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Isolation and Screening of Proteolytic Bacteria Isolated from the Digestive Tract of Pangasius Catfish PATIN (*Pangasius hypophthalmus*)

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ABSTRACT

The demand for protease enzymes in Indonesia has exhibited a significant upward trend; however, domestic large-scale production capacity remains limited. To mitigate dependency on imports, strategic efforts to develop indigenous protease production are essential. This study aims to identify and isolate protease-producing bacteria from the digestive tract of catfish (*Pangasius hypophthalmus*). This research employed an observational method with a cross-sectional design. Samples were obtained from the stomach of the catfish and subsequently inoculated into Brain Heart Infusion (BHI) media. Bacterial isolation was performed using Blood Agar Plate (BAP) and MacConkey Agar. The proteolytic activity of the resulting isolates was evaluated on Skim Milk Agar (SMA), followed by macroscopic and microscopic characterization. The study yielded three pure isolates; however, screening for proteolytic activity revealed that only one isolate, designated as PHS1, exhibited protease production. The proteolytic activity was characterized by the formation of a 2 mm clear zone (lysis zone) with a proteolytic index of 3. Microscopic analysis identified the isolate as a Gram-positive, spore-forming bacterium.

INTRODUCTION

Enzymes play a pivotal role in various industrial and healthcare sectors, with their applications expanding rapidly alongside advancements in biotechnology, fermentation technology, and genetic engineering environments (Hastuty *et al*, 2023). Among these, protease enzymes are of significant importance due to their ability to catalyze the hydrolysis of proteins into amino acids. In Indonesia, the demand for protease enzymes continues to rise across industries such as detergents, leather processing, and food production (Zafrida *et al*, 2024). However, domestic large-scale production remains insufficient, leading to a high dependency on imported enzymes. Therefore, exploring indigenous sources for protease production is a critical step toward achieving national industrial self-sufficiency (Dhayalan & Manivannan, 2022; Sikarina *et al*., 2022; Nursidi *et al*., 2021)

Previous studies have identified various sources of proteolytic bacteria, ranging from soil samples to fermented food products. Recent microbiological research has highlighted that the digestive tracts of aquatic organisms, particularly fish, serve as a rich reservoir for enzyme-producing bacteria due to their specialized metabolic environments (Zafrida *et al*, 2024). While several studies have explored proteolytic activity in marine fish, research

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focusing on local freshwater species remains relatively limited. *Pangasius hypophthalmus*, commonly known as catfish (ikan patin), is a significant freshwater commodity in Indonesia, yet the potential of its digestive system as a source for industrial enzymes has not been fully documented in previous literature (Sikarina *et al*., 2022).

This research addresses this gap by focusing on the isolation and screening of protease-producing bacteria specifically derived from the stomach of *Pangasius hypophthalmus*. The novelty of this study lies in the identification of indigenous bacterial strains that are naturally adapted to the Indonesian aquatic environment, which may offer unique proteolytic characteristics compared to previously studied sources. By characterizing these isolates, this study aims to provide foundational data for the development of locally-sourced protease enzymes, thereby contributing to the reduction of import reliance in the national enzyme industry.

MATERIALS/METHOD

Research Design

This study employed an observational research method with a cross-sectional design to identify and characterize protease-producing bacteria derived from the digestive system of catfish.

Sample Collection and Preparation

The samples consisted of the stomachs of catfish (*Pangasius hypophthalmus*) obtained through random sampling from the Jalan Fajar Market in Pekanbaru. The fish were dissected using sterile knives to retrieve the stomachs, which were then homogenized using a sterile mortar and pestle. A total of 1 gram of the homogenized sample was weighed for further processing.

Inoculation and Bacterial Isolation

The prepared samples were inoculated into Brain Heart Infusion (BHI) broth (Himedia) as an enrichment medium. The mixture was homogenized using a vortex and incubated at 37°C for 24 hours. Bacterial isolation was subsequently performed by streaking the suspension from the BHI media onto Blood Agar Plate (BAP) and MacConkey (MC) Agar (Himedia) using the quadrant streak method to obtain pure cultures.

Screening for Proteolytic Activity

Pure bacterial isolates were screened for proteolytic activity by spot-inoculating them onto Skim Milk Agar (SMA). The plates were incubated at 37°C for 24 hours. The presence of a clear zone (lysis zone) around the colonies indicated the production of extracellular protease enzymes.

Characterization of Isolates

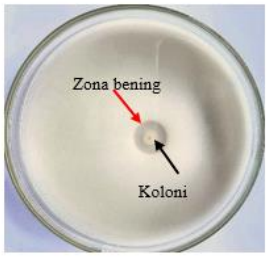
The isolate showing the highest proteolytic potential (PHS1) was characterized through macroscopic and microscopic observations. Macroscopic analysis included colony morphology, color, and elevation on BAP and MC media. Microscopic characterization was conducted using Gram staining (Gentian violet, Lugol, 96% Alcohol, and Safranin) to determine the Gram reaction, cell shape, and the presence of endospores under a light microscope

RESULTS AND DISCUSSION

4.1. Results

The isolation process from the stomach of catfish (*Pangasius hypophthalmus*) yielded three distinct bacterial isolates, designated as PHS1, PHS2, and PHS3. The primary screening for

proteolytic activity on Skim Milk Agar (SMA) revealed that only one isolate, PHS1 (*Pangasius Hypophthalmus Stomach*), was capable of producing extracellular protease enzymes, evidenced by the formation of a clear zone around the colony.



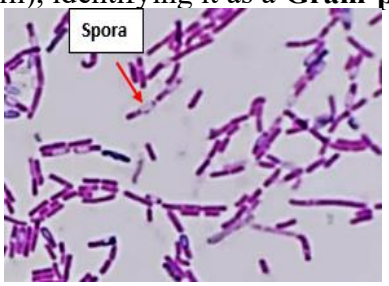
Picture 1

As shown in **Table 1**, isolate PHS1 produced a lysis zone diameter of 2 mm. The calculated Proteolytic Index (PI) for this isolate was 3.0, indicating a high level of enzymatic efficiency. In contrast, isolates PHS2 and PHS3 showed no clear zone formation, suggesting a lack of proteolytic activity under the tested conditions.

Table 1. Proteolytic Activity of Bacterial Isolates from Catfish Stomach

Isolation code	Clear zone diameter	Colony diameter	Proteolytic activity (mm)	Proteolytic index
PHS1	18	6	2 mm	3

Microscopic characterization of the PHS1 isolate through Gram staining revealed purple-colored, rod-shaped cells (bacilli), identifying it as a **Gram-positive** bacterium.



Picture 2.

Furthermore, the presence of endospores was observed, which is a characteristic of survival-resilient bacterial genera such as *Bacillus* sp.

4.2. Discussion

The primary objective of this study was to identify indigenous protease-producing bacteria from the digestive tract of catfish to address the national demand for industrial enzymes. The discovery of isolate PHS1 confirms that the stomach of *Pangasius hypophthalmus* serves as a viable ecological niche for proteolytic microorganisms. This finding aligns with the biological role of these bacteria in assisting the host fish in breaking down complex proteins into absorbable amino acids within the acidic environment of the stomach.

The formation of a 2 mm clear zone on Skim Milk Agar is a direct result of the hydrolysis of casein by the protease enzymes secreted by the bacteria. Scientifically, when protease breaks down the opaque casein protein into smaller peptides and amino acids, the white medium turns transparent, creating the observed "lysis zone." The Proteolytic Index of 3.0 achieved by PHS1 is considered significant, as a PI value greater than 1.0 generally indicates an efficient enzyme producer.

Compared to previous studies, these results are consistent with the findings of Kurniasih et al. (2018) and Putra (2014), which identified that the digestive tracts of freshwater fish are

dominated by Gram-positive, spore-forming bacteria with high enzymatic potential. However, the specific Proteolytic Index found in this study (3.0) is relatively higher than some isolates reported from other freshwater species, suggesting that the indigenous microflora of *Pangasius hypophthalmus* in the Pekanbaru region may have unique metabolic adaptations.

The Gram-positive and spore-forming nature of PHS1 provides a strategic advantage for industrial application. Spore-forming bacteria are generally more resistant to extreme environmental conditions, such as high temperatures and pH fluctuations during industrial fermentation processes. These findings suggest that PHS1 is a promising candidate for further optimization in large-scale protease production to help reduce Indonesia's dependency on enzyme imports.

CONCLUSIONS

Based on the research findings, it can be concluded that the stomach of catfish (*Pangasius hypophthalmus*) serves as a significant source of protease-producing bacteria. The isolation process successfully identified three bacterial isolates, with one specific strain, designated as PHS1, demonstrating high proteolytic activity. Characterization of the PHS1 isolate revealed it to be a Gram-positive, rod-shaped, spore-forming bacterium. The enzymatic efficiency of this isolate was evidenced by a lysis zone of 2 mm and a Proteolytic Index of 3.0 on Skim Milk Agar. These results confirm the potential of indigenous aquatic microflora to contribute to the development of local industrial enzyme production

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REFERENCE

Amoah, K., Huang, Q. C., Tan, B. P., Zhang, S., Chi, S. Y., Yang, Q. H., Liu, H. Y., & Dong, X. H. (2021). Digestive enzyme activity, gut microbiota and health status of *Pangasius hypophthalmus* fed dietary Probiotic *Bacillus subtilis* E20. *Aquaculture Reports*, *19*, 100567. https://doi.org/10.1016/j.aqrep.2020.100567

Dhayalan, V., Sureshkumar, M., & Manivannan, S. (2022). Isolation and screening of protease producing bacteria from fish gut and optimization of enzyme production. *Journal of Applied Biology & Biotechnology*, *10*(2), 125-133.

Hastuty, A., Herawati, I., dan Pikoli, M. R. (2023). Characterization of Protease from Endophytic Fungi Fusarium sp. JE-DP4a. *Jurnal ILMU DASAR*, *24*(2), 129. https://doi.org/10.19184/jid.v24i2.27293

Kurniasih, R., Darmanto, Y. S., & Wijayanti, I. (2018). Screening of proteolytic bacteria from digestive tract of fish for fermented fish paste production. *IOP Conference Series: Earth and Environmental Science*, *116*(1), 012028. https://doi.org/10.1088/1755-1315/116/1/012028

Lestari, S. D., Jubaidah, S., & Gofar, N. (2019). Characterization of protease-producing bacteria from the digestive tract of catfish (*Pangasius* sp.). *Jurnal Teknologi Hasil Perikanan*, *8*(1), 15-22.

Nursidi, N., Ratnasari, R., Alimuddin, A., dan Yusuf, A. (2021). Aktivitas Enzim Amilase, Lipase dan Protease Ikan Nila (*Oreochromis niloticus*) yang dipelihara Pada Air Tawar dan Payau. *Lutjanus*, *25*(2), 60–65. https://doi.org/10.51978/jlpp.v25i2.276

Putra, A. N. (2014). Bakteri probiotik: Isolasi dan karakterisasi dari saluran pencernaan ikan patin (*Pangasius* sp.). *Jurnal Perikanan dan Kelautan*, *4*(1), 21-28.

Ramadhan, W., Marfiani, L., & Safitri, R. (2021). Optimization of protease enzyme production from *Bacillus* sp. isolated from freshwater fish gut. *Journal of Microbiology and Biotechnology*, *6*(2), 45-52.

Savitri, I., Aziz, R., & Santoso, H. (2020). Morphological and biochemical characterization of proteolytic bacteria in aquatic environments. *Indonesian Journal of Tropical Biology*, *4*(3), 88-94.

Sikarina, IIza, M., dan Diharmi, A. (2022). Pengaruh Ph Berbeda Terhadap Aktivitas Ekstrak Kasar Enzim Protease dari Isi Perut Ikan Patin (*Pangasius hypophthalmus*). *Fakultas Perikanan Dan Kelautan Universitas Riau*, 2–8.

Wulandari, T., & Purwani, K. I. (2023). Proteolytic activity of Gram-positive spore-forming bacteria from freshwater fish digestive systems. *Journal of Biological Researches*, *29*(1), 12-19.

Zafrida, S., Yulianto, I., Riana, A. I., & Siahaan, L. P. (2024). Isolasi dan identifikasi molekuler bakteri penghasil enzim protease dari usus ikan selaiis (kryptopterus lais). *Jurnal analis laboratorium medik*, *9*(2), 107-111.