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# Production and Medium Optimization of Amylase by *Bacillus* Spp Using Submerged Fermentation Method

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**Abstract:** Microbial amylases have several industrial applications. A large number of them are available commercially and have almost completely replaced chemical hydrolysis of starch in starch processing industry. The production of extracellular amylase by three selected *Bacillus* species such as *Bacillus subtilis, Bacillus cereus* and *Bacillus megaterium* was screened. From the preliminary trials, *B.megaterium* was found to be the highest amylase producer and was taken for optimization studies using submerged fermentation. The production of enzymes was maximum at 24 hours after incubation, in pH 7 and at 37°C. Among the four carbon sources tested, high production was recorded with starch followed by glucose. The least production of enzyme was noted when sucrose was used as a carbon source. The effect of four nitrogen sources viz; peptone, beef extract, meat extract and yeast extract was assessed and peptone was found to be the ideal nitrogen source for amylase production. Enzyme productions were standardized using various parameters such as pH, incubation time and temperature.

**Key words:** Amylase · Bacillus megaterium · Submerged Fermentation

## INTRODUCTION

Amylases are enzymes that break down starch or glycogen [1]. The production of economically important alpha-amylases essential for the conversion of starches into oligosaccharides [2]. Amylases are extensively used for starch liquefaction and the paper, food, pharmaceutical and sugar industries [3, 4]. Amylases with suitable properties are very useful in specific industries and it has become essential to characterize all available amylolytic microbial strains. Since almost all microorganisms of the *Bacillus* genus synthesise α- amylase, this genus has the potential to dominate the enzyme industry. *Bacillus* spp are heterogeneous and are very versatile in their adaptability to environment. Various factors influence the nature of their metabolic process and the enzymes produced. The composition and concentration of media greatly affect the growth and production of extracellular amylase in bacteria [5]. In this regard, appropriate media components and suitable conditions must be attained for optimal production of the required products. *Bacillus* species and other forms of microorganisms grow at different rates with specificity to different substrates in a culture medium. The growth conditions also influence their enzymatic activities[6]. In this background, the present study was designed to assess the best amylase producer from selected three species of *Bacillus viz*; *B. cereus, B. megaterium* and *B. subtilis* and to carryout optimization studies for the production of amylase enzyme in batch experiments using shake flasks under controlled conditions in a laboratory.

## MATERIALS AND METHODS

**Microorganism:** *Bacillus* spp such as *B. cereus, B. megaterium* and *B. subtilis* were obtained from the culture bank of PG Department of Microbiology, Sri Paramakalyani College, Alwarkurichi and maintained on nutrient agar slants and sub cultured every 10 days.

**Screening and Selection of Species for Optimization Studies:** The selected three *Bacillus* spp were checked for its amylase producing ability by streaking them onto starch agar plates. The *Bacillus* spp, which produced the maximum zone, was chosen for further optimization studies.

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**Inoculum and Fermentation Medium:** The inoculum was prepared by transferring the freshly grown colony on nutrient agar slants, onto nutrient broth and from this 0.5 ml of the cell suspension was inoculated into 100 ml of sterilized fermentation medium and incubated at 35°C for 10 hrs. The composition of the fermentation medium was [g/l] peptone-20g; $MgSO_4$. $7H_2O$-1 g; $K_2HPO_4$-3 g Starch-5 g, pH 7.

**Extraction of Amylase from the Fermentation Medium:** After the incubation fermentation medium was harvested by centrifugation at 10,000 rpm for 10 minutes at 4°C. The supernatant was collected and subjected for the estimation of amylase activity.

**Effect of Temperature:** To study the effect of temperature on amylase production using submerged fermentation was carried out at different temperatures (25°C, 37°C and 55°C).

**Effect of pH:** The fermentation medium was prepared with varying pH values (5.0, 6.0, 7.0, 8.0 and 9.0) and the production of amylase.

**Effect of Incubation Time:** The fermentation medium inoculated with *B. megaterium* was incubated for different time periods (24, 48 and 72 hours)and checked for the production of amylase.

**Effect of Carbon Source:** The fermentation medium was prepared with different carbon sources such as sucrose, starch, glucose and maltose (0.5 % level) and assessed for amylase production.

**Effect of Organic Nitrogen Source:** Different organic nitrogen sources such as peptone, beef extract, meat extract and yeast extract (2.0 % level) were incorporated to the fermentation medium and assessed for their effect on amylase production.

**Assay of Amylase:** The amylase activity was determined following the method of Bernfeld [8]. An assay mixture containing starch as the substrate and DNS as coupling reagent was used. One unit of amylase activity was defined as the number of ìmoles of maltose liberated by 1 ml of enzyme solution per minute.

## RESULTS AND DISCUSSION

Amylase enzymes play an important role in biotechnological industries and has several potential applications in food, fermentation, textile and paper industries [8, 9]. The spectrum of applications of α-amylases has widened in many sectors such as clinical, medicinal and analytical chemistry [10]. Amylases can be obtained from several sources such as plants, animals and microbes [11]. Many microorganisms especially several species belonging *Bacillus* spp are known to produce a variety of extracellular enzymes with a wide range of industrial application [12]. The microbial source of amylase is preferred to other sources because of its plasticity and vast availability [13-15]. Micro-organisms have become increasingly important as producers of industrial enzymes due to their biochemical diversity and the ease of improving the enzyme productivity through environmental optimization and genetic manipulation [11]. There are various reports on starch degrading micro-organisms from different sources and respective amylase activity [14]. Among bacteria, *Bacillus* sp. is widely used for amylase production to meet the industrial needs [2]. Amylase production in different *Bacillus* sp has been reported by several workers [1, 4, 13, 17-21]. In the current study, amylase producing ability of three *Bacillus* sps were checked and all were found to be positive, but *B. megaterium* was found to be the best amylase producer (Table 1). However Vipulverma *et al.* [19] found the maximum amount of amylase production in *B. subtilis* followed by *B. megaterium*, among nine strains tested which included *B. cereus*, *B. megaterium* and *B. subtilis*. These enzymes have traditionally been obtained from submerged cultures because of ease of handling and greater control of environmental factors such as temperature and pH [1, 21-23]. The maximum amylase producing - *B. megaterium* was taken for optimization studies through submerged fermentation by varying the temperature, pH, incubation period, carbon and nitrogen source, since the production of amylase enzymes are influenced by diverse physico-chemical and biological factors[17].

Table 1: Production of amylase by *B.cereus, B. megaterium* and *B. subtilis*

| Name of the strain used | Amylase Activity (U/ml) |
|---|---|
| *B. cereus* | 8.18 |
| *B. megaterium* | 17.53 |
| *B. subtilis* | 10.56 |

**Effect of Temperature:** Temperature is one of the important physical factor influencing the enzyme production [30, 32, 33, 35, 36, 37, 38]. Among the selected three temperatures tested, maximum production of enzyme was noted at 37°C. This could be due to the mesophilic

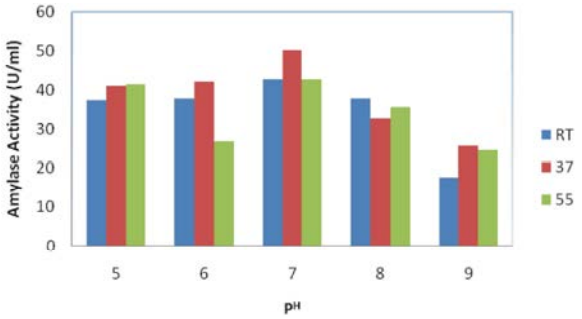


Fig. 1: Effect of Different P[H] on Amylase Activity at 24 hours at various Temperature

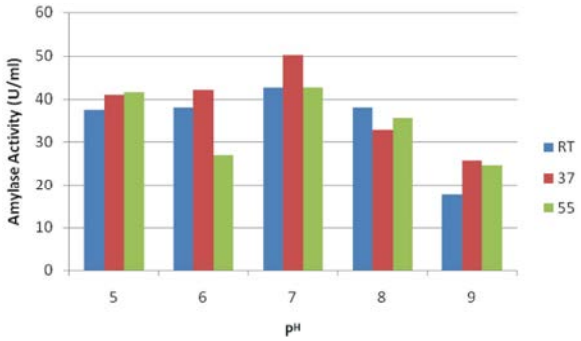


Fig. 2: Effect of Different P[H] on Amylase Activity at 48 hours at various Temperatures

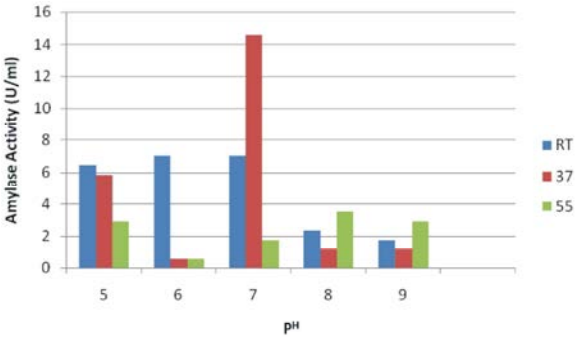


Fig. 3: Effect of Different P[H] on Amylase Activity at 72 hours at various Temperatures

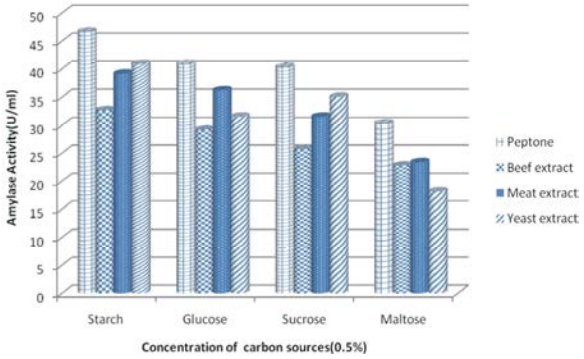


Fig. 4: Effect of Carbon and Nitrogen Source on Amylase Activity

nature of the organism. The optimum temperature was observed for the production of amylase from banana stalk using *B. subtilis* was observed as 35°C reported by Krishna and Chandrasekaran [23]. Increase in incubation temperature, decreased the production of enzyme. The production of the enzyme was greatly inhibited at 40°C, due to lesser growth of the bacteria [8, 23]. Vasantha Raj and Hemashenpagam [17] have also evaluated the influence of temperature on amylase production.

**Effect of pH:** Earlier reports on amylase production indicates greater influence by pH [1, 17, 19, 21, 30, 32, 33, 34, 36, 37, 38]. In our study, amylase production by *B.megaterium* was found to be maximum at pH7.0 (50.24U/ml/min) (Fig. 1). Further increase in the pH decreased the activity of amylase. When pH is altered below or above the optimum the activity appear to be decreased or becomes denatured [15]. Different organisms have different pH optima and decrease or increase in pH on either sides of the optimum value results in poor microbial growth [23].Terui [24] went on to report 6.8 as an optimum pH for the production of amylase by *B. subtilis*.

**Effect of Incubation Period:** Among the three timings tested for amylase production *viz*; 24, 48 and 72 hours, maximum production of amylase was noted at 24 hours and the production found decreased in the rest of the hours (Fig. 2 & 3). Chandrasekhar *et al*. [16] has evaluated the production of amylase at 12, 24, 36, 48 and 60 hours using *B.subtilis* cultured on banana waste and found more production at 24 hours, which corroborate with our results. However Nurullah Akcan [21] noted high production of amylase by *B. subtilis* at 72 hours, when he screened at 12, 24,48,72,96 and 120 hours. It may be due to the nature of the organism, media status and environmental conditions.

The active nature of the cell after 24 hours could prove optimal for better amylase production as currently recorded.

**Effect of Carbon Source:** Different carbon sources have been used for the production of amylase enzyme [4, 21, 30, 38]. Venkatanagaraju and Divakar [25], has used glucose, sucrose, maltose, lactose, galactose, fructose and dextrose at 0.5 % level for producing the enzyme in bacteria and found maltose as the suitable carbon source. In submerged fermentation for the production of amylase,

different carbon sources like glucose, sucrose, starch and maltose were used at 0.5 % level in the current study. Among the varied carbon sources tested, starch was found to be more optimal for amylase enzyme production. The influence of carbon source on the enzyme activity could well be understood from (Fig. 4). Bhuuto *et al.* [27]. tested diverse carbon source such as glucose, fructose, galactose, lactose and maltose at different concentrations and found higher production of enzyme with dextrose at 0.5 % level. The results revealed the different requirements of carbon source for different organisms.

**Effect of Organic Nitrogen Source:** Effectiveness of organic nitrogen compounds on amylase production has been putforth by several workers [28, 29]. The inhibitory effect of inorganic nitrogen has been well demonstrated by Akcan [21]. Among the four organic nitrogen sources tested at 2% level for the production of amylase from *B. megaterium*, highest production (46.74U/ml/min) (Fig. 4) of enzyme was noted with peptone followed by meat extract. Our results are in accordance with the results of Bhutto *et al*. [26] who also recorded the identical results.

## CONCLUSION

From the findings of the current study, it is concluded that *B. megaterium* could be used as a candidate strain for the production of amylase by submerged fermentation. Exposure of the bacteria to other environmental parameters influencing growth and production of economically valuable metabolites through cultural techniques and strain improvement process might aid in providing excellent information for exploiting its biological potential. Moreover the studies also reveals the values as well as the microbial wealth of amylase producing bacteria which can be a boon for the development of biotechnological processes.

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