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Research Article

AMYLASE SYNTHESIS IN ELEVATED LEVEL BY OPTIMIZING FERMENTATION PARAMETERS FROM ASPERGILLUS VERSICOLOR

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ABSTRACT

The production of extracellular amylase synthesis were carried out by using *Aspergillus versicolor* was evaluated under different fermentation parameters by employing submerged fermentation method. The amylase producers detected by the clear zone around the colony by simple plate assay method. *Aspergillus versicolor* is the potential strain among the fungal isolates. The amylase synthesis were increased their yield after the optimization of fermentation parameters. The optimum pH 4.0, temperature 30^0C and inoculum size 1.0 ml and it showed 6.21 IU. This enzyme was growth associated.

KEYWORDS: Aspergillus versicolor, Amylase, submerged fermentation, Plate assay and fermentation parameters

INTRODUCTION

Nowadays, amylases (α-amylases, β-amylases and glucoamylases) represent one of the most important enzyme groups within the field of biotechnology. Microbial amylases for commercial uses about 75 years ago represented a milestone in industrial enzymology. Starch-degrading amylolytic enzymes are of great significance in biotechnological applications ranging from food, fermentation, textile to paper industries (Pandey et al., 2000). The amylases can be derived from several sources such as plants, animals and microbes. The microbial amylases meet industrial demands; a large number of them are available commercially; and, they have almost completely replaced chemical hydrolysis of starch in starch processing industry [Pandey et al., 2000 b].

The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics.

These enzymes are found in animals (saliva, pancreas), plants (malt), bacteria and molds [Abu et al., 2005]. Sources of amylases in yeast, bacteria and molds have been reported and their properties have been described [Akpan et al., 1999; Buzzini and Martini, 2002]. Amylase of fungal origin was found to be more stable than the bacterial enzymes on a commercial scale. Many attempts have been made to optimize culture conditions and suitable strains of fungi [Abu et al., 2005].

In the present work, different fermentation parameters were used for optimization of amylase production under submerged fermentation by using *Aspergillus versicolor*. Hence we made an attempt on amylase production.

MATERIALS AND METHODS

Chemicals

Starch used in the study was procured from Hi-Media Laboratories, Bombay, India; the other ingredients used for the preparation of Czapek Dox's media were also products of Hi-Media Laboratories, Bombay.

Fungal strain

The *Aspergillus versicolor* strains were isolated from different soils. Soils are taken from different regions from Tumkur university campus. Tentatively identified in the laboratory and further the strains were identified at Agarkar research Institute (ARI), Pune.

Screening of amylase producing *Aspergillus versicolor* by plate assay

All the ten isolates were kept for screening of amylase producers by plate assay method as described earlier (Tiwari et al., 2007). *Aspergillus versicolor* was streaked on to the potato dextrose agar medium containing 1% starch. After inoculation the plates were kept for incubation for 48h at 37^0C. After incubation of *Aspergillus versicolor* for 48 h on plates containing starch, plates were flooded with 1 % iodine solution for approximately 5-10 min. starch hydrolysis was evidenced by a clear zone around the fungal colony.

Fermentation Medium

The selected *Aspergillus versicolor* were cultured on production medium. The production medium consists of dextrose 0.1%, yeast extract 0.3%, KCl 0.02%, NaCl, 0.01%, $MgCl_2$ 0.02% and starch 0.5% w/v.

Optimization Studies

The 250 ml Erlenmeyer flasks containing 100 ml of production medium were prepared by mixed with acid/alkali solution to obtain required pH. The pH was adjusted in the range of 3-6 with increments of 0.5. Thus prepared flasks were cotton plugged and autoclaved at 121^0C for 15 min. The flasks were inoculated and incubated. The 100ml of the production medium was separately taken in 250 ml Erlenmeyer flasks and prepared for submerged fermentation. Thus prepared flasks were incubated at different temperatures like 25-35^0C with in increments of 5^0C. The inoculum was prepared separately by reviving the 168h old culture of *Aspergillus versicolor* at different levels i.e., 0.25, 0.50, 0.75, 1.0 and 1.25 ml and then fermentation studies were carried out.

Assay of amylase

Amylase activity was determined as it is described by Okolo and co-workers (Okolo et al., 1995). The reaction mixture consists of 1.25 ml of 1 % soluble starch, 0.5 ml of 0.1 M acetate buffer (pH-5.0), and 0.25 ml of crude enzyme extract. After 10 min of incubation at 50 °C, the liberated reducing sugars (glucose equivalents) were estimated by the dinitrosalicylic acid (DNS) method. The colour developed was read at 510 nm. One unit (IU) of amylase is defined as the amount of enzyme releasing one mol of glucose equivalent per minute under the assay conditions.

RESULTS AND DISCUSSION

Fungal isolates were isolate and identified as *Aspergillus versicolor* in Agrakar Research Institute, Pune. All fifteen strains of *Aspergillus versicolor* produced clear zones on starch plate medium; those were selected from the soil sample. Of the fifteen isolates *Aspergillus versicolor* was considered to be the best and high amylase producing strain. It showed 0.65cm of cleared zone around the colony. The data obtained in the present study on the effect of pH and temperature on submerged fermentation is shown in (Fig. 1 and 2) which reveals that the production of amylase increased with the increase in the pH of the medium up to pH 5.0 temperatures 30^0C and thereafter the decrease of amylase was observed.

The maximum production of amylase 3.89 IU was obtained at pH 5.0 and the minimum production of amylase 2.14 IU was observed at pH 3.0. The production of amylase increased significantly with the increase in fermentation temperature from 25-30^0C and decreased above 30^0C. The maximum amylase production obtained at 30^0C was 4.75 IU and the least production was observed at 25^0 C resulted only 3.41 IU of amylase at 72 hrs of fermentation period. Any temperature beyond the optimum range is found to have some adverse effect on the metabolic activities of the microorganisms and it is also reported by various scientists that the metabolic activities of the microbes become slow at lower or higher temperature (Okolo et al., 1999).

In our study, the data revealed that the pH of 5.0 was found as suitable for maximum production of amylase with *Aspergillus versicolor* strain under submerged fermentation. Our findings are in close agreement with the earlier findings in *Aspergillus* sp. such as *A. oryzae, A. ficuum* and *A. niger* were found to give significant yields of α-amylase at pH 5.0–6.0 in submerged fermentation (Hayashida and Teramamoto 1986 ; Carlsen et al.,1996 ; Djekrif-Dakhmouche 2006). Similar reports are available on among the fungi, most amylase production studies have been done with mesophilic fungi within the temperature range of 25–37 °C (Ramachandran et al., 2004; Francis et al., 2003). A raw starch degrading α-amylase was produced by *Aspergillus ficuum* at 30°C (Hayashida and Teramamoto 1986).

Importance of inoculum size on microbial fermentation process is widely accepted. Out of five inoculum size tested (0.25, 0.50, 0.75, 1.0 and 1.25 ml) and 1.0 ml inoculum was found to be the most suitable for high production of amylase by *Aspergillus versicolor* in submerged fermentation at 72 hrs of fermentation. From Fig. 3, it is clear that the amylase production steadily increased with the increasing in the size of the inoculum until it reaches to the magnitude when enzyme productivity became maximum, thereafter no appreciable change in production of amylase with high inoculum size could be observed. The maximum enzyme activity was showed at

6.21 IU at 1.0 ml inoculum size and least enzyme activity 2.24 IU was showed at 0.25 ml of inoculum size. One ml incoulum was used for the production of amylase by using *Bacillus amyloliquefaciens* (Gangadharan et al., 2006). It is also reported that 10% of inoculum is optimum for amylase production by using *Themophilic lanuginosus* through solid state fermentation (Kunamneni et al., 2005)[14].

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