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Factors affecting alfa Amylase Production on Submerged Fermentation by *Bacillus* sp.

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Abstract
The production of extracellular amylase by *Bacillus spp* was optimized in a submerged fermentation. The production of the enzyme was maximum at 10 h after inoculation. The effect of incubation period, pH of the medium and incubation temperature was optimized. The maximum productions of enzyme were obtained at 35°C and pH 7.

Key-Words: alfa-Amylase production, Submerged fermentation

Introduction
The starch degrading enzyme alpha amylase (α-1,4 glucan-glucanohydrolase *EC 3. 2.1. 1*) is widely distributed in nature. This extracellular enzyme hydrolyses α-1,4 glucosidic linkages randomly throughout the starch molecule in an endo-fashion producing oligosaccharides and monosaccharides including maltose, glucose and alpha limit dextrin (Omemu *et al*., 2005; Bhanja *et al*., 2007; Leman *et al.*, 2009). Alpha amylases are one of most important and widely used enzymes whose spectrumof application has widen in many sectors such as clinical, medicinal and analytical chemistry. Beside their use in starch saccharification they also find applications in food, baking, brewing, detergent, textile and paper industries. These are important enzymes used in starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar constituents. Increasing utility and consumption of alpha amylase in different industries has placed a greater stress on increasing indigenous enzyme production and search of more rapid processes (Carlsen *et al*., 1996; Ramachandran *et al*., 2004; Kathiresan and Manivanan, 2006; Gupta *et al*., 2008).

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Alpha amylase can be derived from several sources such as plants, animals and microorganisms, but production from first two groups is limited for several reasons. The concentration of enzymes in the plant material is generally low so the processing of large amount of plant material is necessary; on the other hand enzyme of animal origin is by- product of meat industry. In contrast, microbial source of alpha amylase can be produced in amount meeting the demands of market. Different fungal and bacterial strains have been extensively used for the enzyme production.

Bacterial starins have been well known for the starch and cellulose degrading enzymes they naturally secrete. The ability of bacterial strains to secrete large amounts of extracellular protein has made them well suited for the industrial enzyme production. The commonly used *Bacillus* sp. is widely used for thermostable production to meet industrial needs. *B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens.* Many species *B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens* have received. Most attention to obtain many kinds of hydrolytic enzymes like alpha amylase, lipase and protease. However, bacillus spp.is the organism of choice because of its ubiquitous nature, non fastidious nutritional requirements and high productivity of alpha amylase

(Abe *et al*., 1988; Archer and Wood, 1995; Agger *et al*., 2001; Zangirolami *et al*., 2002). Similarly, filamentous fungi have been widely used for the production of amylases for centuries. As these moulds are known to be prolific producers of extracellular proteins, they are widely exploited for the production of different enzymes including a-amylase.

Fungi belonging to the genus *Aspergillus* have been most commonly employed for the production of a-amylase. Production of enzymes by solid-state fermentation (SSF) using these moulds turned a cost-effective production technique. Detailed literature is available on various microbial sources for the production of amylases (Vihinen & Mantasala, 1989; Pandey et al, 2005). To meet the demand of industries, low-cost medium is required for the production of α-amylase. Both SSF and submerged fermentation (SmF) could be used for the production of amylases, although traditionally these have been obtained from submerged cultures because of ease of handling and greater control of environmental factors such as temperture and pH. Mostly synthetic media have been used for the production of bacterial amylase through SmF (Bunni, 1989; Haddaoui et al, 1999; McTigue et al, 1995; Haq et al, 1997; Hamilton et al, 1999).

Optimization of various parameters and manipulation of media are one of the most important techniques used for the overproduction of enzymes in large quantities to meet industrial demands (Tanyildizi & Elibol, 2005). Production of α-amylase in fungi is known to depend on both morphological and metabolic state of the culture. Growth of mycelium is crucial for extracellular enzymes like α-amylase(Carlsen, 1996).

Various physical and chemical factors have been known to affect the production of α- amylasesuch as temperature, pH, period of incubation, carbon sources acting as inducers, surfactants, nitrogen sources, phosphate, different metal ions, moisture and agitation with regards to SSF and SmF, respectively. Interactions of these parameters are reported to have a significant influence on the production of the enzyme. Temperature The influence of temperature on amylase production is related to the growth of the organism. Hence, the optimum temperature depends on whether the culture is mesophilic or thermophilic (Ramachandran et al, 2004; Pandey et al, 1999).

Bacterial amylases are produced at a much wider range of temperature. *Bacillus amyloliquefaciens*, *B. subtilis*, *B. licheniformis* and *B. stearothermophilus* are among the most commonly used *Bacillus* sp. reported to produce α-amylase at temperatures 37–60 °C (Syu & Chen, 1997; Mishra et al, 2005; Mendu et al, 2005; Mielenz, 1983).

Material and Methods

Isolation of organism: *Bacillus* spp was isolated from environment and maintained on nutrient agar slants and the sub cultured for every 10 days.

Inoculum and Fermentation Medium: The inoculum was prepared by the addition of sterile distilled water in the freshly grown nutrient agar slants, from this 0.5 ml of cell suspension was inoculated in to 100 ml of sterilized fermentation medium and incubated at 35°C. The composition of the fermentation medium was [g/l] 6.0 g Bacteriological peptone; 0.5 g MgSO .7H O; 0.5 g KCl; 1.0 g Starch-, and maintained pH 7

Extraction of Amylase from the Fermentation Medium: After incubation the fermentation medium was harvested by centrifugation at 5000 rpm for 20 minutes at 4°C.The supernatant was collected and subjected to estimate the amylase activity.

Effect of Temperature: To study the effect of temperature on amylase production the submerged fermentation was carried out at different temperatures (25°C, 30°C, 35°C and 40° C)

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

Assay of Amylase: The alfa amylase activity was determined following the method of Bernfeld [5]. An assay mixture containing, enzyme extract, starch as substrate and DNS as coupling reagent was used. One unit of "- amylase was defined as the number of µ moles of maltose liberated by 1 mL of enzyme solution per minute.

Result and Discussion

Amylase Production in Submerged Fermentation:

In submerged fermentation the production of amylase was reached maximum of 4 U/ml at 10 h of incubation period. Further increase in incubation period did not show any significant increase in enzyme production rather it was decreased. Thus optimum time of enzyme synthesis was to be 10 h after inoculation. Ramesh and Lonsane reported the enzyme production was initiated at about 6 h in the media containing 0.2 or 1.0% soluble starch.

Amylase production in various incubation periods

Incubation period (h)	Amylase activity U/ml
2h	0.4
4h	0.8
6h	1.2
8h	1.8
10h	4.0

12h	3.7
14h	3.2

Effect of Temperature: Results from table shows the effect of different incubation temperature on the production of amylase by *Bacillus spp.*, the maximum production of amylase was obtained at 35°C. The optimum temperature was observed for the production of "- amylase from Banana stalk using *B. subtilis* was also 35°C as reported by Krishna and Chandrasekaran. Increase in incubation temperature, decreased the production of enzyme. The production of the enzyme was greatly inhibited at 40°C. It might be due to that at high temperature, the growth of the bacteria was greatly inhibited and hence, enzyme formation was also prohibited.

Effect of varying incubation temperature on amylase production

Incubation Temperature	Amylase activity U/ml
25	1.9
30	3.0
35	6.8
40	4.6
45	3.8

Effect of pH: In our study the amylase production by *Bacillus spp.*, was found maximum at 7.0 (11 U/ml). Further increase in the pH resulted decrease in the activity of amylase. However, the pH of the fermentation medium was found to be optimum at 7.0. When pH is altered below or above the optimum the activity is decreased or becomes denatured. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth. Terui who reported 6.8 as optimum pH for the production of "-amylase by *B. subtilis.*

Effect of varying pH of the medium on amylase production

pH	Amylase activity U/ml
5.5	3.2
6	4.1
6.5	4.9
7	11.0
7.5	9.0
8	7.0

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