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Production of Alpha Amylase by a Thermophilic Strain of Bacillus Licheniformis

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Abstract: The production of alpha amylase was carried out by thermophilic strains of Bacillus licheniformis. The isolated cultures were screened by solid-state fermentation using wheat bran as substrate, moistened with phosphate buffer (pH 7.5). The culture GHB8 that gave the maximum production of alpha amylase (1277 U/g/min) was used for further studies. The maximum production of alpha amylase (1630 U/g/min) was reached when wheat bran was partially replaced with cottonseed meal in the ratio of 3:1 along with 1 per cent starch. The incubation temperature 40°C was found to be optimum for the production of alpha amylase. The enzyme was most active between 60-70°C.

Key words:- Alpha amylase, Agricultural by-products, Bacillus, wheat bran, starch

Introduction

The production of alpha amylase by fermentation has been a worth praising achievement in the field of industrial microbiology. This enzyme hydrolyzes the alpha1- 4, linkage in starch and related products in an end fashion producing disaccharides, glucose and alpha limit dextrin. (Tengeny *et al.*, 1998, Dobreva *et al.*, 1994 and *et al.*, 2003). Suitable fermentation medium is very essential for the optimum production of alpha amylase. The carbon sources such as glucose or fructose supported much better growth of the bacteria with significant reduction of inducible alpha amylase production (Kell *et al.*, 1995). Wheat bran was found to be the ideal source of carbon and nitrogen for the fermentation of alpha amylase (Prescott and Dunn's 1987 and Ramesh and Lonsane, 1990). The production and activity of the enzyme is very sensitive to temperature. Extensive work has been reported for the production and activity of the enzyme by a thermophilic strain of Bacillus species. (Dobreva *et al.*, 1998, Chang *et al.*, 1995, Weemaes *et al.*, 1996 and Kim *et al.*, 1995).

Pakistan being an agricultural country having many agricultural by-products such as wheat bran, rice bran, rice husk, soybean meal, cottonseed meal etc. These by products which are unprocessed, structurally and nutritionally heterogeneous may be used for their exploitation as substrate for manufacturing of biochemical' s by fermentation process (Babu and Satyanarana 1995, Ramesh and Lonsane 1998).

The present study is concerned with the isolation of suitable strain of Bacillus species, and optimization of cultural conditions for the production of alpha amylase. The parameters studied were selection of substrate, effect of sugars, effect of temperature on the production of enzyme as well as on the activity of enzyme and rate of alpha amylase fermentation.

Materials and Methods

Isolation of Organism: The different thermophilic strains of Bacillus were isolated from soil. The bacteria were isolated by observing clear zone of hydrolyzing starch in the petriplates (Zaghloul *et al.*, 1993).

Inoculum Preparation: The vegetative inoculum was used in the present study. The inoculum was prepared as described by Haq *et al.* (1998).

Fermentation Technique: The solid-state fermentation technique was employed. Ten gram of wheat bran moistened with 10ml of phosphate buffer was transferred to 250 ml cotton plugged conical flask. The flasks were sterilized in the autoclave. After sterilization one ml of inoculum was transferred to each flask. The flasks were then incubated at 40°C for 48 hours. After 48 hours, 100 ml of the phosphate buffer was added in each flask. The flasks were rotated on the rotary shaker for one hour. After one hour, the ingredients of the flasks were filtered and filtrate was used for the estimation of alpha amylase.

Enzyme Assay: Alpha amylase estimation was carried out according to the method of Fisher and Stein (1961). One unit of activity is that amount of enzyme which in 3 min liberates reducing group with 1% Lintner' s soluble starch corresponding to 1mg maltose hydrated.

Statistical Analysis: Treatment effects were compared by the method of Snedecor and Cochrane (1980).

Table 1: Screening of thermophilic bacillus Strains for the production of alpha amylase

Cultures No.	U/g/min
HB1	248 ± 5
HB2	99 ± 3
HB3	50 ± 2
HB4	53 ± 2
HB5	25 ± 4
HB6	36 ± 2
HB7	60 ± 5
HB8	1277 ± 10
HB9	848 ± 12
HB10	110 ± 10
HB11	60 ± 5
HB12	470 ± 15
HB13	150 ± 20
HB14	590 ± 23
HB15	120 ± 5

± indicated the standard deviation from he mean value
Substrate wheat bran
Incubation period 48 h
Temperature for incubation 40°C
Phosphate Buffer pH 7.5.

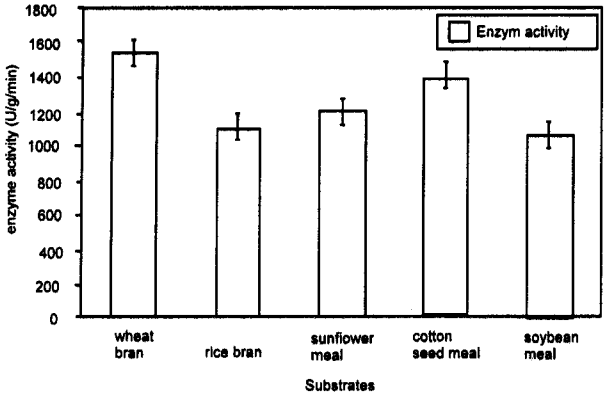

Fig. 1: Selection of substrate for the synthesis of alpha amylase by thermophilic Bacillus licheniformis GHB-8.

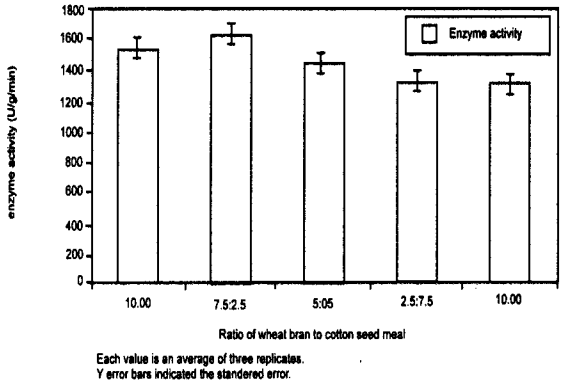

Fig. 2: Effect of different ratios of weatbran with cotton seed meal on the production ofalpha amylase by thermophilic bacillius licheniformis GHB8.

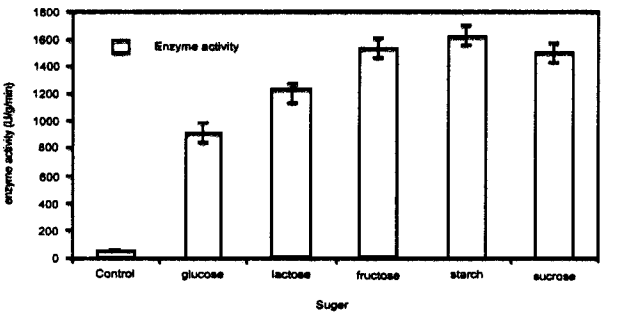

Fig. 3: Effect of different sugers onthe production of alpha amylase by thermophilic bacillius licheniformis GHB8.

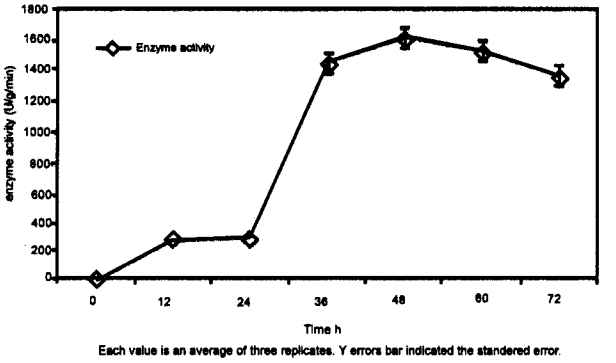

Fig. 4: Rate of alpha amylase production.

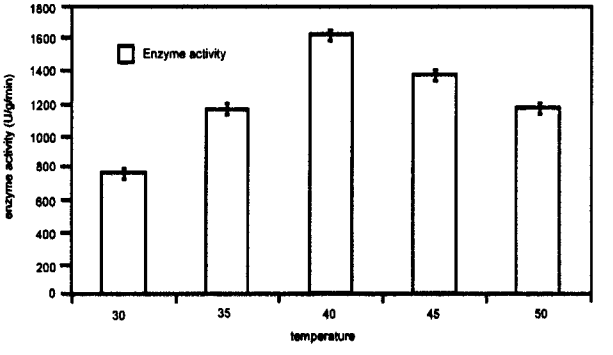

Fig. 5: Effect of incubation temperature on the production of alpha amylase.

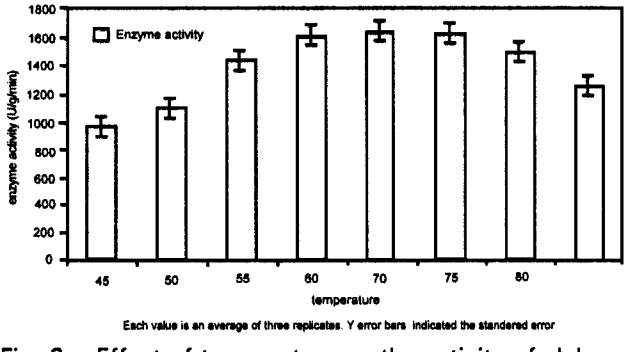

Fig. 6: Effect of temperature on the activity of alpha amylase.

Results

Screening of Organisms: Fifteen isolates of thermophilic Bacillus species were screened for the production of alpha amylase using wheat bran as substrate (Table 1). Of all the isolates examined, the culture no. 8, gave the maximum production of alpha amylase (1277 U/g/min). The culture No. 8 which, gave the maximum production of alpha amylase was identified and assigned the code as Bacillus licheniformis GHB-8. This strain was used for further studies.

Selection of Substrate: Different agricultural byproducts such as wheat bran, rice husk, sunflower meal, cottonseed meal and soybean meal were evaluated for the production of alpha amylase by Bacillus licheniformis GHB8 (Fig. 1). Of all the substrate evaluated, wheat bran was found to be best substrate for the production of alpha amylase (1530 U/g/min). The other substrate rice husk, sunflower meal, cottonseed meal and soybean meal gave 1100, 1200 1400 and 1050 U/g/min of alpha amylase respectively.
The Fig. 2 shows the effect of partial replacement of wheat bran with cottonseed meal. The ratios of wheat bran by cottonseed meals were 10:0, 7.5:2.5, 5:5, 2.5:7.5 and 0:10. Maximum production of alpha amylase (1630 U/g/min) was achieved when wheat bran was partially replaced with cottonseed meal in the ratio of 7.5:2.5. However, when amount of cottonseed meal was increased, the production of enzyme was decreased gradually.

Effect of Sugars: Different sugars such as glucose, lactose, fructose, starch and sucrose were evaluated for the production of alpha amylase (Fig. 3). The sugars were added in the medium at 1% level. The maximum production of alpha amylase (1630 u/g) was reached when starch was added in the medium. In the presence of other sugars however, the production of enzyme was reduced.

Rate of Alpha Amylase Fermentation: Fig.. 4 shows the production of alpha amylase at different time intervals. The cultures were incubated at 40°C for 12, 24, 36, 48, 60 and 72 hours. The amount of enzyme production was maximum (1630 U/g/min) after 48 hours of inoculation. Further increase in the incubation period did not show any increase in enzyme formation rather it was decreased. Thus optimum time of enzyme synthesis was found to be 48 hours after inoculation.

Effect of Temperature: The production and stability of the enzyme was very sensitive to temperature. The effect of temperature on the production and stability of the enzyme was also studied (Fig. 5 and 6). The maximum production of the enzyme was reached at 40°C. Further increase in the incubation temperature, there was gradual reduction in the enzyme formation. However, the enzyme was found to be most active between 60-70°C. The activity of enzyme was found to be insignificant at low temperature. Therefore, 40°C was found to be the best temperature for the production of alpha amylase and 65°C was found to be optimum for the activity of the enzyme.

Discussion

The highly active alpha amylase is very essential for the conversion of starches into oligosaccharides. To obtain higher thermo resistant amylase the selection of suitable strain is necessary. In the present work Bacillus licheniformis GHB8 gave maximum production of alpha amylase as compared with the other strain of Bacillus.
Suitable fermentation medium play very critical role in the production of alpha amylase. In the present work wheat bran was found to be better basal and standardized medium for the production of enzymes as compared with other agricultural by-products. It may be due to that the wheat bran contains maximum amount of starch (12%), which was very necessary for alpha amylase fermentation. Ramesh and Lonsane (1990), Babu and Stanaryana (1995) also selected the wheat bran for the best production of alpha amylase. However in the present study wheat bran was partially replaced with cottonseed meal in the ratio of 3:1 was found to be better producer of alpha amylase. It may be due to wheat bran along with cotton seed provide essential nutrients such as carbon and nitrogen sources for the production of enzyme. The workers Krishnan and Chandra (1982) also have reported that with the addition of cottonseed meal in the fermentation medium there was considerable increase in the production of alpha amylase.
Higher initial concentration of easily available carbon source was required for the maximum production of enzyme. (Terezinha and Iracema, 1994). For this purpose glucose, fructose, lactose, sucrose and starch was added in the fermentation medium. The production of the enzyme was increased with the addition of starch at 1% level. The other sugars such as glucose, fructose, and sucrose produced enzyme in the lower rate. Glucose and fructose was most effective in stimulating the growth and respiration of bacteria (Mei and Chen, 1997) because this carbon source is easily available to the microorganisms and they have inhibitory effect for the amylase formation. The higher starch concentration promoted enzyme formation because the organism used starch much slowly than other sugars for its respiratory activity. Hence it may be speculated that the slow metabolic rate favors amylase production.

The rate of alpha amylase formation by the bacterium was also investigated. The production of the enzyme reached maximum after 48 hours of inoculation. It may be due to that the organism entered in the stationary phase after 48 hours and the alpha amylase accumulation was reached maximum at that time. When the time of fermentation medium was increased the enzyme production was inhibited. It may be due to depletion of the nutrients of the fermentation medium, which resulted decrease in the enzyme formation.

The production and activity of the enzyme was greatly effected with temperature. Babu and Stanaryana (1995) have reported that 50°C was best for the production of thermostable amylase. But in the present work 40°C was found to be optimum for the biosynthesis of enzyme. The activity of the crude enzyme was found to be maximum at 65°C. Lealem and Gashe (1994) have reported that enzyme loses its 50% activity at 70°C. However, in the present work the enzyme was quite stable at 70°C. So our finding is significant than the Lealem and Gashe (1994).

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