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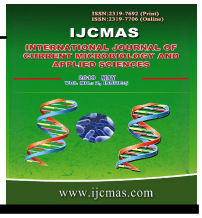


Original Research Article

Isolation and identification of lipase producing organisms from diverse soil samples of Kolli hills

M. Prasanna Rajeshkumar[1,2*], V.S. Mahendran[1,3] and V. Balakrishnan[1,4]

[1]Research and Development Centre, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India
[2]Department of Biotechnology, K S Rangasamy College of Arts and Science (Autonomous), Tiruchengode 637 215, Tamil Nadu, India
[3]Department of Biomedical Engineering, Sri Ramakrishna Engineering College, Coimbatore, Tamil Nadu, India
[4]School of Biotechnology, K S R College of Technology (Autonomous), Tiruchengode - 637 215, Tamil Nadu, India
**Corresponding author e-mail: prasanna4d@gmail.com*

K e y w o r d s

Extracellular enzyme Lipase; Kolli hills; Lipase assay; *Bacillus sp; Pseudomonas;* lipase; 16s rRNA.

A B S T R A C T

Lipases are enzymes which are capable of playing vital role in day to day application. It has various applications which includes from dairy industry to manufacturing detergent. By keeping all those in minds, Kolli hills have been selected as the target site for sample collection. Kolli Hills is known for its rich source of natural biodiversity. From the diverse soil sample microbes have been screened for lipase production and it is assayed for production of lipase. The production rate was compared with standard *Enterococcus faecium* MTCC 5659. And interestingly out of 20 isolate six isolates are capable of producing lipase more than 3 U/ml in 24 hrs of incubation. These microbes have been biochemically and molecularly characterized by using 16s rRNA ribotyping. The sequence was then submitted in NCBI data base.

Introduction

Lipase (Triacylglycerol hydrolases EC 3.1.1.3) are an enzyme which is capable of hydrolyzing triglycerides, diglycerides and monoglycerides into fatty acids and glycerol (Yasuo *et al.,* 2002). Lipases are ubiquitous enzymes which are found in animals, plants, fungi and bacteria. Microbial lipases are high in demand due to their specificity of reaction, stereospecificity and less energy consumption than conventional method (Saxena *et al.,* 1999). These microorganisms have been found in diverse habitat and especially oil processing industries. These enzymes are widely used in numerous biotechnological process such as cosmetic, food, leather, detergent and pharmaceutical industries (Sztajer *et al.,* 1998). Microbial lipases production has increased for the past one

decade, because of its potential application in industries (Hasan *et al.,* 2009). When compared with plant and animal lipases bacterial lipase were well studied. The microorganisms are usually grown in nutrient medium supplemented with carbon source, nitrogen and phosphorous. Glycerol, triglycerides and bile salts are usually used as inducer for the production of lipases (Kishore *et al.,* 2011).

Kolli hill is situated at an ever-so-pleasant altitude ranging from 1000 to 1300 m above mean sea level (11°10'–11°30'N latitude and 78°15'–78°30' E longitude) in the Namakkal district of Tamil Nadu state, South India. Kolli hill has an area of 282.92 sq. km (Kumaran *et al.,* 1998). The Kolli Mountains are covered with evergreen forest and are known for its rich biodiversity.

Kolli hill enjoys a salubrious climate throughout the year. This is fertile pocket in Namakkal district where exotic tropical fruits and medicinal plants grow in plenty. Annual rainfall is 1324 mm, which is received largely between May and December (Meteorological report, 1970 - 1999). Annual mean temperatures of maximum and minimum are 35°C and 18°C respectively (Harikrishnan, 1977). The type of soil is red loamy and black soil. The Kolli is considered to be one of the richest biodiversity. The objective of the present study is to isolate bacteria from soil in various places of Kolli hills and biochemically characterize the microorganisms and to assay the lipase production.

Materials and Methods

Sample collection site

Soil samples were collected in Kolli hills. Eight different soil sites were selected and the entire samples were transferred to the laboratory and stored less than 4°C.

Isolation of Lipolytic Bacteria

Dilution plate method was performed for isolation of lipolytic bacteria from collected soil sample. One gm of soil samples were transferred to 10 ml of 0.85% sterile saline water. Serial dilution was performed by transferring one ml of aliquot from each of the samples to 9 ml of 0.85% saline water upto 10^{-6} dilutions were prepared. From the diluted samples 0.1 ml of 10^{-4} and 10^{-5} was plated on nutrient agar and nutrient agar supplemented with Tween 80. Plates were incubated for 48-72 h at 37°C. Colonies with zone of clearance was picked and stored in to sterile nutrient slant for further studies (Mobarak-Qamsari *et al.,* 2011).

Lipase production medium:

The production medium consist of (%w/v) Peptone 0.2, $NH_4H_2PO_4$ 0.1; NaCl 0.25; $MgSO_4.7H_2O$ 0.04; $CaCl_2.2H_2O$ 0.04; Olive oil 2.0 (v/v); pH 7.0; 1-2 drops of Tween 80 as emulsifier. (Mobarak-Qamsari *et al.,* 2011). Overnight cultures were inoculated into the 250ml Erlenmeyer flasks containing 100ml media and were kept in rotary shaker for 150rpm. Sample were collected after 24 hours and centrifuged at 10,000 rpm for 10mins at 4°C. The cell filtrate was used as a source of extracellular enzyme lipase (Aliyu *et al.,* 2011).

Assay for lipase activity

The activity of lipase was demonstrated by using spectrophotometrically at 30°C by using *p*-nitrophenol palmitate (pNPP) as a substrate (Winkler and Stuckmann, 1979).

The composition of reaction mixture was 700 µl *p*NPP solution and 300 µl of lipase solution. The *p*NPP solution was prepared by adding the solution A (0.001 g *p*NPP in one ml isopropanol) into solution B (0.01 g gum arabic, 0.02 g Sodium deoxycholate, 50 µl Triton X-100 and 9 ml of 50 mM Tris-HCl buffer, pH 8) with stirring until it was dissolved. The absorbance was measured at 410 nm for the first 2 min of reaction. *Enterococcus faecium* MTCC 5695 was used as a standard for lipolytic bacteria. One unit was defined as that amount of enzyme that liberated 1µmol of *p*NP per minute (ε:1500*l*/mol cm) under the test conditions (Karadzic *et al.,* 2006).

Molecular characterization and identification of the Bacteria

The taxonomical characteristics of the bacteria were determined by conventional biochemical test methods (Sneath *et al.,* 1986; Holt *et al.,* 1994; Osterhout *et. al.,* 1998; MacFaddin, 2000). The isolated culture which showed maximum productivity were selected and characterized based on Bergey's manual of systemic bacteriology and by using 16s rDNA analysis. The genomic DNA was extracted by Hosek *et al*., (2006). Then the extracted DNA was then amplified by 16s rRNA specific primer 8F: 5′-AGAGTTTGATCCTGGCTCAG - 3′ 1492R: 5′-ACGGGCGGTGTGTAC-3′. Then the amplified product was sequenced in automated gene sequencer ABI Prism.

Results and Discussion

Screening of isolate for Lipase activity

Lipase producing microbial culture were isolated from different sites of Kolli hills by serially diluting the samples and plated in Tween 80 plate. Lipolytic bacteria were isolated from the plate and enriched in Nutrient broth by periodic sub culturing. Fifty two lipase producers gave positive results in plates showing lipolytic zone. Twenty different bacterial strains were screened which are capable of producing lipolytic zone. The twenty isolates have been given individual strain code for identification (KPL1, KPL2… KPL20) and were used for further studies.

Assay for lipase activity

Even though 20 isolates are capable of producing lipase. Further these isolates were subjected for lipase production along with the standard lipolytic bacteria *Enterococcus faecium* MTCC 5659 in lipase production medium. This *E. faecium* MTCC 5659 shows a maximum lipase activity of 4.280 U/ml (Fig.1). Among the 20 isolates six isolates (KPL1, KPL7, KPL8, KPL9, KPL13 and KPL20) are capable of producing lipase above 3 U/ml in 24hrs of incubation. Based on this production rate six isolates were selected for further studies. Interestingly isolates KPL8 and KPL9 showed maximum activity than the standard *E. faceium* MTCC5659.

Molecular characterization and identification of the Bacteria

After the microscopic examination and biochemical tests the six isolates which has produced maximum lipase is characterized and was identified as *Bacillus tequilensis, Bacillus subtilis, Pseudomonas* sp, *Bacillus subtilis* and *Bacillus flexus* respectively. Akanbi *et al*., (2010) has identified and reported lipase producing *Bacillus cereus* using 16s rRNA sequence analysis. The sequences were submitted in NCBI BLASTN and analyzed

Table.1 Biochemical characterization of six isolates from the soil samples of Kolli Hills

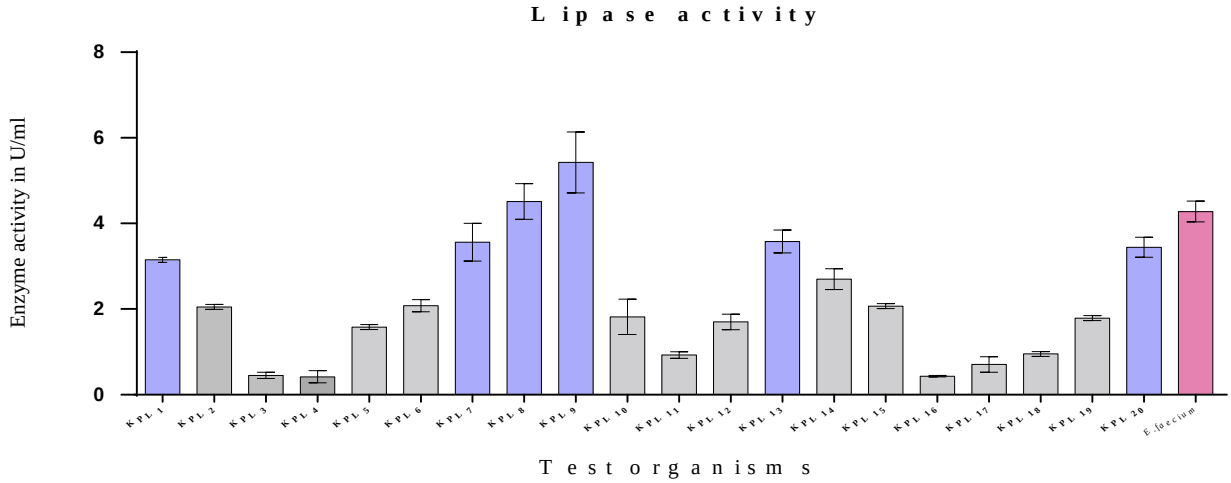
Tests	KPL 1	KPL 7	KPL 8	KPL 9	KPL 13	KPL 20
Grams test	+ Rod	+ Rod	-	+ Rod	+ Rod	+ Rod
Motility	+	+	+	+	+	+
Indole	+	-	-	-	-	-
MR	+	-	-	+	+	-
VP	+	+	-	+	+	-
Citrate	+	-	-	+	+	+
Catalase	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+
Sucrose	+	+		+	+	+
Maltose	+	+	+	+	+	+
Lactose	+	+	+	-	+	+
Fructose	+	-	+	+	+	+
Spore	Central	Central	-	Central	Central	Central
Urease	+	+	+	+	+	+

+ Positive reaction ; - Not detected

Table.2 Selected six isolated organism from the Kolli hill soil

Isolate	Strain code	Organism	NCBI Accession Number
1	KPL 1	*Bacillus tequilensis*	KC822925
7	KPL 7	*Bacillus subtilis*	KC822926
8	KPL 8	*Pseudomonas* sp.	KC823232
9	KPL 9	*Bacillus subtilis*	KC822927
13	KPL 13	*Bacillus subtilis*	KC823231
20	KPL 20	*Bacillus flexus*	KC849389

Figure.1 Lipase assay for 20 different isolate along with control *Enterococcus faecium* MTCC 5659.



for the bacterial class and species with the other sequences. Then it is submitted and accession numbers have been allotted to all the isolates. The details are shows in Table 1 and 2.

From these studies it is very clear that *Bacillus* sp is more predominant in the environmental soil samples of Kolli hills which contribute the production of lipase. Today most of the country relies on microbial enzymes for commercial exploitation. Lipase have diverse role in day to day life. For example lipase are employed in various industries like detergent, dairy foods, beverages, health foods, fats, oils, paper, pharmaceuticals, bakery foods and cosmetics. These isolates can be used for further studies and the gene which is responsible for lipase production can be identified, isolated and cloned in expression vector and can get increased production of lipase.

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