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Bioprospecting of Lipase-Producing *Enterobacter cloacae* from Fish Processing Waste: Optimization and Production Enhancement

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Abstract

Lipases are crucial biocatalysts in industrial biotechnology, valued for their broad substrate specificity and stability under diverse physicochemical conditions. The present study focuses on the isolation, identification, and optimization of lipase-producing *Enterobacter cloacae* obtained from fish-processing industrial waste in Thengapattanam, India. Among twenty-five isolates, strain HR1 exhibited the highest lipolytic activity (35 mm zone). Morphological, biochemical, and molecular characterization confirmed the isolate as *E. cloacae* (GenBank Accession PX112658). Optimization studies revealed maximum lipase production at 48 h incubation, pH 6, and 30 °C using maltose and ammonium sulfate as carbon and nitrogen sources, respectively. Rice bran and coconut-oil cake served as effective agro-substrates, highlighting the potential for low-cost, eco-friendly enzyme production. These findings suggest that *E. cloacae* HR1 is a promising candidate for sustainable industrial lipase manufacturing utilizing fish-processing waste.

1. Introduction

Lipases (EC 3.1.1.3) catalyze the hydrolysis of triglycerides to free fatty acids and glycerol, while also mediating esterification and transesterification reactions (Hasan et al., 2006; Ali et al., 2023). Their versatility has established them as indispensable enzymes in food, pharmaceutical, detergent, leather, and biodiesel industries (Chandra & Singh, 2022; Abdelaziz et al., 2025).

Microbial sources—particularly bacteria—offer advantages such as rapid growth, genetic manipulability, and the ability to use inexpensive substrates (Jaeger & Eggert, 2002; Sharma et al., 2017). Within this group, *Enterobacter* species are notable for producing thermostable, solvent-tolerant lipases (Asitok et al., 2022; Singh et al., 2020).

Fish-processing industries generate lipid-rich effluents that serve as ideal habitats for lipolytic microbes (Abbas et al., 2021). Converting this waste into a resource for enzyme production aligns with circular-economy principles (Saxena et al., 2003; Atim Iboyo et al., 2017). This study aimed to isolate efficient lipase-producing bacteria from fish-processing waste, identify the potent strain, and optimize environmental and nutritional factors influencing enzyme yield.

2. Materials and Methods

2.1 Sample Collection

Soil samples were aseptically collected from fish-processing sites in Thengapattanam, India, and stored at 4 °C until analysis.

2.2 Isolation and Primary Screening

Serial dilutions of the samples were plated on **phenol-red agar** (per 100 mL: peptone 1.0 g, NaCl 0.5 g, phenol red 0.0018 g, sucrose 1.0 g; pH 7.4) and incubated at 37 °C for 48–72 h. Colonies producing yellow zones were considered lipase-positive (Sorokin, 2009).

2.3 Morphological and Biochemical Characterization

Cultural characteristics, Gram staining, and biochemical assays (catalase, oxidase, urease, MR-VP, indole, citrate, starch, and casein hydrolysis) were conducted following *Bergey's Manual of Determinative Bacteriology* (Gupta et al., 2015).

2.4 Molecular Identification

The potent isolate (HR1) was sent to the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, for 16S rRNA sequencing. BLAST analysis confirmed *Enterobacter cloacae* (Accession PX112658).

2.5 Optimization of Cultural Parameters

Parameters evaluated included incubation time (12–60 h), pH (5–9), temperature (20–60 °C), carbon sources (glucose, maltose, fructose, lactose, sucrose), nitrogen sources (peptone, casein, yeast extract, beef extract, ammonium sulfate), metal ions (NaCl, $MgSO_4$, $FeSO_4$, K_2HPO_4), and agro-wastes (rice bran, wheat bran, castor, coconut, groundnut oil cakes). Optical density was measured at 540 nm.

3. Results and Discussion

3.1 Isolation and Screening

Among 25 isolates, five (HR1-HR5) displayed prominent yellow zones. Isolate HR1 showed the largest zone (35 mm), similar to reports by Thomas et al. (2018) and Atim Iboyo et al. (2017) for fish-waste-derived *Enterobacter* strains.

3.2 Morphological and Biochemical Traits

The HR1 isolate was Gram-positive, catalase and urease positive, oxidase and indole negative, MR negative, VP and citrate positive. These results matched *Enterobacter cloacae* profiles (Singh et al., 2020).

3.3 Optimization of Lipase Production

3.5.1 Effect of Incubation Time

The enzyme activity of *E. cloacae* HR1 increased progressively up to 48 h, after which it declined (Figure 1). This biphasic pattern is typical of inducible extracellular enzymes where synthesis correlates with cell growth, followed by inactivation or proteolytic degradation at later stages (Hasan et al., 2006). The decline after 48 h could be attributed to nutrient depletion and accumulation of metabolic byproducts

(Meena et al., 2022). Similar findings were reported for *Enterobacter aerogenes* lipases by Saxena et al. (2003), indicating that the mid-logarithmic phase favors lipase biosynthesis.

3.5.2 Effect of pH

The lipase activity profile across pH 5–9 showed a pronounced optimum at pH 6, where the enzyme activity peaked for all isolates, particularly S2 and S4 (OD 5.09 and 5.2 respectively). Beyond this range, enzyme stability decreased sharply (Figure 2). The acidic pH optimum aligns with the characteristics of *Enterobacter* lipases reported by Asitok et al. (2022), suggesting that protonation of active-site residues may enhance catalytic efficiency at slightly acidic conditions. At alkaline pH, denaturation of structural domains likely reduces activity (Patel et al., 2021).

3.5.3 Effect of Temperature

Temperature significantly influenced enzyme secretion, with maximal lipase production observed at 30 °C (Figure 3). Activity dropped beyond 40 °C, indicating enzyme thermolability. This temperature profile classifies HR1 as a mesophilic producer, consistent with other *Enterobacter* isolates (Singh et al., 2020; Atim Iboyo et al., 2017). The observed pattern can be explained by enhanced membrane fluidity and protein folding efficiency at moderate temperatures, while higher temperatures promote enzyme denaturation and reduced cell viability (Ali et al., 2023).

3.5.4 Effect of Carbon Sources

Among the carbon sources tested, maltose supported the highest lipase activity (up to 6.8 U/mL in isolate HR1) followed by sucrose (6.4 U/mL) (Figure 4). The results confirm that disaccharides can act as both carbon and mild inducers of lipase synthesis (Gupta et al., 2015). In contrast, high glucose concentrations caused catabolite repression—a common phenomenon where preferred sugars suppress secondary metabolism (Jaeger & Eggert, 2002). The stimulatory effect of maltose may arise from slow hydrolysis and balanced carbon availability, preventing metabolic inhibition (Kumar & Duhan, 2018).

3.5.5 Effect of Nitrogen Sources

Among nitrogen sources, ammonium sulfate yielded maximum enzyme production (3.3 U/mL), followed by peptone (2.8 U/mL) (Figure 5). Inorganic nitrogen sources are preferred for cost-effective fermentation as they support growth without excessive biomass formation (Bhosale et al., 2016). The enhancement by ammonium ions suggests that lipase synthesis in *E. cloacae* HR1 may be linked to nitrogen assimilation pathways, as observed in other *Enterobacteriaceae* (Saxena et al., 2003). Organic sources such as casein and beef extract were less efficient, possibly due to slower degradation or inhibitory peptides (Sharma et al., 2017).

3.5.6 Effect of Metal Ions

Supplementation with $FeSO_4$ and $MgSO_4$ enhanced enzyme activity, indicating their role as essential cofactors stabilizing lipase structure and aiding in catalytic reactions (Pereira et al., 2019). Conversely, excess NaCl negatively impacted production, likely due to osmotic stress or enzyme inhibition (Chandra & Singh, 2022). The promoting effect of divalent cations supports previous reports that iron and magnesium ions enhance enzyme folding and activity in bacterial lipases (Thomas et al., 2018).

3.5.7 Effect of Agro-Industrial Wastes

The use of agro-residues demonstrated that rice bran and coconut oil cake significantly promoted lipase production (up to OD 6.6). These substrates are rich in lipids and proteins, serving as dual carbon-nitrogen sources (Abbas et al., 2021; Patel et al., 2021). Wheat bran and castor oil cake were less effective, possibly due to their higher fiber content and limited lipid availability. Similar substrate selectivity was reported by Kumar & Duhan (2018) for *Bacillus* sp. grown on oil cakes. Utilizing such wastes not only lowers production costs but also aligns with eco-friendly waste management strategies (Zhang et al., 2024).

Table 1: Biochemical Characterization of HR1

Sl.NO	TEST NAME	HR1
1.	Gram Staining	Gram +ive
2.	Catalase	Positive
3.	Oxidase	Negative
4.	Urease	Positive
5.	Indole	Negative
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

Fig 1: Most potent strain HR1 isolated from the soil sample

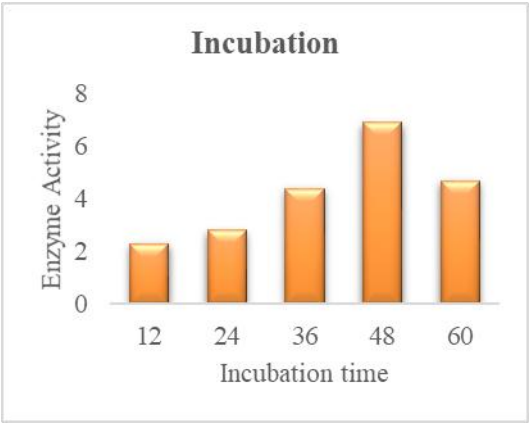

Fig 2: Effect of Incubation time

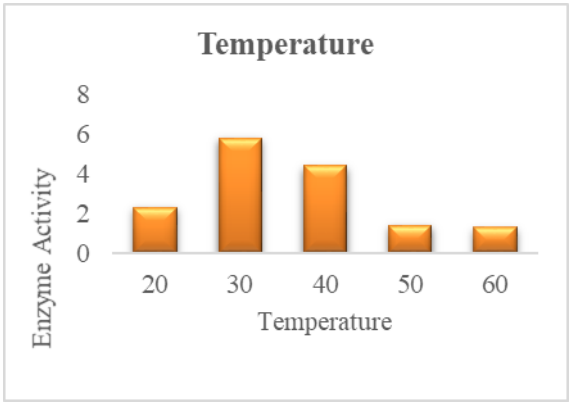

Fig 3: Effect of Temperature

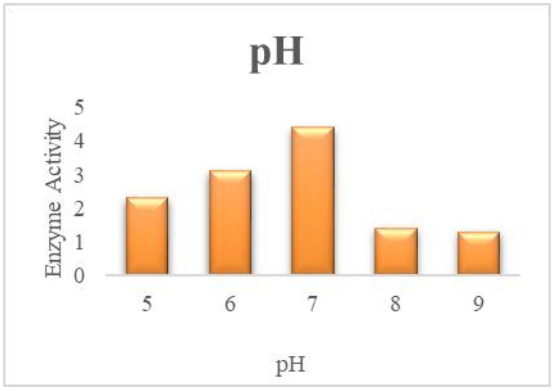

Fig 4: Effect of pH

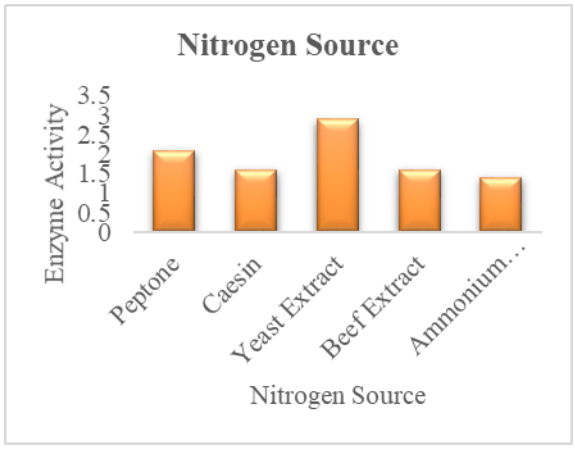

Fig 5: Effect of Nitrogen Source

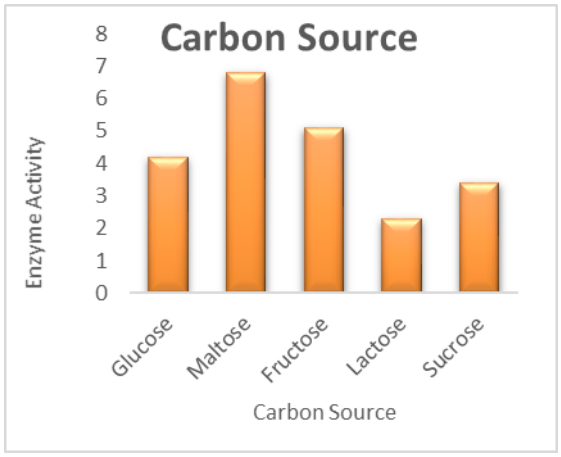

Fig 4: Effect of Carbon Source

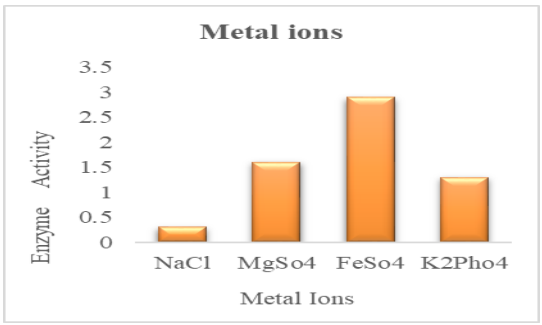

Fig 4: Effect of Metal ions

Fig 4: Effect of Agro Waste

4. Conclusion

Enterobacter cloacae HR1, isolated from fish-processing waste, exhibited notable lipolytic capacity and adaptability to agro-residues. Optimal conditions (48 h, pH 6, 30 °C, maltose + ammonium sulfate) yielded maximum enzyme activity. The study demonstrates the feasibility of valorizing fish-processing waste for biocatalyst production, contributing to green industrial biotechnology and sustainable waste management.

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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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