Studies On Free And Immobilised Cells Of Bacillus Species On The Production Of Alpha-Amylase

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Abstract

Cells of Bacillus species. were immobilized by entrapment in sodium alginate for the production of alpha amylase. Cells growth rate was reduced when the cells were immobilized as compared to free cells. Reduced growth rate of immobilized cells may be attributed to the mass transfer limitation of oxygen. The average percentage of cell growth was reduced of that with free cells. Maximum concentration of alpha amylase was obtained at 48 hours by the isolates DPT 1 and DPT 2 followed by a decline. Immobilized cells did not show any appreciable alpha amylase production. The alpha amylase of Bacillus species had the optimum pH at 6.5 – 7.5 and temperature optima at 45 C. The maximum activity of alpha amylase is shown in substrate concentration of 0.1% to 1.0%. Finally the enzyme was characterized by chromatography and confirmed as alpha amylase.

INTRODUCTION

Alpha amylase from different sources have been purified and many have been crystallized of them, some producing saccharifying and some produce liquefying alpha amylases. Alpha amylase variety of micro organisms including many species of Bacillus like B.subtilis, B.licheniformis, B.amyloliquefaciens etc and molds like, Aspergillus niger, A.oryzae, has shown to be effective in production of alpha amylases.

The enzyme from Bacillus sp. has been isolated and immobilized to produce alpha amylases. Immobilization of Bacillus sp. cells in sodium alginate has been used for the production of alpha amylases. The production of alpha amylases by the immobilized cells was compared with that of free cells. The word reported here deals with the production, and characterization of alpha amylase and its effect of pH on production medium, temperature and substrate concentration on the alpha amylase activity.

MATERIALS AND METHODS

ORGANISMS AND GROWTH CONDITIONS

The soil was suspended in sterile saline and mixed well. Serial dilution of this suspension were made and 0.1ml of each dilution were plated first on nutrient agar plates for colony development .The individual colonies appeared were plated on starch agar plate by middle streak. After 24 hours of incubation bacterial colonies showing clear zone, when the plates were flooded with iodine solution around them were considered to produce extra cellular enzyme.

Using procedures given in Bergy"s manual of determinative bacteriology the partial identification of bacteria was performed. (Sneath et al., 1986)

PREPARATION OF IMMOBILIZED CELLS USING CALCIUM ALGINATE

Equal volume (50ml) of sodium alginate solution and 1ml of cell slurry was gently mixed together. The mixture was extracted drop wise, through a 10ml syringe, from a height of about 2°Cm into a excess of 0.2M calcium chloride solution in a suitable volume for 100ml of mixture. The beads of calcium alginate entrapped cells were left under incubation in the calcium chloride solution, for about 20min.The preparation of control for immobilized cells was also carried out.

CULTURE MEDIUM AND CONDITION

The enzyme production medium contained ammonium dihydrogen phosphate (0.02), manganese chloride (0.05), soluble starch (0.2), beef extract (0.02), distilled water (100ml). The pH of the medium is adjusted to 6.5.

The cells were entrapped by calcium alginate method. Two sets of production medium were prepared. To one of it entrapped gel beads were added. To the second unimmobilized cells were added control was set up by the

medium containing calcium alginate without cells. After incubation the enzyme was assayed at different pH, at different temperature, at different time interval and also with different substrate concentrations.

AMYLASE ACTIVITY AT VARIOUS INTERVALS DURING GROWTH.

0.1ml of 24 hours old culture was inoculated into 50ml of production media and incubated in the incubator at 37°C.At regular intervals, 1.5ml of the culture were removed for amylase assay.

The effect of pH of the medium on the alpha amylase production is observed by inoculating 0.1ml of 24 hours old culture into 50ml of production media of various pH range 6.5 – 8.5 and incubated at 37°C .At regular intervals 1.5ml of culture was with drawn and used for alpha amylase activity.

The effect of temperature was done by incubating the centrifuged culture supernatant of both immobilized and immobilized cells at various temperatures like 29°C, 37°C, 55°C and 70°C for 30 minutes. After incubation this crude enzyme is assayed.

The effect of substrate is done by inoculating 0.1ml of immobilized and unimmobilized cells of 24 hours old culture into 50ml of production medium of various substrate concentration via 0.1 – 1.0. It is incubated at 37C. At regular intervals 1.5ml of culture was withdrawn and used for alpha amylase assay.

The characterization of the type of amylase is performed by thin layer chromatography

RESULTS AND DISCUSSION

SCREENING OF AMYLOLYTIC ORGANISMS

Samples collected were screened for amylase producing micro organisms on starch agar plates with clear zone was seen after flooding the plate with iodine. The samples were streaked on nutrient agar plates.

CHARACTERISTICS OF ISOLATES

These two isolates are identified by Bergey"s manual of systemic bacteriology (Sneath,et.al, 1986). They were identified as genus Bacillus. Further investigation is essential to identify these at species level. Emanuilova, et al., (1984), reported that the Bacillus sp. Produce the acidic and alkaline amylase.

KINETICS OF ALPHA AMYLASE PRODUCTION

The enzyme production by the two isolates was studied. The values are tabulated in table after 48 hours. Chabb, et.al. (1997) reports that the alpha amylase is maximum near 48hours.

Figure 1

Table 1: Alpha amylase production from Bacillus isolates

Bacillus Isolate	Alpha amylase activity µ moles / ml/min	
	Unimmobized	Immobilized
DPT 1	2.12	2.26
DPT 2	1.98	2.08

The effect of pH.

The isolates grown at pH 6.5 and 7.5 at 24hours and 72hours were found to produce enzyme, but it was less when compared to 48hours of incubation. Even at 24 hours and 48 hours with increase in pH 8.5, there was decrease in enzyme production.

Figure 2

Table 2: Effect of pH of production medium on alpha amylase activity after 24 hours.

Bacillus Isolate	Alpha amylase activity µ moles /ml/min					
	pH values of unimmobilized			pH values of immobilized		
	6.5	7.5	8.5	6.5	7.5	8.5
DPT 1	1.80	1.91	1.43	1.77	1.89	1.53
DPT 2	1.66	1.83	1.40	1.81	1.97	1.51

EFFECT OF TEMPERATURE

The isolates showed maximum enzyme activity at 45°C, whereas nearly 30% of activity was decreased on increase of temperature at 55°C.

Figure 3

Table 3: Effect of temperature on alpha amylase production

Bacillus isolate	Alpha amylase activity µ moles /ml/min							
	Unimmobilized temperature				Immobilized temperature			
	27ºC	37ºC	45º C	55ºC	27ºC	37º C	45ºC	55º C
DPT 1	1.34	1.66	2.22	1.80	1.57	1.89	2.44	1.75
DPT 2	1.29	1.61	2.17	1.66	1.52	1.80	2.35	1.80

THE EFFECT OF SUBSTRATE CONCENTRATION.

The substrate concentration has major effect on amylase activity. The isolates showed increased activity from 0.1% to 1.0% at 48 hours of incubation. But during 24 hours and 72 hours of incubation there was decreased enzyme activity when compared to 48 hours of incubation.

Figure 4

Table 4: Effect of substrate concentration on alpha amylase production.

Bacillus isolate	Alpha amylase activity µ mole/ml/min							
	Substrate concentration (%)				Substrate concentration (%)			
	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8
DPT 1	1.06	1.29	1.43	1.61	1.24	1.52	1.71	1.85
DPT 2	1.01	1.20	1.38	1.56	1.20	1.43	1.61	1.80

CHARACTERIZATION OF AMYLASES

The enzyme separated from Bacillus species were separated in thin layer chromatography. The Bacillus sp. produced maltose and maltotriose.

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