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Original Research Article

Screening of thermostable lipase producers from alkaline lake

S.S.Lokre[1*] and D.G.Kadam[2]

[1]Department of Microbiology, Dr.Rafiq Zakaria Centre for Higher Learning and Advanced Research, Aurangabad, 431001, Maharashtra state, India
[2]Department of Microbiology, D.B.P. College of Science, Solapur, Maharashtra tate, India

**Corresponding author*

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Thermostable lipase, Screening, Lipase activity, pNPP.

A B S T R A C T

Lipases are the class of enzymes, which catalyzes the hydrolysis of long chain triglycerides. Rapid development in enzyme technology has diverted the attention of researchers towards microbial lipases. Thermostable lipases have tremendous industrial potential. As they have widespread applications in chemical, pharmaceutical, medical, cosmetic and leather industry, bio surfactant synthesis and agrichemicals. The present work focuses on isolation, identification, screening for lipase activity and selection of a suitable media and conditions for maximum production of lipase. The cultures were isolated by spread plate technique and were identified by relevant biochemical tests as mentioned in Bergey's manual to be as *Bacillus spp*. and *Aeribacillus spp*. Screening for lipase production was done by plate assay method using tributyrin agar. Both the cultures showed maximum activity in Luria Bertani broth supplemented with olive oil under stationary conditions, 50^0C, pH 8.0 and 9.0, respectively. Lipase activity was determined spectrophotometrically using p-nitrophenyl palmitate (pNPP) as a synthetic substrate.

Introduction

Lipases (Triacylglycerol acylhydrolase; EC 3.1.1.3) a group of enzymes active at interface of aqueous and non aqueous phases and hydrolyze long chain acylglycerol (Heravi *et al.*, 2008, Bayoumi *et al.*, 2007). Most microbial lipases are mesophilic in nature which cannot hydrolyze a substrate that exist in solid form at room temperature. Thermophilic lipases shows thermo-stability at elevated temperature and also resistant to chemical denaturation. At least 75% of all industrial enzymes are hydrolytic in action including lipases. Lipases remain enzymatically active in organic solvents, various physical and environmental conditions (Sharma *et al.*, 2011). Due to versatile reaction properties of lipases they have been widely used in many industrial applications such as food, chemical, detergents, pulp and paper, organic

synthesis, bioconversions in organic and aqueous media, resolution of racemic acids and alcohol, ester synthesis, oleochemical industries, etc. (Rohit Sharma *et al.,* 2001). Many microorganisms are known as good producers of extracellular lipases such as strains of *Bacillus, Pseudomonas, Candida, Rhodococcus, Staphylococcus,* etc. (El-moniem Abada, 2008).

The present study reports the isolation of thermostable lipase producing microorganisms from alkaline lake and the identification of those using morphological, biochemical characteristics. Also the focus of this study is on the selection of media for the maximum production of thermostable lipase in alkaline condition and the effect of submerged and stationary condition during fermentation also a point of interest.

Materials and Methods

Sample collection and processing

Water samples were collected in sterile container from Lonar lake, Buldhana district, Maharashtra state, India. Sampling was done at temperature range of 35˚C–37˚C and pH of water was around 10.3. The collected water samples were serially diluted up to 10^{-1} to 10^{-10} and were spread on agar plate followed by incubation at 50˚C.

Screening of lipolytic enzyme producing bacteria

Several methods have been proposed for screening of lipase production. In present investigation, author followed the screening of lipase producing bacteria on tributyrin agar plates (Heravi *et al.,* 2008). The composition of tributyrin agar (g/l) was, peptone-2.5, yeast extract-3.0, agar-15.0, and tributyrin 10 ml, pH-8.0. Each culture was streaked onto tributyrin agar plate and incubated at 50˚C for 2 days. The plate detection method was used for observation of lipolysis through the presence of clear zones around bacterial streak on tributyrin agar plates.

Optimisation of lipase production media

Twenty two different strains of *Bacillus* were isolated from the Lonar lake water sample, Buldhana district, Maharashtra state, India. These were qualitatively tested for the growth on different media for lipase production. The media used were nutrient agar (Anurag Sekhon *et al.,* 2006) incorporated with olive oil, nutrient agar with tween 80, Luria Bertani agar with tween 80, Luria Bertani agar with olive oil, tributyrin agar (Bayoumi *et al.,* 2007; Heravi *et al.,* 2008; Sirisha *et al.,* 2010), rhodamine B agar (Heravi *et al.,* 2008), modified G9+Y agar (Heravi *et al.,* 2008) with tributyrin, modified minimal medium (Anurag Sekhon *et al.,* 2006) with tributyrine and nutrient agar with tributyrine. Each culture was spot inoculated and incubated at 50˚C for 48 hrs. The large zone of hydrolysis around the colony was used for further study.

Identification of isolate

A morphological, physiological and biochemical characterization of lipase producers were done according to Bergey's manual of systematic bacteriology

Production of lipase from isolate

The selected cultures were grown in modified liquid medium containing casein enzyme hydrolysate 1%, yeast extract 0.5%, sodium chloride 1%, and olive oil 5%, pH 8.0 by surface and submerged (Longo *et al.,* 2010) condition. The rate of revolution was selected as 100 rpm (Baharun *et al.,* 2003). The temperature selected as 50˚C for 48 hrs. The broth was tested for the extracellular

lipase activity by a spectrophotometric method.

Assay of lipase activity

The spectrophotometric assay of extra cellular lipase was done according to the method of Rakesh Kumar *et al.*, 2012 with slight modification.

Results and Discussion

Thermophilic bacteria have several molecular modifications at cellular and sub cellular level to survive at high temperature and alkaline conditions. These organisms secrete such enzymes which are thermostable and resistant to high temperature and pH. Totally 22 bacterial strains were isolated from the Lonar lake water.

Identification of lipase producing thermophilic bacteria

Out of 22 bacterial isolates two strains (8.0-4 and 9.0-4) were identified as potent degrader of oil and showed clear zone on tributyrin agar plate at 50˚C (Figure 1). The zone of clearance around the streaks was due to hydrolysis of tributyrin by the secretion of extracellular lipase by an isolates.

Optimisation of lipase production media

The growth of isolates on a different media was shown in Table 1, showing Luria Bertani medium was effective for lipase production for all the 22 isolates. The growth of isolates in submerged and surface production were shown in Figure 2, which showed bacterial strain 8.0-4 and 9.0-4 for maximum production of lipase in surface condition than submerged.

Identification of isolate

The two bacterial isolates were characterized on the basis of cultural characteristics, microscopic appearance and biochemical tests (Table 2). Both the cultures are Gram- positive, non-motile giving small, round, regular, creamy, fast growing, butyrous colonies and non-spore forming. After morphological, physiological, biochemical identification, these two bacterial isolates were identified as species of *Bacillus* (8.0-4) and *Aeribacillus* (9.0-4)

The *Bacillus* sp. showed resistance towards antibiotic cefotaxime and cefadroxil whereas *Aeribacillus* sp. showed resistance towards antibiotic Cefotaxime and cefuroxime.

Figure.1 Screening of lipase producing thermophilic bacteria at 50^0C, clear zone indicates the hydrolysis of tributyrin as a result of lipase production

Table.1 The growth of isolates on a different media and showing Luria Bertani medium was effective for lipase production for all the 22 isolates

Medium Culture	NA+T80	NA+OO	LB+T80	LB+OO	TBA	G9+Y	Minimal	Rhodamine B	NA+TB
8-1	+	+	+	+	+	+	+	+	+
8-2	+	+	+	+	+	+	+	+	+
8-3	+	+	+	+	+	+	+	+	+
8-4	+	+	+	+	+	+	+	+	+
8-5	+	+	+	+	+	+	+	+	+
8-6	+	+	+	+	+	+	+	+	+
8-7	+	-	+	+	-	-	+	-	-
8-8	+	-	+	+	+	+	+	+	+
9-1	+	+	+	+	+	+	-	+	+
9-2	+	+	+	+	+	-	+	+	+
9-3	+	+	+	+	+	+	+	+	+
9-4	+	+	+	+	+	+	+	+	+
9-5	+	+	+	+	-	+	-	+	-
10-1	+	+	+	+	-	-	+	-	-
10-2	+	+	+	+	+	+	+	+	+
10-3	+	-	+	+	+	+	+	-	+
10-4	+	-	+	+	+	+	-	-	+
11-1	+	+	+	+	+	+	-	+	+
11-2	+	-	+	+	-	-	+	+	-
11-3	+	-	+	+	-	-	+	+	-
12-1	+	-	+	+	+	-	-	-	+
12-2	+	+	+	+	+	+	-	-	+

+ positive; - negative

Figure.2 Surface and submerged production of thermophilic lipase using bacterial isolates

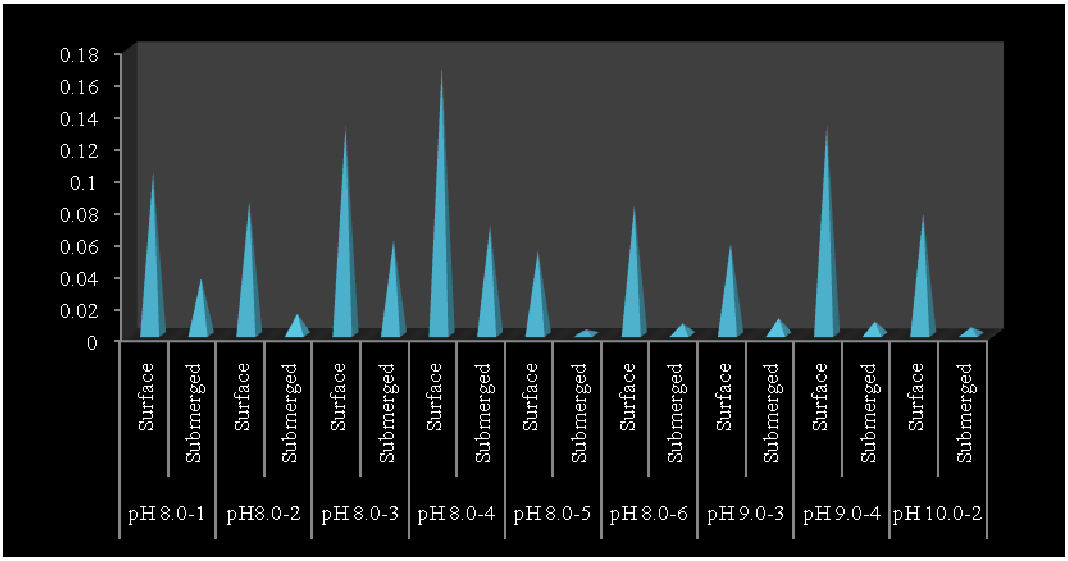

Table.2 Cultural and biochemical characteristics

Test	*Bacillus* sp.	*Aeribacillus* sp.
Amylase	+	-
Protease	+	-
Lecithinase	-	-
Urease	++	+
Cellulase	-	-
Gelatinase	+	-
Lipase	+	+
Catalase	++	+
Oxidase	+	+
SUGAR		
Rhamnose	-	-
Mannitol	A +++	A +
Cellobiose	AG ++	A +
Sucrose	A +++	A ++
Raffinose	-	-
Glucose	A +++	A +++
Arabinose	A ++	-
Xylose	A +++	A ++
Fructose	A ++	A ++
Lactose	-	-
Dulcitol	A ++	A+
OTHER TESTS		
H_2S production test	-	-
TSI test	Y, Y, No gas, H_2S -ve	Y, Y, No gas, H_2S -ve
Indol test	-	+
Methyl red test	+	+
Voges Proskaur test	+	-
Citrate utilization test	-	-
7% NaCl growth	++	+
Growth at 65^0 C	+	+
Nitrate reduction test	-	-
Motility test	+	+
Lysine decarboxylase test	-	-
Arginine decarboxylase test		
Ornithine decarboxylase test		
ONPG test	-	-

+ positive; - negative; A acid; AG Acid and gas; Y yellow

Figure.3 Antibiotic sensitivity test for the isolated *Bacillus* sp and *Aeribacillus* sp

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