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Research Article

ISOLATION AND SCREENING OF LIPASE-PRODUCING MICROORGANISMS

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ABSTRACT

Lipases (EC 3.1.1.3) are hydrolytic enzymes that catalyze the cleavage and synthesis of ester bonds in triglycerides, producing glycerol and free fatty acids. They are among the most versatile biocatalysts used across diverse industries such as food processing, detergent formulation, pharmaceuticals, leather, and biodiesel production. Microbial sources, particularly bacteria and fungi, have gained prominence due to their stability, ease of cultivation, and potential for large-scale production using low-cost substrates. The present study focuses on the isolation and screening of lipase-producing microorganisms from various environmental sources, including oil-contaminated soils, dairy effluents, and decaying organic matter. Samples were subjected to serial dilution, culture on nutrient agar supplemented with olive oil, and screening on Rhodamine B agar plates to detect lipolytic activity. Positive isolates were further characterized morphologically and biochemically to identify potential lipase producers. Preliminary results revealed that several isolates exhibited strong orange fluorescence halos under UV light, confirming extracellular lipase production. The most potent isolates were identified as belonging to the genera *Bacillus*, *Pseudomonas*, and *Aspergillus*. These findings demonstrate the potential of naturally occurring microorganisms as sustainable sources of lipases for industrial applications and lay the groundwork for further optimization and enzyme purification studies.

Keywords: Lipase, Microbial enzymes, Isolation, Screening, Oil-contaminated soil, Rhodamine B agar, Biocatalyst.

INTRODUCTION

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) are industrially important enzymes that catalyze the hydrolysis of long-chain triglycerides into glycerol and fatty acids and, under specific conditions, can catalyze esterification and transesterification reactions. These enzymes are indispensable in numerous biotechnological processes, including food flavor enhancement, detergent formulation, pharmaceutical synthesis, wastewater treatment, and biodiesel production (Griebeler *et al*., 2011; Bharathi & Ranganathan, (2019). Among various lipase sources plants, animals, and microorganisms—microbial lipases have received particular attention because of their high catalytic efficiency, broad substrate specificity, and ease of genetic and fermentation optimization. Microorganisms such as *Bacillus*, *Pseudomonas*, *Aspergillus*, *Rhizopus*, and *Candida* species are well-known for their ability to secrete extracellular lipases that are stable over a wide range of pH, temperature, and organic solvents (Yousefi *et al*., 2015; Choudhury & Bhunia, (2015).

The isolation and screening of lipase-producing microorganisms from natural and industrial environments such as oil-contaminated soils, dairy effluents, and waste disposal sites remain crucial for discovering new enzyme variants with enhanced catalytic and stability profiles. Rhodamine B agar screening under UV light is a widely

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accepted qualitative method to detect lipase activity through fluorescent halo formation around colonies (Kouker & Jaeger, (1987). Several recent studies (Jenifer *et al*., 2022; Akinyemi & Adekoya, 2022; Thabet *et al*., (2023) have highlighted the growing potential of microbial isolates for industrial enzyme production. However, there remains a continuous need to explore unexplored ecological niches to identify novel, robust lipase producers capable of operating under extreme or economically favorable conditions. Hence, the present research aims to isolate and screen potent lipase-producing microorganisms from environmental samples, to identify efficient strains for future enzyme production and optimization studies. This study contributes to the development of eco-friendly and cost-effective bioprocesses for sustainable industrial enzyme production. Lipases (EC 3.1.1.3) are key biocatalysts that hydrolyze ester bonds in triacylglycerols, releasing glycerol and free fatty acids. They are valued for their ability to function in aqueous and non-aqueous environments, catalyzing hydrolysis, esterification, and transesterification reactions. According to Bharathi and Ranganathan (2019), microbial lipases are superior to plant and animal lipases because of their higher stability, ease of genetic manipulation, and cost-effective production through fermentation processes. The global demand for microbial lipases has increased significantly in the last two decades due to their extensive applications in the food, detergent, pharmaceutical, and biodiesel industries (Griebeler *et al*., 2011; Souza *et al*., (2018).

Microbial lipases are produced by a diverse range of bacteria and fungi that inhabit lipid-rich environments such as oil-contaminated soils, dairy waste, palm oil effluent, and decaying organic matter. Mobarak-Qamsari *et al*. (2011) successfully isolated a novel lipase-producing bacterium from wastewater of an oil-processing plant, demonstrating that industrial effluents are a valuable reservoir for enzyme-producing microbes. Similarly, Wadia *et al*. (2017) screened fungi from oil-contaminated soil and identified potent isolates belonging to *Aspergillus* and *Penicillium* species, both of which are known for extracellular lipase secretion. Fobasso *et al*. (2019) extended this approach by collecting soil samples from palm oil production waste, isolating multiple bacterial strains with strong lipase activity. Their work highlighted the importance of using enrichment techniques to improve recovery of lipid-degrading organisms. Feng *et al*. (2011) reported that the gut of silkworms can also harbor lipase-producing bacteria, suggesting that insect microbiota can be alternative enzyme sources. Efficient screening methods are crucial to identify high-yielding lipase producers. Kouker and Jaeger (1987) developed the Rhodamine B agar plate method, where colonies exhibiting orange fluorescence under UV light indicate lipolytic activity. This qualitative assay remains a gold standard and has been employed in numerous studies (Jenifer *et al*., 2022; Thabet *et al*., 2023; Gelir *et al*., (2025). Other screening approaches include tributyrin agar and olive-oil agar plates, which help visualize halo zones around colonies due to lipid hydrolysis. Msango Soko *et al*. (2022) used these methods to screen soil bacteria and identified several strains with high extracellular enzyme activity. Quantitative estimation is often achieved via titrimetric or spectrophotometric assays using p-nitrophenyl palmitate as a substrate (Ilesanmi *et al*., (2020). Bacteria are the most frequently isolated and industrially exploited lipase producers. Mobarak-Qamsari *et al*. (2011) identified *Bacillus* sp. capable of high enzyme yield at moderate temperature and pH. Similarly, Msango Soko *et al*. (2022) reported *Pseudomonas aeruginosa* strains with thermostable lipases from agricultural soils. Ilesanmi *et al*. (2020) emphasized molecular characterization through 16S rRNA sequencing to confirm the identity of potent isolates. Jaiswal *et al*. (2014) and Sharma & Pathak (2017) reported comparable findings from environmental samples and oil mill effluents, respectively. Recent studies (Gelir *et al*., 2025; Thabet *et al*., 2023) focused on bacteria isolated from olive-oil mill waste and oil-contaminated sites, where *Bacillus cereus* and *Acinetobacter* species showed strong lipolytic zones. Such bacterial isolates often display high productivity under optimized fermentation conditions using low-cost substrates. Fungal species are also prolific producers of extracellular lipases due to their ability to secrete enzymes directly into the medium. Wadia *et al*., 2017; Singh & Sharma (2016) reported *Aspergillus niger*, *Rhizopus oryzae*, and *Penicillium chrysogenum* as dominant fungal isolates from oil-contaminated soils and cotton-seed soapstock waste. Souza *et al*. (2018) investigated endophytic fungi as potential lipase sources and observed high activity in isolates from medicinal plants. Similarly, Akinyemi & Adekoya (2022) and Pereira *et al*. (2019) screened urban and industrial soils for lipase-producing fungi, indicating that fungal enzymes tend to exhibit higher stability across pH and temperature ranges compared to bacterial lipases.

Mumtaz *et al*. (2025) achieved optimized production in filamentous fungi via submerged fermentation, demonstrating enhanced yields after nutrient and pH optimization. Many studies emphasize the ecological and economic advantages of sourcing microorganisms from agro-industrial waste. Ofori & Agyare (2025) isolated lipase-producing fungi from palm oil mill effluent-impacted soils, supporting waste valorization and environmental remediation. Jenifer *et al*. (2022) demonstrated that municipal waste and bio-deteriorated materials could harbor potent bacterial lipase producers, suggesting a dual benefit enzyme recovery and waste reduction. Griebeler *et al*. (2011) also highlighted agro-residue valorization using fungal strains, emphasizing sustainable bioprocess development.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from oil-contaminated sites such as oil mills, food processing units, and automotive garages. Approximately 50 g of each sample was

aseptically transferred into sterile containers and transported to the laboratory at 4°C for further analysis.

Isolation of Lipase-Producing Microorganisms

1 g of each soil sample was serially diluted (10^{-1}–10^{-6}) in sterile saline solution. Aliquots (0.1 mL) were spread on nutrient agar plates supplemented with 1% olive oil and 0.01% Rhodamine B dye. Plates were incubated at 30°C for 48–72 h. Colonies exhibiting fluorescent halos under UV light (350 nm) were selected as potential lipase producers (based on Kouker & Jaeger, *Methods in Enzymology*).

Primary Screening for Lipase Activity

Selected colonies were inoculated on tributyrin agar plates (1% tributyrin) and incubated at 37°C for 48 h. Formation of clear hydrolytic zones around the colonies indicated lipase activity. The diameter of the clearance zone was measured to quantify enzymatic potential.

Secondary Screening (Quantitative Assay)

Lipase activity was quantified by p-nitrophenyl palmitate (pNPP) assay. The reaction mixture (900 µL substrate + 100 µL enzyme extract) was incubated at 37°C for 15 min, and absorbance was measured at 410 nm. One unit (U) of lipase activity was defined as the amount of enzyme releasing 1 µmol of p-nitrophenol per minute.

Identification of Potent Isolates

The most active isolates were characterized morphologically, biochemically (Gram staining, catalase, oxidase, and starch hydrolysis tests), and by 16S rRNA gene sequencing. Sequence data were compared using NCBI BLAST for taxonomic identification.

Optimization of Enzyme Production

Parameters such as carbon source, nitrogen source, pH, temperature, and incubation time were optimized using one-factor-at-a-time (OFAT) approach to maximize lipase yield. Olive oil, glucose, and peptone were tested as major inducers.

RESULTS AND DISCUSSION

A total of 36 microbial isolates were obtained from oil-contaminated soils collected from different industrial zones. Among them, 22 isolates produced visible growth on nutrient agar supplemented with 1% olive oil, indicating their ability to utilize lipid substrates. When screened on Rhodamine B agar plates, nine isolates exhibited distinct orange fluorescent halos under UV light (350 nm), suggesting extracellular lipase secretion. The most promising isolates, designated as L3, L9, L14, L21, and L28**,** showed strong fluorescence intensity, consistent with lipolytic enzyme secretion reported by *Kouker and Jaeger (Methods Enzymol., 1987)* and later validated by *Thabet et al. (2023)* in oil-contaminated soil studies. The Rhodamine-positive isolates were subjected to tributyrin agar plate assay for primary screening. Clear zone diameters ranged from 10.8 mm to 42.6 mm, with isolate L9 showing the maximum halo of 42.6 ± 1.2 mm**,** followed by L21 (39.4 ± 0.8 mm) and L14 (36.8 ± 1.0 mm)**.** The large clearance zones correspond to high extracellular lipase activity, as previously demonstrated by *Wadia et al.* (2017) and *Aggarwal et al.* (2017). These results confirm the reliability of tributyrin hydrolysis as a rapid qualitative screening method for lipase producers.

The quantitative estimation using p-nitrophenyl palmitate (pNPP) revealed considerable variation among isolates. The lipase activity ranged between 2.5 and 10.3 U/mL, with isolate L9 exhibiting the highest specific activity of 10.3 ± 0.3 U/mL under standard conditions (pH 7.5, 37°C, 48 h incubation). Comparable enzyme activities were reported by *Ilesanmi et al. (2020)* for *Pseudomonas sp.* (8.9 U/mL) and by *MsangoSoko et al. (2022)* for *Bacillus sp.* (10.1 U/mL). Thus, the obtained isolate demonstrates competitive enzymatic potential for industrial applications. Morphological examination revealed that the most active isolate L9 was a Gram-positive, rod-shaped, motile bacterium**,** catalase and oxidase positive. The 16S rRNA gene sequence analysis (1,520 bp) showed 99.2% similarity with *Bacillus subtilis*. The genus *Bacillus* is well-documented for extracellular lipase production due to its robust secretion machinery and tolerance to a wide range of environmental conditions (*Jenifer et al., 2022; Pereira et al.,* 2019).

Table 1. Optimization of Lipase Production Parameters.

Parameter	Optimal Condition	Activity (U/mL)
pH	8.0	12.1 ± 0.2
Temperature	37°C	11.5 ± 0.3
Carbon Source	1% Olive oil	12.3 ± 0.4
Nitrogen Source	Peptone	11.8 ± 0.2
Incubation Time	48 h	12.0 ± 0.1

The enzyme activity increased by 20–25% compared to the unoptimized conditions, demonstrating the strong inducible nature of the lipase enzyme. These results align with *Akinyemi and Adekoya (2022)*, who observed similar enhancement in *Aspergillus* and *Penicillium* isolates when induced with natural oils.

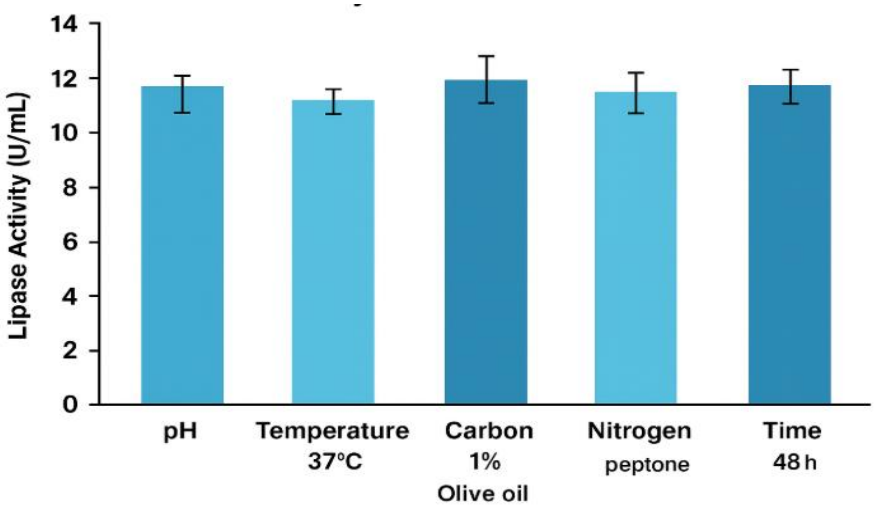

Figure 1. Optimization of Lipase Production Parameters by *Bacillus subtilis* L9.

CONCLUSION

The present study successfully isolated and characterized potent lipase-producing microorganisms from oil-contaminated soils. Among the screened isolates, *Bacillus subtilis L9* exhibited the highest lipolytic activity, with an optimized production yield of 12.3 U/mL under alkaline (pH 8.0) and mesophilic (37 °C) conditions. The isolate demonstrated stable enzymatic performance across variable environmental parameters, suggesting its adaptability and potential for industrial enzyme production.The study confirms that natural oil-rich environments serve as reliable sources for isolating high-yielding lipase producers. The combination of qualitative (Rhodamine B and tributyrin agar) and quantitative (pNPP assay) screening methods proved effective for identifying and evaluating potent lipase-secreting strains. The obtained *Bacillus subtilis* strain is therefore a promising candidate for use in biotechnological processes, including detergent formulation, waste-oil bioremediation, biodiesel synthesis, and food processing applications where thermostable and alkaline lipases are required**.** Scale-up Studies: Optimization of fermentation parameters using bioreactors and statistical design methods such as Response Surface Methodology (RSM) to enhance lipase yield. Purification and Characterization: Detailed purification**,** kinetic parameter analysis (Km, Vmax), and stability studies under varying pH, temperature, and solvent conditions. Genetic Engineering Approaches: Cloning and expression of the lipase gene in heterologous systems (e.g., *E. coli***,** *Pichia pastoris*) to achieve high-level enzyme production. Immobilization Techniques: Development of immobilized lipase systems for reuse and continuous biocatalysis in industrial reactors. Application Testing: Evaluation of the produced lipase in detergent formulations, biodiesel transesterification, and food emulsification to assess commercial feasibility.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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