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EFFECT OF DIFFERENT CARBON AND NITROGEN SOURCES ON α- AMYLASE PRODUCTION FROM *BACILLUS AMYLOLIQUEFACIENS*

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ABSTRACT

Amylases are among the most important enzymes in present-day biotechnology. The effects of various carbon and nitrogen sources on α-amylase production by submerged fermentation (smF) from *Bacillus amyloliquefaciens* was investigated. The study revealed that the levels of the α- amylase production varied greatly with the type and concentration of carbon and nitrogen sources used. The highest α-amylase production was obtained in medium supplemented with lactose at 5g/l concentration. The other carbon sources like sucrose and mannitol also favored the α-amylase production. Maximum enzyme production was found with Ammonium chloride (NH_4Cl) as the nitrogen source at 6g/l concentration. Yeast extract and peptone also seems to be suitable for the production of α-amylase from *Bacillus amyloliquefaciens*.

INTRODUCTION: *Bacillus sp*. are considered to be the most important sources of amylase and have been used for its production [1, 2]. *Bacillus amyloliquefaciens* is responsible for much of the world production of α-amylase and protease. The Starch degrading amylolytic enzymes have a great commercial value in biotechnological applications ranging from food, fermentation, textile to paper industries [3, 4].

Although Amylases can be derived from several sources such as plants, animals and microbes, however, the microbial amylases meet industrial demands and have almost completely replaced chemical hydrolysis of starch in starch processing industries [4]. Both submerged fermentation (SmF) and solid state fermentation (SSF) could be used for the production of amylase, although traditionally have been obtained from submerged cultures [5].

Many researchers have studied amylase production with a wide variety of substrates and microorganisms like bacteria, yeast and fungus. Due to the ever increasing demand of this enzyme, people are still trying to increase the productivity of amylase by a variety of approaches like selection of a high enzyme producing strain, process optimization, usage of cheaper substrates, effective downstream processing. The enzymes of amylase family have great significance due to its wide area of potential application [6]. *Bacillus amyloliquefaciens* mostly produce carbon dioxide, butanediol, acetate and a small amount of lactate [7].

MATERIAL AND METHODS:

Bacterial Strain: Pure culture of the bacteria *Bacillus amyloliquefaciens* (MTCC-1488) obtained from the Institute of Microbial Technology, Chandigarh, India, was used for fermentation purpose.

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 1xPBS. 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.

RESULT AND DISCUSSION: Application of carbon and nitrogen sources at different concentration enhanced the α-amylase production from *Bacillus amyloliquefaciens.* Different fermentation parameters were optimized for α-amylase production by conducting a series of experiments. The study revealed that the levels of the α- amylase production varied greatly with the type and concentration of carbon and nitrogen sources used.

Effect of Carbon source (Sugars) on α-amylase production: To investigate the effects of various carbon sources on α-amylase production**,** *Bacillus amyloliquefaciens* strain was grown in different media containing lactose, sucrose and mannitol as carbon sources. The highest α-amylase production was obtained in medium supplemented with lactose at 5g/l concentration. The other carbon sources like sucrose and mannitol also favored the α-amylase production. The mannitol showed high production of α-amylase at 3 g/l (**Fig. 1 and Table 1**).

Kelly *et al.,* (1997) [8] reported in case of *B. flavothermus* the highest amylase activity with maximum biomass was obtained when lactose was used as carbon source. Similar results were also observed by [1, 6] in *Bacillus spp.* 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 [3].

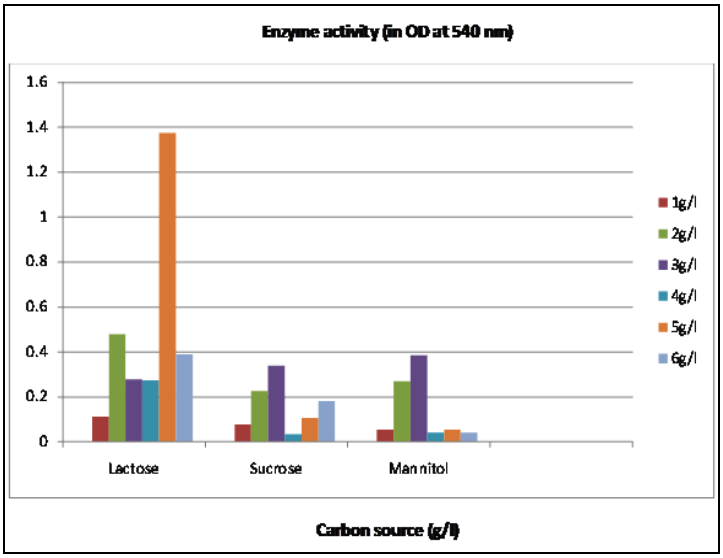

FIG. 1: EFFECT OF DIFFERENT CARBON SOURCES AT VARIOUS CONCENTRATIONS ON ENZYME PRODUCTION

TABLE 1: EFFECT OF DIFFERENT CARBON SOURCES (SUGARS) AND NITROGEN SOURCES AT VARIOUS CONCENTRATION ON α-AMYLASE SYNTHESIS FROM *BACILLUS AMYLOLIQUEFACIENS*

Sugars	Conc. (g/l)	OD at 540nm	Nitrogen sources	Con. (g/l)	OD at 540nm
Control	-	0.003	Control	-	0.003
Lactose	1	0.113	Ammonium chloride	1	0.057
	2	0.479		2	0.063
	3	0.278		3	0.164
	4	0.275		4	0.363
	5	1.376		5	0.153
	6	0.390		6	0.553
Sucrose	1	0.077	Peptone	1	0.037
	2	0.227		2	0.056
	3	0.339		3	0.080
	4	0.035		4	0.135
	5	0.107		5	0.048
	6	0.182		6	0.034
Mannitol	1	0.056	Yeast extract	1	0.034
	2	0.271		2	0.010
	3	0.385		3	0.024
	4	0.042		4	0.176
	5	0.055		5	0.058
	6	0.042		6	0.089

Effect of Nitrogen source on α-Amylase production: Three nitrogen sources (ammonium chloride, peptone and yeast extract) were applied at 1, 2, 3, 4, 5 & 6 g/l concentration for the production of α-amylase from *Bacillus amyloliquefaciens.* The study revealed that at the concentration of 1, 2, 3, 4, 5 & 6 g/l of 3 nitrogen sources (NH_4Cl, peptone and yeast extract) maximum α-amylase production was obtained with NH_4Cl at 6g/l concentration (**Fig. 2** & Table 1). However, peptone and yeast extract showed good production of α-amylase compared with control conditions. Above study indicated that NH_4Cl is the best nitrogen source for maximum production of α-amylase from *Bacillus amyloliquefaciens.*

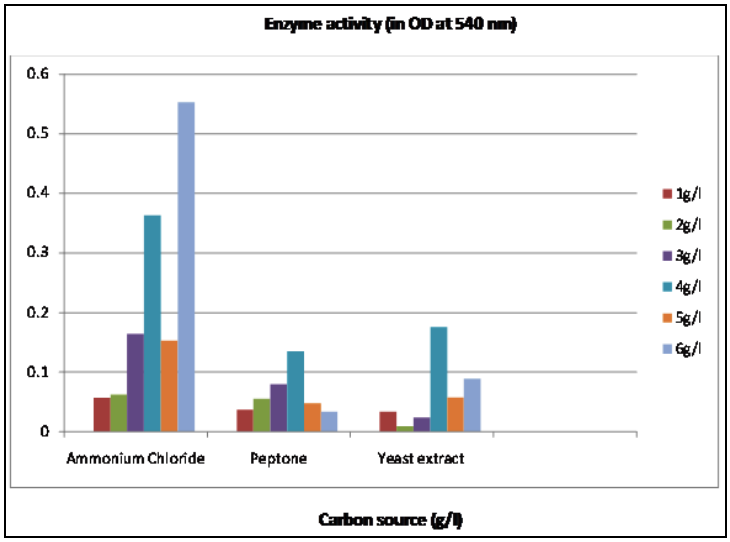

FIG. 2: EFFECT OF DIFFERENT NITROGEN SOURCES AT VARIOUS CONCENTRATIONS ON ENZYME PRODUCTION

Magee RJ and N Kosaric [9] also observed the ammonium chloride as the best nitrogen source for the production of α -amylase from *B. amyloliquefaciens.* α-amylase yield was high in media containing ammonium chloride as sole nitrogen source. Peptone and yeast extract also served as good substrate for enzyme synthesis. The nature and relative concentration of nitrogen sources are important in the formation of α- amylase. Lower levels of nitrogen are inadequate for the enzyme production and excess nitrogen is equally detrimental causing enzyme inhibition.

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