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Partial purification and effect of metal ions on the activity of amylase from thermophilic *Bacillus subtilis* AR-27

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Abstract: This study reports the production of amylase enzyme from *Bacillus subtilis* AR-27. Studies on the enzyme production revealed that optimum conditions were pH 7.0 and at temperature 60ºC. Cells were cultivated in LB broth medium supplemented with 1% starch for 24 hrs. Enzyme was partially purified by 85% ammonium sulphate precipitation. It was found that Na^+ and Ca^{2+} have stimulatory effects on enzyme activity whereas Mg^{2+} at concentration 3mM enhanced enzyme activity but it inhibits activity at high concentrations.

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INTRODUCTION

Extracellular enzymes produced by genus Bacillus are used in many industrial applications[1-3]. Among them amylases are one of the most important enzymes as they are used in various industrial processes including brewing, baking, textile & detergent[4,5].

The amylase enzymes with desirable properties, for instance, low pH stability, raw starch digestibility, thermo stability & utilization of high concentration of starch can be very useful in related industrial applications[6].

Amylases can be derived from various sources, such as plants, animals and microorganisms[7,8]. Although at present, Bacillus, Aspergillus & Rhizopus species are considered to be the most important sources of industrial amylases[9], it remains a challenging task to obtain a strain with suitable characteristics[10].

Since thermo stability is a feature of most of the enzymes sold in bulk for industrial applications, thermophilic microorganisms are of special interest for the production of amylases[11,12].

This study reports the production of amylase enzyme from a thermophilic strain of *Bacillus*, its partial purification & particularly its behavior towards various metal ions.

MATERIALS AND METHODS

Culture incubation

Culture was cultivated in two litre batches. Four conical flasks of 500ml capacity were taken, each containing 250ml LB broth supplemented with 1% starch. Flasks were autoclaved and then inoculated with 1% 18 hrs incubated culture. Flasks were then incubated at 60^0 C in a shakubator at 120rpm for 24 hrs.

Centrifugation and precipitation of proteins

To obtain clear, Cell Free Fluid (CFF), 24 hrs grown culture was centrifuged at 5000rpm. The pellet was discarded and the supernatant (containing enzyme) was collected. For precipitation and fractionation of proteins, cell free fluid was treated with different concentration of ammonium sulphate. First fractionation was done by adding ammonium sulphate salt up to 40% concentration. Precipitates were separated by centrifugation at 5000rpm. The supernatant was treated for 60% and then 85% precipitation. The collected precipitates were tested on starch agar plates for the presence of amylase. Precipitates showing amylase activity were dissolved in 50mM Tris- acetate buffer (pH 7.0). Total protein was estimated by the method of Lowry[13].

Desalting of protein

For desalting, sample dissolved in Tris-acetate buffer was transferred in a dialysis bag and then immersed in 500 ml of dialysis buffer (50mM Tris-acetate pH 7.0). It was left on slow stirring at 4°C for 24 hrs. The dialysis buffer was changed continuously after every 4 hrs. The dialysis sample was then concentrated to half of its original volume.

Concentration of the sample

Dialyzed sample was concentrated with the help of Centricon Ultracel YM-10 (Millipore) 10 KDa filter membrane. Sample was kept in Centricon column and was centrifuged at 4000rpm for 15 minutes at 4ºC.

Effect of different metal ions on enzyme activity

To evaluate the stimulatory or inhibitory effects of metal ions on enzyme activity chloride salts of different ions were used at the concentrations of 3mM to 9mM. Partially purified enzyme was mixed with salt solutions in a ratio of 1:1 and kept at 30ºC for 15 minutes and then enzyme assay was performed[14]. Salts used for the study were NaCl, $CaCl_2$ and $MgCl_2$.

RESULTS AND DISCUSSION

Under optimized conditions, culture was grown in two litre batches. Centrifugation was performed to obtain cell free fluid and then protein was precipitated out by 40%, 60% and 85% ammonium sulphate precipitation. Enzyme activity was found in 85% precipitates. The effects of various metal ions on the activity of α-amylase produced by *Bacillus subtilis* AR-27 were investigated by incubating enzyme with metal ion solutions of different concentrations. Metals are considered as agents that stabilize and activate enzymes by either taking part directly in catalysis or involving in structural modifications. Na^+ was found to be stimulatory for enzyme activity at concentration 6mM (Figure 1) which is contrary to another findings where Na^+ was found to be strongly inhibitory for enzyme activity[15].

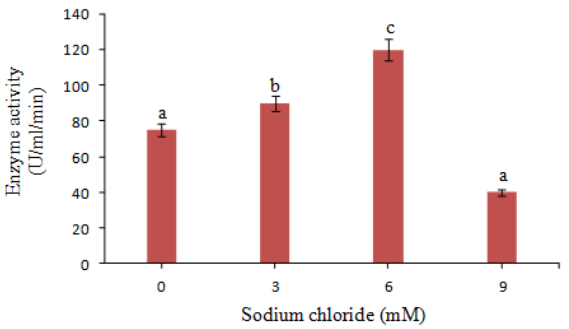

Figure 1: Effect of NaCl on α-amylase activity. Symbols (mean± SEM, n=6) having similar letters are not significantly different from each other (Bonferroni test, P<0.05).

Mg^{2+} at concentration 3mM found to be stimulatory (Figure 2) however high concentrations strongly inhibit enzyme activity. Ca^{2+} also stimulates the enzyme activity (Figure 3).

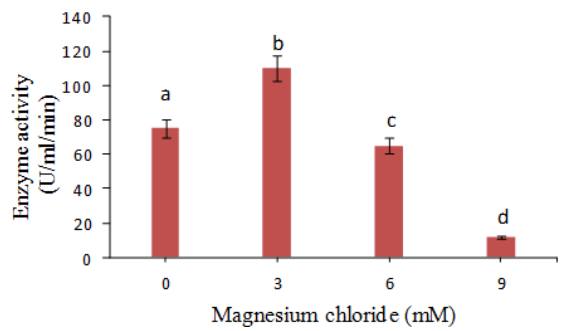

Figure 2: Effect of $MgCl_2$ on α-amylase activity. Symbols (mean±SEM, n=6) having similar letters are not significantly different from each other (Bonferroni test, P<0.05).

In general amylases are known as metalloenzymes and contain at least one Ca^{2+} ion as an essential constituent for enzyme activity. Likewise, to enhance, stabilize and extend the half-life of enzyme, Ca^{2+} is the preferred cation[16]. Thermostable α-amylases show more thermostability in the presence of Ca^{2+}[17]. Studies for purification and characterization are in progress.

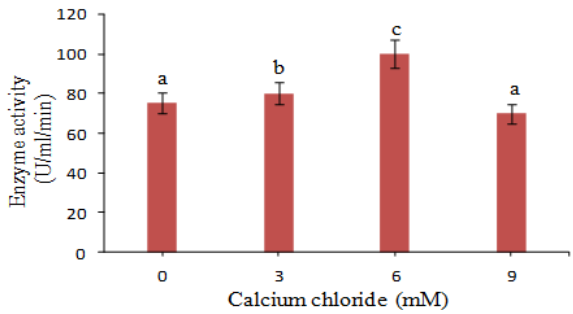

Figure 3: Effect of $CaCl_2$ on α-amylase activity. Symbols (mean±SEM, n=6) having similar letters are not significantly different from each other (Bonferroni test, P< .05).

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