Overexpression of Lysin B in LestyG Bacteriophage

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Abstract

The purpose of this research was to isolate LestyG (a bacteriophage discovered by Caleb Manu at Hampden-Sydney College in 2020), extract the lysin B gene, and insert the gene in the PQE-9 inducible promoter. The resulting gene and promoter were inserted in TOPO *E. coli* bacteria and stored in the -80° freezer for future research.

Background Information

Bacteriophages are viruses that infect bacteria. Researchers believe that there are more than 10^{31} bacteriophages on earth, outnumbering every organism combined.[1] Bacteriophages use bacteria to replicate, following the lytic cycle. This cycle is a five step cycle, in which the bacteriophage attaches to the host bacteria, injects its DNA, hijacks the bacteria to create new phage proteins, assembles the proteins into new phages, and the host cell undergoing lysis to release the newly mature bacteriophages.

Historically, bacteriophages have been used in industry for several purposes. In 2006, the FDA first approved a bacteriophage to be used in the treatment of food. Since then, bacteriophages have been developed for use in targeting *Samonella, E. coli,* and *L. monocytogenes.* This method of food treatment is environmentally-friendly, can be made to target only specific bacterial agents, and does not require the use of unnatural additives.[2]

Bacteriophages have also been used historically in medicine. After the discovery of bacteriophages in 1917, scientists quickly realized that bacteriophages had potential therapeutic uses. Bacteriophages were first used in testing on avian typhosis in 1919, and was used to prevent gangrene in Soviet field hospitals during World War II.[3] More recently, bacteriophages have been used in the treatment of certain types of multi-drug resistant bacteria. One patient with cystic fibrosis and treatment-resistant *M. abscessus* was treated and cured with bacteriophages in 2022, potentially opening the door to future treatments.[4]

In 2008, the Howard Hughes Medical Institute developed a program to allow undergraduate institutions to engage in the isolation and identification of bacteriophages. This initiative, called the SEA-PHAGES program, now has over two-hundred partnering institutions. Hampden-Sydney College joined the program in 2011, and has discovered thirty-two phages, with six of them having been sequenced. Among these phages is LestyG, discovered by Caleb Manu during the summer of 2020.[6] This bacteriophage attacks *M. smegmatis,* a close relative of tuberculosis. By sequencing and annotating the genome, several genes were discovered that are believed to be related to the lysis of *M. smegmatis* cells.

Methods and materials

DNA extraction.

DNA extraction was done using the QIAprep Spin Miniprep kit. The instructions provided were followed without modifications.

Polymerase Chain Reaction (PCR).

Primers were designed using SnapGene and ordered from Integrated DNA Technologies. PCR was done using the Biology 151 lab protocols, with the extension step modified to 90 seconds, run at 35 cycles.

Cloning.

The TOPO TA Cloning Kit from Invitrogen was used to clone the lysin B gene. The instructions provided were followed without modifications.

Results

The central goal of this research was to insert the lysin B gene in LestyG into an inducible promoter, and then insert this into *E. coli* for storage and future research. This required several intermediate steps. First, the DNA of LestyG was extracted, then the gene of interest was isolated using PCR. The success of this extraction and isolation was confirmed with gel electrophoresis. The gene was cloned into TOPO TA *E. coli,* and the process of DNA extraction, isolation, and confirmation through gel electrophoresis was repeated. Restriction sites were added to the gene. Finally, the gene was inserted into the PQE-9 vector. The vector was reinserted into TOPO *E. coli* and stored for future research.

Conclusion

The work over the summer of cloning lysin B and inserting the gene into a promoter opens the door for several avenues of future research. The most likely next step is to assess whether the over-expressed gene increases the pathogenicity of LestyG against *M. smegmatis.* Because of the new facilities in Pauley Science Center, there is also the possibility of research with other types of mycobacteria with similar cell walls to *M. smegmatis*, including potentially tuberculosis.

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