

UNIVERSITI TEKNOLOGI MARA

**POTENTIAL OF LACTIC ACID
BACTERIA AS PROBIOTIC FROM
MALAYSIAN STINGLESS BEE'S
GUT**

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ABSTRACT

Two hundred and twenty six lactic acid bacteria (LAB) were isolated from the gut of four species of Malaysian stingless bee *Heterotrigona itama*, *Geniotrigona thoracica*, *Tetragonula laeviceps* and *Tetrigona Melanoleuca* in order to identify the potential probiotic strains. These isolates fit the classification of lactic acid bacteria as Gram-positive bacteria, catalase negative and oxidase negative. Among all the LAB isolates, there are 7 isolates exhibited as potential probiotic with high antimicrobial properties, tolerance to acid pH 2.5, simulated gastric juice and 0.3% bile salt conditions after incubation within 3 hours which are the key criteria in potential probiotic selection. Those 7 LAB isolates are name as TIS 5, TIS 25, TID 18, TTD 6, TLH 13, TLH 16 and TMH 2. The 7 LAB isolates exhibited high antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Eschericia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028. Isolate TID 18 is significantly had high inhibition against the pathogenic bacteria compare to other isolated LAB. Isolate TID 18 demonstrated significantly high percentage tolerance to acid and simulated gastric juice while TIS 5 demonstrated high tolerance to bile salt. As for antibiotic resistance, all LAB isolates are less susceptible to antibiotic streptomycin, gentamycin, tetracycline, kanamycin and ampicillin except for isolates TIS 5 and TIS 25 which were inhibit by ampicillin. Interestingly, all LAB isolates also be able to grow in condition of medium supplemented 0.2% and 0.5% phenol. Isolates TID 18 and TTD 6 were found significantly have high ability to adhere to solvents such as n-hexane, chloroform and ethyl acetate. All LAB isolates also had high aggregation ability which related to cell adherence properties within 3 hours exposing to PBS solution. Results indicated that among the all isolated LAB, TID 18 and TTD 6 had higher percentages of coaggregation along with high autoaggregation abilities. These isolate could possibly prevent colonization of pathogenic bacteria especially *E. coli*, *P. aeruginosa*, *S. aureus*, *S. typhimurium*, *B. cereus* and *B. subtilis* in the stomach. From the 16S rRNA gene sequencing analysis data, isolates TIS 5 and TIS 25 identified as *Lactobacillus plantarum* with sequences similarity 100%, while isolates TID 18 and TTD 6 were identified as *Fructobacillus trophaeli* (with 99% similarity). As for LAB isolates TLH 13, TLH 16 and TMH 2, the results identified these three isolates as *Pediococcus pentosaceus* with sequences similarity 100%.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Many insects are known to have microorganisms in the gut which play an important role for their food and nutrition. The intestinal floras of most organisms play a crucial role in nutrient assimilation and immune function (Mahesh et al., 2012). There is rising awareness on the importance of the composition of intestinal microflora for health and growth of the bees (Dillon & Dillon, 2004). One of the most common groups of microorganism associated with multicellular organisms is bacteria, which is particularly widespread across insects. Mutualistic bacteria served range of ecological benefits to their insect hosts, including nutritional upgrading of deficient diets, degradation of dietary polymers, and defence against antagonists (Feldhaar, 2011).

Bacterial symbionts of insects have received increasing attention due to their outstanding role in nutrients acquisition and defence (Leonhardt & Kaltenpoth, 2014). A unique lactic acid bacteria (LAB) present in large amount in bee gut had reported to have symbiosis with honeybees (Olofsson et al., 2014). Lactic acid bacteria were found producing not only common metabolites such as formic acid and lactic acid but also a wide variety of other interesting metabolites such as benzene and 2-heptanone (De Vuyst & Leroy, 2007). Lactic acid bacteria symbionts with honeybees are the sources to the factors contributing to the honey's antimicrobial properties against a variety of severe chronic wound pathogens.

According to Leonhardt and Kaltenpoth (2014), lactic acid bacteria primarily belongs to the genera *Lactobacillus* and *Bifidobacterium* in several bee species, including honeybees (*Apis mellifera*), bumblebees, stingless bees and several solitary bee species (*Xylocopa* sp.). McFrederick et al. (2013) through their findings suggested that host-specificity of lactic acid bacteria is rare in Hymenoptera and may be maintained in social bees by spreading the symbionts among nestmates and transmitting them from one generation to the next via workers during colony