RESEARCH PAPER | Microbiology | Volume : 6 | Issue : 6 | June 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Thermophilic Lipase from *Lysinibacillus mangiferihumi* : Screening and Partial Characterization

KEYWORDS: Lonar Lake, Haloalkaliphiles, Bacillus, Lipase

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ABSTRACT *Alkaline Lonar Lake, a unique ecosystem situated in Buldhana District of Maharashtra State, India, harbors various haloalkaliphilic bacterial species which produces biotechnologically important thermo-haloalkaliphilic enzymes such as lipase. Lipases are diversified enzymes in their properties and substrate specificity, which make them attractive tools for various industrial applications. In this study, an alkalinethermostable lipase producing bacterium was isolated from Lonar Lake and ccharacterized morphologically, culturally and biochemically and identified as Lysinibacillus mangiferihumi by 16S rRNA sequencing. Alkaline lipase production was optimum at pH 9 and at 600C and enzyme activity was maximum at 1.54 unit/mL to 1.66 unit/mL. Lipase from this bacterium was active at higher temperature and pH and finds potential applications in food, pharmaceutical and detergent industries.*

INTRODUCTION

Alkaline Lonar Lake is a unique ecosystem situated in the Buldhana District of the Maharashtra State, India, harbors various haloalkaliphilic bacterial species which produces biotechnologically important thermohaloalkaliphilic enzymes (Tambekar *et al*, 2010; Joshi *et al.,* 2005). Lipases are diversified enzymes in their properties and substrate specificity, which make them attractive tools for various industrial applications. In this study, an alkaline thermostable lipase producing bacteria were isolated from Lonar lake (Tambekar and Tambekar, 2012). Lipases have been employed in a wide array of industrial applications, such as food technology, detergent, chemical industry and biomedical sciences (Gupta *et al,* 2004; Shuen-Fuh Lin *et al.,* 1996; Tambekar and Tambekar, 2011). Thermophilic microorganisms that are important sources for thermophilic enzyme are normally isolated from the soil of areas with special temperature conditions (Tambekar *et al*., 2013)). Among the available sources, only certain species have acceptable biosynthetic capabilities for use in organic reactions. These species include *Achromobacter, Arthrobacter, Bacillus* and *Pseudomonas*. Different genera of bacteria including *Streptomyces* spp. are known to produce lipase but *Achromobacter* spp, *Alcaligenes* spp, *Arthrobacter* spp, *Pseudomonas* spp, *Chromobacterium* spp and *Lysinibacillus* spp have been well exploited for lipase production (Tambekar and Dhundale, 2012; Joshi *et al.,* 2002). However, attempt was made to isolate new species of *Bacillus*, which can produce good quality of lipase useful in the detergent and leather industry (Horikoshi, 1971, 1999). Therefore, the present study was aim to deal with the isolation, screening, partial characterization and production of lipase from bacterium from alkaline Lonar Lake.

MATERIALS AND METHODS

Screening and Identification of Lipolytic Alkaliphilic Bacteria: Total 12 samples (water, sediment and matt) were collected from 4 different sites of Lonar lake and transported to laboratory for isolation and identification of bacteria with lipase production. A specific individual bacterial colonies were screened for lipolytic activities on egg yolk agar plate (egg yolk 1%, Peptone 1%, yeast extract 1%, beef extract 1%, sodium chloride 0.5%, Agar-Agar 2%, pH 10). The pH of the medium was adjusted by using pH meter with addition of 1 N NaOH before and after sterilization (Joshi *et al.,* 2007). The inoculated plates were incubated at 37^{0}C for 72 h. The halozone upper near the colony indicates as the lipid was hydrolyzed by bacteria. The bacterial strain with prominent zone of clearance on egg yolk agar medium was processed for identifications based on morphological, cultural and biochemical characteristics. The isolates were also tested for their growth at different temperature, pH and NaCl concentration. These isolates were identified in accordance with the methods recommended in Bergey's Manual of Systematic Bacteriology (Sneath *et al*.). The selected strains were then analyzed by 16S rRNA sequencing at Agharkar Institute, Pune (Maharashtra).

Optimization and assay of Enzyme Lipase: Egg yolk (1mL /100ml) containing sterile alkaline nutrient broth was inoculated with bacterial cultures and incubated for 72 h and then centrifuged at 5000 rpm for 15 min. The supernatant served as crude enzyme sources for extracellular lipase. Assay of lipase was carried out by standard titrimetric method (Kempka *et al.,* 2008)). Assay mixture contains 5mL oil emulsion and 5mL 0.1 M tris buffer and 1 mL enzyme suspension was added and incubated for 30 min at room temperature. After incubation, the reaction was by addition of acetone and methanol mixture and titration was done against 0.025N NaOH by addition of 1% phenolphthalein indicator. Assay mixture containing 180mL of distilled water, 20mL olive oil, 0.4g sodium benzoate with 1g gum arabic, 5 mL 0.1M tris buffer and add 1 mL culture supernatant at pH 10 such type of master mixture was incubated at 40^{0}C for 30 min and the reaction was stopped with 10 mL of acetone and methanol mixture (1:1). Liberated fatty acids were titrated with 0.025N NaOH using 1% phenophthalein as in-

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dicator. 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.

Lipase Unit Calculation:	Where- $\Delta V = V_2 - V_1$
Lipase activity = $\frac{\Delta V \times N}{V_{(sample)}} \times \frac{1000}{30}$	V_1 = Volume of NaOH used against control flask V_2= Volume of NaOH used against experimental Flask. N = Normality of NaOH

Effect of various parameters on Alkaline Lipase Activity: The effect of pH on alkaline lipase produce from *Lysinibacillus mangiferihumi* was determined by assaying the enzyme activity at different pH ranging from 7.0 to 10.5 and the effect of temperature determined at temperature ranging from 40°C to 100°C. The effect of substrate concentration on alkaline lipase activity was determined by incubating the reaction mixture (at pH 10.5) for 30 min with different substrate concentration, ranging from 0.5 mL to 8.0 mL and the effect of enzyme concentration was determined by incubating the reaction mixture (pH 0.5) for 30 min at various enzymes concentration ranging from 1 mL to 8 mL of supernatant. The activity of the lipase was then measured as per assay procedure.

RESULTS AND DISCUSSION

In the present study, a total of 12 different bacterial species were isolated from water, sediment and matt samples of Lonar Lake. Out of these one was selected for further analysis on the basis of its optimum lipid hydrolysis capacity (21 mm zone of hydrolysis). In present study DHT 17 revealed Gram-positive, highly motile and spore forming (Table 1). Phylogenetic analysis based on 16S rRNA gene sequencing indicated that strain DHT17 was affiliated with the genus *Lysinibacillus mangiferihumi*. The effect of temperature on enzyme activity was measured at different temperatures. As the temperature increases the activity also increases and it declined after attaining optimum temperature and maximum activity was recorded at 60°C (Fig.1). As the substrate concentration increases the enzyme activity also increases up to the maximum level after which it decreases. Optimum substrate concentration of *Lysinibacillus mangiferihumi* on enzyme activity of lipase was found to be 1.54 unit/mL at 5 unit/mL substrate concentration (Fig.2). As the pH increases the activity of enzyme also increases and at optimum pH 10, the maximum activity of lipase 3.87 unit/mL was recorded (Fig. 3) and also it showed optimum activity at the enzyme concentration of 3.14 unit/mL (Fig. 4); Both alkali-tolerant and obligate alkaliphiles were found and identified by phylogenetic analysis as the microbial species found in soda lake microbial population and known for being good lipase producers (Tambekar and Dhundale, 2012; Vargas *et al.*, 2004; Arpigny and Jaeger, 1999). Microorganisms that survive in extreme condition especially at elevated pH (10-12) are able to produce alkaline enzymes that in general show high catalytic activity at the optimal growth condition (Tambekar *et al.*, 2013).

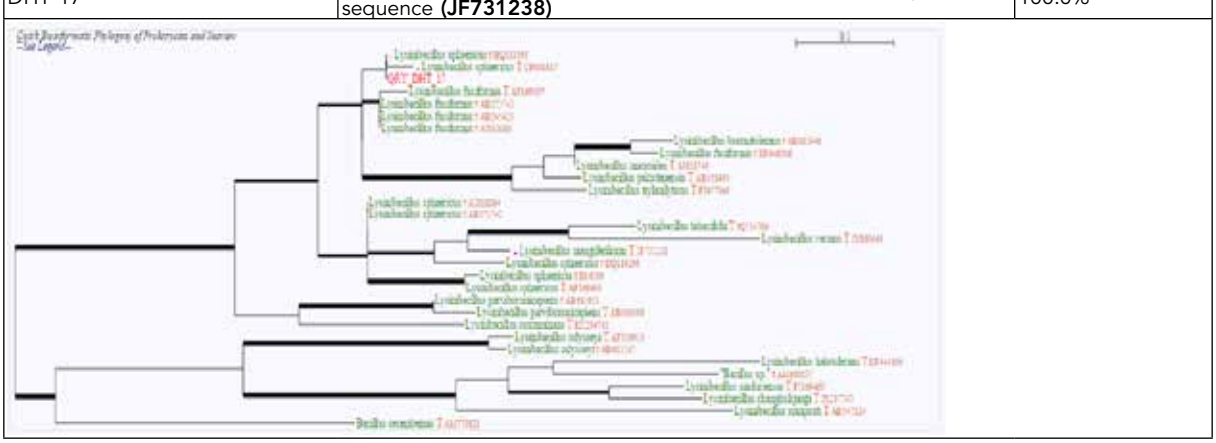
Table: 1. Cultural, morphological and biochemical characteristics of lipase producing *Lysinibacillus mangiferihumi*

TEST	RESULT	TEST	RESULT	TEST	RESULT
Colony shape	Circular	ONPG	+	Rhamnose	-
Colour of colony	Cream	Esculin hydrolysis	-	Glucose	+
Gram staining	Gm+ve rod	Adonitol	-	Lactose	-
Arrangement	Single	Meliboise	+	Arabinose	+
Motility	Motile	Reffinose	-	Trehalose	+
Catalase	+	α-Methyl-D-glucoside	-	α -Methyl-D-annoside	-
Oxidase	+	Malonate	-	Melezitose	-
Nitrate reduction	+	Voges Proskauer's	-	Xylose	-
Citrate	-	Arginine	+	Cellobiose	+
Sorbitol	-	Cellobiose	+	Erythritol	-
Maltose	+	Sucrose	+	Sodium Gluconate	-
Fructose	+	L-Arabinose	+	Glycerol	+
Dextrose	+	Mannose	-	Salicin	+
Galactose	+	Insulin	+	Dulsitol	-
Inositol	-	Mannitol	+	Arabitol	-

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

Table: 2. The 16S rRNA gene sequencing closest phylogenetic affiliation and ribosomal database project report of isolated Lipase producing organism DHT 17 from Lonar Lake

Strain Designation	Closest phylogenetic affiliation	Max ident
DHT 17	***Lysinibacillus mangiferihumi*** (T) 16S ribosomal RNA gene partial sequence **(JF731238)**	100.0%



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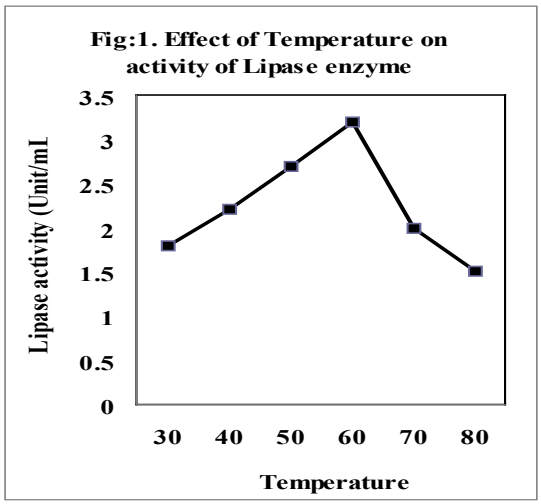

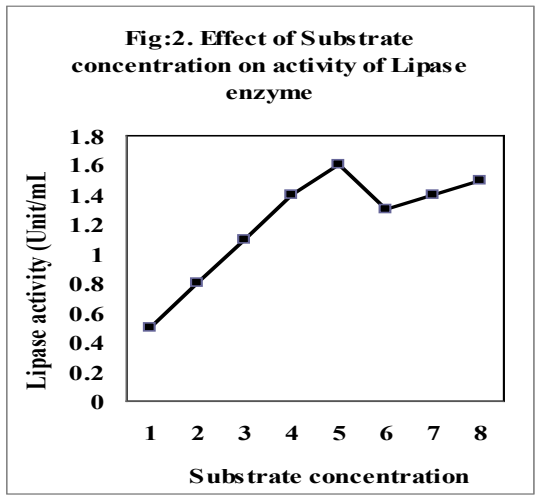

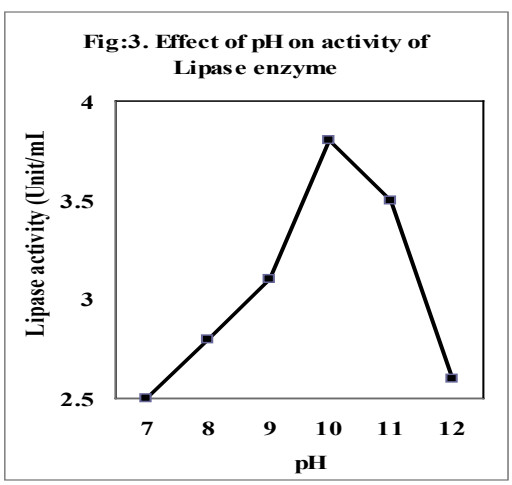

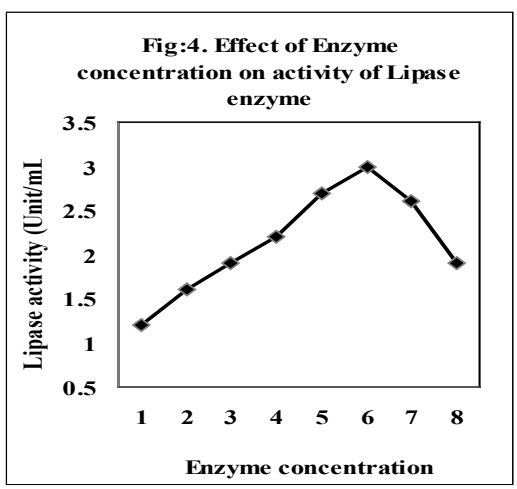

Conclusion

In the present study, different bacterial species were isolated from water, sediment and matt sample of Lonar Lake. Out of them, one bacterial strain was found lipase producer. The bacterial strains DHT 17 was isolated and screened for production and the partial characterizations of lipase. The bacterial isolates were characterized and identified as *Lysinibacillus mangiferihumi* (DHT 17). Alkaline lipase production was optimum at pH 9 and temperature active at 60^{0}C and enzyme concentration active at 6 and substrate concentration active at 5 the activity was 1.54unit/mL to 1.66 unit/mL. The isolated *Lysinibacillus mangiferihumi* strain produces the lipase enzymes which was thermostable, alkaliphilic and has potential to produce good quality lipases which can be used in food, pharmaceutical and the detergent industries.

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