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EFFECT OF DIFFERENT HEAVY METALS AND PH ON A-AMYLASE PRODUCTION FROM BACILLUS AMYLOLIQUEFACIENS

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ABSTRACT

Microbial enzymes are used in the many industries like textile industries, food industries, pharmaceutical industries, paper industries etc. Alpha- amylase is one of these enzymes. The effects of different parameters like different pH and heavy metals concentration on alpha-amylase production by Submerged fermentation were examined. The present investigation concerned with the production of α-amylase by *Bacillus amyloliquefaciens.* The fermentation was carried out in by continuous shaking containing 50 ml of medium in 250 ml flask. The incubation time was 72 h; incubation temperature (37°C) and size of inoculums (0.05ml) were also optimized. The optimum pH for production of α-amylase was detected at pH 7.5. Effects of different heavy metals were noted and it was found that production of α-amylase was strongly inhibited by Cu^{2+} but less affected by Mg^{2+}, Fe^{2+}, and Mn^{2+}at higher concentrations.

KEYWORDS

α-amylase, *Bacillus amyloliquefaciens,* Heavy Metals, pH, Submerged fermentation.

INTRODUCTION

α-amylase hydrolyses the internal α-1, 4 linkages in starch and related substrates in an endo- fashion producing oligosaccharides including maltodextrins, maltose, and glucose[1]. This enzyme is extensively used in starch liquefaction, brewing, food, paper, textile and pharmaceutical industries [2-6]. The production of microbial alpha amylase by bacteria dependent on the type of strain, composition of medium, methods of cultivation, cell growth, nutrient requirement, metal ions, pH, temperature, time of incubation and thermostability[7]. Microorganisms like fungi and bacteria have been extensively screened for α-amylase production[8]. Almost all microorganisms of the *Bacillus genus* synthesized alpha amylase. This genus has the potential to dominate the enzyme industry[9].The industrially important Bacillus strains, which are extensively used to produce alpha amylase are *B. amyloliquefaciens*, *B. licheniformis*[10], *B. stearothermophilus*[11]. But the *Bacillus* species such as *B. subtilis*, *B. licheniformis* and *B. sterothermophilic* can be used for the better production of α-amylase in shake flask[12]. The amylolytic bacterial cultures normally grow at pH ranging from 4.5 to 10.5 while enzyme activity remains optimal at 5.5 to 8.0[13]. Heavy metals also affect the production of α-amylase. It was strongly inhibited by Co2+, Cu2+, and Hg2+ but less affected by Mg2+, Zn2+, Ni2+, Fe2+, and Mn2+ [14].

MATERIALS AND METHODS

Bacterial Strain:

Pure culture of the bacteria *Bacillus amyloliquefaciens* obtained from Institute of Microbial Technology, Chandigarh.

Inoculum Preparation

The pure bacterial culture was raised on amylase production medium. The medium was composed of (g/l): 1.0 Starch, 6.0 Peptone, 0.5 $MgSo_4$ and 0.5 KCl. The pH 7.0 of the medium was adjusted with 1N NaOH and was autoclaved at 121°C for 15 minutes. After inoculation it was then incubated at 37°C for 72 hours and was used as inoculum.

Substrate

Banana Peel was used as substrate for amylase production. It was obtained from fruit market and chopped into small pieces of uniform sizes.

Culture cultivation

The enzyme was produced at larger scale for extraction under continuous shaking conditions (120 rpm) at optimized conditions of temperature, medium pH, incubation time and substrate i.e. Banana Peel.

Enzyme Extraction

After optimum incubation period the experimental flask was harvested. The fermented biomass sample was filtered and centrifuged at 5000 rpm for 15minutes at 10°C temperature in the centrifuge to remove the spores of the organism. The supernatant was carefully collected and the crude enzyme, thus obtained, was subjected to enzyme assay.

Enzyme assay

0.5 ml of appropriately diluted enzyme solution was incubated for 15 minutes with 0.5 ml of mixture of 1% starch (substrate) and 1x PBS. The reaction was terminated by adding 1 ml of

DNS reagent and the mixture was boiled for 15 minutes in water bath and diluted with 8 ml water. The absorbance was read at 540 nm. This absorbance was translated by plotting against standard curve to get µmoles of maltose to calculate units of enzyme activity. One unit of enzyme activity is defined as the amount of maltose (µ moles) released per ml of enzyme solution per minute.

$$\text{Enzyme Activity (U/ml)} = \frac{\mu \text{ moles of maltose released}}{\text{ml. of enzyme used x incubation time (min.)}}$$

Where, U is enzyme unit in µ moles/min.

RESULTS AND DISCUSSION

Effect of different pH:

Effect of different pH (4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9) on alpha amylase production by the strain of *Bacillus amyloliquefaciens* was investigated. Fig. 1& Table 1 show the effect of pH of reaction mixture for the activity of alpha amylase. The enzyme activity is extremely low at pH 4.0. pH 4.0-8.0 is more suitable for alpha amylase activity and maximum at pH 7.5. Further increase in the pH resulted decrease in the activity of alpha amylase. Thus, pH was found to be optimum at 7.5. Similar results were also observed by [15] in *Bacillus spp.* The hydrolysis of starch by α-amylase is highly affected by pH. In present investigation, the different pH (4.0-9.0) was tested for the activity of α-amylase. The maximum activity of α-amylase was found at slightly alkaline pH 7.5 while at acidic pH activity of α-amylase was extremely low. It might be due to the α-amylase was inactive in the acidic medium [16].

***Effect of different heavy metals*:**

The concentration of heavy metals affects the production of α-amylase from *Bacillus spp*. Fig. 2 & Table 2 show the effect of heavy metals for the activity of alpha amylase. Effect of heavy metals on α-amylase production investigated and it was reported that production of α-amylase was strongly inhibited at 0.4 g/l concentration by Cu^{2+} but at 0.4 g/l concentration of Mg^{2+}, Fe^{2+}, and Mn^{2+} were less affected. Similar results were also observed in *Bacillus spp*[14, 17]. It has been reported that the synthesis of carbohydrate degrading enzymes in most species of the genus *Bacillus* is subjected to catabolic repression by readily metabolisable substrates[18].

aTable 1.

Effect of different pH of the medium on production of ά -amylase

S.No.	pH	OD at 540 nm
1.	Control	0.001
2.	4	0.010
3.	4.5	0.012
4.	5	0.021
5.	5.5	0.036
6.	6	0.034
7.	6.5	0.061
8.	7	0.058
9.	7.5	0.066
10.	8	0.032
11.	8.5	0.021
12.	9	0.017

Table 2.

Effect of different heavy metals on the production of α-amylase (conc. 0.4 g/l):

Conc. In g/l	Heavy Metals	OD at 540 nm
0.4	Control	0.001
0.4	$MgSO_4$	0.018
0.4	$MnSO_4$	0.052
0.4	$CuSO_4$	0.009
0.4	$FeSO_4$	0.218

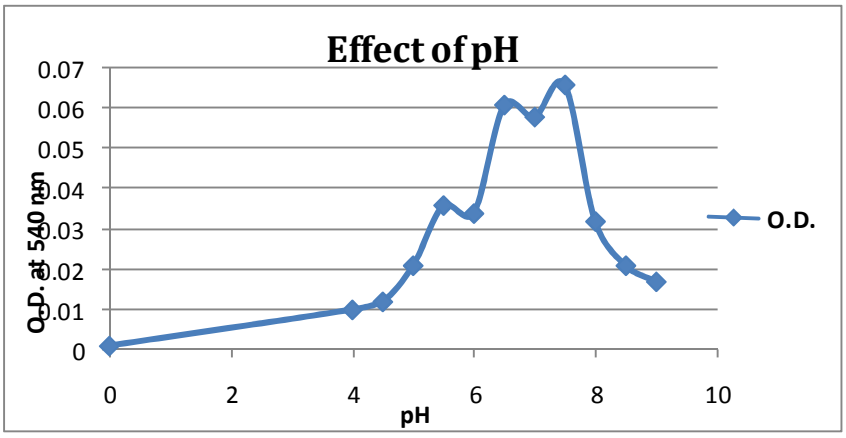

Figure.1:

Effect of different pH on alpha-amylase production

Graph represented that pH 7.5 is optimum for alpha-amylase production from B.amyloliquefaciens

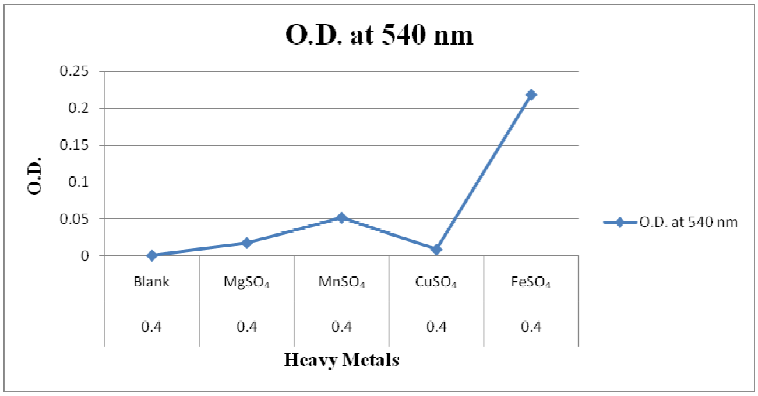

Figure. 2:
Effect of Heavy Metals on the production of alpha-amylase

Graph represented that $CuSO_4$ inhibit amylase production while other are less affected amylase production

REFERENCES

1. Calik P and T H Ozdamar, Carbon sources affect metabolic capacities of Bacillus species for the production of industrial enzymes. Biochem. Eng. J, 8:61-81, (2001).
2. Rasias I A and B H Rehm, One-step production of immobilized alpha-amylase in recombinant *Escherichia coli*. Appl. Environ. Microbiol, 75(7): 2012-6, (2009) .
3. Rajagopalan G and C Krishnan, Alpha-amylase production from catabolite depressed *Bacillus subtilis* KCC103 utilizing sugarcane bagasse hydrolysate. Biores. Technol, 99:3044-3050, (2008).
4. Gangadharan D, K M Nampoothiri, S Sivaramakrishnan and A Pandey, Biochemical characterization of Raw-starch-digesting alpha-amylase purified from *Bacillus amyloliquefaciens.* Appl. Biochem. Biotechnol, DOI 10.1007/s12010-008-8347-4, (2006).
5. Arikan B, Highly thermostable, alkaline, SDS and chelator resistant amylase from a thermophilic *Bacillus sp*. isolate A3-15. Bioresource Technology, 99:3071-3076, (2008).
6. Thippeswami S, K Girigowda and V H Mulimani, Isolation and identification of alpha amylse producing Bacillus sp., from dhal industry waste. Ind. J. Biochem. Biophys, 43:295-298, (2006).
7. Prescott S and A Dunn's, Industrial Microbiology, 4th Edn, CBS Publishers and Distributors, New Delhi, India: 550-565, (1987).
8. Ivanova V, D Yancov, L Kabaivanova and D Pashkoulov,. Simultaneous biosynthesis and purification of two extracellular Bacillus hydrolases in aqueous two α-amylase. Biochem. Eng. J, 8:61-81, (2001).
9. Pretorius I S, de Kock M J, Britz H J, Potgieter H J and Lategen P M, Numerical taxonomy of α-amylase producing *Bacillus species*. J.Appl. Bacteriol, 60:351-360, (1986).
10. Fogarty W M and Kelly C T, Amylase, amyloglucosidase and related glucanases. Rose A.H. Economic Microbiology, Microbial enzymes and Bioconversion. Academic Press Inc, New York. 5:115-170, (1980).
11. Wind R D, Buitelaar R M G, Huizing H J and Dijkhuizen L, Appl. Microbiol. Biotechnol. 41:155-162, (1994).
12. Mamo G, B A Gashe and A Gessesse, A highly thermostable amylase from a newly isolated thermophilic Bacillus sp. WN11. J. Appl. Microbiol. 86:557-560, (1999).

13. Goyal N, G S Sindhu, S T Chakraburti and J K Gupta, Thermostability of alpha-amylase produced by *Bacillus species*., E2-a thermophilic mutant. J. Micobiol. Biotechnol., 314:251-257, (1995).
14. Asgher M, M J Asad, S U Rahman and R L Legge, A thermostable amylase from a moderately thermophilic Bacillus subtilis strain for starch processing. J. Food Eng, 79:950-955, (2007).
15. N Riaz, Ikram-ul-haq and M A Qadeer, Characterization of α-amylase by *Bacillus subtilis*., 03:249-252, (2003).
16. Castro G R, M A Ferrero, B S Mendez and F Sineriz, Screening and selection of bacteria with high amylolytic activity. Acta Biotechnol., 13:197-201, (1993).
17. Farouk H Akel and R Natshi, Characteristics of a Novel Highly Thermostable and Extremely ThermophilicAlkalitolerant Amylase from Hyperthermophilic *Bacillus* Strain HUTBS71, J. Biologi. Scien, 9 (3): 67-74, (2009).
18. Babu K and Satyanarayan T, process biochem, 30: 305-309, (1995).