene 40 into plasmid pExTra, an inducible expression plasmid that could be inserted into both E. coli and M. smegmatis. The new pExTra plasmid containing gene 40 was then transformed into E. Coli before being purified and prepared from E. coli culture for transformation into M. smegmatis. After inserting the pExTra containing gene 40 into M. smegmatis through a second bacterial transformation and plating on 7H10Kan10 and 7H10Kan20 plates, cytotoxicity and immunity assays were performed. To perform the cytotoxicity assay and immunity assays, colonies were picked from the 7H10Kan10 plate. Different concentrations of ATC, ATC10 and ATC100 and no ATC, were used to induce pExTra into expressing gene 40 in the assays. The results of the cytotoxicity assay showed that the expression of gene 40 had no effect on the growth of M. smegmatis, suggesting that gene 40 may not be toxic to M. smegmatis. The results of the immunity assay showed that there was no difference in the titer of phage Girr or phage Larva, a K5 cluster phage that also infects M. smegmatis, suggesting that gene 40 has no effect on the ability to protect M. smegmatis from infection by another phage. Although the results of these two assays suggest that gene 40 has no cytotoxic or immunity function, further experimentation using proteomics to identify protein-protein interactions that may be required to induce the cytotoxic or immunity functions of gene 40 could be explored. In addition, DNA sequencing of the pExTra plasmid including the gene 40 insert could have been performed to ensure that the gene was actually expressed, or the addition of a His tag onto our gene to detect for expression and immunoblotting to check expression levels could be performed to further improve this research. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.