# ISOLATION, SCREENING, AND CHARACTERIZATION OF LIPOLYTIC BACTERIA & APPLICATION

**Ajaj Ahmed*[1], Gautam Kumar Meghwanshi*[2]**

[1]Ph.D Scholar, Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan
[2]Assistant Professor, Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan.

**Abstract: Lipase from bacterial culture LB11 , LB9, and LB 4 were isolated from soil and dairy effluent located in Bikaner regions. Isolated bacterial culture LB11 , LB9, and LB 4 showed maximum lipase activity on TBA (Tributyrin Agar) medium.Total of 30 isolated bacterial cultures were examined for lipase activity on TBA medium. Result showed that out of 30 bacterial isolated LB11 , LB9, and LB 4 produced good lipase activity in the Production medium. But LB11 isolated Bacterial culture was maximum lipase activity. The lipase was analyzed for Biodegradation of 2T oil (Two-stroke oil) in the presence of LB11 isolated bacterial culture more effectively as compared to other bacterial isolated LB9 and LB4.**

**Keywords:- Lipase, Effectively, Production, Activity, 2T-oil**

## Introduction

Lipase ( Triacylglycerol acyl hydrolase EC (3.1.1.3) is an enzyme group which is capable hydrolyzing triglycerides, diglycerides and monoglycerides into fatty acid and glycerol under aqueous environment but can carry out reverse reaction of synthesis under microorganism aqueous environment. Lipase is ubiquitous enzyme which is found in animals, plants, fungi and Microorganisms. Microbial lipases production has increased for the past one decade, because of its potential application in industries . From these studies it is very clear the *Bacillus* sp.,(Priyanka et al.,2019; Bharathi and Rajalakshmi,2019). is more predominant in the environmental soil samples of Bikaner. Lipase have diverse applications For example lipases are employed in various industries like detergent, dairy, food, beverage, health and biodiesel production, oleo chemical, food& flavor, pharmaceutical, agrochemical, pulp and paper, polymer synthesis and detergent aid (Bharathi and Rajalakshmi,2019; Martinez-Ruis et al.,2018;Navvabi et al.,2018) lack of lipase studies from the desert regions, especially Bikaner,The present investigations was designed, wherein soil samples collected from Bikaner region were investigated. The bacterial lipases obtained in the present study can further be cloned in a suitable vector and its production can be enhanced to meet the industrial demand. The treatment of hazardous petroleum wastes heavily relies on biodegradation.The use of bioprocesses to treat hazardous wastes is a technology that shows promise because it is both affordable and capable of completely eliminating the danger and mineralizing it.Nature uses biodegradation to recycle wastes, break down organic matter into nutrients that can be used by other species, or change it into compounds that are less hazardous or non-toxic. Using these innate biodegradational factors, certain environmental pollutants can be diminished or entirely eliminated (Samir Eskander and Hosam Saleh,2017) However, it may be technically challenging to use microorganisms directly in the biodegradation of oil-polluted areas as a result, the necessity to concentrate on microbial enzymes is taken into account as an alternative (Kareem et al., 2017) . This study aimed to identify suitable lipase-producing microorganisms, optimise the conditions for lipase production, and use lipase from *Providencia stuartii* to biodegrade petroleum hydrocarbons.

## Materials and Methods

**(1) Media and reagents**: - Composition of Tributyrin Agar Medium (TBA) (Composition g/l: Peptone- 5g, Yeast extract – 3.0, Nacl-5.0, tributyrin-10.0, Agar-Agar Powder- 20.0, pH-7.0±0.2, Double distilled water to make the final volume to 1000 ml), Nutrient Broth (NB)( Composition g/l: Peptone- 5g, Yeast extract – 3.0, Nacl-5.0, pH-7.0±0.2, Double distilled water to make the final volume to 1000 ml), Nutrient agar(NA) was prepared by adding agar-agar at a concentration of 1.5% to Nutrient broth (NB). Lipase production medium (Composition g/l:-Peptone -10.0, Yeast extract -5.0, Sodium Sulphate – 2.0 , $KH_2PO_4$ – 1.0, $K_2HPO_4$- 3.0, $MgSO_4.7H_2O$- 0.1, Dextrose- 2.0, Olive oil -5.0, pH- 7.0, Double distilled water to make the final volume to 1000 ml)

**(2) Sample collection**: - Soil samples were taken from different locations in Bikaner county. Soil samples were taken with a drill to excavate to a depth of 5 inches and transferred the soil samples into sterile plastic zipper bags and brought to the laboratory of the Department of Microbiology of M.G.S University, Bikaner for further processing.

**(3) Isolation and Screening of Lipolytic Bacteria**:- - The screening and isolation of dissociated bacteria was performed on TBA plate (Tributryin agar) (Lawrence et al.,1967) by Pour plate technique. Serial dilutions were made to $10^{-7}$ and 100 µl of the diluted sample from the dilutions marked $10^{-3}$, $10^{-5}$, $10^{-6}$ were plated on TBA plates. Plates were incubated at 37°C for 24 h. The isolates were then cultured on TBA plates using the quadrant striped plate method to obtain pure cultures. Pure isolates were maintained on nutrient agar slants stored at 4ºC for further studies.

**(4) Qualitative screening** :- Perform qualitative screening on TBA medium. Bacterial strains were isolated on TBA plates. Striped TBA plates were incubated at 37 °C for 24 h and then the hydrolyzed zone was observed.

**(5) Culture and Biochemical characterization of the selected Bacterial isolate:-** The selected bacterial strain was characterized for morphological and biochemical properties to assign it a provisional genus.

**(6) Production of lipase and its application:- (a) Production of seed culture :-** The isolate bacterial culture was inoculated into an Erlenmeyer flask (250 ml capacity) containing 50 ml of nutrient broth. Inoculated NB flasks were incubated at 37 °C and 120 rpm for 24 h. These 24-hour-old explants were used as seed culture medium or as inoculum for inoculating lipase-producing media.
**(b) Inoculation of Production Medium:-** One percent (1%) of the seed culture was inoculated into lipase-producing medium contained in a 250 ml Erlenmeyer flask. Inoculated flasks were incubated at 37°C and 150 rpm for 24 h.
**(c) Biomass removal and enzyme recovery by centrifugation:-** The medium was incubated in a rotary shaker at 200 rpm and 37ºC for 24 h. Cells were removed by centrifugation at 10,000 rpm for 10 min and the supernatant was collected to determine lipase activity.
**(d) Application of lipase in biodegrdation of 2T oil (Two stroke oil):-** Lipase being a hydrolase has the ability to biodegrade of 2T oil from hydrocarbon medium. The extracellular lipase from the selected bacterial isolate was therefore used for biodegrade oil.
**Observation and Results :-**
**1.Sample collection**:- Soil samples were taken from different locations in Bikaner district as shown in **Table 1**. Some sample collection sites are shown in **Fig 1.**

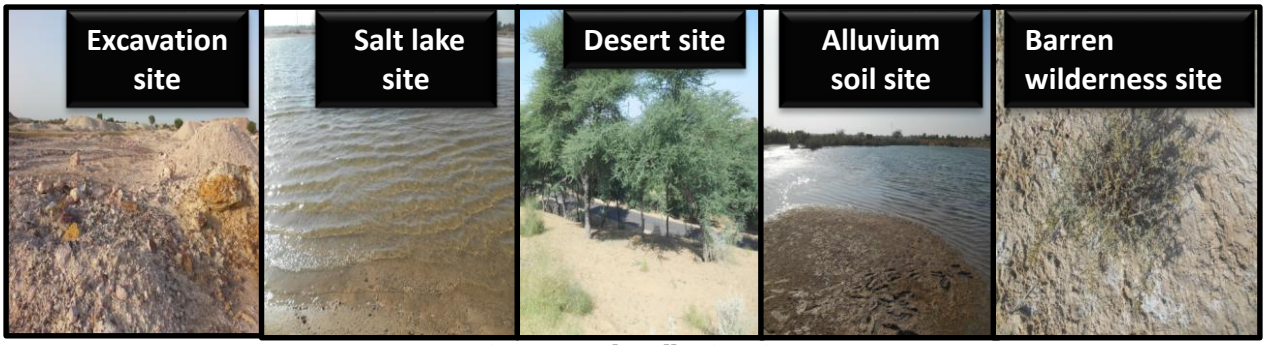


**Fig:-1 Sample collection sites**

**2. Screening** :- A total of 30 bacterial isolates were tested for lipase activity on TBA medium (Tributyrin agar) **(Table 1).** The results showed that among the 30 bacterial isolates, LB11, LB9 and LB4 produced good lipase activity in lipase-producing media **Table 1.** Prominent and stable was the 2.1 cm hydrolysis region by lipase enzyme in 24 h incubation on TBA plate and temperature at 37ºC **(Figure 2).** On the other hand, other cultured bacterial isolates show negligible or incomplete hydrolysis area on TBA medium. But the culture of the LB11 isolate was more stable than that of other isolates (LB9,LB4) While LB11 was selected for further study and outstanding application.

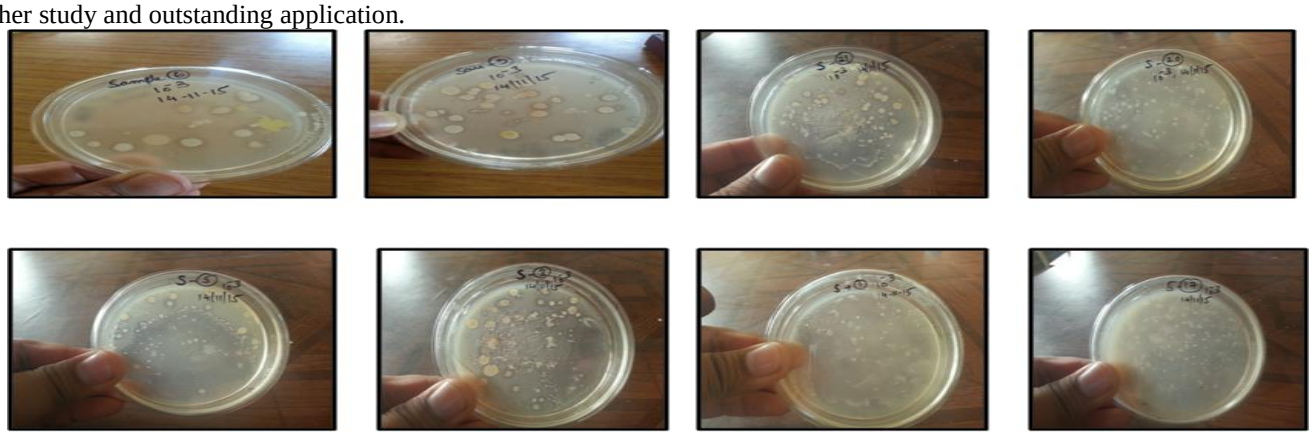

**Fig:-2 Qualitative screening on TBA medium (Pour plate method)**


**Fig:-3 Bacterial cultures Isolated on Rotary flask shaker& maximum lipase production (LB11)**

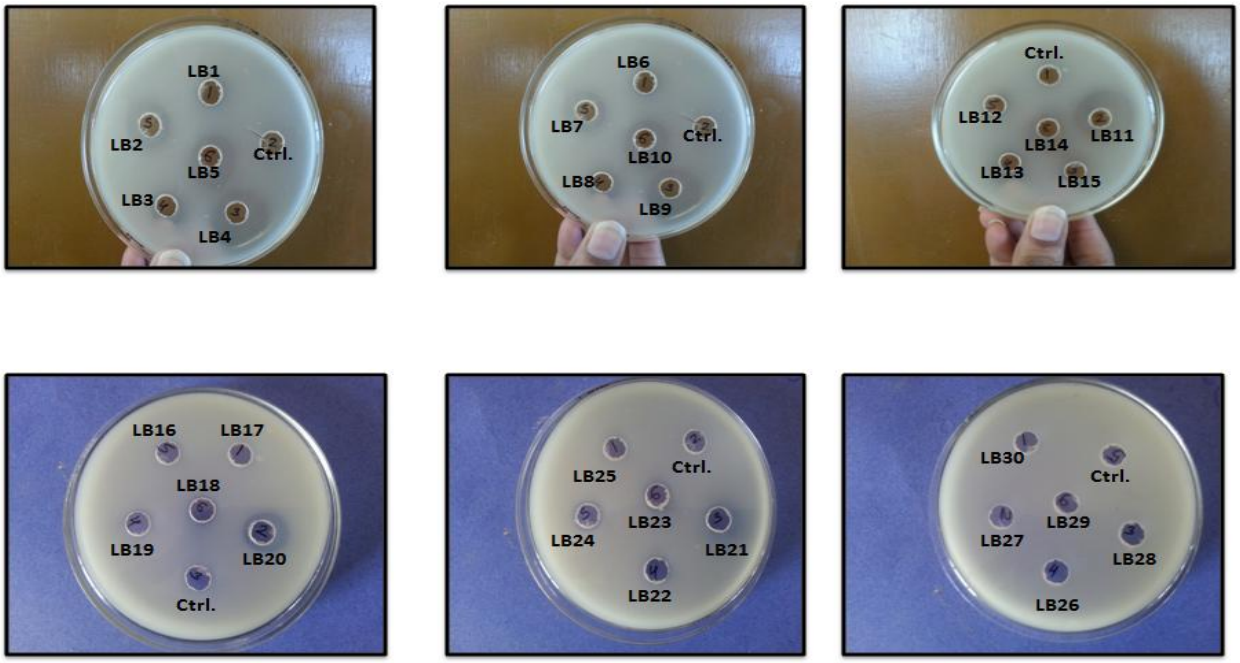


**Fig 4:- Qualitative Screening: - Agar diffusion assay of lipase enzyme (LB4,LB9,LB11) And other Isolated bacterial culture on TBA medium**

| Sr.No. | Bacterial isolate | Site of collection (Bikaner district) | Lipase status | Sample type |
|---|---|---|---|---|
| 1 | LB1 | MGSU Campus | ++ | Sand |
| 2 | LB2 | Gajner | + | Sand |
| 3 | LB3 | Techri phanta | ++ | Sand |
| 4 | **LB4** | **Barren site** | **++++++** | **Sand** |
| 5 | LB5 | Local chaipati | + | Sand |
| 6 | LB6 | udasar | ++ | Sand |
| 7 | LB7 | Gangasaher | ++ | Sand |
| 8 | LB8 | Naal village | + | Sand |
| 9 | **LB9** | **Excavation site** | **+++++++** | **Sand** |
| 10 | LB10 | Bholasar | ++ | Sand |
| 11 | **LB11** | **Dairy effluent/industry** | **++++++++++** | **waste dairy water** |
| 12 | LB12 | Lakhusar | + | Sand |
| 13 | LB13 | Satasar | ++ | Sand |
| 14 | LB14 | Chattargarh | ++ | Sand |
| 15 | LB15 | Mahajan | ++ | Sand |
| 16 | LB16 | Jamsar | + | Sand |
| 17 | LB17 | kalu village | - | Sand |
| 18 | LB18 | Satasar | + | Sand |
| 19 | LB19 | Naal Badi | + | Sand |
| 20 | LB20 | Sheethal Village | + | water |
| 21 | LB21 | Ridmalsar | + | Sand |
| 22 | LB22 | Sagar village | - | Sand |
| 23 | LB23 | Gusainsar | + | Sand |
| 24 | LB24 | Napasar | - | Sand |
| 25 | LB25 | Naurnagdesar | + | Sand |
| 26 | LB26 | Murlidhar colony | - | water |
| 27 | LB27 | Jaimalsar | + | Sand |
| 28 | LB28 | Lunkarnsar village | - | Sand |
| 29 | LB29 | Akhasar village | - | Sand |
| 30 | LB30 | Bacchasar | + | Sand |

**Table 1:- Results of Qualitative Screening of Bacterial Cultures Isolated from the Bikaner regions**

**3. Culture and Biochemical characterization of LB11Bacterial isolates:-** The bacterial culture was characterized for morphological & biochemical features as presented in T**able 2** and **Fig 5.** On the basis of culture and biochemical characteristics analysis the selected bacterial isolate was identified as a strain of *Bacillus* sp. production of lipase by bacterial species *Bacillus cerus C7* (Dutta et al.,2009), *Bacillus alcalophilus* (Ghanem et al.,2000) *Bacillus pumilus* (Ismael et al.,2013) *Bacillus thermoleovorans ID-1* (Cho et al.,2000).

**Table 2:- Morhological and biochemical characterization of LB11 bacterial isolate**

(A) Morphological Characterization

| Sample | Morphology on TBA medium | Growth in broth | Grams test | Sporulation | Pigment Production | Cell shape |
|---|---|---|---|---|---|---|
| LB11 | Circular, Creamy, white, irrregular | Normal | + | + | - | Short Rod shaped |

(b) Biochemical characterization

| Biochemical test | Sample (LB11) |
|---|---|
| Catalase | + |
| Lactose | + |
| Indole | - |
| MR | - |
| VP | + |
| Motility | - |
| Sporulation | + |
| Urease | + |

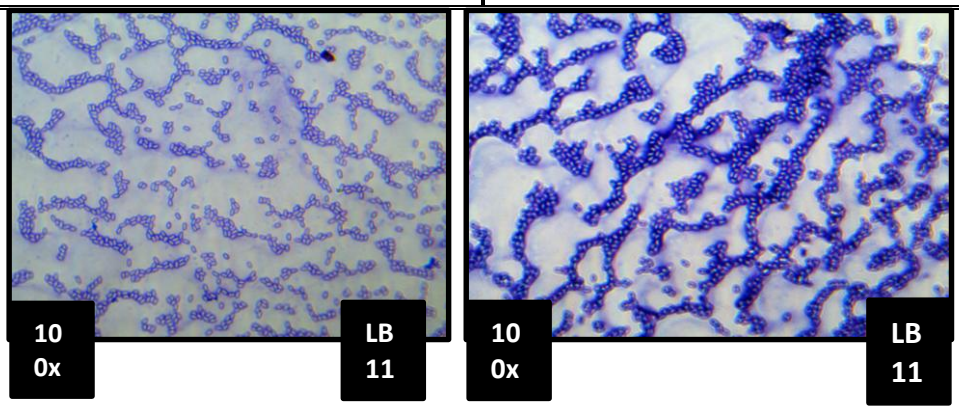


**Fig 5:- Gram & endospore stained of selected bacterial isolated culture (LB11)**

**4.Application of lipase in biodegrdation of 2T oil :-** Microorganisms that break down hydrocarbons are widely present in soil, freshwater, and marine habitats. It is frequently assumed that the capacity to isolate specific oil-degrading microorganisms from an environment indicates that such organisms are the active degraders of the environment's contents. The biodegradation/bioremediation of hydrocarbon pollutants has been the subject of numerous scientific projects in recent years.The destruction of hydrocarbons has been attributed to bacteria belonging to the genera *Achromobacter, Acinetobacter, Arthrobacter, Azoarcus, Brevibacterium, Cellulomonas, Corynebacterium, Flavobacterium, Marinobacter, Micrococcus, Nocardia, Ochrobactrum, Pseudomonas, Stenotrophomaonas, and Vibrio* (Varjani et al.,2017) Only a few number of thermophilic bacteria, mostly *Bacillaceae* species like *Geobacillus pallidus* and *thermodenitrificans* (Sakthipriya et al.,2015; Zheng et al.,2011; Wang et al.,2006; Elumalai et al., 2019) have been identified and adapted to biodegradation.

**Result and analysis :-** The result of biodegradation of 2T oil analysis as observed from Fig 6.show that the lipase from bacterial isolates LB11 culture completely biodegrade oil from hydrocarbon medium**.** 1 ml of broth culture of LB11(Isolated bacterial culture) + 2 ml 2T oil inoculate in hydrocarbon media **(Table3)** with the help of sterlize micropipette and keep at incubated rotary shaker for 120 h.,Temperature 45ºC and rpm 200 after 120h observed the results. The results are presented below in **(Fig 6).** After 120h. Isolated bacterial culture (LB11) utilised 2T oil as a carbon source.

**Table 3:- Compostition of Hydrocarbon medium**

| Component | gm/ltr. |
|---|---|
| Sodium chloride | 154 |
| Di-potassium hydrogen phosphate | 4.84 |
| Potassium hydrogen phosphate | 1.606 |
| Ammonium Sulphate | 2.2 |
| Magnesium sulphate heptahydrate | 0.44 |
| pH | 7.0 |
| Distilled water | 1000 ml |

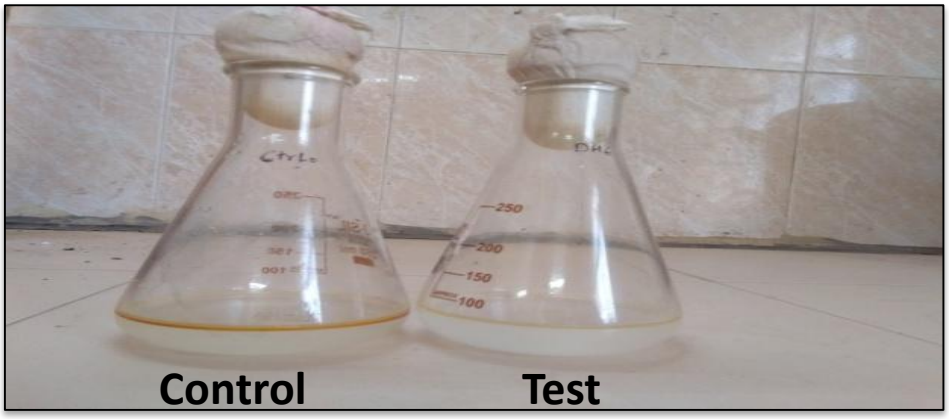


**Fig 6:- Biodegradation of 2T oil by *Bacillus* sp.LB11 Isolated from dairy effluent**

**Conclusion** :- This research resulted in the lipase enzyme.Lipases are ubiquitous enzymes that can degrade fat into free fatty acids and glycerol.This method is environmentally beneficial.

Furthermore, this enzyme is appropriate for biodegradation of 2T oil. (LB11) isolates bacterial culture was more stable than other isolates bacterial culture. Because LB11 showed good lipase activity on TBA agar medium. This bacterial culture isolates (LB11) will be best for further studies.

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**Conflict of interest : None**

**Financial Support** : **None**

**Supplmentary data :** E- supplementary data for this work can be found in e-version of this paper online

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