

## **Production of Thermostable and Ca<sup>2+</sup> Independent $\alpha$ -Amylases from Halophilic Bacteria**

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### **Abstract**

In the present study, moderately halophilic isolates ND1 and ND2 were used for amylase production. The activity of amylase in isolate ND2 is 0.2-0.25  $\mu\text{mole/ml/min}$  and in ND1 is 0.1-0.15  $\mu\text{mole/ml/min}$ . even at 60°C, confirming their thermostable nature. Additionally, they are also active at acidic-neutral pH. Our findings also substantiated that no metal ions are actually needed for their optimum activity and this property may add to the cost effectiveness when the enzyme production at industrial scale is sought.

**Keywords:** halophilic bacterial isolates, amylase, thermo stability

### **INTRODUCTION**

Halophilic bacteria obtain special attention as they grow and develop in wide range of salty environments, and capable to produce a great source of enzymes. The enzymes from halophilic bacteria are active at elevated saline conditions and harsh industrialized method where several enzymatic reactions will be inhibited by high saline conditions. In industries, Halophilic bacteria were used in the production of exopolysaccharides, in the treatment of waste water, bioelectronic materials such as bacteriarhodopsin and enzymes like amylases, esterase, protease and xylanase [1].

The amylases can be acquired from many living resources like plants, animals and bacteria. The main benefit of using bacteria to produce amylase is economic as they are easy to manipulate to obtain enzymes with desirable characters [2]. The amylases from microorganisms have industrial importance. They are commercially available and have replaced completely in starch processing industries. The most commonly

used industrial applicable microorganisms are *Aspergillus niger*, *Bacillus licheniformis*, *Bacillus amyloliquifaciens* [3].

Besides starch saccharification, amylases have extensive applications in clinical study, medicinal chemistry and starch chemistry [4]. As amylase is an industrially important enzyme, there is increasing interest in the isolation of new amylases suitable for wider industrialized importance. In this study, it is aimed to isolate and characterize amylases from halophilic bacteria.

## **MATERIALS AND METHODS**

### **Collection of samples and isolation of halophilic bacteria**

Soil and water samples were collected from Dwarka (Gujarat, India). The enrichment of halophilic bacterial isolates was done on the media with 5% NaCl, 0.5% Tryptone, 0.1% Glucose, 0.25% Yeast extract and 3% agar (STGYA).

### **Amylase production**

The isolates positive in starch hydrolysis were selected for amylase production and optimization studies. The growth curve was performed to check the growth and generation time of selected bacterial isolates. STGYA broth was taken with different concentration of NaCl from 5% to 25%, 37°C, 36-48hrs and absorbance was measured at 600nm according to Miller, 1959 [5]. The amylase activity (U) was determined by the reducing sugar liberated by amylase is equivalent to one  $\mu$ mole of D-glucose from starch. The standard of glucose solution was also performed by DNS method.

### **Effect of carbon source and various parameters on enzyme production**

Different carbon sources were applied to check maximum enzyme production. Various process factors like temperature, pH, incubation time and substrate concentration, affecting the activity of enzyme were optimized.

### **Determination of effect of metal ions and chemical reagents on enzyme activity**

The effect of different metal ions like  $\text{Ca}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Fe}^{3+}$  and chemical reagents like ethylene-diaminetetraacetic acid (EDTA), sodium dodecyl sulfate (SDS), Triton X-100,  $\beta$ -mercaptoethanol and Tween 80 on enzyme activity was studied by preincubating the crude enzyme with them and then assaying the amylolytic activity.

## **RESULTS AND DISCUSSION**

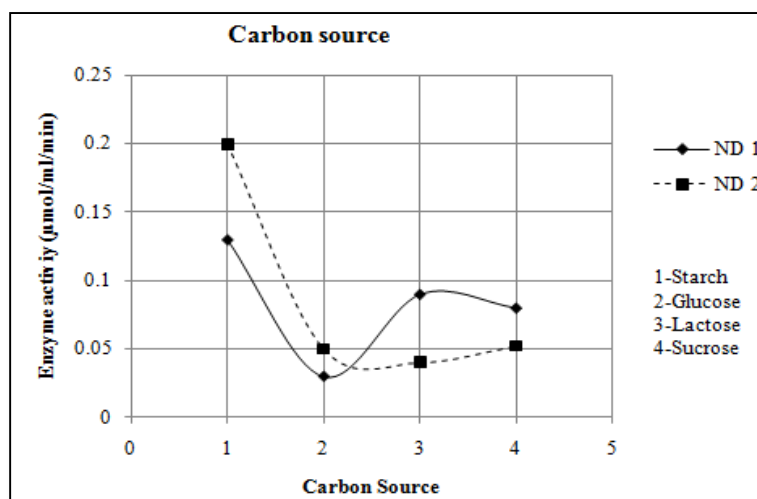
### **Screening and selection of amylase producing bacteria**

Among all isolated and purified halophilic bacteria, ND1 and ND2 were selected for further enzyme studies based on starch hydrolysis test. Both the organisms were gram positive, rod shaped and non spore producer. The highest biomass and density for both the isolates were attained in the media with NaCl upto 25%. Both the isolates showed maximum growth in the media having 5% NaCl and growth was decreased as

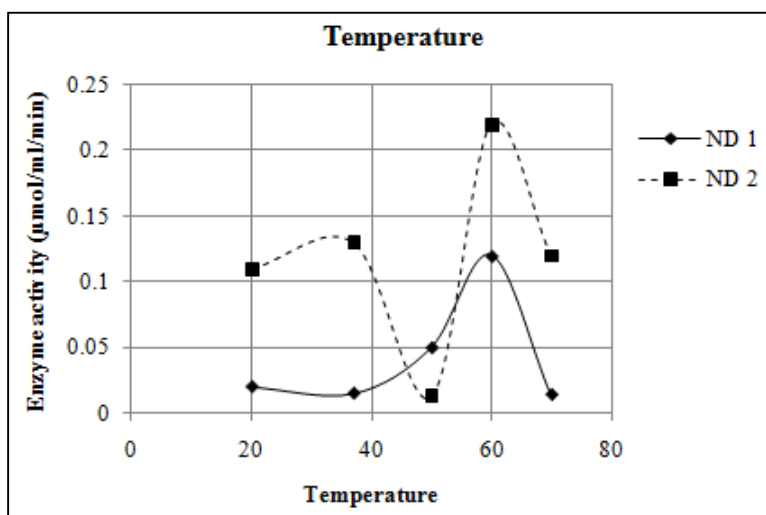
NaCl concentration increased, confirmed their moderately halophilic nature. The reducing sugar released from the starch by amylase determined the amylase activity [6].

### Effect of carbon source on amylase production

Among all the nutrients, the carbon source is very important factor in the media to produce extracellular amylases. To induce amyolytic enzymes, Starch is usually a conventional nutritional component.



(A)



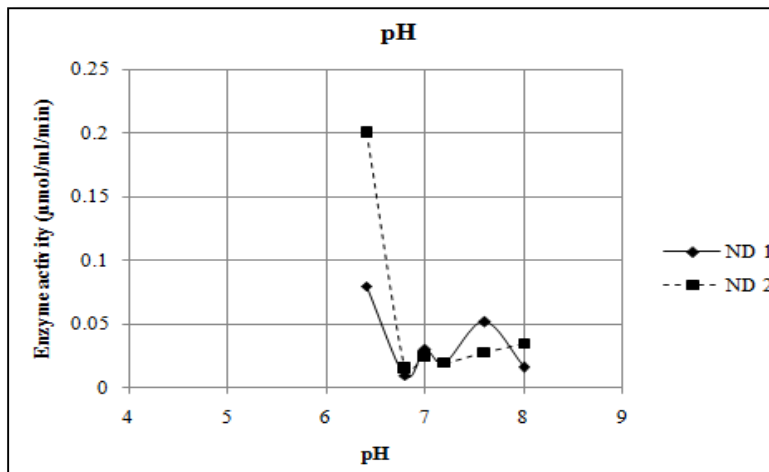
(B)

**Figure 1:** Amylase activity using different carbon sources (A) and at different temperatures (B)

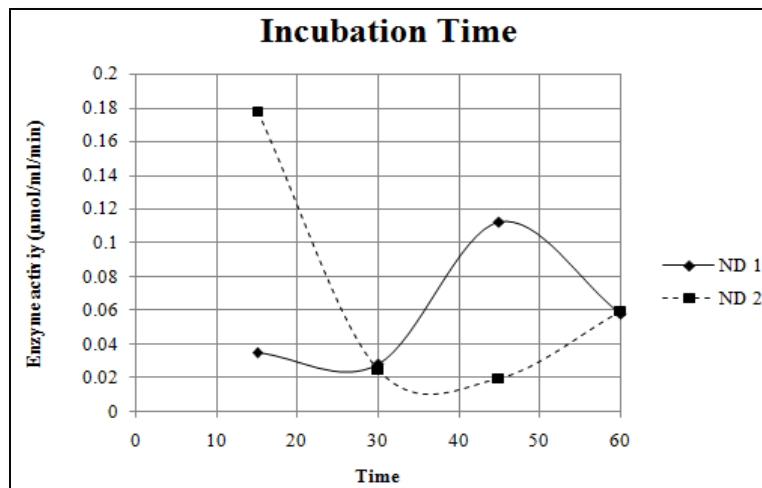
Fig.1 (A) shows that highest amylase production was obtained in medium containing starch for the isolate ND1. ND2 also showed maximum enzyme activity with starch and least activity with lactose and sucrose. The best carbon source for the production of  $\alpha$ -amylase from halophilic strain MA-2 was dextrin and starch was found as the second best carbon source [7].

### Optimization of various process parameters for amylase activity

In isolates ND1 and ND2, the amylase activity was increased as temperature increases and it showed highest at temperature 60°C for both the isolates (Fig.1 B).  $\alpha$ -Amylases from different *Bacillus* species like *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus licheniformis* etc reported to produce at a wide range of temperatures from 37 to 60°C [8].



(A)



(B)

**Figure 2:** Enzyme activity at different pH (A) and at different time interval (B)

Among all significant physical parameters, the pH of the medium plays a vital role in the organism which induces morphological changes, enzyme secretion and also enzyme stability [9]. Isolates ND1 and ND2 showed maximum enzyme activity at pH 6.0 (Fig.2 A) near acidic-neutral, which is indicative of the fact that these amylases can be applied in industries where acidic as well as neutral condition prevails. In isolate ND1, the reaction time for maximum amylase activity was found at 45 mins, whereas ND2 showed maximum enzyme activity after incubation for 15 mins (Fig.2 B). The enzyme activity of ND1 was maximum at 0.2ml substrate concentration while ND2 showed the maximum enzyme activity at 0.1ml substrate concentration.

### Effects of metal ions and chemical reagents

$\alpha$ -amylases are mostly known as metal ion-dependent, as they are dependent on some divalent ions like  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ca}^{2+}$  etc. [10]. The study on influential effect of different chelators and metal ions shown that the amylase was usually activated by  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  and mostly inhibited by EDTA and  $\text{HgCl}_2$  [11]. In this study, the results contradict these observations, where for ND1, these metal ions inhibited the enzyme activity and  $\text{Fe}^{3+}$  totally inhibited the enzyme activity of ND2. It is observed that these isolates do not need metal ions as cofactors for their activity.

**Table 1:** %Residual activity in presence of metal ions (A) and chemical reagents (B)

% Residual activity (B)			% Residual activity (A)		
Chemical reagents	ND1	ND2	Metal ions	ND1	ND2
Control	100	100	Control	100	100
SDS	-	-	$\text{Zn}^{2+}$	63.7	50
EDTA	33.15	19.07	$\text{Mg}^{2+}$	39.1	8.7
Tween 80	64.73	43.81	$\text{Mn}^{2+}$	40.0	10.0
Triton X-100	34.73	-	$\text{Ca}^{2+}$	87.4	61.5
$\beta$ mercaptoethanol	35.26	41.75	$\text{Fe}^{3+}$	69.0	-

In the presence of SDS, isolate ND1 and ND2 showed complete inhibition in amylase activity. EDTA, anion chelate and Triton X-100 were also not useful for the activity of amylase. On the other hand, Tween 80 and Tween 20 caused decrease in amylase activity at concentration of 10 mM, which could be due to predominant presence of oleic acid in Tween 80 and Lauric acid in Tween 20 [12].

## CONCLUSION

Amylase is regarded as the most significant enzyme at industrial level. Interestingly, the amylases produced by these strains were thermo stable as they are efficiently active at 60°C. The characterization study of amylase showed that the enzyme remained unaffected in the presence of metal ions, which contributes to the fact that no metal ions are actually needed for their optimum activity and this property may add to the cost effectiveness when the enzyme production will be carried out at industrial scale. These properties can be utilized for the future potential industrial applications and they can also be further explored for other ample applications.

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