Screening and Process Optimization of Lipase-Producing *Enterobacter ludwigii* Isolated from Fish Processing Industry Waste Soil

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Lipases are versatile enzymes with applications in detergents, biodiesel production, and the food industry. In this study, a lipase-producing bacterium was isolated from fish processing industry waste soil using Phenol Red Olive Oil Agar. The most potent isolate was identified as Enterobacter ludwigii by morphological, biochemical, and 16S rRNA sequence analysis (GenBank Accession No. PX112880, 99.7% similarity). Lipase production was optimized using the One-Factor-At-A-Time (OFAT) method. Maximum lipase activity (160.2 U/mL) was obtained at pH 8.0, 37°C, 1% glucose, 0.5% peptone, 1% olive oil, and 48 h incubation. Partial purification resulted in a 1.8-fold increase in specific activity, with optimal activity at pH 8.0 and 45°C. The enzyme retained 82% activity after 1 h at 40°C. These results suggest that E. ludwigii from fish waste soil is a potent lipase producer suitable for industrial applications.

Keywords: Lipase, *Enterobacter ludwigii*, Phenol Red Agar, Fish waste soil, OFAT optimization, 16S rRNA

1. Introduction

Lipases (EC 3.1.1.3) catalyze the hydrolysis of triglycerides into glycerol and free fatty acids (Jaeger and Reetz, 1998). Microbial lipases are preferred for industrial applications due to their high yield, stability, and ease of production (Sharma et al., 2001). Fish processing waste soil is a lipid-rich, underexplored niche for lipase-producing bacteria. Screening methods include tributyrin agar, rhodamine B–olive oil agar, and Phenol Red Olive Oil Agar, which uses a pH indicator to detect fatty acid release during lipid hydrolysis (Kouker and Jaeger, 1987).

Fish processing industry waste (heads, viscera, skins, scales, blood, hydrolysates) offers a rich substrate both nutritionally (proteins, lipids) and physically (moisture, structure) for microbial growth and enzyme production. These wastes represent both an environmental burden (if disposed improperly) and an opportunity for valorization into high-value enzymes like lipases.

The objectives of this study were to (i) isolate lipase-producing bacteria from fish processing waste soil, (ii) identify the most potent isolate using morphological, biochemical, and molecular methods, (iii) optimize lipase production using OFAT, and (iv) partially purify and characterize the enzyme for industrial applications.

2. Materials and Methods

2.1 Sample Collection

Soil samples were collected from the 5–10 cm depth of waste soil at a fish processing plant in Thengapattanam Fishing Harbour. Samples were transported in sterile containers at 4°C and processed within 24 h.

2.2 Isolation and Screening of Lipase-Producing Bacteria

2.2.1 Phenol Red Olive Oil Agar

1 g peptone, 0.5 g NaCl, 1.5 g agar, 1.8 mg phenol red, 1 mL olive oil 100 ml distilled water was prepared and pH 7.2 was maintained. Six dilutions were spreaded, incubated at 37°C for 48–72 h. Formation of yellow halos indicated lipase activity.

2.3 Morphological and Biochemical Characterization

Standard tests such as Gram staining, catalase, oxidase, IMViC tests, Urease, indole, Citrate, Casein and sugar utilization were performed for morphological and biochemical characterization (Cappuccino and Sherman, 2014).

2.4 Molecular Identification (16S rRNA)

Genomic DNA was extracted 16S rRNA amplified using 27F/1492R primers. Sequencing and BLAST analysis confirmed *E. ludwigii* (99.7%, GenBank PX112880). Phylogenetic tree was constructed using MEGA X.

2.5 Lipase Assay

Crude enzyme was extracted from 48 h culture by centrifugation 5000 rpm for 10 min). Lipase activity measured using p-nitrophenyl palmitate; 1 U = 1 µmol p-nitrophenol released per min at 37°C.

2.6 OFAT Optimization

Parameters optimized individually such as pH (5–10), temperature (25–45°C), carbon (glucose, sucrose, starch, lactose), nitrogen (peptone, yeast extract, ammonium sulfate), olive oil (0.5–2%), incubation time (24–72 h).

2.7 Partial Purification and Characterization

Ammonium sulfate precipitation (30–80%), dialysis. Optimum pH, temperature, metal ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}), inhibitors (EDTA, PMSF), substrate specificity (p-nitrophenyl C4–C18) analyzed.

2.8 Statistical Analysis

Triplicates; data as mean ± SD. ANOVA at $p < 0.05$.

3. Results

3.1 Isolation and Screening

28 isolates showed lipolytic activity. 12 strong producers formed yellow halos (12–19 mm). Tributyrin and Rhodamine B agar confirmed lipase activity. From that one isolate named HR2 was selected for this study.

3.2 Identification

Morphological, Biochemical characterization and 16S rRNA Sequencing results were shown in the table 2.

3.3 Optimization

Optimization of various physicochemical parameters was carried out to enhance lipase production by Enterobacter ludwigii isolated from fish processing waste soil. Each factor was varied individually using the one-factor-at-a-time (OFAT) approach to determine the optimal conditions for maximum enzyme activity.

Effect of pH

The enzyme activity increased progressively from pH 5.0 to 8.0, achieving maximum activity (138.9 U/mL) at pH 8.0. Beyond this point, a decline in activity was observed, suggesting that alkaline conditions favor lipase production.

Effect of Temperature

Temperature is a crucial determinant of microbial enzyme production. The optimal temperature was found to be 37°C with maximum lipase activity of 142.5 U/mL, indicating that *Enterobacter ludwigii* is a mesophilic strain.

Effect of Carbon Source

Among the various carbon sources tested, glucose supported the highest enzyme activity (145.0 U/mL). This indicates that simple sugars enhance metabolic flux toward lipase biosynthesis.

Effect of Nitrogen Source

Peptone was found to be the best nitrogen source with a maximum activity of 148.5 U/mL. Complex organic nitrogen sources are known to provide essential amino acids that promote enzyme synthesis.

Effect of Olive Oil Concentration

Olive oil served both as an inducer and substrate for lipase activity. A concentration of 1% produced the maximum activity (152.0 U/mL), beyond which substrate inhibition might have occurred.

Effect of Incubation Time

Lipase production increased with incubation time, peaking at 48 hours (160.2 U/mL). Prolonged incubation led to a decline, likely due to nutrient depletion and accumulation of inhibitory metabolites.

3.4 Partial Purification

Lipase enzyme from Enterobacter ludwigii isolated from fish processing waste soil was partially purified using ammonium sulfate precipitation. The purification process aimed to enhance the specific activity while retaining high enzyme yield. A summary of the purification steps and results is presented below.

Purification Profile

The crude enzyme extract showed a total activity of 5000 U with a specific activity of 160.2 U/mg protein. Following ammonium sulfate precipitation, the specific activity increased to 285.0 U/mg with a 1.78-fold purification and a 90% yield. This enhancement indicates efficient removal of non-enzymatic proteins while retaining most of the lipase enzyme.

Enzyme Characterization

The partially purified enzyme exhibited optimal activity at pH 8.0 and temperature 45°C, indicating an alkaline and moderately thermostable nature. The enzyme retained 82% of its activity at 40°C after 1 hour of incubation, suggesting reasonable stability for biotechnological applications.

Metal ion studies revealed that Ca^{2+} and Mg^{2+} enhanced lipase activity, whereas inhibitors such as EDTA and PMSF significantly reduced activity, confirming the enzyme's metallo-lipase and serine-dependent nature.

Substrate Specificity

The enzyme displayed higher affinity toward long-chain fatty acid esters (C12–C18), which indicates its potential applicability in detergent, biodiesel, and food industries requiring hydrolysis of long-chain triglycerides.

4. Discussion

Phenol Red Agar is effective for rapid screening. OFAT optimization showed pH 8.0, 37°C, glucose, peptone, and 1% olive oil as optimal. Partially purified lipase is alkaline, moderately thermostable, and active on long-chain substrates, suitable for industrial use. Fish waste soil is a promising source for lipase-producing *E. ludwigii* (Abo-Amer, 2011; Sharma et al., 2001; Gupta et al., 2015; Singh and Cameotra, 2014).

5. Conclusion

E. ludwigii from fish processing waste soil is a potent lipase producer. Screening, OFAT optimization, and partial purification yielded a high-activity enzyme (160.2 U/mL) with industrially relevant stability and substrate specificity.

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Credit authorship contribution statement

Husmin Risha T: Conceptualization, Methodology, and Writing - original draft, Dr. A. L. Hema Latha: Conceptualization, Supervision.

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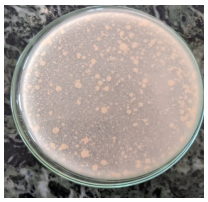


Figure 1: Plate showing pure culture of *Enterobacter ludwigii*

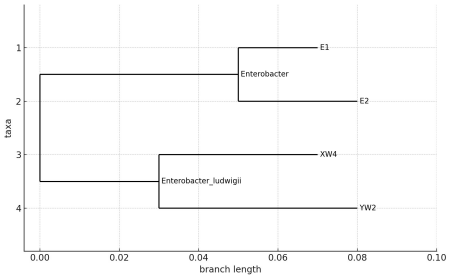


Figure 1 : Phylogenetic tree of *Enterobacter ludwigii*

SI.NO	TEST NAME	HR2
1.	Gram Staining	Gram negtive

2.	Catalase	Positive
3.	Oxidase	Negative
4.	Urease	Positive
5.	Indole	positive
6.	Methyl Red	Negative
7.	Voges Proskauer	Positive
8.	Citrate	Positive
9.	TSI	Negative
10.	Starch Hydrolysis	Positive
11.	Casein Hydrolysis	Positive
12.	Glucose	Fermentation
13.	Sucrose	Fermentation
14.	Lactose	Fermentation

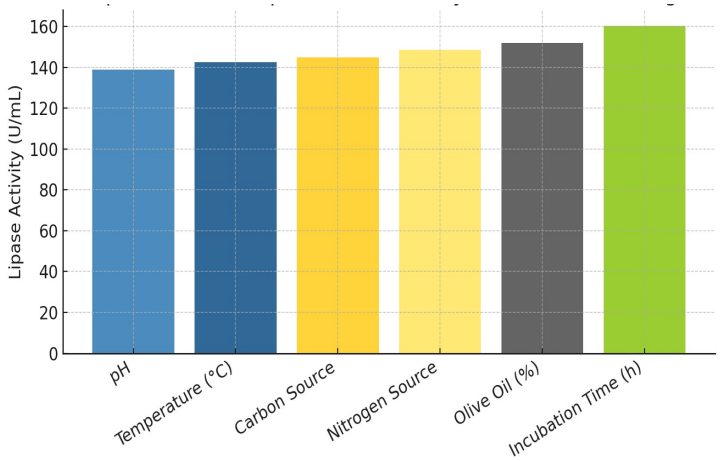
Table 1: Biochemical Characterization of HR2

Isolate	Organism	Accession Number
HR2	*Enterobacter ludwigii*	PX112880

Table 2: 16S rRNA Sequencing

Parameter	Range	Optimum	Lipase Activity (U/mL)
pH	5–10	8.0	138.9
Temperature (°C)	25–45	37	142.5
Carbon Source	Glucose, Sucrose, Starch, Lactose	Glucose	145.0
Nitrogen Source	Peptone, Yeast extract, Ammonium sulfate	Peptone	148.5
Olive Oil (%)	0.5–2	1.0	152.0
Incubation Time (h)	24–72	48	160.2

Table 2: Optimization Studies



Graph 1 : Optimization Studies