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Short Communication

Effect of different concentrations of metal ions on alpha amylase production by *Bacillus amyloliquefaciens*

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Bacillus amyloliquefaciens **is obtained from soil which produces extracellular alpha amylase enzyme. The present study is concerned with effect of metal ions on alpha amylase production. Metal ions are Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} at different concentrations 2g/l, 5g/l and 7g/l. Supplementations of salts of certain ions provide good growth of microorganism and production of alpha amylase. $Ca^{2+}$and Mg^{2+}exhibit positive influence on alpha-amylase production. Our results show that the amylase production is higher in the presence of Ca^{2+} (0.439) IU/ml/min at 7g/l concentration in comparison of other metal ions. The enzyme activity of Mg^{2+}(0.321) IU/ml/min at 2g/l concentration. The study focuses on supplementation of metal ions increase the production of amylase.**

Keywords: *Bacillus amyloliquefaciens,* metal ions, amylase, enzyme activity

Alpha-Amylases (E.C. 3.2.1.1.) are starch-degrading enzymes that catalyze the hydrolysis of internal 1, 4-*O*-glycosidic bonds in polysaccharides with the retention of alpha-anomeric configuration in the products. Many enzymes require metal ions for maximal activity. If the enzyme binds the metal very tightly or requires the metal ion to maintain its stable, native state, it is referred to as a metalloenzyme. Enzymes that bind metal ions more weakly, perhaps only during the catalytic cycle, are referred to as metal activated. One role for metals in metal-activated enzymes and metalloenzymes is to act as electrophilic catalysts, stabilizing the increased electron density or negative charge that can develop during reactions. Most of the alpha-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes (Bordbar et al., 2005). Alpha amylase is a glycoprotein. Its single polypeptide chain of about 475 residues has SH group and 4 disulphide bridges and contains a tightly bound Ca^{2+}. It exists in two forms (I &II) which have identical enzymatic properties, differing only in electrophoretic mobility. Ca^{2+} ions are reported to be present in majority of these enzymes. Addition of $CaCl_2$ to the fermentation media increased the enzyme production (Patel et al, 2005). Metal ions can be considered as good examples, different metals exhibiting different behaviours in their ability to act as effectors

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(Li et al., 2005).In previous reports, most amylases activity were inhibited in the presence of Ag+, Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Fe^{2+}, Ni^{2+}, Mn^{2+} and Zn^{2+} (Pandey et al., 2000; Gupta et al., 2003; Sun et al., 2010).The enzyme activity of alpha amylase from *P. citrinum* HBF62 was strongly activated by Mn^{2+}, Ca^{2+}, Co^{2+}, Fe^{3+} and Ba^{2+}, however, the enzyme was slightly stimulated by NH^{4+} and Al^{3+}. The HBF62 amylase was poorly affected by the other metal ions tested since the relative activity was higher than 85%, but partially inhibited by Hg^{2+} (29%). The effects of metal ions have been well studied on several alpha amylases from fungi and yeast (Metin et al., 2010).

The aim of study is to investigate, effect of metal ions on amylase production at different concentration.

Material & Methods:

Selection of Microorganism

Bacillus amyloliquefaciens were obtained from the culture collection of Clonegen Biotech, Noida.

Testing for amylase activity (Starch Hydrolysis)

Bacteria were inoculated on Amylase assay media as a spot in centre of plate and incubated over night. After that plates were tested for the presence of amylase enzyme on the starch agar plates as follows: flood plates with iodine test solution and observe color. If starch is present, the iodine will react to form blue-black color. If amylase is present in the extract, it breaks down or digests the starch and clear areas will appear in the agar (Krishna et al., 2011).

Fermentation medium

The inoculum was prepared by inoculating the loopful of strain in to nutrient broth and it was incubated in shaker for 24 hrs. 100µl of this 24 hr old inoculum was transferred aseptically to 100 ml production medium (g/l) containing: Bacteriological peptone-6g /L, $MgSO_4.7H_2O$-0.5g/L, KCl-0.5g/L, Starch-1g/L, distilled water 1000 ml and incubated in shaker for 48 hrs.

Extraction of enzyme

Two ml of production media culture was transferred into centrifuge tubes and spinned for 20 minutes at 5000 rpm. After 20 minutes, the supernatant portion was decanted, which is the crude enzyme extract.

Estimation of enzyme

The DNS method used involved estimating the amount of reducing sugar produced (Miller 1972), using 1% soluble starch as substrate. The reaction mixtures consist of 0.5 ml of substrate solution (1% soluble starch in 0.05 M phosphate buffer, pH 6.9) and 0.5 ml of the cell free extract. The reaction mixture was incubated for 3 mins at 30 degree C. The reaction was terminated by the addition of 1 ml of dinitrosalicylic (DNSA) reagent (Miller et al., 1972). The mixture was heated at 100 degree C for 5 mins and cooled. The optical density was read at 540 nm in spectrophotometer. Maltose was used as standard. One unit of enzyme activity was defined as the amount of enzyme that formed 1 mg of reducing sugar in 1 min.

Amount of reducing sugar = Absorbance at 540 / Slope of maltose standard

$$\text{Enzyme activity (IU/ml/min)} = \frac{\text{Amount of reducing sugar} \times 1000}{\text{Molecular weight of maltose} \times \text{time}}$$

Effect of metal ions on amylase

Medium of different concentration (2g/l, 5g/l &7g/l) was prepared. The effect of salts ($CaCl_2$, $CuSO_4$, $MgSO_4$, $FeCl_2$ & $MnCl_2$) on enzyme activity was determined. The estimation of enzyme was determined in the presence of 1% soluble starch as substrate. The relative enzyme activity was measured under standard assay conditions.

Result & Discussion:

Effect of metal ions on amylase activity

Metal ions Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+} at different concentration were tested. Mg^{2+} showed amylase activity 0.321 IU/ml/min at 2g/l concentration and then a gradual decline with increasing concentrations. Ca^{2+} was found 0.412 IU/ml/min activity of amylase at 2g/l concentration, 0.418 IU/ml/min at 5 g/l conc., and 0.439 IU/ml at 7g/l concentration in medium but Cu^{2+}, Fe^{2+} & Mn^{2+} exhibited less activity than Mg^{2+} and Ca^{2+}. The optical density of metal ions showed in (Fig. 1). At the concentration of 2g/l, Ca^{2+} and Mg^{2+} showed better activity but rest of three ions gave less activity. Increasing in concentration Mg^{2+}, the enzyme activity decreased but Ca^{2+} showed gradually increased activity at different concentrations. The activity of Cu^{2+}, Fe^{2+} & Mn^{2+} inhibited with increasing the concentration. The results in Fig. 3 showed that activity of metal ions in (IU/ml/min).The effects of metal ions have been well studied on several amylases from fungi and yeast. Amylase is a metalloenzyme which contains at least one activating Ca^{2+} ion. The affinity of Ca^{2+} to amylase is much stronger than that of other ions (Gupta et al., 2003). Enhancement of amylase activity such as Mn^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+} and Ba^{2+} ions could be based on its ability to interact with negatively charged amino acid residues such as aspartic and glutamic acid (Linden et al., 2003).

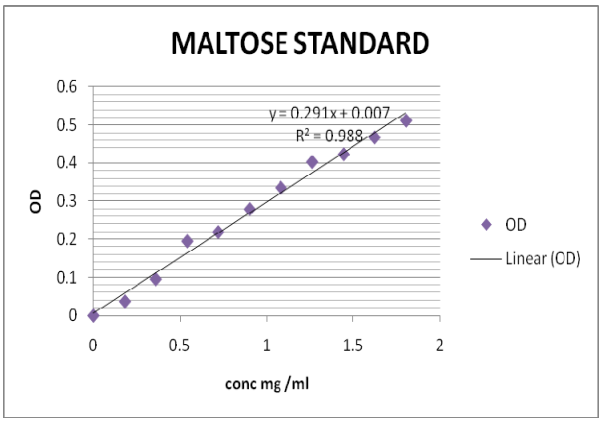

Figure 1. Standard graph for DNS Assay

Table 2. Optical density of metal ions at different concentrations

Metal ions	OD at different conc. of metal ions		
	2g/l	5g/l	7g/l
Ca^{2+}	0.400	0.405	0.426
Cu^{2+}	0.102	0.087	0.099
Mg^{2+}	0.312	0.210	0.153
Fe^{2+}	0.221	0.100	0.123
Mn^{2+}	0.286	0.101	0.106

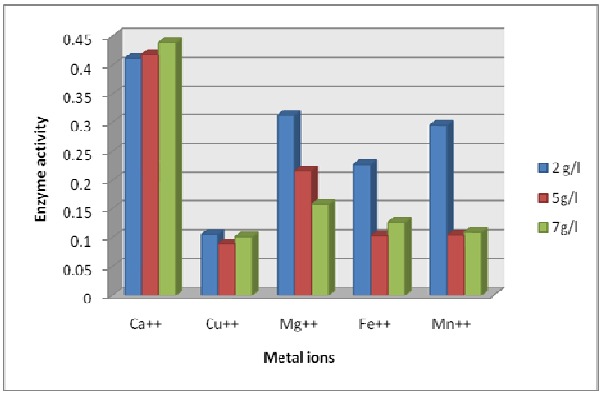

Figure 2. Amylase activity of metal ions at different concentration

Most of amylases are known to be metal ion-dependent enzymes, namely divalent ions like Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, *etc*. was reported to increase a-amylase activity of an alkaliphilic *Bacillus* sp. ANT-6 (Pandey et al., 2000; Burhan et al., 2003). The stabilizing effect of Ca^{2+} on thermo stability of the enzyme can be explained due to the salting out of hydrophobic residues by Ca^{2+} in the protein, thus, causing the adoption of a compact structure (Goyal et al., 2005). Concerning the effect of some ions on the specific activity of purified alpha amylase from *Penicillum olsonii*, the result demonstrated that Mn^{2+} (1 mM) and Mg^{2+} (1 mM) enhanced the enzyme activity by values range between 23.0 and 12.0%. Less stimulation was occurred in presence of Ca^{2+} (1 mM). It was found that metal ions may stimulate the enzyme activity by acting as a binding link between enzyme and substrate combining with both and so holding the substrate and the active site of the enzyme. The results are also showed that $Co^{2+,}$ Zn^{2+}, Fe^{3+}, Na^{2+}, and K^{+} couldn't affect the enzyme activity at all (Afifi et al., 2008).

Conclusion:

In the present study an attempt has been made by amylase producing strain *Bacillus amyloliquefaciens* and studied the impact of various metal ions on enzyme activity at different concentrations. Ca^{2+} and Mg^{2+} exhibited better activity while Cu^{2+}, Fe^{2+} & Mn^{2+} showed less amylase activity. The presence of metal ions influences the production of alpha amylase.

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