CALIFORNIA STATE SCIENCE FAIR
2011 PROJECT SUMMARY

Name(s)
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Project Number
S0510

Project Title
The Effect of Single Amino Acid Mutations on Engineering Thermally Stable Enzymes for Bioethanol Production

Abstract

Objectives/Goals
The goal of my project was to enhance the thermal stability of an enzyme involved in breaking down cellulose so as to increase the efficiency of converting plant biomass into biofuel.

Methods/Materials
1. Analyzed beta-glucosidase (BG) protein with an algorithm (PoPMuSiC) designed to predict single-site mutations that enhance stability.
2. The native BG gene was PCR-amplified from genomic DNA of lyophilized Paenibacillus polymyxa (soil bacteria) and cloned into an E. coli expression vector.
3. Wild-type (WT) BG protein was expressed in and purified from E. coli and confirmed to have enzymatic activity using a chromogenic substrate.
4. The top six mutations identified from PoPMuSiC were incorporated into the BG gene.
6. All six mutants (Mut1-Mut6) were sequence verified and protein expression confirmed from five.
7. Following scale-up of expression, each mutant was purified and its residual enzymatic activity measured at 37°C, 45°C, and 55°C relative to WT protein.
8. The T50 values (temperature at which 50% of the activity remains) of two mutants exhibiting increased thermal tolerance were determined in comparison to WT protein.

Results
1. The 5 BG mutants (Mut2-Mut6) show activity nearly identical to wild-type protein at 37°C.
2. Following pre-incubation at 45°C, Mut3-Mut7 show activity nearly equal to wild-type protein. Mut2 shows ~15% reduction in activity compared to WT following 45°C pre-incubation and ~30% reduction in activity compared to WT following 55°C pre-incubation.
3. Mut3 and Mut4 exhibit significantly more residual activity (30-60%) relative to WT following 55°C pre-treatment suggesting that these may be more thermally stable compared to WT BG.
4. The T50 values of WT, Mut3, and Mut4 BG were determined to be 48°C, 51°C, and 53°C, respectively.

Conclusions/Discussion
1. Not all mutations identified by the algorithm resulted in enhanced thermal stability.
2. Of the 6 mutations actually generated and tested, only two (Mut3 & Mut4) enhanced thermal stability (between 3-5°C) compared to WT protein.
3. The PoPMuSiC algorithm is clearly not 100% accurate and only serves as a starting point to begin the rational approach to increase the thermal stability of BG.

Summary Statement
The project is about engineering more stable enzymes that can facilitate the process of bioethanol production from plant biomass.

Help Received
My science teachers, Mr. Smay and Ms. Levensailor, provided encouragement and critical evaluation of my project over last few months. My father helped me purchase the necessary reagents and provided me access to the instruments, kits, and supplies and technical guidance.