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Production of amylase from fruit peel using *Aspergillus Niger* by Solid State Fermentation

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ABSTRACT

Solid state fermentation holds tremendous potential for the production of the amylase by Aspergillus Niger. In the present study Aspergillus species was isolated from an onion sample from the local market and it was allowed to grow in a PDA plates. Then the inoculum for SSF was prepared by the extract of sweet lime peel. For amylase production through SSF, sweet lime peel, along with MSM medium were taken in conical flask and inoculated with Aspergillus spp. After 96 hours, the growth of Aspergillus spp was found to be maximum at 28°C and at pH 6.2. Amylase was harvested through phosphate buffer at 6.2 pH. Amylase produced showed the activity of 90 U/ml/min.

Keywords: Amylase, *Aspergillus niger*, Solid State Fermentation, Fruit peel, Sweet Lime.

INTRODUCTION

Alpha-Amylase (EC 3.2.1.1) also named as 4-α-D-glucanglucanohydrolase, has extended its application in a wide range of industries including food, brewing, distilling industry, textile, paper pharmaceutical and bioconversion of solid waste etc.[1, 2]. Large range of applications of amylase has led to the extensive industrial production of alpha amylase all over the world. Although amylases have been reported to be produced by various sources such as plant, animal and microbial sources, most of the reports showed that microbial amylase production have been the most effective one. The first industrially produced enzyme was an amylase from a fungal source in 1994, which was used for the treatment of digestive disorders [3].

Nowadays, *Bacillus*, *Aspergillus*, *Rhizopus* and *Rhizobia* isolates are specified and considered to be the most important sources of industrial amylases. Nevertheless, various other sources of microbial amylases have also been investigated throughout the world. Growth parameters and other nutrients which promote high yields of microbial amylases were also studied extensively. The usual carbon sources such as dextrin, fructose, glucose, lactose, maltose and starch are very expensive for commercial production of amylase. These expensive products can be replaced in the medium with economically available agricultural by-products or industrial amylaceous substances as carbon substrates which are cost effective [4].

Thus the present study was designed in the search of cheaper carbon sources for the production of alpha amylase enzyme by using fungal strains. Amylase produced from the fungal cultures was found to be more stable than that produced by bacteria on a commercial scale. Many suitable strains of fungi have been optimized for their culture conditions [5]. Molds are capable of producing high amounts of amylase. *Aspergillus niger* is used for commercial

production of amylase. Studies on fungal amylases especially in developing countries have concentrated mainly on *Aspergillus niger*, probably because of their ubiquitous nature and non-fastidious nutritional requirements. *Aspergillus wentii* was used for the production of cellulose using lingo cellulose as carbon source [6]. Chitinase enzyme was produced from crustatian shells by using Bacillus species [7].

Solid State Fermentation holds tremendous potentials for the production of enzymes. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source [8, 9].

The free water is indispensable to the microorganism's growth and is adsorbed on a solid support or complexed into the interior of a solid matrix. This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials [10, 11].

Solid state fermentation (SSF) has been reported to be cheaper because of the simple enzyme extraction procedures [12]. In case of SSF the cost of the substrate also plays a keyrole in deciding the cost of production. Agro industrial wastes have been reported to be good substrates for the cost effective production of alpha amylases and are thus attracting researchers for using agro industrial waste as a substrate for alpha amylase production [13]. Fungal species have been studied a lot for the production of alpha amylase because of the low cost of substrates used for the production of alpha amylases [14, 15, 16].

MATERIALS AND METHODS

2 a. Glassware

2b. Instrument used

Centrifuge machine, Autoclave, Shaker Incubator, Laminar Air Flow (LAF), Calorimeter, Mixer grinder

2 c. Chemicals used

Potassium Chloride(KCl), Ferrous sulphate, Calcium chloride, Sodium chloride, Ethanol, Sodium sulphat, Sodium hydroxide, Crystal phenol, Iodine solution, Starch Soluble Glucose, DNS (Dinitrosalicylic acid), 1% starch solution, Rochelle salt, Magnesium chloride, and Ammonium chloride.

2 d. Isolation of *Aspergillus Niger*

A piece of onion was kept in moist condition at room temperature for 2 days. The onion sample was serially diluted and different dilutions were plated on potato dextrose agar (PDA) (Potato – 200g, Starch – 200g, Dextrose – 20g, Agar – 20g, Distilled water – 1000ml) medium. The Petri plates were incubated at room temperature for 4 days. Fungal cultures were observed on PDA medium. All the fungal cultures were confirmed as *Aspergillus niger* by studying the morphology and the spore color.

2 e. Determination of Amylase Activity

All the *Aspergillus niger i*solates were tested for amylase production by starch hydrolysis.When starch agar medium (Peptone – 0.5g, Beef Extract – 0.15g, Yeast extract – .15g, NaCl 0.5g, Starch – 1g, Agar – 2g, Distilled water – 100ml) was inoculated with the organism and subsequently flooded with iodine solution(Iodine – 0.2%, Potassium Iodide – 0.4%, Distilled water – 100ml), the zone of clearance around the microbial growth indicated the production of amylase.

2f. Cheap Substrates Used

Sweet lime peel.

2 g. Enzyme Production

Production of amylase was carried out by Sweet lime peel by SSF using the above substrate of zero cost. For SSF 20gm of powdered peel were taken in 250ml flasks and moistened with nearly 50ml of MSM containing the following in gm/l (0.8 g NaCl, 0.8 g KCl , 0.1 g $CaCl_2$, 2.0 g Na_2HPO_4 , 0.2g $MgSO_4$, 0.1 g $FeSO_4$, 8.0 g Glucose, 2.0 g NH_4Cl pH 6.2). Flasks were autoclaved, cooled to room temperature, inoculated with1ml of 48 hour old grown broth culture and incubated at 28°C for 4 days.

2 h. Extraction of Crude Enzyme

Crude enzyme was extracted from fermented media by adding 100ml of phosphate buffer at 6.2 pH, agitating the flask in shaker at 180rpm for 1 hr, the mixture was filtered through cheese cloth and centrifuged at 8000 rpm at 4^{o}C for 5 min. The supernatant was collected and treated as crude enzyme.

2 i. Enzyme assay in Crude Enzyme

2 i. i. Dinitrosalicylic acid (DNS) method:

This is a convenient and sensitive method to estimate reducing sugars, particularly when large number of samples are to be analyzed.

Reagents

a. DNS: One gm of Dinitrosalicylic acid, 200mg of crystalline phenol and 50mg of sodium sulphites were placed in a beaker with 1 ml of 1% solution of NaOH and stirred well. The reagent was stored in a stopper bottle at 4^{0}C.
b.40% solution of Rochelle salt (sodium potassium tartarate)

2 i. b. Assay

0.5 ml aliquot of the enzyme extract was added with 0.5 ml of 1 % starch solution into a test tube and allowed to incubate for 10 minutes. Then 1 ml of DNS reagent was added to the mixture to stop the reaction. The mixtures were heated for 5 minutes in a boiling water bath. After the colour has developed, 1ml of 40% Rochelle salt solution was added when the contents of the tubes were still warm. The tubes were cooled under running tap. The O.D values were measured at 575 nm in a colorimeter. The amount of reducing sugar was calculated using a standard graph prepared from glucose.

$$\textbf{Amylase Activity (U/ml)} = \frac{\textbf{microgram of glucose produced}}{\textbf{(Volume of enzyme solution) X Incubation time}}$$

RESULTS AND DISCUSSION

The fungal strain was isolated from onion and allowed to grow in PDA plates for 48 hours. Based upon the morphology it was identified as *Aspergillus spp.* Then the inoculum for solid state fermentation was prepared by ten grams of dried ground fruit peel with 100ml of hot distilled water with NH_4NO_3 and filtered. Later the extract was inoculated by *Aspergillus spp*. from the PDA plates. This inoculum was kept for incubation at 28 ^{0} c for 48 hours as shown in Fig: 1.

Fig : 1 Culture of *Aspergillus* on sweet lime peel.

3.1 Solid State Fermentation

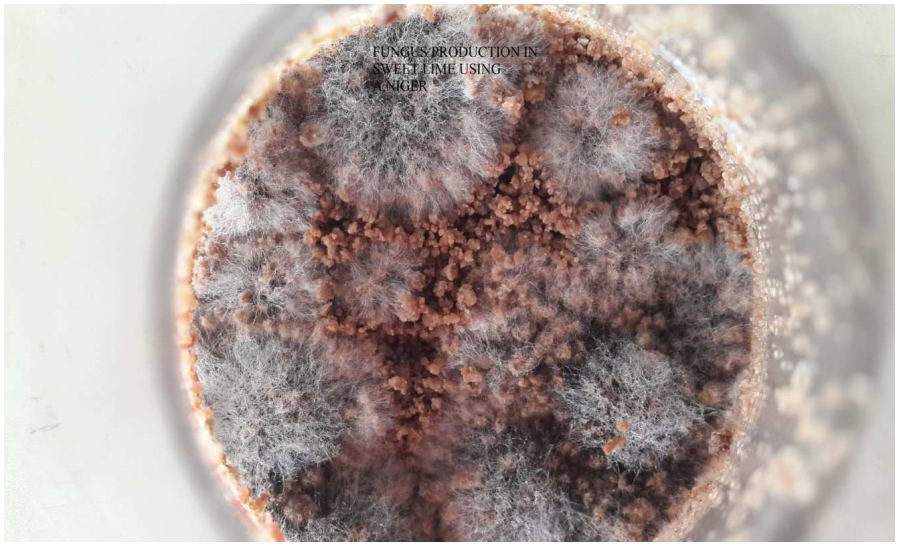

Fig: 2 Culture of *Aspergillus* on sweet lime peel.

Production of amylase was carried out by Sweet lime peel by SSF for zero cost. For SSF 20gm of powdered peel were taken in 250ml flasks and moistened with nearly 50ml of MSM containing the following in gm/l (0.8 g NaCl, 0.8 g KCl , 0.1 g $CaCl_2$, 2.0 g Na_2HPO_4 , 0.2g $MgSO_4$, 0.1 g $FeSO_4$, 8.0 g Glucose, 2.0 g NH_4Cl, pH 6.2). Flasks were autoclaved, cooled to room temperature, inoculated with1ml of 48 hour old grown broth culture and incubated at 28°C for 4 days as shown in the Fig 2.

The amylase which was produced in the flask was harvested by adding 100ml of phosphate buffer and kept for agitation in the shaker at 180 rpm for 15 minutes. Later the extracts were filtered with a cheese cloth. The filtered extracts were centrifuged at 8000 rpm for 15 minutes. The supernatant was decanted and used for amylase assay.

DNS method was carried out for the assay of amylase and it was found that the activity of amylase is 90 U/ml. min.

CONCLUSION

Effective production of amylase was studied through solid state fermentation by using cost free substrate from *Aspergillus spp.* In future, production of amylase through various cheap sources has to be studied with several parameters.

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