Junior Science Communications
Faculty of Applied Sciences, UiTM Shah Alam
https://journal.uitm.edu.my/ojs/index.php/JSC

E-ISSN 2637-0689
Vol 68 2026

Colloquium on Applied Sciences 2 2025
19-22 January 2026, Faculty of Applied Sciences, UiTM Shah Alam, Malaysia

Isolation and Characterization of Protease-Producing Bacteria from Soil

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Structured Abstract

Background: Proteases are commercially significant enzymes accounting for approximately 60% of the global enzyme market due to their extensive applications in the detergent, leather, and pharmaceutical sectors. In Malaysia, the industrial sector relies heavily on high-cost imported enzymes, making the discovery of indigenous, robust microbial strains a priority for sustainable local production. Soil ecosystems serve as primary reservoirs for microorganisms with unique metabolic capabilities, evolved to secrete extracellular enzymes for nutrient cycling. This study utilises indigenous soil samples to identify bacteria capable of producing stable protease under varying environmental conditions. The purpose of this study is to isolate protease-producing bacteria from lake and garden soils, characterise their morphological and biochemical properties, and quantitatively measure their relative protease activity by casein hydrolysis.

Methods: Soil samples were collected from lake and garden sites, followed by serial dilution and pour plating on nutrient agar. Proteolytic potential was screened on Skim Milk Agar (SMA) by observing the halo zones. Isolated strains underwent Gram staining and biochemical tests, including catalase test, Simmons citrate test, indole test, methyl red test, Voges-Proskauer Test, Carbohydrate Fermentation Test (glucose, sucrose, lactose), and motility test. Finally, the casein digestion assay, measured at 660 nm, was used to quantify relative protease activity based on tyrosine release.

Results: This study successfully isolated Gram-positive, rod-shaped *Bacillus* species from both soil types. All lake isolates showed positive halo zones on SMA, indicating high proteolytic potential. Biochemical tests confirmed all isolates were catalase-positive and motile. The casein digestion assay revealed that both source productive, garden soil isolates exhibited the highest average absorbance, indicating superior proteolytic potency.

Conclusion: Lake and garden soils in Malaysia harbour specialised *Bacillus* strains with significant potential for industrial protease production. These findings provide a foundational step toward reducing economic dependency on imported enzymes. Future research should utilise 16S DNA sequencing for precise identification and evaluate the thermal stability of these enzymes for industrial application.

Keywords: Proteases, *Bacillus,* Soil Bacteria, Casein Hydrolysis, Enzyme Characterisation

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