

Isolation and Antimicrobial Properties of Lactic Acid Bacteria from *Budu* and *Tapai Ubi*

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Structured Abstract

Background: Lactic Acid Bacteria (LAB) are Gram-positive, non-spore-forming bacteria that appear as cocci or rod-shaped cells, catalase-negative, utilize carbohydrates, and produce lactic acid as a metabolic byproduct. LAB commonly found in both dairy and non-dairy fermented foods, act as probiotic that help enhancing digestive system and inhibiting enteric pathogens. Despite the common consumption of fermented foods in Malaysia, research on LAB from non-dairy Malaysian fermented foods remains limited. Variations in fermentation methods and recipes also influence the LAB composition. Thus, the study aims to isolate and characterize LAB from traditional fermented foods like *budu* (anchovy sauce) and *tapai ubi* (fermented tapioca), and evaluate their antimicrobial properties against pathogens such as, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*.

Methods: LAB were isolated on MRS agar with addition of bromocresol green as pH indicator. A pure culture that exhibiting typical LAB colonies morphology were selected to perform gram staining and biochemical tests to confirm the LAB identity. The antimicrobial activity of LAB was assessed using the agar well diffusion method. Finally, molecular characterization was conducted through 16s rRNA gene sequencing, and Basic Local Alignment Search Tool (BLAST) were performed.

Results: A total of five distinct colonies were successfully isolated. However, after gram staining and chemical testing, only three were identified as LAB, while the remaining two were classified as yeast. The isolated LAB hence forward known as BM, BS, and Tu1. Antimicrobial test shown that Tu1 significantly inhibits *E. coli*, *B. cereus*, and *S. aureus*, while BM against *E. coli* and *S. aureus*, BS only against *S. aureus*. Further test were done to determine whether the inhibition zone was due to bacteriocin or lactic acids, and results confirmed that only Tu1 produce bacteriocin. PCR amplification was performed, and Tu1 was selected for Sanger sequencing. The BLAST analysis identified Tu1 as *Limosilactobacillus fermentum*.

Conclusion: In conclusion, the finding of this study indicated that *Limosilactobacillus fermentum* can be isolated from tapai ubi. It also produced bacteriocin that capable to inhibits foodborne pathogens like *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*. Thus, this LAB can be considered to use as probiotic or starter culture or food preservative.

Keywords: LAB, antimicrobial, enteric pathogens

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