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Alkalothermophilic Amylase from Bacillus Stearothermophilus

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A B S T R A C T

Alkalothermophilic amylase producing *Bacillus Stearothermophilus* from water sample.(velar estuary).The growth kinetics of the strain was optimized by using different substrate concentration with different pH and different temperature. With the study on starch utilization, the maximum growth rate was obtained at 1.5% .Starch was used as a carbon source .The maximum growth rate was obtained at pH 11 & the temperature at 50 ºC. The growth and substrate utilization rate in the fermentor was marginally higher than compared to those in the shake flask. In fermentor OD of *Bacillus Stearothermophilus* was found to be 2.986,biomas was 0.088g and substrate utilization was 1.27g at 24 hours time. The maximum amylase activity was obtained at pH 10 and at 100 º C and it was estimated as 1635.48 U/ml. The maximum enzyme activity was observed with Ca 2+,Hg 2+,Fe 2+ and the respective activity was found to be 2267,7 u/ml,2338.7 u/ml,2177,4 u/ml. Thus the present study revealed that this alkhapolic & thermophile is highly suitable for industrial application especially in detergent & starch based food industries.

Keywords: *Bacillus stearothermophilus*, Growth kinetics, Alkalophilic, Thermophilic Enzyme activity, Metal ions.

A R T I C L E I N F O

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1. Introduction

Number of Starch converting enzyme that are used in the production of maltodextrin, glucose and fructose syrups belong to α-amylase family which comprised approximately 30% of the world's enzymes (Pandey et al,2000).With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened to too many other fields, such as clinical, medical & analytical chemistries, in addition to the use in the textile, laundry and

detergents ,food, brewing, baking and distilling industries etc. α-amylase are having high temperature, which are critical parameters to be used in detergent and food industries. Screening of microorganisms with α-amylase activity could facilitate the discovery of novel amylase for varying applications. Marine environment with enormously varying parameters in an ideal habitat to look for such enzyme. The reason is the adaptability of source organisms to varying parameters including extreme conditions. The special living conditions force the microbes to produce a vast number of enzymes with unique activities. Hence the present study to search for an amylase enzyme producing potential strain with unique properties.

2. Experimental

Collection of Samples

The Vellar Estuary surface water samples were collected using Presterilized sample bottle allowing enough air space in the bottles to facilitate through mixing. Precautionary measures were taken to minimize the contamination.

Isolation of the Marine Amylolytic bacteria

The water sample was serially diluted and plated on starch agar medium with pH (Lin et al.,1998) and the plates were incubated at 45° c for 48hours. The plates were incubated at 45°c were prepared with 2.5% agar content to prevent melting of the medium and incubated in an incubator fitted with fan.

Figure 1: Amylase Producing Marine Bacteria

Screening for Amylase activity

Best producers were selected through screening in starch agar plates. The identification was done according to Bergey's manual of determination bacteriology (Buchanan et al., 1974)

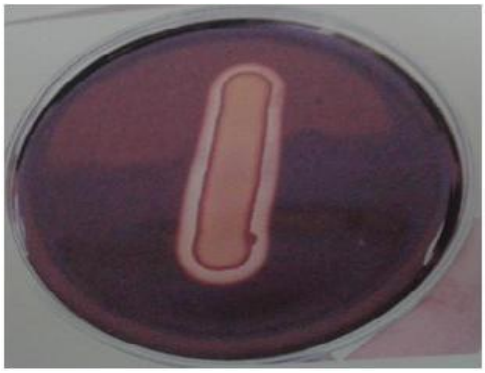

Figure 2: Primary Screening of Amylase

Estimation of α-amylase activity

1.5% starch mineral medium was selected for the production of α-amylase. To assay the crude enzyme activity the culture was harvested at the end of 48 hours from the fermentor. The cell free supernatant was separated by centrifuging at 10,000 rpm for 15minutes at 4°c. The supernatant was used to assay the crude enzyme activity by DNS method.

In a test tube 500µl of crude enzyme ,250 µl of TrisHcl buffer(pH8) were added and incubated for 10 minutes at 60°c. The reaction was stopped by adding 1ml of dinitrosalicylic acid reagent & optical density was measured at 540nm. One unit of amylase activity was defined as the amount of enzyme that catalyzed the liberation of reducing sugars equivalent to one µmol of maltose per minute under the assay conditions. The total protein content was determined by the method of Lowry et al., 1951.

Purification of α- Amylase

The cell free supernatant was saturated with 60% ammonium sulphate and allowed to stand for 24hours at 4°c.The precipitated enzyme protein was collected by centrifugation at 10,000rpm for 10minutes at 4°c.The precipitated protein was dissolved in 100 mM Tris Hcl buffer with pH 8. It was dialyzed against distilled water for 24hr and lyophilized (Stamford et al 2001). The partially purified amylase was determined by estimating the maltose liberated in µmoles /minute. When treated with 1mg of protein using 1ml of 3-5 Dinitrosalicylic acid (Miller,1959 and Lin et al.,1998). The total protein content was determined by the method Lowry et al., 1951.

Estimation of the Purified Amylase (With Different Parameter) and characterization of the enzyme

a. Effect of Ph and Temperature

The pH of the enzyme was determined by varying pH of reaction mixing using the following buffers 100mM Sodium phosphate (pH 6.0 & 7.0) ; Tris Hcl (pH 8.0 & 9.0); carbonate bicarbonate buffer (pH 10& 11). The reaction was performed at different temperature such as 40°c, 50°c, 60°c, 70°c, 80°c, 90°c, 100°c and five different pH ranges (7,8,9,10 & 11). In a test tube 500µl of amylase enzyme, 250µl of 1% starch solution and 250µl of Tris Hcl buffer (pH8) was added and incubated for 10 minutes at different temperature ranges (40°c-100°c). The reaction was stopped by adding the 1ml DNS reagent and optical density was measured at 540nm.

b. Effect on Metal Ions

The enzyme was incubated with different ions like zn^{2+}, ca^{2+}, Na^{2+}, Mg^{2+}, Fe^{2+},Cu^{2+}, &Hg^{2+} at a concentration of 1mM in 20µl assay buffer (pH 10). Incubation temperature kept at 100°c and the activity was assayed by using the DNS method.

3. Results and Discussion

The maximum amylase activity was obtained at pH 10 and 100°c and it was estimated as 1635.48U/ml (fig.3). Relatively better amylase activity was also obtained in pH 11 at 90°c(1522.5U/ml). At pH 11 when the temperature

was increased to 100°c enzyme activity was reduced to 1393.5 U/ml (fig 4) ie the optimum production of amylase was found to be pH 10 and 100°c.

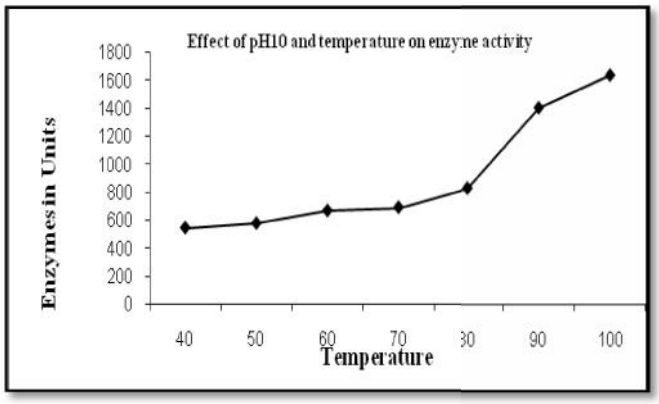

Figure 1: Effect of pH10 and temperature on enzyme activity

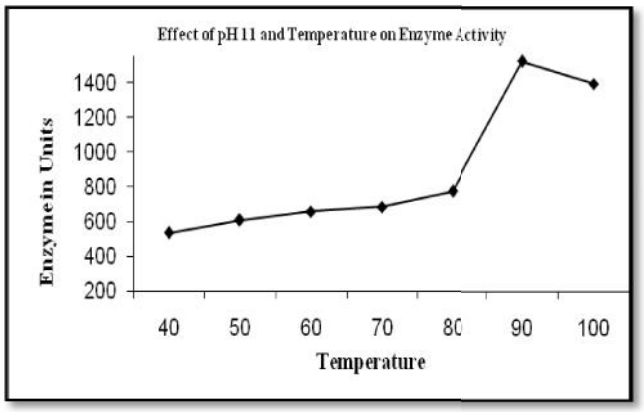

Figure 2: Effect of pH 11 and Temperature on Enzyme Activity

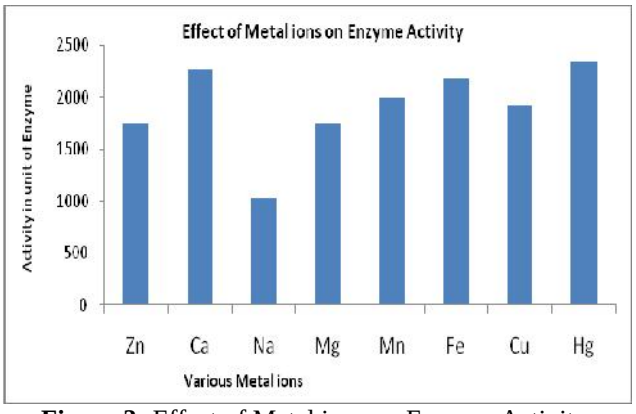

Figure 3: Effect of Metal ions on Enzyme Activity

With metal ions interesting result were obtained. The maximum enzyme activity was observed with ca^{2+}, Hg^{2+}, Fe^{2+} ,and the respective activity was found to be 2267.7 U/ml,2338.7U/ml,2177.4U/ml (fig 5).The binding of ca^{2+} ions had been shown to increase the α helical structure of α-amylase in *B.amyloliquefaciens* α-amylase, leading to increased stability (Kim et al,1991). Most of the amylases are known to be metal ion dependent enzymes (Pandey et al., 2000).

Thus the present study revealed that this alkalophilic & thermophilic strain is highly suitable for industrial applications especially in detergent & starch based food industries.

4. Conclusion

As there is a great industrial demand for the alkalothermophilis α amylase exists, it prompted the present study to hunt a promising strain with the desired nature from vellar estuary. The srrain screened was identified as Bacillus stearothermophilus. Maximum growth of the strain flask was obtained at 1.5% starch, at pH11 and at 50°c. Marginal increase in biomass and substrate utilization was noted in fermentor study. When the enzyme was partially purified it showed a highest activity of 1635.48 Unit/ml at pH 10 and at 100°c. When metal ions were used, enzyme activity increased to 2267.7U/ml,2338.4U/ml and 2177.4U/ml respectively with divalent ions like ca^{2+}, Hg^{2+} and Fe^{2+}. Thus the present study revealed that this alkalophilic and thermophilic strain is highly suitable for industrial applications especially in detergent and starch based food industries.

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