CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)

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Project Number

S0509

Project Title

Creating a More Efficient Cellulase for Biofuel Production

Abstract

Objectives/Goals

The objective of this project is to create a more efficient cellulase for biofuel production, using random PCR mutagenesis to randomly mutate the gene sequence of a cellulose degrading enzyme, or cellulase, and utilizing my previously developed Trypan Blue agar plate method to screen for mutants that possess increased efficiency.

Methods/Materials

1. Develop a more efficient cellulase screening method (Trypan Blue plates)
2. Select cellulase gene
3. Synthesize the selected cellulase in expression vector
4. Establish PCR conditions for amplifying the cellulase gene
5. Confirm cellulase gene expression and protein secretion
6. Demonstrate that cellulase transformed E. coli can produce halos on a Trypan Blue plate for screening (Current Step)
7. Perform random PCR mutagenesis, and screen for more efficient mutants

Results

The Cel5Z cellulase was identified and selected from literature due to its small size, expression in gram negative bacteria, and strong presence in the biofuel industry. The Cel5Z+HylA fusion gene was designed simultaneously with a purpose of enhancing Cel5Z cellulase secretion. The fusion gene was synthesized by GenScript in expression vector pET21a. Using Cel5Z+HylA as a template, I successfully designed PCR primers to amplify the exact Cel5Z gene, which was then re-cloned into pET21a. Importantly, both Cel5Z and Cel5Z+HylA were successfully expressed in E. coli, but unfortunately, neither protein was efficiently secreted, leading to insufficient halo formation on the Trypan Blue plates for screening.

Conclusions/Discussion

Analysis of protein expression indicates that the Cel5Z cellulase is likely toxic to E. coli, reducing secretion. The inability of the bacteria to secrete a sufficient quantity of cellulases has stalled my project, and prevented it from advancing to the mutagenesis step. At present, I am exploring several options to help me get around this roadblock. Ultimately, although I have not achieved the project's final goal, I am still proud of what I have accomplished and I believe that what I have learned will be of great benefit to me as I pursue future success.

Summary Statement

I aimed to create a more efficient biofuel cellulase, advancing through multiple steps of the project. Unfortunately, I ran into an unexpected challenge (cellulase toxicity in E. coli), and I am working hard to address the roadblock.

Help Received

I would like to thank Dr. Joel Cohen, my mentor, who aided me with valuable advice and feedback, and Johns Hopkins University's Cogito Research Award, which provided me with funding and an opportunity to pursue my research.