

## Isolation and Characterization of Amylase from *Lysinibacillus Xylanilyticus* from Alkaline Environment

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**Abstract:** The uniqueness of the Lonar Lake water is its salinity and alkalinity. Most of the industrial processes are carried out at high temperature and at high pH and needs enzyme having high temperature and pH stability. The present study deals with isolation, production and dynamics of amylase from bacterial strain isolated from the alkaline Lonar Lake. Isolation of bacteria was done by using Horikoshi medium and screened for production and partial characterization of amylase. A total of five bacterial cultures were isolated and a bacterial isolate DHT 18 selected for study was characterized by cultural, morphological, biochemical and 16S rRNA gene sequencing resulted it into *Lysinibacillus xylanilyticus* which showed optimum activity at temperature 60°C and pH 10 indicating thermo-stability which is most useful in food, pharmaceutical and detergent industries and can be exploited for biotechnological potential.

**Keywords:** Lonar Lake, Haloalkaliphiles, *Bacillus*, Amylase

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### 1. INTRODUCTION

Natural alkaline environment occurred in Lonar Lake, an Indian soda Lake situated in Lonar, District Buldhana, Maharashtra, India which is a unique basaltic rock meteorite impact crater, ranking third in the world and filled with saline water having an average pH of 9.5-10 [1]. Haloalkaliphiles, in particular *Bacillus* species are discovering for their industrial application such as food, pharmaceutical and detergent industries which have ability to produce extracellular enzymes at high pH and temperature [2]. Microbial enzymes are widely used in industrial processes and  $\alpha$ -amylase is one of the most important industrial enzymes, having applications in industrial processes such as brewing, baking, textiles, pharmaceuticals, starch processing, and detergents.  $\alpha$ -amylases are some of the most versatile enzymes in the industrial enzyme sector and account for approximately 25% of the enzyme market [3]. Strains of *Bacillus* have been some of the workhorses of enzyme production for decades, mainly because of their ability to overproduce amylase [4]. *B. subtilis*, *B. stearothermophilus*, *B. licheniformis*, and *B. amyloliquefaciens* are known to be good producers of  $\alpha$ -amylase, and they have been widely used for commercial production of the enzyme for various applications. These alkaline amylase producing bacteria are of great importance for its high thermo-stability, pH stability and most important industrial enzymes production [5, 6, 7]. Therefore, attempt was made to study the isolation, production and dynamics of amylase from bacterial strain isolated from the alkaline Lonar Lake.

### 2. MATERIALS AND METHODS

#### 2.1. Collection, Enrichment, Isolation and Identification of Amylase Producing Bacteria

A total of 12 samples of sediment, matt and water were collected from Lonar Lake and transferred to 100mL sterilized distilled water in 250 mL conical flask and agitated (100 rpm) at 37°C for 15 min on rotary shaker. The sample was then heated at 80°C for 15 min to destroy all the vegetative microbial cells. One mL of each diluted sample was inoculated in Horikoshi medium (A, B, C and D) and incubated at 37°C for 72h and four time repeated sub-culturing was made in the same medium. After enrichment, culture was inoculated on Nutrient agar (pH 10) and incubated at 37°C for 24h and well distinct colonies were selected and maintained as a stock on nutrient agar slant for screening. Individual bacterial colonies were screened for amyolytic activities on Starch agar medium and zone hydrolysis was recorded. Amylase producer were identified based on morphological, cultural and biochemical characteristics and 16S rRNA gene sequencing of culture was performed at Agharkar Research Institute, Pune.

## 2.2. Amylase Assay

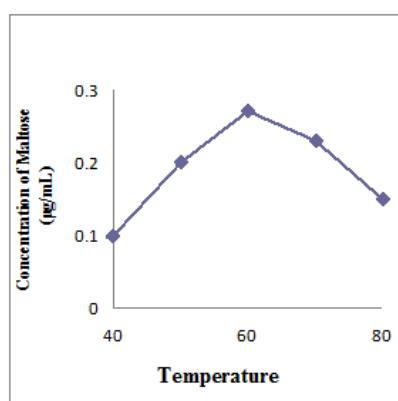
The 100 mL Starch nutrient medium was inoculated with culture and incubated for 48h at 37°C in incubator. After 48h incubation, centrifuged the broth at 5000 rpm for 15 min. The supernatant served as crude enzyme source. The standard graph of maltose was prepared using 3,5-dinitrosylisilic acid reagent. Estimation of amylase was carried out with 2.5 mL of (1%) starch solution; 2.5 mL of PO<sub>4</sub> buffer, 1mL of NaCl and 1 mL of enzyme in a test tube and incubated in boiling water bath for 5 min as per DNS method.

## 2.3. Characterization of Amylase

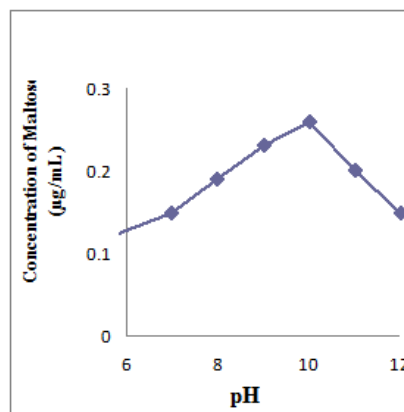
The effect of pH on alkaline amylase was determined by assaying the enzyme activity at different pH ranging from 6.0 to 12, effect of temperature by incubating from 40°C to 80°C using the PO<sub>4</sub> buffer (0.2 M). The effect of substrate concentration on alkaline amylase activity was determined by incubating the reaction mixture for 15 min. with different substrate concentration, ranging from 0.5mg/mL to 4mg/mL. The effect of enzyme concentration on alkaline amylase activity was determined by incubating the reaction mixture (pH 10) for 15 min. at different enzyme concentration ranging from 0.5mL to 4mL. The activity of the amylase was then measured as per assay procedure.

## 3. RESULTS AND DISCUSSION

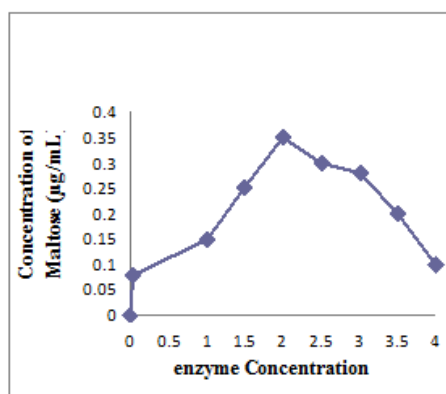
In the present study, a total of 10 different bacterial species were isolated from water, sediment and matt samples from Lonar Lake. Out of 10, four isolates were showed maximum starch hydrolysis activity on starch agar medium at pH 10. Out of them one isolate DHT18 was selected for further study since it showed prominent amyolytic zone of 23mm. This isolate was characterized based on cultural, morphological and biochemically by commercially available Hi-media Rapid detection kit. The isolate DHT 18 was Gram positive, long rod shape and motile. Growth was detected at different pH (7 to 12) and salt concentration of NaCl (1 to 8%). The growth of isolate DHT 18 was found to be optimum at 50°C to 60°C temperature. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain DHT18 is *Lysinibacillus xylanilyticus*.



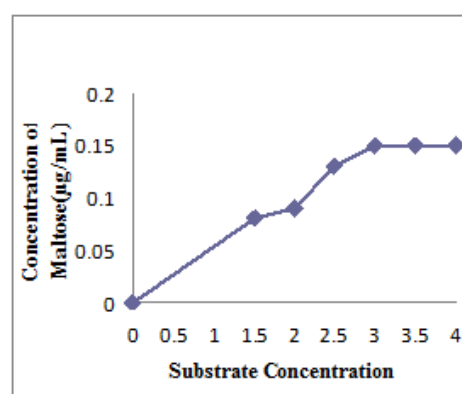
**Fig1.** Effect of temperature on the activity of Enzyme Amylase



**Fig2.** Effect of pH on the activity of enzyme Amylase



**Fig3.** Effect of enzyme concentration on the Activity of enzyme Amylase



**Fig4.** Effect of Substrate on the activity of enzyme Amylase

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**Table1.** Cultural, morphological and biochemical characteristics of amylase producing *Lysinibacillus xylanilyticus* (T) ( FJ477040)

Gram character	+	Glucose	-	$\alpha$ -Methyl-D-glucoside	-
Shape of Bacteria	LR	Dextrose	+	Rhamnose	-
Arrangement of Cell	Single	Galactose	-	Cellobiose	-
Spore	+	Raffinose	-	Melezitose	-
Motility	+	Trehalose	+	$\alpha$ -Methyl-D-mannoside	-
Catalase	-	Meliboise	-	Xylitol	-
Oxidase	-	Sucrose	-	ONPG	-
Voges Proskauer	-	L- Arabinose	-	Esculin hydrolysis	+
Citrate utilization	-	Mannose	-	D-Arabinose	-
Nitrate reduction	-	Inositol	-	Malonate Utilization	-
Arginine	-	Sorbitol	-	Sucrose	-
Lactose	-	Mannitol	-	Inulin	-
Xylose	-	Adonitol	-	Sodium gluconate	+
Maltose	+	Arabitol	-	Glycerol	-
Fructose	-	Erythritol	-	Salicin	-

**Note:** + = Positive, - = Negative

From the data it showed that, the maximum amyolytic activity was observed at temperature 60°C (Fig.1) and pH 10 (Fig. 2) by *Lysinibacillus xylanilyticus*. Optimum pH for amyolytic activity of amylase producing bacteria was observed between pH 8-10.5. The optimum enzyme concentration required for maximum activity of amylase 2  $\mu$ g/mL (Fig.3) and substrate concentration was found 1.5  $\mu$ g/mL (Fig.4). The isolated bacteria *Lysinibacillus xylanilyticus* produce the amylase enzyme which has thermophilic, alkalophilic and has potential is used in industry. Same results reported by Singh *et al*, [8]. Annamalai *et al*. [9], reported on amylase production at optimum activity was found at 8 and maintained at pH 11. Tambekar *et al*.,[10] isolates amylase producing *Bacillus sp.* and optimum activity was found to be on 50°C, pH 10 and substrate concentration 1.5  $\mu$ g/mL of amylase

**Table2.** The 16S rRNA gene sequencing closest phylogenetic affiliation, pair similarity and ribosomal database project report of isolated amylase producing organism DHT 18 from Lonar lake

Strain Designation	Closest phylogenetic affiliation	Max ident
DHT 18	<i>Lysinibacillus xylanilyticus</i> (T) 16S ribosomal RNA gene partial sequence ( FJ477040)	100.0%

Quick Bioinformatic Phylogeny of Prokaryotes and Seaview  
--See Legend--

## 4. CONCLUSION

The haloalkaliphilic *Lysinibacillus xylanilyticus* isolated from the alkaline Lonar Lake, India and exhibited amylase activity at extremophilic condition. However, novel features of the enzyme such as stability over the wide range of pH 6-12, temperature 40-80°C and salt concentration 0.5-10% make it an attractive candidate for future studies and development process. The production of the enzyme with these sources would be economically attractive proposition. This is valuable information for enzyme production and optimization by extremophilic *Lysinibacillus xylanilyticus*. Amylase from

*Lysinibacillus xylanilyticus* has bright future towards the improvement and production of novel enzymes for entirely new areas of industrial and biotechnology application.

#### REFERENCES

- [1] Tambekar DH and Dhundale VR. (2012). Studies on the physiological and cultural diversity of *Bacilli* characterized from Lonar Lake (MS) India. *Biosci Disco*, 3(1): 34-39.
- [2] Joshi AA, Kanekar pp, Kelkar AS, Shouche YS, Wani AA, Borgave SB and Sarnaik SS, (2008). Cultivable bacterial diversity of alkaline Lonar Lake in India. *Microb Ecol*, 55(2):163-72.
- [3] Shanmughapriya S, Kiran GS, Selvin J, Gandhimathi R, Baskar TB, Manilal A and Sujith S. (2009), Optimization, production and partial characterization of an alkaliphilic amylase produced by sponge associated marine bacterium *Halobacterium Salinarum*.MMD047. *Biotechnology and Bioprocess Engineering*, 14: 67-75.
- [4] Soenshein AL, Hoch JA and Lasik R (1993). *Bacillus subtilis* and other Gram-positive Bacteria: Biochemistry, Physiology and Molecular Genetics, ASM, New York.
- [5] Borsodi AK, Micsinai A, Rusznyak A, Vladar P, Kovacs G, Toth EM, and Marialigeti K. (2005). Diversity of alkaliphilic and alkali tolerant bacteria cultivated from decomposing reed rhizomes in Hungarian Soda Lake. *Microb ecol*, 50: 9-18.
- [6] Srinivasan TR, Das S, Bal KV, Philip R and Kannan N. (2009). Isolation and characterization of thermostable protease producing bacteria from tannery industry effluent. *Recent Res Sci Technol*, 1(2), 63-66.
- [7] Dhundale VR, More VR, Nagarkarand RD, Hemke VM and Tambekar DH. (2014). Isolation and characterization of a novel amylase from *Bacillus pseudofirmus*. *Indian J.L.Sci*, 4 (1): 69-76.
- [8] Singh R, Kumar V and Kapoor V (2014). Partial purification and characterization of a heat stable  $\alpha$ -amylase enzyme from a Thermophilic Actinobacteria, *Streptomyces* sp. MSC702. *Enzyme Res*. 10(6):363-368.
- [9] Annamalai N, Thavasi R, Vijayalakshmi S and Balasubramanian T (2011). Extraction, purification and characterization of thermostable, alkaline tolerant a-Amylase from *Bacillus cereus*. *Ind J Microbio*, 51(4): 424–429.
- [10] Tambekar DH, Chandurkar AY and Tambekar SD (2014). Partial characterization and optimization of alkaline Amylase from *Bacillus* spp. from Lonar Crater. *Int J Adv Pharm Biol Chem*, 3(2): 481-486.