Research Article

Biotechnology

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

INVITRO OPTIMIZATION AND COMPARATIVE ANALYSIS OF BACTERIAL AND FUNGAL AMYLASE

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ABSTRACT

The present study was conducted for the isolation of a suitable Amylase producing Bacterial and Fungal strain and optimization of the cultural conditions for the production of amylase enzyme. Studies on the optimum conditions for the production of amylase were performed with isolated and identified bacterial (*Bacillus*) and fungal (*Aspergillus*) species. The optimum temperature for amylase production by bacterial species was detected as 35^0C and by fungal species was detected as 25^0C. Amylase production was observed at pH 5-9 with maximum at pH 7 for bacteria and pH 6 for fungus. The levels of amylase production varied greatly with the addition of carbon source. Effect of different nitrogen sources revealed that protease peptone and urea were the better nitrogen sources and showed increased enzyme yield. The amylase enzyme was purified by Ammonium sulphate precipitation followed by Dialysis and then column chromatography. The purified Amylase was then analyzed by SDS-PAGE.

KEYWARDS: **Amylase, Optimization, Enzyme production, Enzyme purification**

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INTRODUCTION

Amylases are of great significant and among the most important enzymes in biotechnology and are commercially significant in various starch processing industries[1]. Amylase has wide spectrum application in many sectors such as clinical, medicinal and analytical chemistry. They also find applications in baking, brewing, detergent, textile, paper and distilling industry[2, 3, 4]. Amylases are unanimously distributed throughout the animal, plant and microbial kingdoms. The main microbial sources for amylase production are *Bacillus* species[5, 6] and *Aspergillus* species[7, 8]. Two major classes of amylases have been identified in microorganisms, namely α-amylase and glucoamylase. α-Amylases (endo-1,4-a-D-glucan glucohydrolase, E.C. 3.2.1.1) are extracellular enzymes that randomly cleave the 1,4-a-D-glucosidic linkages between adjacent glucose units in the linear amylose chain. Glucoamylase (exo-1,4-α-D-glucan glucanohydrolase, E.C. 3.2.1.3) hydrolyzes single glucose units from the non reducing ends of amylose and amylopectin in a stepwise manner[9]. Low yield of enzyme has always been a problem in the commercial production of amylases. It is well known that extracellular enzyme production in microorganisms is greatly influenced by nutritional factors like carbon sources, nitrogen sources and mineral salts[10-13]. The present investigation dealt with the optimization of culture media parameters for maximum production of amylase by bacterial and fungal species. Optimum utilization of environmental conditions, such as temperature and pH was carried out for achieving high yield of amylase enzyme. Additives play an important role in fermentation processes; hence, investigation was also carried out for the effect of various additives such as carbon sources and nitrogen sources on production of amylase. Also Amylase was partially purified by column chromatography and then analyzed on by SPS-PAGE.

MATERIALS AND METHODS

Collection and Identification of microorganism

The Amylase producing microorganisms were isolated from soil collected from community dump sites. The isolated microorganisms were identified by morphological and biochemical characterization for bacteria and morphological and conidial characterization for fungi. The bacterial culture was maintained on NA (Nutrient Agar) slants containing 1 % starch and the fungal culture was maintained on PDA (Potato dextrose agar) slant.

Enzyme production

The bacterial culture was transferred from stock to 100 ml nutrient broth and the inoculated Erlenmeyer flasks were incubated overnight at 37 ^{0}C. The fungal culture of 1 ml spore suspension was inoculated in Erlenmeyer flask with 100 ml Potato dextrose broth and incubated at 30 ^{0}C for 5 days. Enzyme was extracted in 50 mL of 0.1 M phosphate buffer (pH=7) on a rotary shaker at 250 rpm for 30 min. The content was filtered and the filtrate was centrifuged at 8000 rpm for 10 min and supernatant was used as the enzyme source.

Enzyme assay

Amylase activity was determined by incubating a mixture of 1 ml of supernatant and 1 ml of 1 % soluble starch at 50 ^{0}C for 20 min. The reaction was stopped by adding 1 ml of DNS (3-5-dinitrosalicylic acid) followed by boiling for 10 min. The final volume was made up to 5 ml with distilled water and the reducing sugar released was measured at 540 nm.

Optimization for enzyme production

The production media was incubated at different temperature (8, 15, 20, 25, 30, 35, 37 and 40) and the amylase production was estimated. The production medium was adjusted to different pH ranging from 4 to 9 with 1 variations keeping remaining media composition constant to check the effect of pH on Amylase production. The effect of carbon

source on Amylase production was studied using (1%) Rice bran, wheat bran, tapioca, potato and corn waste as sole carbon source in production medium. The effect of nitrogen source on Amylase production was studied using (1%) peptone, ammonium nitrate, sodium nitrate, urea, ammonium phosphate as sole nitrogen source in production medium.

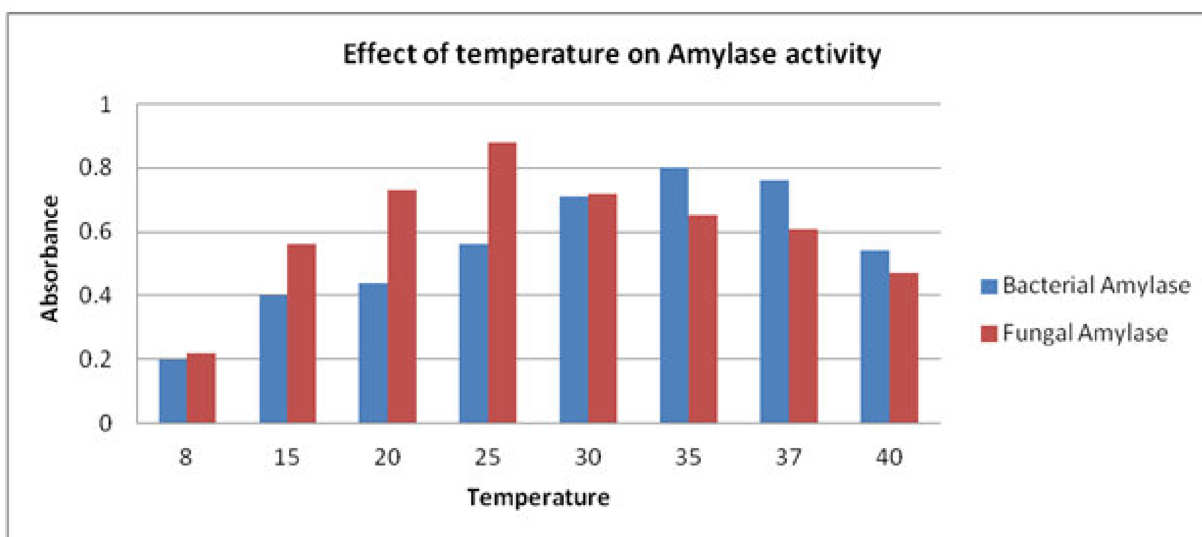
Enzyme Purification and SDS-PAGE

Total proteins were isolated from cultures by ammonium sulfate precipitation and then dialysis was performed for removal of ammonium sulfate. The isolated total proteins were partially purified by size exclusion (Sephadex G-100) column chromatography. Protein content of eluted fractions was determined by spectro-photometric measurement. SDS-PAGE was performed as described earlier [14]. After the electrophoresis, the gel was stained with Comassie Brilliant Blue.

RESULTS AND DISCUSSION

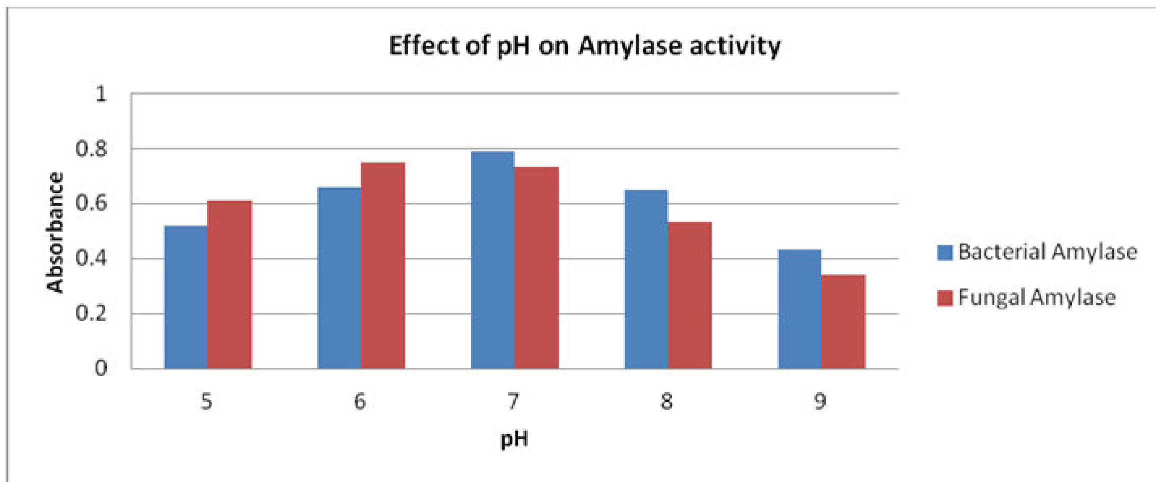
The isolated bacterial was identified as *Bacillus* species and fungal culture was identified as *Aspergillus* species. The effect of Temperature on the production of amylase from Bacterial and Fungal species was investigated and showed in (Figure 1).The temperature was maintained at 8, 15, 20, 25, 30, 35, 37 and 40^0 C. The production of amylase was maximum at 35 and 37^0 C for Bacterial and at 25^0 C for fungal culture. In case of Bacterial culture, there was gradual reduction in production of amylase below 35^0C and above 37^0 C. The fungal growth and the production of amylase were inhibited at higher temperature above 30^0 C.

Figure 1
Effect of Temperature on Amylase production and activity



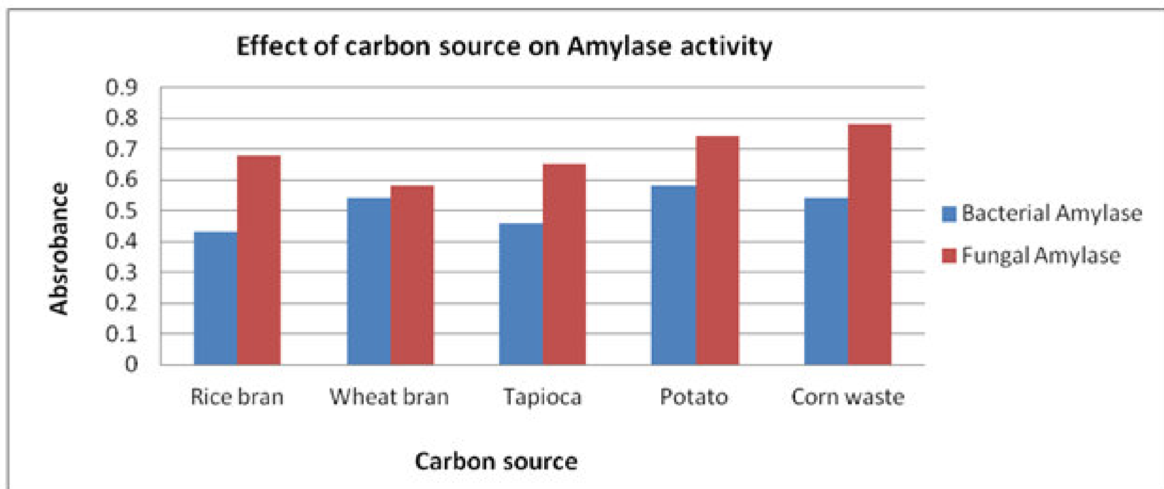
The pH of the media was varied from 5-9. The production of amylase was found to be optimum when the pH of the medium was maintained at 6 and 7 for both bacterial and fungal culture as shown in (Figure 2). The further increase or decrease in the pH resulted in gradual reduction in amylase production.

Figure 2
Effect of pH on Production and activity of Amylase



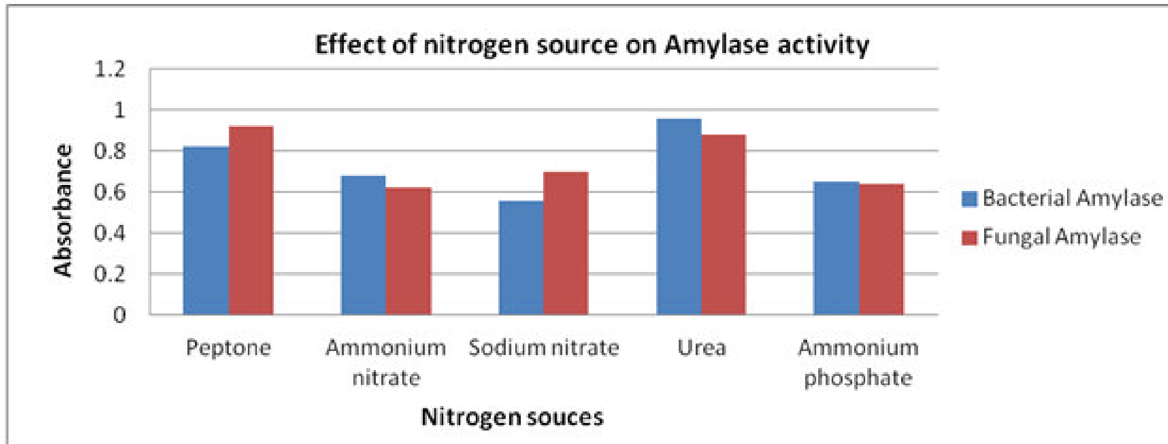
To investigate the effect of various carbon sources on amylase production, bacterial and fungal species was grown in different media containing natural carbon source such as Rice bran, Wheat bran, Tapioca, Potato and Corn waste. Maximum amylase production was observed with potato in bacterial culture and other sources showed considerable amylase production. Corn waste proved to be a better carbon source than other carbon source for fungal culture (Figure 3).

Figure3
Effect of Carbon on production and activity of amylase



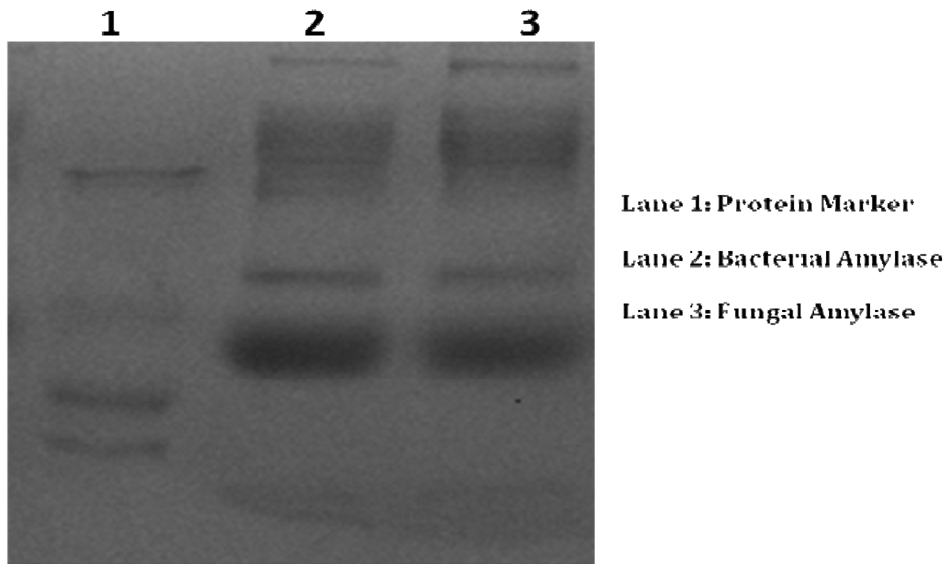
The influence of nitrogen sources on amylase production was determined (Figure 4). More amylase was produced when urea was used for bacterial amylase production and maximum amylase production was found with protease peptone as a nitrogen source.

Figure 4
Effect of Nitrogen on production and activity of Amylase



Total proteins were isolated from cultures by ammonium sulfate precipitation and then dialysis was performed for removal of ammonium sulfate. The isolated total proteins was partially purified by column chromatography and then detected by SDS-PAGE (Figure 5).

Figure 5
SDS-PAGE of bacterial and fungal Amylase



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