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PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA (LAB) ISOLATED FROM STINGLESS BEES IN MALAYSIA

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Stingless bee is a natural type of bee that exists in almost every continent. The honey produced by this bee has been widely used across time and space. There are approximately 50 species of stingless bee have been identified in Malaysia. Despite the extensive studies on lactic acid bacteria (LAB), the data on LAB isolated from stingless bee is limited. Moreover, there is a huge demand for novel LAB strains as probiotic and it is on the rise. This study reports the isolation and identification of LAB from 3 species of stingless bee which were *Heterotrigona itama*, *Geniotrigona thoracica* and *Tetragonula laeviceps* and also assessing the *in vitro* probiotic activity such as acid tolerance, bile salt tolerance, tolerance to simulated gastric juice and surface hydrophobicity. Twenty eight LAB isolates were successfully isolated from the three species of stingless bees and were further tested by *in vitro* probiotic characterization. The cell morphology of all 28 LAB isolates showed 60.71% and 21.43% of rod and cocci respectively. As for Gram staining, all 28 LAB isolates revealed Gram negative bacteria. Among the 28 LAB isolates, 4 isolates (HIT11, GTH3, GTH6 and TLA4) revealed good probiotic and antimicrobial properties. The GTH6 isolates showed excellent antimicrobial activity (24 mm) against *P. vulgaris* with inhibition zone higher than 6 mm as compared to others LAB isolates. Acidity tolerance to pH 2 and pH 3 from GTH6 isolates showed highest survivability with 5.20 ± 0.42 log cfu/ml and 4.25 ± 0.70 log cfu/ml respectively. As for bile salt and simulated gastric juice tolerance, GTH3 and HIT11 revealed good result with 5.59 ± 0.07 log cfu/ml and 5.15 ± 1.55 log cfu/ml respectively. This study reported that TLA4 exhibited high percentage survival in surface hydrophobicity test with 87.96 ± 0.02%. These 4 LAB isolates were further identified by molecular identification using 16s rRNA gene sequencing. Results from the analysis identified isolates HIT11 and GTH3 as *Fructobacillus tropaeoli* with sequences similarity 99.51% and 98.80% respectively. Isolate GTH6 was identified as *Weisella paramesenteroides* (with 99.24% similarity) while isolate TLA4 was identified as *Lactobacillus plantarum* with 99.25%. This study demonstrated that Malaysian stingless bees are a highly potential valuable reservoir for discovering new strains of LAB as probiotic candidates.

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TABLE OF CONTENTS