



Isolation and screening of halotolerant protease-producing bacteria from lampung fermented shrimp paste

Khaerunissa Anbar Istiadi*, Ningsih, Erma Suryanti, Lisana Husna Imaniar

Departement of Biology, Faculty of Science, Institut Teknologi Sumatera, Jl. Terusan Ryacudu, Way Huwi, Kec. Jati Agung, Kabupaten Lampung Selatan, Lampung 35365, Indonesia

*Corresponding e-mail: khaerunissa.istiadi@bi.itera.ac.id

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Article info	Abstract
<p>Article History: Received: 12 December 2025, Revised: 31 December 2025, Available Online: 31 March 2026</p> <p>Keywords: Halotolerant, Lampung, Proteolytic Index, Protease, Terasi.</p> <p>©2026 Bioeksperimen. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 (CC-BY-NC) International (https://creativecommons.org/licenses/by-nc/4.0/).</p>	<p>Protease are class of degradative enzymes that hydrolyze proteins, breaking its peptide bond, into smaller peptides and amino acids. Microbial proteases have gained increasing attention due to its productivity and utilization in various industries. Proteolytic bacteria derived from fermented shrimp paste from Lampung have great potential to meet this need. However, studies on these bacteria are still limited, so further research is needed to explore and optimally utilize their potential. This study aims to explore the diversity of proteolytic bacteria from Lampung terasi.. The methods employed included isolation stages using Nutrient Agar supplemented with 5% and 10% NaCl (w/v), followed by proteolytic screening properties using Skim Milk Agar media. A total of 20 bacterial isolates were obtained from terasi using Nutrient Agar supplemented with 5% (w/v) NaCl, and 15 isolates were obtained from Nutrient Agar (NA) supplemented with 10% (w/v) NaCl. Screening results showed that 85% and 67% of the isolates obtained from NA supplemented with 5% and 10% NaCl, respectively, exhibited proteolytic activity. Halotolerant bacterial isolates isolated using NA + 5% NaCl exhibited a proteolytic index ranging from 0.3 – 3.0, while those isolated with NA + 10% NaCl showed values ranging from 0.18 to 3.06. The screening results revealed that isolates BTCL5-07 and BTCL10-05 exhibited the highest proteolytic activity, with proteolytic indices of 3.0 and 3.06, respectively. These findings indicate that halotolerant bacteria from Lampung terasi represent a promising source of potential proteolytic enzymes. Further research is required to optimize enzyme production.</p>

Introduction

Protease are class of degradative enzymes that hydrolyze proteins, breaking its peptide bond, into smaller peptides and amino acids (Dos Santos Aguilar & Sato, 2018). Proteases represent one of the most economically significant groups of industrial enzymes, contributing to over 60% of total enzyme sales worldwide (Singh & Panesar, 2023). Microbial proteases have attracted considerable interest because they offer advantages such as lower production costs, higher yields, shorter and more sustainable production cycles, reduced space requirements, and greater ease of genetic manipulation compared with proteases derived from plant and animal sources (Barzkar et al., 2022). Moreover, microbial proteases can be cultured on a large scale using inexpensive substrates, including agricultural waste, and their extracellular nature simplifies downstream processes (Solanki et al., 2021).

Interest of halotolerant and halophilic protease from halotolerant microbes has been raising, as these enzymes retain stability and activity under high-salt conditions. These properties support their application in industrial processes involving high-salt environment (Kochhar et al., 2022). Halotolerant and halophilic



proteases have been used in detergent industries ([Mokashe et al., 2017](#)), leather industry for dehairing ([Li et al., 2022](#)), and food processing ([Naveed et al., 2021](#)).

Previous research have explored halotolerant and halophilic protease-producing bacteria from various food source such as fish sauce ([Abd Samad et al., 2017](#)), several Thai fermented fish ([Taprig, 2013](#)), anchovy paste ([Oktavia et al., 2018](#)), Chinese fermented catfish paste ([Yuan et al., 2022](#)), and fermented shrimp paste ([Yao et al., 2025](#)). Beyond food products, environmental samples are being explored as sources of proteolytic bacteria, including seagrasses ([Asar et al., 2016](#)), lakes ([Benmebarek & Kharroub, 2023](#); [Ibrahim et al., 2019](#); [Ruginescu et al., 2020](#)), hypersaline water ([Proca et al., 2020](#)), mud volcano ([Rohman et al., 2012](#)), and salt mines ([Ali et al., 2016](#)).

Terasi, fermented shrimp paste from Indonesia, traditionally produced through spontaneous fermentation of shrimp with salt addition, generating habitat dominated by halotolerant and halophilic bacteria ([Kobayashi et al., 2003](#)). These bacteria secrete extracellular enzymes, including protease to hydrolyze protein in shrimp as raw material ([Prihanto et al., 2021](#)). This condition makes terasi a potential source for isolating halotolerant, protease-producing bacteria.

Lampung is one of Indonesia's terasi producers, with exports reaching US\$50,000 per year ([BPS Lampung, 2025](#)). Lampung's naturally fermented terasi represents promising source of novel halotolerant proteolytic bacterial isolates. However, studies on halotolerant bacteria from Lampung's terasi with proteolytic activities remain limited, indicating the need for further exploration to optimize their potential applications. The potential of halotolerant bacterial enzymes underscores the importance of exploring protease-producing bacteria from fermented foods, one of which is Lampung's shrimp paste.

Materials and methods

Sampling and Isolation of Halotolerant Bacteria from Terasi Lampung

Wet shrimp paste samples were collected from local producers in Labuhan Maringgai, East Lampung. One gram of sample was homogenized in 9 mL of sterile physiological saline solution (0.85% NaCl). The homogenate was subjected to serial dilution up to 10^{-5} , and 1 mL of the final dilution was spread-plated onto Nutrient Agar supplemented with 5% (w/v) and 10% (w/v) NaCl. The plates were incubated at 37 °C for 24 h. Bacterial colonies that developed on the media were subsequently screened for proteolytic activity.

Screening Test for Protease Activity using a Plate Assay

Proteolytic activity was assessed by inoculating a loopful of the selected isolates onto Skim Milk Agar, followed by incubation at 37°C for 72 hours ([Afifah et al., 2014](#)). Positive proteolytic activity was indicated by the appearance of a clear zone surrounding the colonies, and the diameter of this zone was measured to calculate the enzyme activity index.

$$\text{Proteolytic Index} = \frac{a \text{ (mm)} - b \text{ (mm)}}{b \text{ (mm)}}$$

a = clear zone diameter (mm)

b = bacterial colony diameter (mm)

Macroscopic characterization of potential bacterial isolates

Macroscopic characterization of bacterial isolates was performed based on shape, color, elevation, and border shape ([Cappuccino & Sherman, 2008](#)).

Data Analysis

The obtained data are presented descriptively, including macroscopic characteristics and qualitative and quantitative enzyme activity tests.

Results and discussion

Proteolytic bacteria were isolated from wet terasi collected in Labuhan Maringgai using two media types: Nutrient Agar supplemented with 5% NaCl (w/v) (NA+5% NaCl) and Nutrient Agar supplemented with 10% NaCl (w/v) (NA+10% NaCl). As shown in Tables 1 and 2, a total of 20 and 15 bacterial isolates exhibiting distinct colony morphologies were obtained from NA+5% NaCl and NA+10% NaCl,



respectively. Macroscopic morphological characteristics of the isolates, including colony color, shape, elevation, and margin, were recorded according to standard methods ([Cappuccino & Sherman, 2008](#)).

Table 1. Colony Morphological Characteristics of Halotolerant Bacteria Isolated from Lampung Terasi on NA + 5% NaCl

No.	Code	Form	Colour	Elevation	Margin
1.	BTCL5-01	Round	White	Convex	Entire
2.	BTCL5-02	Round	White	Convex	Entire
3.	BTCL5-03	Round	White	Convex	Entire
4.	BTCL5-04	Round	White	Convex	Entire
5.	BTCL5-05	Irregular	cream	Convex	Lobate
6.	BTCL5-06	Irregular	white	Convex	Lobate
7.	BTCL5-07	Round	Yellowish white	Convex	Entire
8.	BTCL5-08	Round	Yellow	Convex	Entire
9.	BTCL5-09	Round	Yellow	Convex	Entire
10.	BTCL5-10	wrinkled	Cream-White	Flat	Undulate
11.	BTCL5-11	Irregular	White	Convex	Entire
12.	BTCL5-12	Round	White	Convex	Entire
13.	BTCL5-13	Round	cream	Convex	Entire
14.	BTCL5-14	Round	White	Convex	Entire
15.	BTCL5-15	Round	cream	Raised	Entire
16.	BTCL5-16	Round	Yellow	Convex	Entire
17.	BTCL5-17	Irregular	White	Convex	Entire
18.	BTCL5-18	Irregular	White	Convex	Entire
19.	BTCL5-19	Round	Yellow	Convex	Entire
20.	BTCL5-20	Round	Yellow	Convex	Entire

Table 2. Colony Morphological Characteristics of Halotolerant Bacteria Isolated from Lampung Terasi on NA + 10% NaCl

No.	Code	Form	Colour	Elevation	Margin
1.	BTCL10-01	Round	White	Convex	Entire
2.	BTCL10-02	Round	White	Convex	Entire
3.	BTCL10-03	Round	cream	Convex	Entire
4.	BTCL10-04	Round	cream	Convex	Entire
5.	BTCL10-05	Round	Yellowish white	Convex	Entire
6.	BTCL10-06	Round	Yellow	Convex	Entire
7.	BTCL10-07	Round	Milky white	Convex	Entire
8.	BTCL10-08	Round	Milky white	Convex	Entire
9.	BTCL10-09	Round	Milky white	Convex	Entire
10.	BTCL10-10	Round	Milky white	Convex	Entire
11.	BTCL10-11	Round	Milky white	Convex	Entire
12.	BTCL10-12	Round	Milky white	Convex	Entire
13.	BTCL10-13	Round	Milky white	Convex	Entire
14.	BTCL10-14	Round	Milky white	Convex	Entire
15.	BTCL10-15	Irregular	cream	Convex	Lobate

All isolates that grew were then purified and inoculated on Skim Milk Agar (SMA) and incubated for 24 hours to observe the protease enzyme activity of each isolate. Skim Milk Agar is a differential medium used to observe the qualitative activity of protease because it contains casein as the main substrate of the protease enzyme. Bacteria that are able to hydrolyse and have proteolytic activity against casein will have a clear zone around the isolate (Figure 1) ([Chung et al., 2021](#); [Decimo et al., 2014](#)). Based on the screening results, 85% of the bacterial isolates obtained from NA + 5% NaCl exhibited proteolytic activity, whereas 67% of the isolates derived from NA + 10% NaCl showed similar activity.

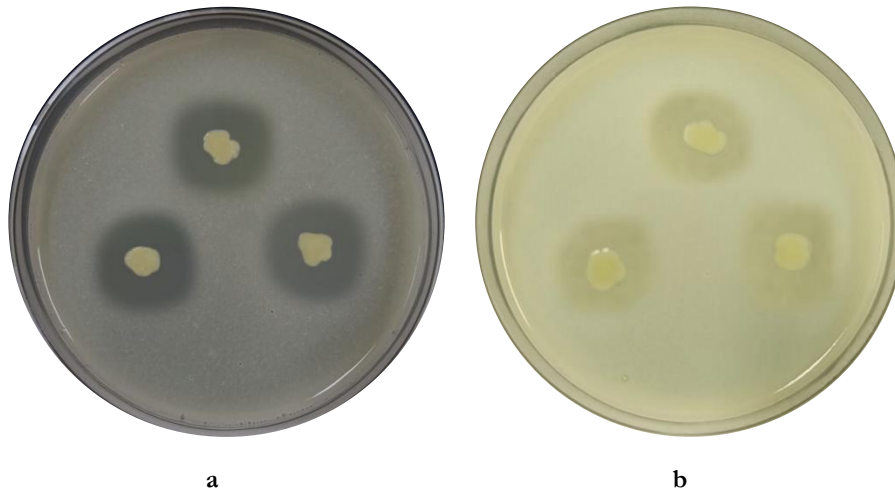


Figure 1. Growth of isolated bacteria a) BTCL5-15 and b) BTCL10-3 from Terasi. Clear zone represents proteolytic activity of the bacteria.

Qualitative activity of protease enzymes was assessed by measuring the diameter of the clear zone formed around the bacterial colonies grown on SM agar media. Casein, the major protein component of skim milk, consists of phosphoproteins that bind calcium and form calcium caseinate salts, resulting white appearance of the medium (Amin, 2018). Protease enzyme produced by bacteria hydrolyze casein to soluble amino acids, causing local decolorization of the medium and producing transparent and clear zone surrounding the colony (Mamangkey & Suryanto, 2021). The clear zone indicates the protease activity produced by the bacterial isolate. The larger the clear zone indicates higher extracellular protease enzyme activity (Cahyaningrum et al., 2021).

Based on the clear zone measurements depicts in Figure 1 and Figure 2, the bacterial isolates exhibited varying levels of protease activity. The formation of these clear zones confirms that the isolates release protease into the growth medium and are capable of degrading the casein in SM agar as their primary protein substrate (Artha et al., 2019; Ramadhan et al., 2021). All isolates showed the ability to produce protease enzyme with varying activity. Based on the proteolytic index category, index value less than 1.5 is classified as low, IP 1.5 to 3.5 is classified as medium, and IP values above 3.5 are classified as high (Ayuningrum et al., 2022). Majority of the bacterial isolates isolated from two media showed a low index.

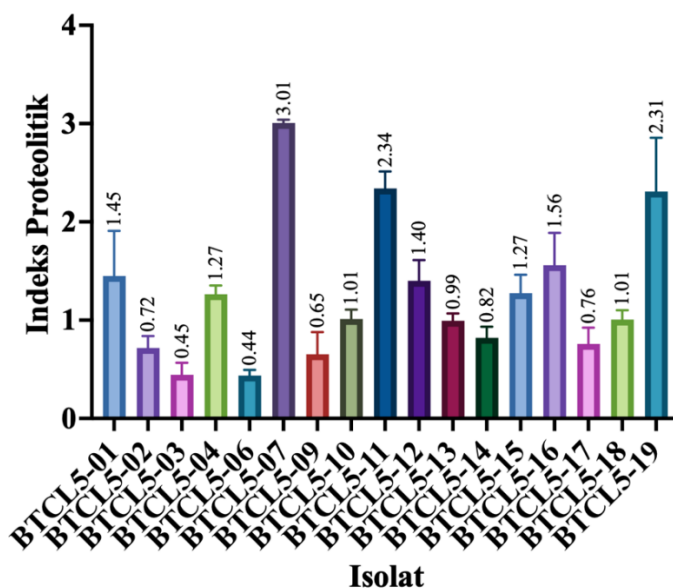


Figure 2. Proteolytic index of bacterial isolates grown on nutrient agar supplemented with 5% NaCl.

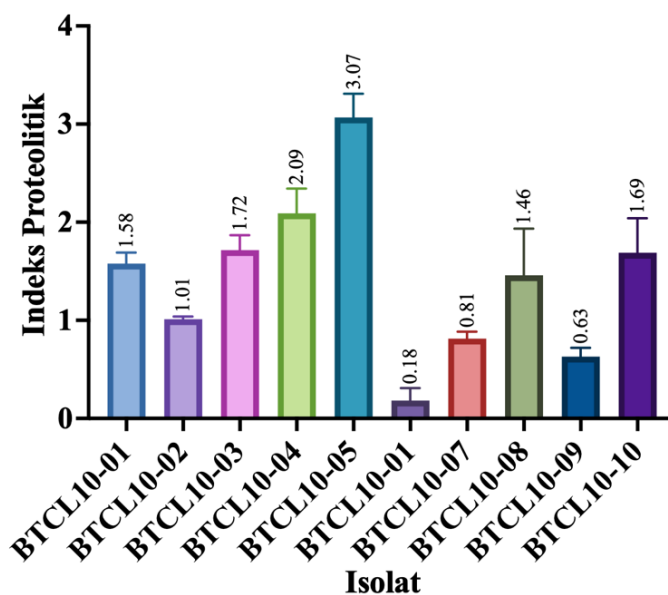


Figure 3. Proteolytic index of bacterial isolates grown on Nutrient Agar supplemented with 10% NaCl.

As shown in Figure 1 and Figure 2, isolates obtained from NA + 5% NaCl exhibited a proteolytic index of 0.3 – 3.0, while those isolated with NA + 10% NaCl showed an activity of 0.18 - 3.06. In NA+ 5% NaCl and NA+10% NaCl media, the highest proteolytic index was shown by isolates BTCL5-07 and BTCL10-05 with values of 3.0 and 3.06, respectively. Based on the proteolytic index classification, both isolates fall within the medium-activity category. Previous studies on proteolytic bacteria from shrimp paste have also reported diverse levels of activity. For example, [Handayani et al., \(2025\)](#) identified 41 isolates from seven shrimp paste samples had proteolytic activity with a clear zone greater than 2 cm. Another study by [Susanti et al., \(2021\)](#) found 16 proteolytic bacteria with proteolytic index ranging from 1.60-3.32. These findings indicate that halotolerant bacteria from Terasi Lampung represent a promising source of potential proteolytic enzymes. Further research is required to optimize enzyme production.

Conclusion

Isolates BTCL5-07 and BTCL10-05 from Lampung fermented shrimp paste (terasi) exhibited the highest proteolytic activity, with proteolytic indices of 3.0 and 3.06, respectively. These findings indicate that halotolerant bacteria from Lampung terasi represent a promising source of potential proteolytic enzymes. Further research is required to optimize enzyme production.

Author Statements

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Author's contributions: Khaerunissa Anbar Istiadi: Conceptualization, research design, data collection, data analysis, and manuscript writing. Ningsih: field support, data collection, and data analysis. Erma Suryanti: research design, critical revision of the manuscript, and final manuscript approval. Lisana Husna Imaniar: critical revision of the manuscript and final manuscript approval.

Generative AI: Grammarly was used to enhance grammar of the manuscript.

Data availability: Data sets generated during the current study are available from the corresponding author on reasonable request.



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