Designing a Thermostable Cellobiohydrolase: A Novel Approach to Sustainable Ethanol Production

Samavedi, Sagarika

Due to depleting oil reserves and the threat of global warming, renewable energy sources, such as the biofuel ethanol, are being explored as alternatives to petroleum. Cellulose, the most common naturally occurring polymer on Earth, can be a feedstock for cellulosic ethanol. Cellulose is converted to glucose by cellulase enzymes, one of which is cellobiohydrolase. Cellobiohydrolase does not, however, catalyze at a rate fast enough for sustainable ethanol production. The goal of this project is to create a modified, thermostable Cellobiohydrolase Cel7a from Hypocrea jecorina that can catalyze cellulose at a higher rate at a higher temperature. The enzyme was modified by selectively replacing amino acids using site directed mutagenesis. Using UGENE 1.25, multiple cellobiohydrolase FASTA sequences were analyzed to identify conserved domains, active sites and potential targets for modification. Each of the amino acid replaced was carefully chosen for beneficial changes such as extra hydrogen, cation pi, disulfide, and ion bonds. Using the protein modelling software I-TASSER 5.0 and CHIMERA 1.2, a 3-D model of the enzyme was created to verify that the active site is not affected by these changes. Using STRUM, the change in thermal stability for each substitution was calculated using a ΔΔG score. A positive ΔΔG score corresponds to increased thermal stability. The final 15 mutations made to the enzyme resulted in a net increase of hydrogen bonds, cation pi interactions, disulfide bonds, and salt bridges. This will lead to a significant increase in thermal stability of Cellobiohydrolase, improving the efficiency of ethanol production.