Biofuels from Plant Cell Biomass: Characterization of a Novel Enzyme

Shulami, Noa

Purpose of the Experiment: Plant biomass is a renewable energy source, which can be converted into biofuels for transportation. Biofuels are more environmentally friendly than conventional fossil oil since they have lower CO2 emissions. However, the main challenge in converting biomass to biofuels is to develop an efficient degradation process of plant cell polysaccharides to monosaccharides that can be fermented to ethanol (biofuel) by microorganisms. In nature, microorganisms degrade the plant cell polysaccharides by using specific enzymes - Glycoside Hydrolases (GH). Identification of new and uncharacterized GH's is a key step toward an efficient and economical degradation of biomass to fermentable sugars. Procedures Used: An uncharacterized gene, araN, from a thermophilic bacterium Geobacillus stearothermophilus was over expressed in Escherichia coli BL21(DE3) and the protein was purified in a two-step process: heat treatment and nickel affinity chromatography. The purity of the protein was inspected using SDS-PAGE. Purified Enzyme activity was tested on different synthetic substrates, p-nitrophenyl-glycosides. Results: Following the purification process the enzyme was above 90% pure and activity screening revealed that the enzyme specifically cleaved pNP-?-L-arabinofuranoside. The activity was metal-dependent, indicating a unique catalytic mechanism for glycoside hydrolases. The purified enzyme was suitable for crystallization and currently the crystal structure is being solved. Conclusions: The characterized enzyme was found to hydrolyze ?-L bond between arabinofuranose units, thereby it can be used to liberate arabinose sugars from the plant cell polysaccharides and participate in transforming biomass to fermentable sugars.

Awards Won: