

Effect of Alternative Carbon and Nitrogen Sources on Production of Alpha-amylase by *Bacillus megaterium*

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Abstract: Amylases are one of the most important industrial enzymes that account for about 30% of the world's enzyme production and have a wide variety of applications ranging from conversion of starch to sugar syrups, food, feed fermentation, textile, detergent and paper industries. In present work, *Bacillus megaterium* was isolated from soil on starch agar medium by zone analysis method. Work was carried out for the optimization of α -amylase production by *Bacillus megaterium* grown on different carbon source such as glucose, fructose, galactose, lactose, maltose, sucrose, starch, dextrose, molasses, date syrup, rice husk and sugarcane bagasse with different concentration (0.5 and 1.0 %). Maximum production of α -amylase was obtained on 0.5 % Dextrose (1015 Units/ml). α -amylase production was also optimized by using different nitrogen sources such as peptone (control), tryptone, yeast extract, corn steep liquor, casein hydrolyzed, casein soluble, urea, sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride and ammonium sulphate and the maximum production of α -amylase were found in the presence of 1.5% peptone (1722 Units/ml).

Key word: alpha-amylase • *Bacillus megaterium* • Carbon source • Nitrogen source

INTRODUCTION

Amylases are one of the most important industrial enzymes that account for about 30% of the world's enzyme production [1] and have a wide variety of applications ranging from conversion of starch to sugar syrups, food, feed fermentation, textile, detergent and paper industries [2, 3]. A significant increase in amylases production and utilization occurred in the early 1960s when *B.subtilis* α - amylase and *Aspergillus niger* glucoamylase were used to replace acid catalysis in the production of dextrose from starch [4]. Bacterial amylases are generally preferred over fungal amylases due to several characteristics advantages that it offers [5]. *Bacillus* species are heterogeneous forms of organisms and they are very versatile in the adaptability to the environment. There are various factors that influence the nature of their metabolic process and enzyme production [6]. Almost all organisms of the bacillus genus synthesized alpha amylase, thus this genus has the potential to dominate the enzyme industries [7].

The production of amylases by fermentation has been thoroughly investigated and it's affected by a

variety of physiochemical factors. Most notable among these are the composition of the growth medium, inoculum age, pH, temperature, carbon and nitrogen sources [8-11].

In present work, *Bacillus megaterium* newly isolated from soil was optimized for amylase production grown on different carbon and nitrogen sources.

MATERIAL METHODS

Isolation and Screening of Microorganism: *Bacillus megaterium* Aq-2007 was isolated from the soil sample collected from the vicinity of University of Sindh, Jamshoro, Pakistan. Strains capable of producing alpha amylase were screened by allowing them to grow for 24-48 hours on nutrient agar plates containing 1% (w/v) starch at 37°C. The plates were stained with Gram's iodine solution and largest halo-forming zone was considered as the most promising strain. Later microbiology of the isolated strain was determine according to the methods describe in Barges manual of systematic bacteriology [12] and strain was maintain on nutrient agar slant at 4°C for further studies.

Enzyme Production Medium: The enzyme production was carried out in the basal medium composed in %, such as starch 1%, peptone 0.5%, K_2HPO_4 0.6%, $MgSO_4 \cdot 7H_2O$ 0.02%, $CaCl_2 \cdot 2H_2O$ 0.05%. The initial pH of the medium was adjusted to 7.0 with 1N NaOH and was autoclaved at 121°C for 15 minutes. 5.0ml nutrient broth inoculated with a loop-full of growing culture of *B.megaterium* and was incubated at 37°C for 24 hours. After 24 hours inoculated nutrient broth (starter) was transferred into 45ml of sterile basal medium and incubated at 37°C for 12 hour. At regular interval of every 1 hour, the triplets sample were harvested and organism were separated by centrifugation at 8000 rpm for 10 minutes at 4°C [Kubota-8700] and the cell free filtrate was used for enzyme assay and characterization.

α -amylases Assay Method: α -Amylase was determined by spectrophotometric method as described by Fisher and Stein [13]. According to procedure 1.0 ml of culture broth was taken in test tube in duplicate and 1.0 ml of substrate (starch) was added in test tube. Appropriate controls were also taken for blank enzyme and blank substrate. The test tubes were covered with aluminum foil and incubate at 35°C for 15 minutes in water bath. Then 2.0ml DNS reagent was added in each tube to stop the reaction and kept in boiling water bath for 5 minutes. After cooling at room temperature, the absorbance was read at 540 nm by spectrophotometer (VIS-7220).

A unit of α -amylase activity was defined as the amount of α -amylase required to catalyze the liberation of reducing sugar equivalent to one μ mol of D-glucose per minute under the assay conditions.

Effect of Carbon Sources: Different pure carbon sources like glucose, fructose, galactose, lactose, maltose, sucrose, dextrose, industrial waste (molasses and date syrup) and agricultural waste (sugarcane bagasse and rice husk) were incorporated with basal medium by replacing starch with two different concentration (0.5% and 1%) to analyze their effect on amylases production. The inoculated flasks were incubated at $37 \pm 2^\circ C$ for different time period (1-12 hours).

Effect of Nitrogen Source: Enzyme production was optimized by using different nitrogen sources such as tryptone, yeast extract, corn steep liquor, casein hydrolyzed, casein soluble, urea, sodium nitrate, potassium nitrate, ammonium nitrate, ammonium chloride and ammonium sulphate incorporated in basal medium by

replacing peptone (control).

Effect of chloride: Different concentrations of sodium chloride were incorporated in basal medium ranging from 0.3- 2.1% to analyze their effect on amylases production by *Bacillus megaterium* when incubated at 37°C, with initial pH 7.0.

RESULTS AND DISCUSSION

The production of amylases by fermentation has been thoroughly investigated and it is affected by a variety of physiochemical factors. The organism was isolated from soil by serial dilution and screened by zone hydrolysis method [14] and later identified as *Bacillus megaterium* Aq-2007 according to Bergey's Manual of Determinative Bacteriology [15]. The *Bacillus megaterium* Aq-2007 strain was found gram positive, catalase positive, motile, endospore positive and glucose positive. The best pH for growth was 7.0 where as the optimum temperature was 40°C and strain was capable to produce α -amylase by hydrolyzing starch.

To investigate the effect of various carbon sources such as glucose, fructose, galactose, lactose, maltose, sucrose, starch, dextrose, molasses, date syrup, rice husk and sugarcane bagasse with different concentration (0.5 and 1.0 %) for the growth of *B.megaterium* for 12 hours and production of amylase. After every 1 hour interval, samples were analyzed for amylase activity. Growth and enzyme production were varying in each carbon source and the results of α amylase activity μ /ml per minute produced by *B.megaterium* are shown in Figure 1. Among the carbon sources tested dextrose was found to enhance α amylase production but the other carbon sources showed repressive effect on enzyme synthesis. The maximum yield was obtained in the medium containing dextrose (0.5%) at 7 hours of incubation. Bajpair and Bajpair [16] concluded that the synthesis of α amylases was greatly suppressed when the bacterium was grown on sucrose, glucose or fructose and amylase production was enhanced when the bacterium was grown on starch and Dextrin. Similar finding were also found by Lin *et al.*, [15] and Asghar [17]. Teodoro and Martins [18] have also found that amylase synthesis was diminished when glucose 0.5% was added to medium. The same behavior of the organism was observed when 1% carbon source used for amylase production as shown in Figure 2.

The various concentration of peptone (0.25-2.0) used for the amylase production and 1.5% has given the

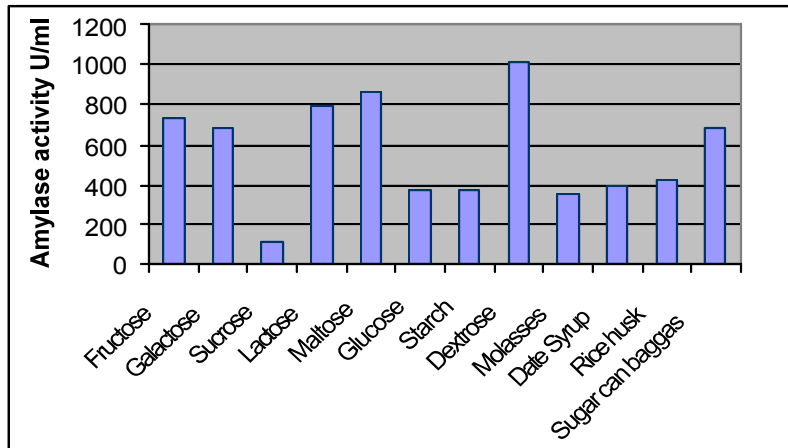


Fig. 1: Maximum production of Amylase on 0.5 % different carbon sources by *Bacillus megaterium*

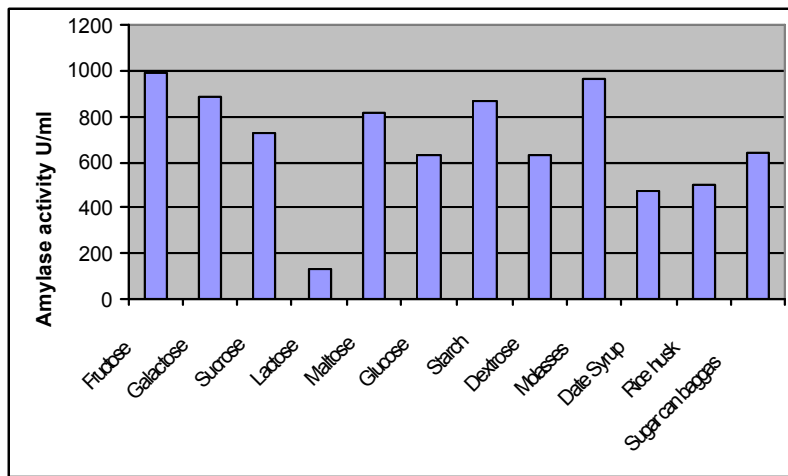


Fig. 2: Maximum production of Amylase activity on 1.0 % different carbon sources by *Bacillus megaterium*

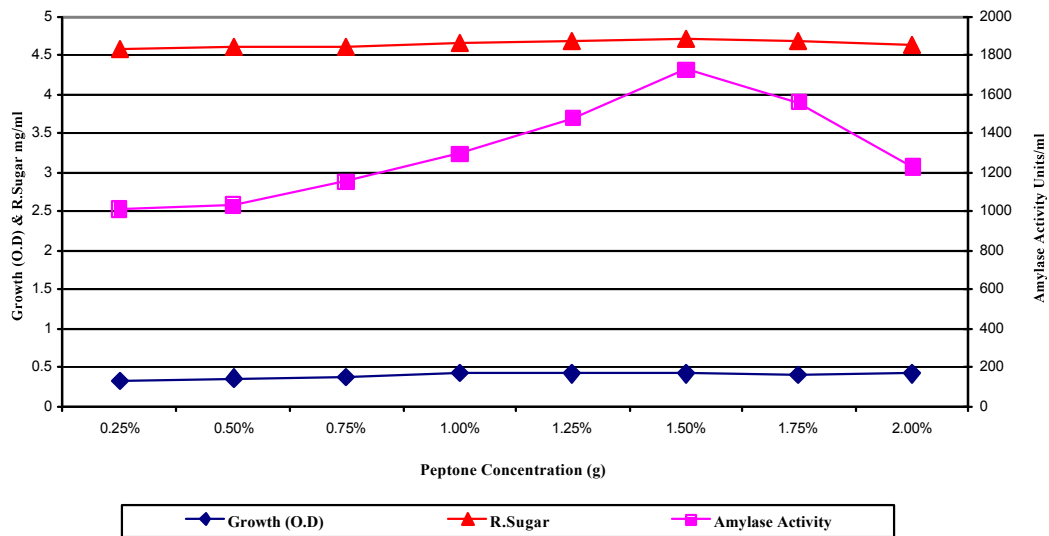


Fig. 3: Effect of different concentrations of Peptone on Amylase Production by *B. megaterium* using 0.5% Dextrose as a carbon source when initial pH was 7.0 and incubated at 37°C for 7 hours.

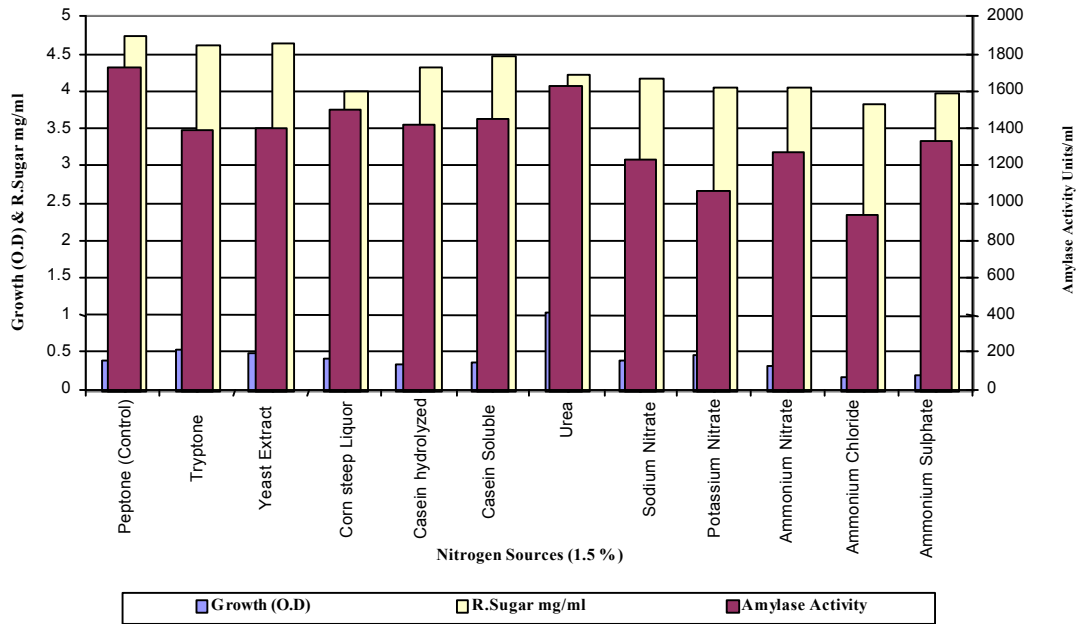


Fig. 4: Effect of different nitrogen sources on Amylase Production by *B. megaterium* using 0.5% Dextrose as carbon source and 1.5 % different nitrogen sources when initial pH was 7.0 and incubated at 37°C for 7 hours

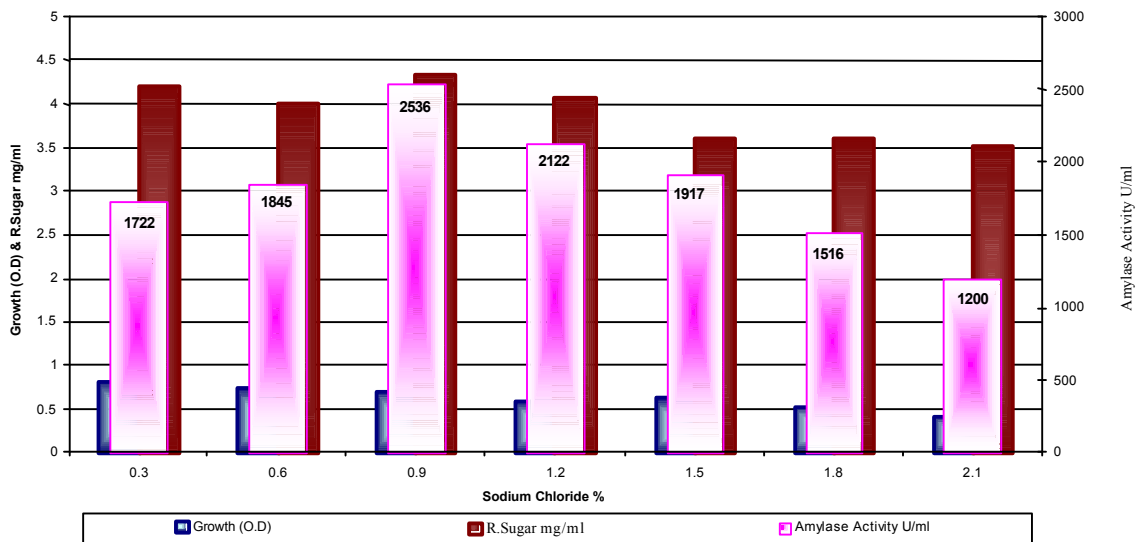


Fig. 5: Effect of different Sodium chloride (NaCl) Concentration on Amylase Production by *B. megaterium* using 0.5% Dextrose as carbon source and 1.5 % Peptone as nitrogen sources when initial pH was 7.0 and incubated at 37°C for 7 hours.

highest activity 1722 μ /ml as shown in Figure 3 and then peptone was replaced with 1.5% of different organic and inorganic compound used as nitrogen sources keeping the rest of the media composition same and it was observed that among all the nitrogen sources, peptone was the best candidate for the maximum production

(Figure 4). It has been reported that organic nitrogen sources like peptone, yeast extract usually have stimulating effect [19-21]. Figure 5 shows the effect of different concentrations of sodium chloride incorporated in basal medium ranging from 0.3- 2.1% and the production of enzyme was enhanced (2536 u/ml) with

addition of 0.9% NaCl when incubated at 37°C for 7 hours, with initial pH 7.0.

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