UNIVERSITI PUTRA MALAYSIA

COMPARISON OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA FROM HONEY STOMACHS AND HONEYCOMBS OF GIANT HONEYBEE (APIS DORSATA) IN KEDAH AND TERENGGANU, MALAYSIA

NASER TAJABADI

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COMPARISON OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA FROM HONEY STOMACHS AND HONEYCOMBS OF GIANT HONEYBEE (*APIS DORSATA)* IN KEDAH AND TERENGGANU, MALAYSIA

By

NASER TAJABADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

October 2010

DEDICATION

I wish to dedicate this thesis to my beloved family, my father, my mother, my son Hadi and my wife Faegheh who always understand and give me loving support.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

COMPARISON OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA FROM HONEY STOMACHS AND HONEYCOMBS OF GIANT HONEYBEE (*APIS DORSATA)* IN KEDAH AND TERENGGANU, MALAYSIA

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October 2010

Chairman: Professor Dato Makhdzir Mardan, PhD

Faculty: Agriculture

The isolation and identification of lactic acid bacteria (LAB) in the honey stomachs and honeycombs of Giant honeybee (*Apis dorsata*) from different areas in Malaysia were performed to prospect for beneficial bacteria or probiotics. Honey stomach samples of honeybee and honeycomb filled with honey were collected from *Apis dorsata* colonies in different bee trees from the rainforests of Pedu Lake in Kedah state and the agricultural region of Marang in Terengganu, Malaysia. The isolates were cultured anaerobically in different media of MRS agar, MRS broth and TPY broth at 37°C. Three hundred and fourteen isolates were obtained and identified based on biochemical tests, as LAB. In addition, 20 uncultured samples were identified as *Bifidobacterium* by using the nested polymerase chain reaction (PCR). The 16S rRNA genes from extracted DNA of bacterial colonies were amplified with PCR and nested PCR using universal

primers of 27F and 1492R for LAB, and for *Bifidobacterium* two pairs of genus specific primers (Lm26 paired with Lm3 and Bif164-F paired with Bif662-R) were utilized. All bacterial 16S rRNA genes were sequenced and entrusted in GenBank and were given specific accession numbers. Phylogenetic analysis of the 16S rRNA sequences showed that the novel LAB and *Bifidobacterium* isolates could be grouped into four different phylotypes. They were found to be composed of 37 *Lactobacillus*, 6 *Enterococcus,* 2 *Bifidobacterium* and 1 *Weissella* phylotypes. The results showed that among 334 isolates and sequences, *Lactobacillus* spp with 64.97% were found to represent the most common LAB in *Apis dorsata* honey stomachs and honeycombs followed by *Enterococcus* spp with 28.74%, *Bifidobacterium* with 5.99% and the rest 0.3% *Weissilla*. Overall, the predominant lactobacilli species found in the total samples constituting approximately 40.55% of *Lactobacillus kunkeei*. *Lactobacillus kunkeei* (YH-15) related sequences (88) were the predominant lactobacilli followed by 33.18% of other *Lactobacillus* sp with related sequences (72). In honey stomach samples, the prevalence of *Lactobacillus* spp. was 53.21%. Interestingly, the predominant *Lactobacillus* species found in all honey stomach samples was *Lactobacillus plantarum*. (30.12%) followed by *Lactobacillus pentosus* (27.71%), *Lactobacillus kunkeei* (22.89%), *Lactobacillus* sp (15.66%), *Lactobacillus vermiform* (2.41%) and *Lactobacillus fermentum* (1.21%). Samples from the honey-filled honeycomb showed a prevalence of *Lactobacillus* spp. at 75.28 %. The predominant *Lactobacillus* species from all honeycomb samples constituting of *Lactobacillus kunkeei* (51.49%), *Lactobacillus* sp (44.03%) and *Lactobacillus alvei* (4.48%). *Enterococcus* spp. was isolated from every honey stomach (40.38%) and honeycomb (18.54%) of Kedah and

Terengganu samples. The predominant *Enterococcus* species from all honey stomach and honeycomb samples, constituting approximately 88.54% of total isolated *Enterococcus* spp., was *Enterococcus* sp and the rest was *Enterococcus faecalis* (11.46%). Whereas, Bifidobacteria and *Weissilla* account for about 5.99% and 0.3% respectively.

In conclusion, the diversity of total bacterial contents of honey stomachs and honeycombs in highland were somewhat different from the lowland data. This discrepancy reflects the corresponding difference associated with the high diversity of flower, nectar and pollen in forest area of Kedah state versus a low diversity of flower, nectar and pollen in agriculture area of Terengganu state.

Abstrak tesis yang dilemukan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan untuk ijasoh Master Sains

PERBANDINGAN BAKTERIA ASID LAKTIK DAN BIFIDOBACTERIA DARI MADU DAN SARANG LEBAH MADU APIS DORSATA DARI KEDAH DAN TERENGGANU, MALAYSIA

Oleh

NASER TAJABADI

Oktober 2010

Pengerusi: Profesor Dato Makhdzir Mardan, PhD

Fakulti: Pertanian

Pemencilan dan pencirian bakteria asid laktik (LAB) dari perut madu dan sarang *Apis dorsata* dari pelbagai kawasan di Malaysia telah dijalankan bagi mendapatkan bakteria baik atau probiotik. Sampel perut madu dan sarang yang dipenuhi madu telah dikumpulkan dari koloni *Apis dorsata* dari pokok hutan hujan di Kedah dan kawasan pertanian di daerah Marang, Terengganu. Semua isolat dibiakkan secara anaerobik space media MRS agar, MRS broth dan TPY broth pada suhu 37^{o}C. Sebanyak 314 isolat telah diperolehi dan dikenalpasti sebagai LAB menggunakan ujian biokimia. Space itu, sebanyak 20 sampel yang tidak boleh dibiakkan telah dikenaplasti sebagai *Bifidobacterium* dengan menggunakan PCR. Gen 16S rRNA dari DNA yang telak diekstrak dari koloni bakteria telah digandakan dengan kaedah PCR dan *nested* PCR

menggunakan primer *universal* 27F dan 1492R bagi LAB dan untuk *Bifidobacterium*, 2 pasang primer yang spesifik kepada genus Bifidobacterium (Lm26 berpasangan dengan Lm3 dan Bif164-F berpasangan dengan Bif662-R) telah digunakan. Semua urutan DNA gen 16S rRNA telah dijujukkan dan disimpan di bank gen dengan diberi nombor capaian tertentu. Analisis pilogenetik untuk urutan 16S rDNA menunjukkan bahawa LAB dan *Bifidobakterium* boleh dibahagikan kepada 4 kumpulan pilotipik. Mereka terdiri dari 37 *Laktobasillus*, 6 *Enterokokkus*, 2 *Bifidobakterium* dan 1 *Weisella.* Keputusan menunjukkan bahawa diantara 334 isolat dan urutan, *Laktobasillus* spp dengan 64.97% adalah yang paling dominan LAB space perut madu dan sarang lebah *Apis dorsata* diikuti oleh *Enterococcus* spp dengan 28.74%, *Bifidobacterium* dengan 5.99% dan selebihnya 0.3% *Weissilla*. Secara keseluruhan, nya spesis *Lactobasillus* yang paling banyak ialah *L. kunkeei* (40.53%), diikuti oleh *Lactobasillus sp*. (33.18%). Didalam sampel perut madu, *Lactobacillus* spp. Hadir sebanyak 53.21%. Menariknya, *Lactobasillus* spesis yang dominan dari sampel perut madu adalah *Lactobasillus plantarum* (31.12%), diikuti oleh *Lactobacillus pentosus* (27.71%), *Lactobacillus kunkeei* (22.89%), *Lactobacillus* sp (15.66%), *Lactobacullus vermiform* (2.41%) dan *Lactobacillus fermentum* (1.21%). Sampel dari sarang yang dipenuhi madu pula menunjukkan kehadiran *Lactobacillus* spp. Sebanyak 75.28%. *Lactobacillus* yang dominan didalam sampel ini ialah *Lactobacillus kunkeei* (51.49%), *Lactobacillus* sp. (44.48%) dan *Lactobacillus alvei* (4.48%). Enterococcus telah dipencilkan dari setiap perut madu (40.38%) dan sarang (18.54%) dari sampel Kedah dan Terengganu. Enterococcus yang dominan dari semua sampel perut madu dan sarang yang mewakili sebanyak 88.54% ialah *Enterococcus* sp dan selebihnya ialah *Enterococcus faecalis*

(11.46%). Manakala, *Bifidobacterium* dan *Weisella* pula masing-masing sebanyak 5.99% dan 0.3%. Keputusan kami menunjukkan bahawa terdapat kepelbagaian yang besar space spesis bakteria dari perut madu dan sarang lebah *Apis dorsata*.

Kesimpulannya, kepelbagaian kandungan bakteria didalam perut madu dan sarang madu pada lebah dari tanah tinggi dan tanah rendah adalah berbeza. Perbezaan ini adalah saling berkait dan disebabkan oleh taburan atau kepelbagaian bunga, nektar dan polen yang berbeza diantara tanah tinggi di Kedah dan kawasan pertanian di Terengganu.

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I certify that an Examination Committee has met on 1 October 2010 to conduct the final examination of Naser Tajabadi on his thesis entitled "Comparison of Lactic Acid Bacteria and Bifidobacteria from Honey Stomachs and Honeycombs of Giant Honeybee (*Apis dorsata* F.) of Kedah and Terengganu, Malaysia" in accordance with the University colleges Act 1971 and the Constitution of Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Halimi Mohd Saud, PhD
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BUJANG BIN KIM HUAT, PhD
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Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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NASER TAJABADI

Date 1 October 2010

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