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Research Article

Production and Partial Characterization of Lipase from Halo-alkaliphilic *Pseudomonas aeruginosa*

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ABSTRACT

Halo-alkaliphiles grow high pH and high salt concentration can be a source of novel enzymes. The enzymes produced by these bacteria have great importance in industry due to its high thermo and pH stability. The alkaline lipase producing bacteria are generally found in sea water or alkaline lakes such as Lonar Lake. A total of 17 bacterial cultures were isolated as methylotrophs from alkaline Lonar lake, 6 showed lipolytic activity and one strain DHT12 was prominent lipase producer which was studied further for its phenotypic and biochemical characters. The bacterium DHT12 was screened for production and partial characterizations of lipase, and 16SrRNA sequencings identified as *Pseudomonas aeruginosa.* The maximum lipase production was reported at pH 9, the activity was 3.7 unit/mL and optimally active at 60°C. Thus the enzyme produced by this bacterium have promising applications in many industries like leather, soaps and detergents, pharmaceuticals, biofuel, oleochemical, food and textile.

Keywords: Halo-alkaliphiles, alkaline lipase, *pseudomonas,* Lonar Lake.

INTRODUCTION

Halo-alkaliphilic lipolytic bacteria have been recognized for many years, and enzyme lipases (E.C. 3.1.1.3) have emerged as key product of rapidly growing biotechnology industries, such as food, detergent, chemical and have biomedical applications[1]. Lipase catalyzes lipids and liberates fatty acids and glycerol additionally; they also catalyze the synthesis and transesterification of glyceride[2]. Lipases are serine hydrolases which act at the lipid water interface. Different genera of bacteria including *Streptomyces* spp. are known to produce lipase but *Achromobacter* spp, *Alcaligenes* spp, *Arthrobacter* spp, *Pseudomonas* spp and *Chromobacterium* spp have been well exploited for lipase production. The important species of *Pseudomonas* i.e. *P. fragi*, *P. fluorescens* and *P. aeruginosa* have been extensively exploited to produce lipase[3,4]. Lipases from various species of *Pseudomonas* have been proved to be useful both in organic reactions and in many industries. Most of the well-studied microbial lipases are inducible extra cellular enzymes. They are synthesized within the cell and exerted externally in environment. The very small work or study had been reported on lipase producing bacteria from halo-alkaliphilic Lonar Lake, which have ability to produce thermo and alkali tolerant lipase[5]. Therefore, the present study was aim to deal with the isolation, screening of lipase producing bacterium and partial characterization and production of a lipase from it.

MATERIALS AND METHODS

Collection, Enrichment and Isolation of microbes:

A total of 12 (water, sediment and matt) samples were collected from 4 different sites of Lonar lake in September, 2014. Enrichment of isolates was carried out in Horikoshi I (A, B, C and D) medium, incubated at room temperature on a rotary shaker (100 rpm) for 7 days. After enrichment, the organisms were isolated on respective agar medium and incubated at 37° C. Well isolated and differentiated colonies from these enrichment media

were transferred on slants and maintained as stocks for further study[6] .

Screening and Identification of Lipolytic Bacterial Alkaliphiles: The isolated bacterial colonies were screened for lipolytic activities on egg yolk agar medium (pH 10) at 37^0C for 72 h. The halozone appears around the colony indicated lipid hydrolysis and lipolytic bacterium[5] (Joshi *et al.,* 2005). The bacteria with prominent zone of clearance on egg yolk agar medium were processed for identifications based on morphological, cultural and biochemical characteristics and identified in accordance with the methods recommended in Bergey's Manual of Systematic Bacteriology. The 16S rRNA sequencing and BLAST identification was performed by Agharkar Research Institute, Pune (Maharashtra).

Production, Optimization and assay of Crude Enzyme Extracts: In 100 mL of sterile alkaline nutrient broth, 1 mL egg yolk was added and the broth was inoculated with cultures and after for 72 h incubation on rotary shaker, centrifuged the broth at 5000 rpm for 15 min. The supernatant served as crude enzyme sources for extracellular lipase. Lipolytic activity in the synthetic media was determined titrimetrically on the basis of olive oil hydrolysis by the slightly modified method of assay mixture containing 180 mL of distilled water, 20 mL olive oil, 0.4 g sodium benzoate with 1g gum arabic. Assay mixture contains 5 mL oil emulsion, 5 mL 0.1 M tris buffer, added with 1 mL enzyme suspension was incubated for 30 min at room temperature. After incubation the reaction was stopped by addition of acetone and methanol mixture and liberated fatty acids were titrated with 0.025 N NaOH using 1% phenolphthalein as indicator[7]. The one unit of Lipolytic activity was defined as the amount of enzyme that produced fatty acid in (µmL–1) unit per mL under the standard assay conditions and determined as under:

Lipase Unit Calculation:

$$\text{Lipase activity} = \frac{\Delta V \times N}{V_{(\text{sample})}} \times \frac{1000}{30}$$

Where- $\Delta V = V_2 - V_1$

V_1 = Volume of NaOH used against control flask

V_2= Volume of NaOH used against experimental Flask.

N = Normality of NaOH

Enzyme kinetics alkaline Lipase: The effect of pH on alkaline lipase from *Pseudomonas* spp. was determined by assaying the enzyme activity at different pH ranging from 7.0 to 10.5 and effect of temperature at different temperature ranging from 40^0C to 100^0C. The effect of substrate and enzyme concentration on alkaline lipase activity was determined by incubating the reaction mixture (pH 10.5) for 30 min with variable substrate and enzyme concentrations.

RESULTS AND DISCUSSION

The presence of both alkali-tolerant and obligate alkaliphilic lipase producers were recorded and identified by phylogenetic analysis in Lonar Lake8[8,9]. In the present study, a total of 17 different bacterial species were isolated from water, sediment and matt samples of Lonar Lake. Out of 17 bacterial methylotrophs, 6 were lipase producer and the one which was prominent lipase produce (25 mm zone of hydrolysis) was selected for detail study (Fig.1). The selected bacterium (DHT12) is long rod (5.0 µm), 1.0 µm in width Gram-negative and highly motile (Table 1). The 16s rRNA sequencings and blast analysis identified this bacterium as *Pseudomonas aeruginosa* JCM 5962 (T) (Table 2).

The lipolytic activity of lipase produced by *Pseudomonas aeruginosa* was pH sensitive and optimum activity at pH 9 (Fig.2). In the present experiment, temperature increases enzyme activity and maximum recorded at 60°C and there after it declined (Fig.3). Tambekar *et al*[10], isolates *Bacillus cereus* OCW3 (1) strain from Lonar lake and recorded optimum lipase production at pH 9 and at 60ºC As the substrate concentration increases, the enzyme activity also increases up to the maximum level after which it decreases. Optimum substrate concentration for activity of lipase was found to be 2.37unit/mL at 6 mL (Fig.4). Optimum lipolytic activity was recorded at 3.7unit/mL enzyme at 6mL of substrate concentration (fig. 5). The concurrent results was recorded by Tambekar *et al*[11], who isolated three strains of *Bacillus pseudofirmus* and showed lipase production was maximum at pH 9 and activity was 1.54 unit/mL to 1.66 unit/mL.

CONCLUSION

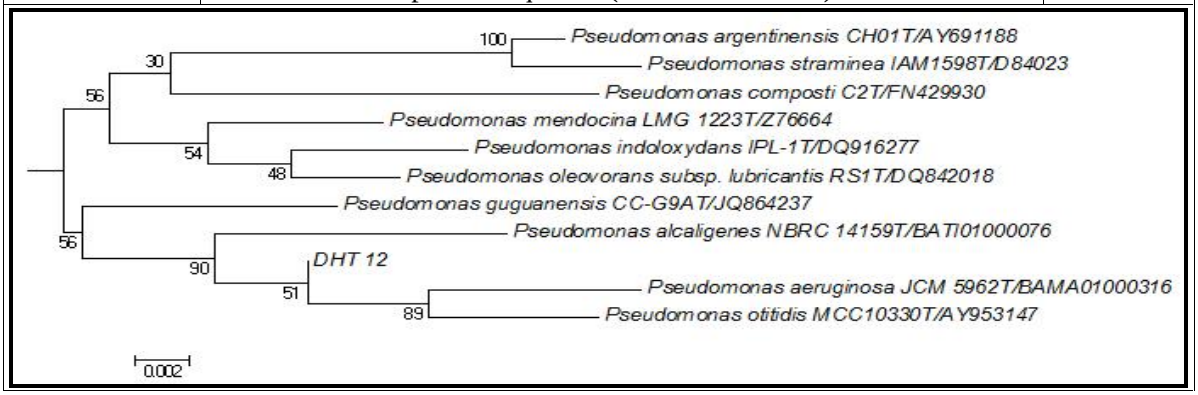
The *Pseudomonas aeruginosa* is prominent alkaline thermostable lipase producer and optimally active at high alkaline pH 9 and thermostable at 60°C and can be used in the industrial level. Thus the enzyme produced by this bacterium have promising applications in many industries like leather, soaps and detergents, pharmaceuticals, biofuel, oleo-chemical, food and textile.

Table 1
Morphological and biochemical characteristics of *Pseudomonas aeruginosa*

Test	Results	Test	Results
Morphological Character		Biochemical characters	
Gram character	-ve	Nitrate reduction	-
Shape	LR	Catalase	+
Size	5x1µm	Oxidase	+
Arrangement	Single	Indol	-
Spore	-	MR	-
Colony	Green	VP	-
Capsule	+	Citrate	+
Motility	+	Utilization	
Growth at temperature		Glucose	+
37° C	+	Arabinose	+
45° C	+	Mannitol	+
50°C	+	Xylose	+
55° C	+	Lactose	-
Growth at pH		Trehalose	-
pH7	+	Sucrose	+
pH8	+	Cellobiose	-
pH9	+	Galactose	+
pH10	+	Maltose	+
pH11	+	Fructose	+
Growth at NaCl		Salicin	-
1% NaCl	+	Sorbitol	-
2% NaCl	+	Raffinose	-
3% NaCl	+	Hydrolysis	
4% NaCl	+	Starch	+
5% NaCl	+	Lipid	+
6% NaCl	+	Casein	+
Note: LR = Long rod; + = Positive; - = Negative			

Table 2
Molecular identification and Closest phylogeny of haloalkaliphilic lipase producer

DHT 12	*Pseudomonas aeruginosa* JCM 5962(T)16S ribosomal RNA gene partial sequence (BAMA01000316)	100%



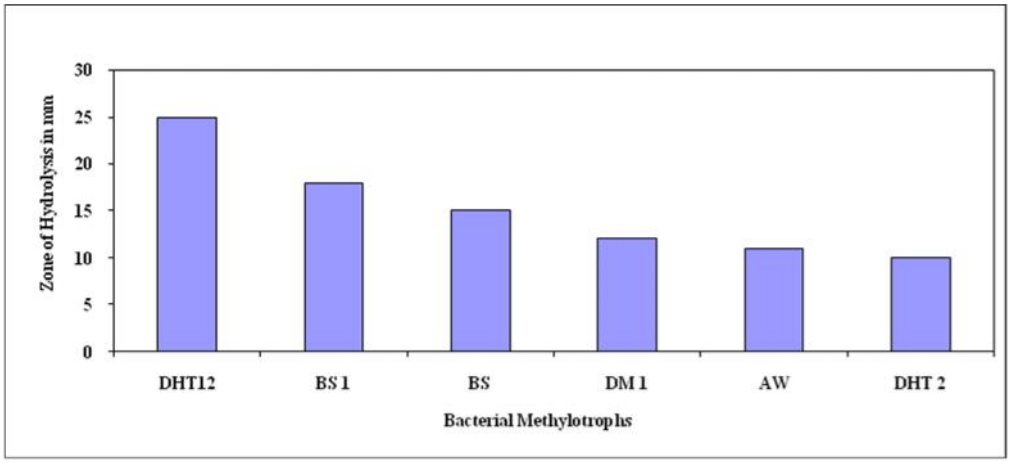


Fig. 1
Lipolytic Activities of Various bacteria isolated from Lonar Lake

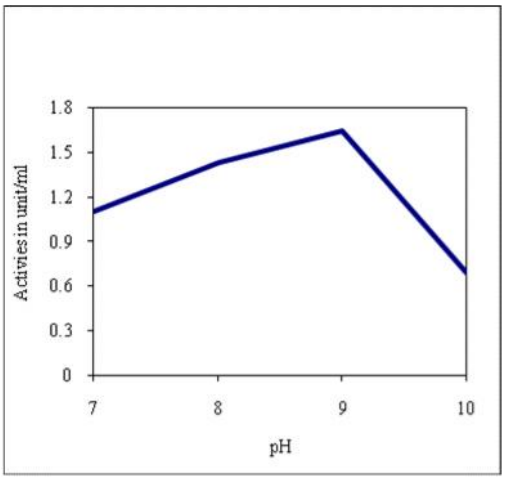


Fig.2
Lipolytic acivities *Pseudomanas aeruginosa* (DHT12) at various pH

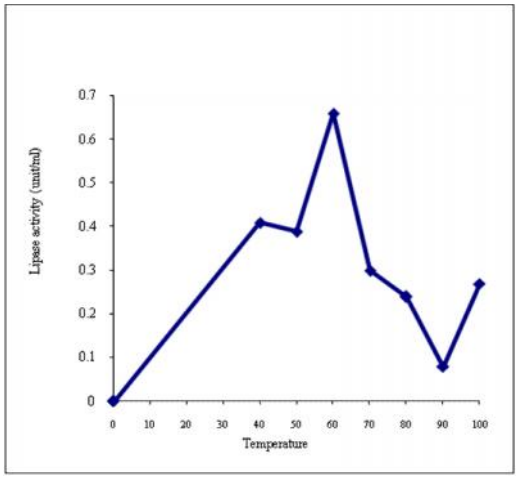


Fig. 3
Effect of Temperature on Lipase Enzyme

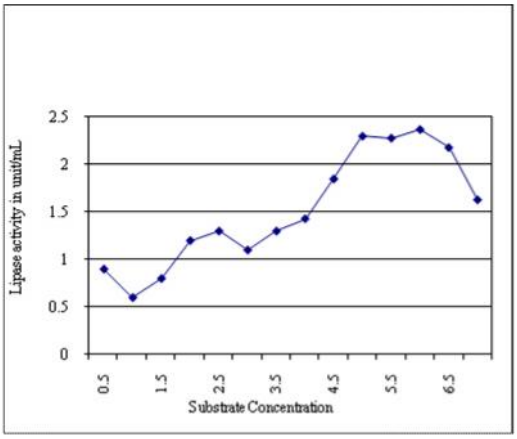


Fig. 4

Lipolytic activies of *Pseudomonas auruginosa* (DHT 12) at various substrate concentrations

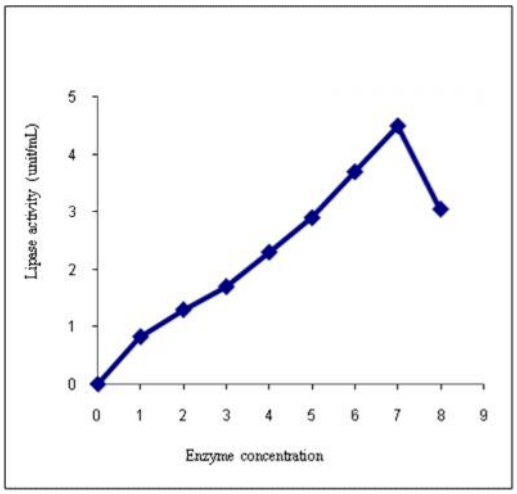


Fig. 5

Effect of Enzyme concentration on lipolytic activity

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