Production, Partial Purification and Characterization of Glucoamylase from *Aspergillus niger*

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INTRODUCTION

Glucoamylase is an indispensable enzyme for industries. It is an exo-amylase that liberates glucose sequentially from the non-reducing end of the starch molecule. It can cleave both α-1,4 as well as α-1,6 linkages, giving almost complete hydrolysis of starch into dextrose. Glucoamylase is the second most important industrial enzyme and is used in saccharification, textiles, ethanol production, pharmaceuticals, baking, etc. [1, 2]. Numerous microbes produce glucoamylase but *Aspergillus* and *Rhizopus* species are major industrial producers of glucoamylase due to several reasons.

Most of the industrial processes operate at high temperatures and are also prone to chemical contaminants, both of these conditions are unsuitable for many enzymes. Therefore, industries tend to protect enzymes from both of these conditions, which costs them a great deal of capital. Both *Aspergillus* and *Rhizopus* species generally produce mesophilic enzymes that cannot withstand high temperatures. This condition is problematic for many industries, like the sugar-producing industry, as it has to expend much energy and thus cost on lowering the temperature. Researchers are also experimenting to genetically transform organisms to meet these demands of industries. However, the search for novel strains of *Aspergillus* and *Rhizopus* that produce thermophilic enzymes, which are also less sensitive to denaturating agents is still appealing for researchers and industrialists, therefore much work is going on in this area.

OBJECTIVES

The aim of our study was to produce glucoamylase from *Aspergillus niger* CP, to partially purify the enzyme and to characterize the partially purified enzyme (i.e. to determine the effect of temperature, pH, time, metal ions, industrial chemicals, and organic solvents on the activity of partially purified enzyme).

METHODOLOGY

Previously, *Aspergillus niger* CP was isolated from rotten *Capsicum annum*. In present study, *Aspergillus niger* CP was inoculated in fermentation medium of pH 5 containing soluble starch (1%), magnesium sulfate (0.05%), yeast extract (0.2%), and peptone (1%) and was fermented via submerged fermentation for 5 days at ambient temperature. The cell-free filtrate was collected by filtration. To partially purify the enzyme, fractional Ammonium sulfate precipitation was employed. The CFF was saturated first to 40% saturation of Ammonium sulfate and then centrifuged at 10,000 rpm for 10 min. to separate out the precipitates. Then, more Ammonium sulfate was added in the supernatant until 80% saturation was achieved, which is the reported concentration of Ammonium sulfate for precipitation of *Aspergillus* glucoamylase [3]. The precipitates were again separated by centrifugation at 10,000 rpm for 10 min. Both precipitates were resuspended separately in minimal volume of 100 mM Acetate buffer of pH 5 and stored at -18°C before further use.

Starch plate assay was carried out first to determine the fraction with maximum activity. After that, quantitative enzyme assay was carried out, for which 2% soluble starch solution of pH 5 was used as substrate. The partially purified enzyme was mixed with substrate in the ratio of 1:9 and incubated at 50°C for 5 minutes. The tubes were boiled to halt the enzymatic reaction. GOD/PAP method was employed to estimate the liberated glucose against 100 mg/dl glucose standard [4]. The absorbance was measured at 546 nm, using spectrophotometer. The enzyme was then characterized. The effect of reaction pH on the activity of partially purified enzyme was analyzed by incubating the enzyme at different pH ranging from 3-8. The effect of reaction temperature was analyzed by incubating the enzyme at different temperatures ranging from 40-90°C. Similarly, the effect of reaction time, ranging from 10 min to 2 hrs, was analyzed. Iodine test was also performed in relation with the reaction time study to determine when starch began to decline in the reaction mixture. The effects of organic solvents (Ethanol, Propanol, and Acetone), industrial chemicals (EDTA, Triton X-100, Tween-20, Tween-80, and SDS), and metal ions (Na^{+}, Mg^{++}, Ni^{+++}, Ca^{++}, Ba^{++}, and Fe^{+++}) were also analyzed.

CONCLUSION/RESULTS

Our study shows that this strain of *Aspergillus niger* is capable of producing great amounts of glucoamylase via submerged fermentation. The 80% fraction was found to be most rich in glucoamylase. By quantitative enzyme assay, the yield was found to be 41 kU/ml. This is incredible because such higher enzyme units are produced by molds primarily through solid state fermentation. However, submerged fermentation is easier than SSF and allows more control over environmental parameters. Therefore, this work can be of great significance as this strain of *A. niger* gives adequate enzyme units even by submerged fermentation and thus could prove quite beneficial for industries by giving greater enzyme units, while strengthening the control over environmental parameters as well as media sterilization and downstream processing.

The optimum reaction pH of the enzyme was found to be 4, while the optimum reaction temperature resulted to be 75°C. The enzyme was also able to perform well at 90°C, but its activity was comparatively low around 40-50°C. This shows that the enzyme falls in the category of thermophilic enzymes. The optimal reaction time was found to be 25 minutes and after that enzyme activity began to decrease. Iodine test revealed that after 25 minutes, starch was almost completely digested and was not essentially present in the reaction mixture. Organic solvents did not significantly affect enzyme activity and some industrial chemicals including Tween-20, Triton X-100 and EDTA also didn't have any significant effect on enzyme activity in low concentrations, however SDS considerably reduced enzyme activity and Tween-80 greatly increased enzyme activity. All the metal ions tested were found to reduce enzyme activity to a significant level. Previously, Hanif *et al*. also reported similar effect of metal ions on the activity of glucoamylase [5].

Therefore, this strain of *Aspergillus niger* could prove to be a potent producer of thermophilic glucoamylase in bulk quantities, which can be quite beneficial for industries in short run and can provide great benefit to the country in long run. However, further studies regarding optimization of growth conditions for maximal production of glucoamylase and thermostability of the enzyme are still required.

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