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Research Article

Heterologous, Expression, and Characterization of Thermostable Glucoamylase Derived from *Aspergillus flavus* NSH9 in *Pichia pastoris*

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A novel thermostable glucoamylase cDNA without starch binding domain (SBD) of *Aspergillus flavus* NSH9 was successfully identified, isolated, and overexpressed in *Pichia pastoris* GS115. The complete open reading frame of glucoamylase from *Aspergillus flavus* NSH9 was identified by employing PCR that encodes 493 amino acids lacking in the SBD. The first 17 amino acids were presumed to be a signal peptide. The cDNA was cloned into *Pichia pastoris* and the highest expression of recombinant glucoamylase (rGA) was observed after 8 days of incubation period with 1% methanol. The molecular weight of the purified rGA was about 78 kDa and exhibited optimum catalytic activity at pH 5.0 and temperature of 70°C. The enzyme was stable at higher temperature with 50% of residual activity observed after 20 min at 90°C and 100°C. Low concentration of metal (Mg^{++}, Fe^{++}, Zn^{++}, Cu^{++}, and Pb^{++}) had positive effect on rGA activity. This rGA has the potential for use and application in the saccharification steps, due to its thermostability, in the starch processing industries.

1. Introduction

Glucoamylase (1,4-α-D-glucan glucohydrolase, EC 3.2.1.3, GA) is an exoacting enzyme that yields β-D-glucose from the nonreducing ends of starch and related oligo- and polysaccharide chains by hydrolyzing α-1,4 and α-1,6 linkages [1]. This enzyme is able to completely hydrolyze starch if incubated for extended periods of time and hence called the saccharifying enzyme. Glucoamylase is an important group of enzymes in starch processing in the food industries, as it is used for the production of glucose and fructose syrup from liquefied starch [2]. It is also used in baking, juice, beverage, pharmaceuticals, confectionery, and many fermented foodstuffs industries for commercial production [3]. It is the most important type of industrial enzyme because of its widespread uses together with α-amylases and debranching enzymes, in the saccharification of starch to yield soluble sugars, which are widely used by many food industries and in the production of bioethanol [4].

From a structural point of view, glucoamylase enzymes constitute the bulk of family GH15 of glycoside hydrolases [5]. Glucoamylase (GA) can be derived mostly in microorganisms and also in animals and plants. A large number of microbes, including bacteria, yeast, and fungi are capable of producing glucoamylase. Filamentous fungi constitute the major source of all microorganisms. However, the exclusive