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Process optimization, purification and characterization of glucoamylase from different *Sorghum* varieties

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ABSTRACT

Glucoamylase production was carried out under solid state fermentation by Aspergillus niger NCIM 616 has been investigated using three different Sorghum vulgare varieties as substrates. The solid state fermentation medium containing Sample-1 as a substrate yielded the highest enzyme activity. We purified enzyme from the culture supernant by ammonium sulfate precipitation, DEAE- Sepharose column chromatography. The molecular weight of purified enzyme was estimated to be 74KDa by sodium dodecyl sulfate- polyacrylamide gel electrophoresis. Maximum enzyme activity 82±2 U/g of dry Sample-1 was achieved under optimum growth conditions. The optimum conditions were incubation period 72h, inoculums size 15% (by mass per volume) having 10^7-10^8 spores/ml, incubation temperature of 40^0C and pH of 5.0.

Key words: Glucoamylase, *A. niger*, *Sorghum vulgare*, Solid state fermentation, SDS-PAGE.

INTRODUCTION

Glucoamylase (1, 4-α-D-glucan glucohydrolase; EC 3.2.1.3), which is an industrial enzyme that hydrolyzes1, 4-linked α-D-glucosyl residues successively from the non reducing end of oligo and polysaccharide chains with the release of D-Glucose [1]. It is an extracellular enzyme and is secreted to the culture medium. Many investigators has been purified and characterized from various fungi. Glucoamylase used commercially from strains of either *Aspergillus sp* or *Rhizopus*

sp. It is also identified in some bacteria and some yeast [2]. On the basis of hydrolysis of starch glucoamylase classified in to two groups, one total hydrolysis of starch yielding β-limit dextrins and second 80% hydrolysis of starch in which 40% conversion of β-limit dextrins to glucose [3]. Glucoamylase has received considerable industrial usefulness. It has extensive biotechnical applications like production of glucose, high conversion syrups, fermented ethanol and BOD biosensor for starch containing waste water [4]. Thermostability is a desired characteristic of most of the industrial enzymes and each application of industrial enzymes requires optimum properties like specificity, stability, temperature and pH dependence [5]. Recently solid state fermentation (SSF) processes have been applied largely for the production of glucoamylase as compare to submerged fermentation (SmF) [6]. In fungal cultures glucoamylase rarely occurs without alpha amylase [7]. Other amylolytic enzymes such as alpha glucosidase are also likely to be concomitantly produced [8]. In present investigation, process optimization of glucoamylase production from *Aspergillus niger* NCIM 616 was carried out by using different Sorghum varieties as substrates.

EXPERIMENTAL SECTION

Microorganisms- *Aspergillus niger* NCIM 616 used in the present study was obtained from NCIM, Pune. The culture was maintained on potato dextrose agar (PDA) slants containing 2% starch at 4^0C.

Substrates- Barshi (Sample-1), Suwarna (Sample-2) and Waradhi (Sample-3) these are local varieties of *Sorghum vulgare* in Maharashtra, India.

Solid state fermentation (SSF)

The fermentation was carried out in 250ml Erlenmeyer flask containing 10g of substrate moistened with aqueous mineral salts solution [$MgSO_4.7H_2O$, 1%; KH_2PO_4,0.1%; $CaCl_2$,0.1%; $FeSO_4$.0.05% and $(NH_4)_2SO_4$,0.1%] [9, 10]. Sterilization was at 121^0C for 15min. Then after cooling the flasks were inoculated with 1ml of spore suspension of *A. niger* NCIM 616 (10^7-10^8 spore/ml) and incubated (40^0C, 72h)

Extraction of Crude glucoamylase

Fermented substrate were treated with 50ml of distilled water and agitated on orbital shaker at 200rpm for 15 min. The precipitate was removed by centrifugation at 18000rpm (39200 X g) for 30 min at remove suspended particles [11]. Supernant was used for purification of the glucoamylase.

Purification of glucoamylase

The supernant was brought to 80% ammonium sulfate saturation by adding solid ammonium sulfate to an ice bath. After the supernant was left standing overnight at 4^0C, the precipitate was collected by centrifuging at 15000 X g for 15 min, and dissolved in 0.1M acetate buffer (pH=5.0). The enzyme solution was dialyzed overnight at 4^0C against the same buffer. After dialysis the enzyme solution was centrifuged at 15000 X g for 15 min to remove precipitate. 10ml of enzyme solution was applied to column chromatography on DEAE-Sepharose. Column of DEAE-Sepharose equilibrated with 0.1M acetate buffer (pH=5.0). The column was washed with 150ml of the same buffer and elution was done with a 300 ml linear NaCl gradient (0 to

0.1M). Each fraction of 5 ml was collected at a flow rate 20ml/h. The fractions that contained the enzyme activity were collected [12].

Electrophoresis and Molecular mass determination
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out with a 15% polyacrylamide gel [10]. Proteins on polyacrylamide gel were stained with 0.2% coomassie brilliant blue.

Enzyme assay
Glucoamylase activity was measured by; 5 ml of 0.1% soluble starch, 1ml of 0.1M acetate buffer at pH=5, 1 ml of D/W and 1 ml of enzyme solution were incubated at 40^0C in water bath for 10 min. The reaction was stopped by addition of 0.5ml dinitrosalicylic acid reagent. The absorbance was recorded at 540 nm [13].

Optimization of culture conditions
Effect of incubation time, temperature and pH
To ascertain the effect of culture condition the present study was carried out at different incubation time (24, 48, 72, 96, 120 hrs) [14]. The relative glucoamylase activity was determined at several pH values (pH 3.0 to 7.0) with 50mM citrate-sodium citrate buffer [15]. The optimal temperature was determined by assaying the enzyme activity at various reaction temperatures (30, 40, 50, 60 and 70^0C) [16].

RESULT AND DISCUSSION

The production of extracellular glucoamylase by *Aspergillus niger* NCIM 616 was studied in solid state fermentation (SSF) [17, 18]. In the present study three different substrates, viz. Barshi (Sample-1), Suwarna (Sample-2), Waradhi (Sample-3) were used for glucoamylase production. This fermentation has numerous advantages including simple technique, superior productivity, low capital investment, low energy requirement, less waste water out put and better product recovery [19, 20 and 21]. Culture media containing 10g of each substrate were subjected to fermentation for 120hrs at pH=5, temperature 40^0C and inoculums level 10% (by mass per volume). All the substrates supported growth and enzyme formation by the cultures. Among the three substrates tested highest enzyme production was observed with Sample-1((86±2) U/g) after 72h, followed by Sample-2 ((54±2) U/g). Sample-3 gave the minimum enzyme yield ((25±2) U/g). The biosynthesis of glucoamylase decreased after 72h (fig.1). Sample-1 gave good enzyme production and further studies are required for efficient utilization of this substrate generated in large quantities. In our subsequent optimization studies Sample-1 was used as substrate for production of glucoamylase. [22] Reported that 72h is the optimum period for glucoamylase synthesis by SSF.

Effect of pH on the medium is shown in fig.2. The maximum glucoamylase production was obtained at pH=5.0 after 72h of incubation at 40^0C. Optimum pH is very important, the composition of cell wall and plasma membrane of microorganism is known to be affected by the culture pH. The change in the initial pH of the medium may lead to change in the nature of the cell wall and cell membrane and hence affecting the glucoamylase production and growth of organisms. These effects may be dependent on the ionic environment around the active site ot the

enzyme bound to the carrier [23]. Optimization of the culture conditions for glucoamylase production by *Aspergillus sp* under SSF and optimum enzyme yield noted at pH=5 [24].

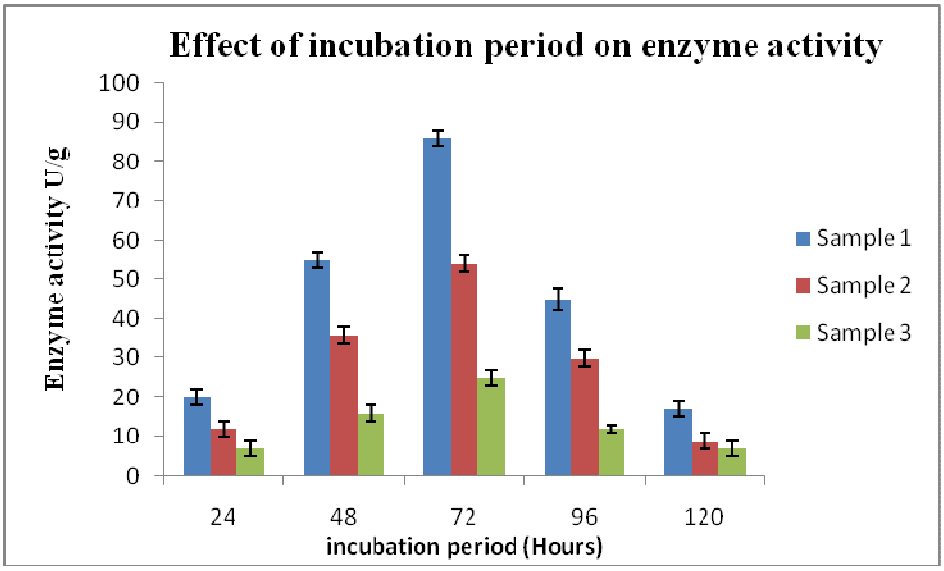

Fig 1: Effect of incubation period on enzyme activity of Sample 1, Sample 2 and Sample 3

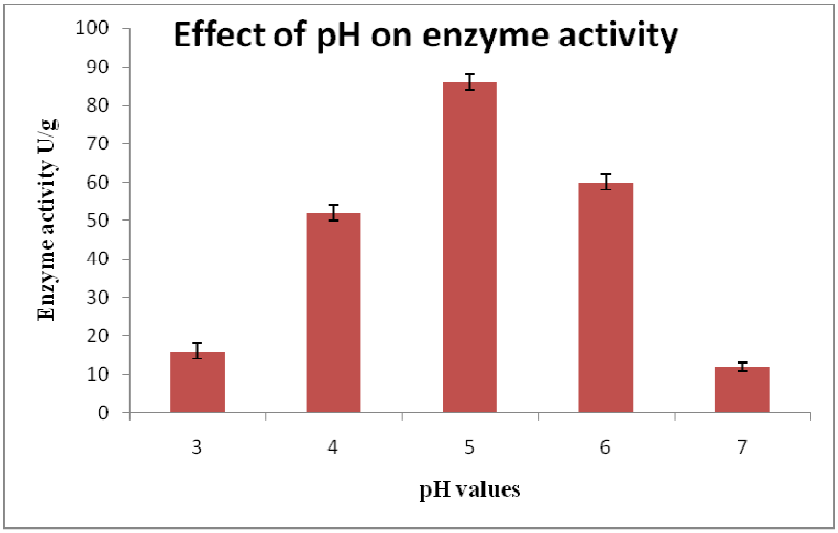

Fig 2: Effect of pH on enzyme activity of amylase (Sample 1)

Fig.3 shows the effect of temperature on glucoamylase production by optimized conditions of *A. niger* NCIM 616. Maximum glucoamylase yield was observed in the medium incubated at 40^0C. Growth temperature is a very critical parameter which varies from organism to organism and slight changes in growth temperature may affect glucoamylase production. Organisms have various mechanisms that allow them strictly to control excretion.

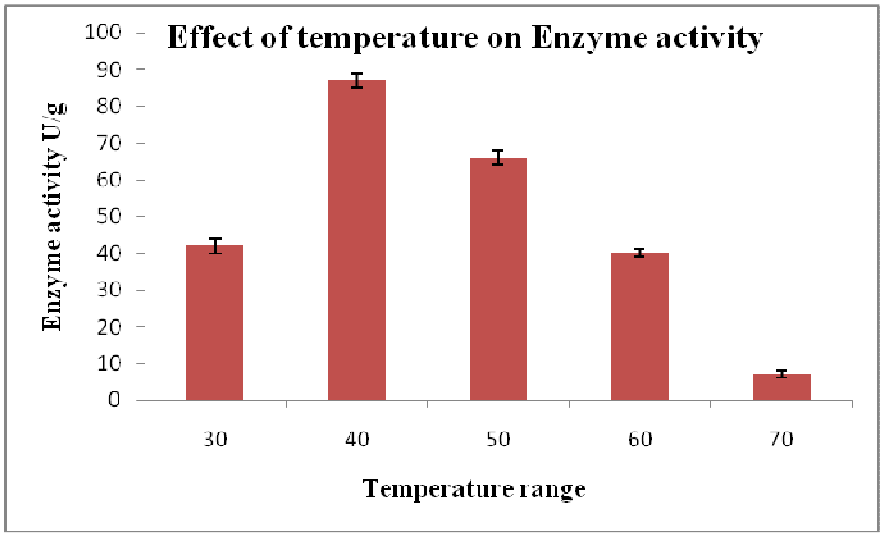

Fig 3: Effect of temperature on enzyme activity of amylase (Sample 1)

Change in the nature of cell envelop can affect the release of extracellular enzymes to the culture medium. Temperature is one of the factor that induces such changes on cell membrane and cell wall [25, 26] Crude enzyme was obtained from the culture supernant by ammonium sulfate precipitation (80% saturation) and dialysis. To minimize the proteolytic degradation of enzymes, all of the purification steps were carried out at 4^0C. Enzyme was purified serially by column chromatography on DEAE-Sepharose. The molecular weight of purified glucoamylase was estimated to be approximately 74KDa from the band pattern on gel. The result of SDS-polyacrylamide gel electrophoresis of the enzyme shown in fig.4. From those results, it is concluded that the enzyme consist of a single polypeptide chain.

Fig 4: SDS-PAGE of the purified amylase (Sample 1)

The result obtained in the present study indicated that *Aspergillus niger* NCIM 616 as a potential strain, for glucoamylase production using solid state fermentation with Barshi (Sample-1) as substrate. Interesting observation was that it showed and attained maximum enzyme activity at

50^0C. Furthermore the enzyme was found to show optimum activity under acidic condition (pH=5.0). This makes the glucoamylase as useful for various industrial applications like starch liquefaction.

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