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Cloning, Over-expression & purification of recombinant proteins and studying their functional properties

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Abstract:

Extremophiles are organisms that thrive in conditions of high temperature, alkaline pH, and other such extremes. They are classified as thermophiles and hyperthermophiles (growth at a high temperature), psychrophiles (growth at a low temperature), acidophiles and alkaliphiles (growth at an acidic or basic pH), barophiles (growth at high pressure), and halophiles (growth at a low temperature, high salt concentration). Their unique adaptation mechanism and the high stability of their proteins fascinate scientists. Due to their resistance to proteases, detergents, and chaotropic agents, these proteins or enzymes are used in industrial and basic research. A model organism for Gram-positive bacteria, Bacillus subtilis is one of the most well-studied bacteria. It's a rod-shaped bacterium and it makes endospores, which allow it to survive in harsh conditions like high heat and desiccation. Aminopeptidase plays a critical role in protein hydrolysis by removing amino acid residues from the N-terminal end of the protein or peptide. It is a subclass of exopeptidase and is also known as proteolytic enzymes. Additionally, it is used to synthesize active peptides and analyze protein sequences. In this study, we cloned and expressed putative Bacillus aminopeptidase from Bacillus subtilis. After that, it was purified and described. Bioenergy is one of many different resources that can help us get the energy we need. It is a type of renewable energy that comes from organic materials. Biomass can be used to make transportation fuels, heat, electricity, and other things. Industrial applications for plant biomass are numerous. As a byproduct of the pulp and paper industry, a large amount of energy-dense plant biomass is generated. Due to the complexity of their structure, numerous chemical reactions are required to generate simple components such as simple sugars. Alternatives to these chemical treatments include the use of various enzymes from different hyperthermophiles involved in biomass degradation. The primary function of biomaterialtransforming enzymes is to convert polymeric cellulose or hemicellulose to common saccharides, sugars that can be fermented by microorganisms or used as building blocks for the synthesis of valuable biofuels. In this study, we cloned and expressed Cellulase I from Clostridium thermocellum and presumed βgalactosidase, presumed Beta-glucosidase, and presumed Beta-mannosidase from Pyrococcus furiosus. Following that, attempts were made to purify and characterize them.

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