



**UNIVERSITI PUTRA MALAYSIA**

**EFFECTS OF PREBIOTIC ACTIVITY OF *GANODERMA LUCIDUM*  
EXTRACTS ON *BIFIDOBACTERIUM* SPECIES AND FAECAL  
MICROFLORA *IN VITRO***

**MOHD YAMIN BIN SHAARI**

**FBSB 2012 3**

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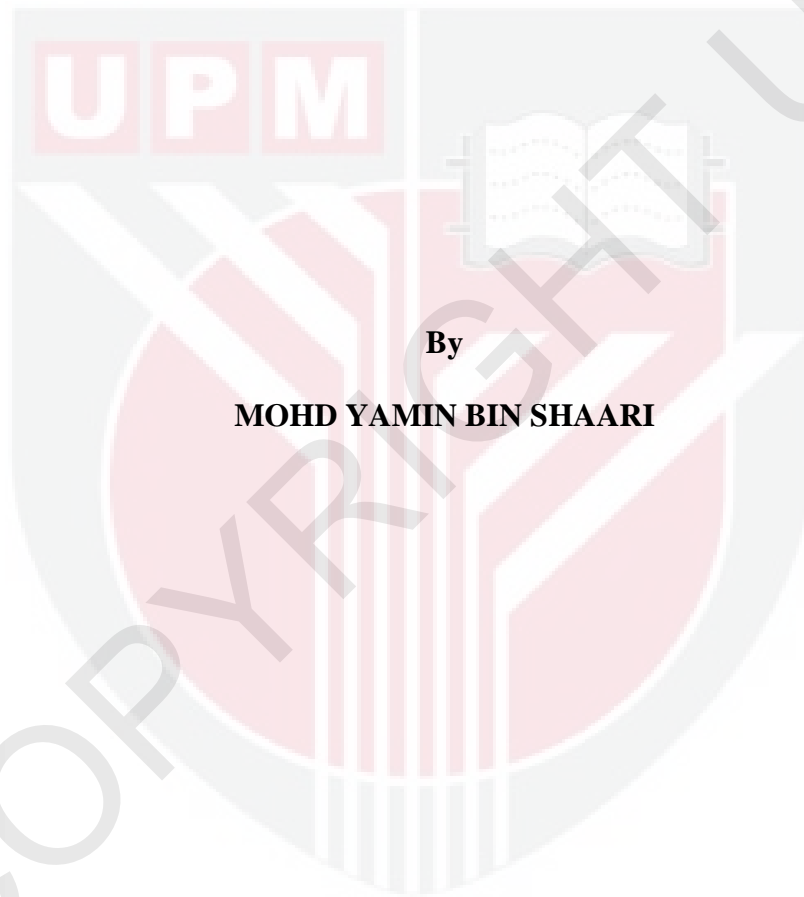
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MICROFLORA *IN VITRO***



**By**

**MOHD YAMIN BIN SHAARI**

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
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**EFFECTS OF PREBIOTIC ACTIVITY OF *GANODERMA LUCIDUM* EXTRACTS ON *BIFIDOBACTERIUM* SPECIES AND FAECAL MICROFLORA *IN VITRO***

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**September 2011**

**Chairman : Assoc. Prof. Shuhaimi Mustafa, PhD**  
**Faculty : Biotechnology and Biomolecular Sciences**

*Ganoderma lucidum* is a fungus that has long history in Japan and China as traditional medicine with many claims about its health-stimulating properties. Many researches conducted by scientists have proven the medical significance of polysaccharides extracted from *G. lucidum* such as inhibition of tumour cell, effectiveness in hyperglycaemic and hypoglycaemia, and hypertension treatment. The polysaccharides were also reported to have potential to act as prebiotics to support the growth of probiotic bacteria. Thus, in this study, the effects of polysaccharides extracted from *G. lucidum* and its fraction were tested on selected *Bifidobacterium* strains and bacterial microflora from faeces *in vitro*. Three *Bifidobacterium* strains used were revived from stock cultures and confirmed as pure culture through microscopic test. Crude polysaccharides from *G. lucidum* (GLCP) were successfully obtained and fractionated

into four types of fraction named as Polysaccharides fraction 1 (PF-1), Polysaccharides fraction 2 (PF-2), Polysaccharides fraction 3 (PF-3) and Polysaccharides fraction 4 (PF-4). PF-2 showed promising results in previous studies (Hamim, 2009) was selected for further test in this study. The growth rate of three types of *Bifidobacterium* strains was tested in pure culture fermentation. *B. pseudocatenulatum* G4 grew at the growth rate of  $0.67 \pm 0.09 \text{ h}^{-1}$ . *B. breve* ATCC 15700 and *B. longum* BB536 grew at  $0.60 \pm 0.09 \text{ h}^{-1}$  and  $0.64 \pm 0.05 \text{ h}^{-1}$ , respectively. Glucose was served as control. In pure culture fermentation, 5 batch cultures of tryptone peptone yeast (TPY) medium were supplemented with different carbon sources namely glucose, fructooligosaccharides (FOS), inulin, GLCP and PF-2. The growth patterns of all *Bifidobacterium* strains increased in each carbon sources used with the highest growth was in FOS and followed by inulin, glucose, PF-2 and GLCP. In mixed culture fermentation, two prebiotic candidates (GLCP and PF-2) and commercial prebiotics (inulin and FOS) were tested to determine their impact to the bacterial microflora simulated using faecal culture. The increment pattern was shown in *Bifidobacterium* genus with the maximum growth at 24 h were  $8.3 \pm 0.3 \log_{10}$  cells/ml for FOS. Meanwhile, maximum growth up to  $8.3 \pm 0.3$ ,  $8.0 \pm 0.4$ ,  $7.9 \pm 0.1$  and  $7.5 \pm 0.2 \log_{10}$  cells/ml were recorded for inulin, glucose, PF-2 and GLCP, respectively. Growth increase was also shown in *Lactobacillus* genus and higher than *Bifidobacterium* genus for FOS fermentation. However, the results were positive as *Bifidobacterium* and *Lactobacillus* genus were considered as beneficial bacteria for the host. For GLCP and PF-2, the increased growth showed promising result as the increases were 0.3 and 0.7  $\log_{10}$  cells/ml for *Bifidobacterium* genus and 0.7 and 1.0  $\log_{10}$  cells/ml for *Lactobacillus* genus at 24 h, respectively. On the other hand, the

inhibition pattern could still be observed on *Salmonella* and *E. faecalis* when compared to glucose (control) in all tests conducted. HPLC result showed that the pattern of lactic acid and acetic acid production might directly result in antimicrobial action. From this research, it can be concluded that *Ganoderma lucidum* extracts (GLCP and PF-2) have prebiotic capability and can be considered for commercialization in the future.



Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN AKTIVITI PREBIOTIK DARIPADA EKSTRAK *GANODERMA LUCIDUM* KE ATAS *BIFIDOBACTERIUM* SPESIS DAN MIKROFLORA DI DALAM NAJIS *IN VITRO***

Oleh

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*Ganoderma lucidum* adalah kulat yang mempunyai sejarah yang lama di Jepun dan China sebagai ubat tradisional yang dikatakan mempunyai bahan yang membantu meningkatkan kesihatan tubuh badan. Kajian demi kajian yang dilakukan oleh para saintis membuktikan keberkesanan perubatan menggunakan polisakarida yang diekstrak daripada *Ganoderma lucidum* seperti perencatan sel kanser dan juga berkesan di dalam merawat hiperglisemik, hipoglisemik dan hipertensi. Polisakarida juga dilaporkan berpotensi bertindak sebagai prebiotik yang menyokong pertumbuhan bakteria probiotik. Maka, di dalam kajian ini, kesan-kesan polisakarida yang diekstrak daripada *Ganoderma lucidum* dan pecahannya yang diuji ke atas spesies *Bifidobacterium* yang terpilih dan mikroflora dari najis dinilai secara *in vitro*. Tiga *Bifidobacterium* strain digunakan telah diaktifkan semula dari kultur simpanan dan disahkan kultur tulen melalui ujian mikroskopik. Polisakarida mentah daripada *Ganoderma lucidum* (GLCP)

telah berjaya diperolehi dan dipecahkan kepada empat jenis pecahan dinamakan sebagai Pecahan Polisakarida 1 (PF-1), Pecahan Polisakarida 2 (PF-2), Pecahan Polisakarida 3 (PF-3) dan Pecahan Polisakarida 4 (PF-4). Tetapi, PF-2 yang mana telah menunjukkan keputusan memberansangkan di dalam penyelidikan terdahulu (Hamim, 2009) telah dipilih untuk ujian seterusnya di dalam penyelidikan ini. Kadar pertumbuhan untuk ketiga-tiga jenis *Bifidobacterium* strain yang di gunakan telah diuji di dalam fermentasi kultur tulen. *B. pseudocatenulatum* G4 telah tumbuh pada kadar  $0.67 \pm 0.09 \text{ jam}^{-1}$ . Manakala, *B. breve* ATCC 15700, dan *B. longum* BB536 telah tumbuh pada kadar  $0.60 \pm 0.09 \text{ jam}^{-1}$ , dan  $0.64 \pm 0.05 \text{ jam}^{-1}$  bagi masing-masing di dalam glukosa sebagai control. Dalam fermentasi kultur tulen, 5 kultur kelompok Tryptone Peptone Yeast (TPY) medium telah ditambah dengan sumber-sumber karbon berbeza iaitu glukosa, fructooligosaccharides (FOS), inulin, GLCP dan PF-2. Pola-pola pertumbuhan semua *Bifidobacterium* strain telah meningkat di dalam setiap sumber-sumber karbon digunakan di mana pertumbuhan tertinggi adalah di dalam FOS dan diikuti oleh inulin, glukosa, PF-2 and GLCP. Dalam fermentasi yang bercampur, dua calon prebiotik (GLCP dan PF-2) dan prebiotik komersial (inulin dan FOS), telah diuji untuk menentukan kesan mereka kepada mikroflora bakteria dalam medium najis. Corak pertumbuhan telah ditunjukkan untuk genus *Bifidobacterium* dengan pertumbuhan maksimum pada 24 jam iaitu  $8.3 \pm 0.3 \log_{10} \text{ sel/ml}$  untuk FOS. Manakala, pertumbuhan maksimum sebanyak  $8.3 \pm 0.3$ ,  $8.0 \pm 0.4$ ,  $7.9 \pm 0.1$  and  $7.5 \pm 0.2 \log_{10} \text{ sel/ml}$  telah ditunjukkan di dalam inulin, glukosa, PF-2 and GLCP. Penambahan pertumbuhan juga ditunjukkan dalam genus *Lactobacillus* dan lebih tinggi daripada jenis *Bifidobacterium* bagi fermentasi FOS. Bagaimanapun, ini dianggap sebagai keputusan positif untuk



kedua-dua jenis bakteria kerana *Bifidobacterium* and *Lactobacillus* adalah bakteria yang berfaedah untuk tuan rumah. Untuk GLCP dan PF-2, pertumbuhan tambahan menunjukkan hasil yang baik dengan pertambahan 0.3 dan 0.7 log<sub>10</sub> sel/ml untuk genus *Bifidobacterium* dan 0.7 dan 1.0 log<sub>10</sub> sel/ml untuk genus *Lactobacillus* pada 24 jam. Sebaliknya corak perencatan masih boleh diperhatikan pada genus *Salmonella* and *E. faecalis* apabila dibandingkan dengan glukosa (kawalan) untuk semua eksperimen dijalankan. Keputusan HPLC menunjukkan corak yang mana pengeluaran laktik dan asetik asid mungkin secara langsung mengakibatkan tindakan agen antimikrob. Daripada penyelidikan ini, dapat disimpulkan bahawa ekstrak daripada *Ganoderma lucidum* (GLCP dan PF-2) mempunyai kebolehan prebiotik dan boleh dipertimbangkan untuk proses pengkomersilan pada masa hadapan.

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I certify that an Examination Committee has met on **date of viva** to conduct the final examination of Mohd Yamin bin Shaari on his Master of Science thesis entitled "**EFFECTS OF PREBIOTIC ACTIVITY OF *GANODERMA LUCIDUM* EXTRACTS ON *BIFIDOBACTERIUM* SPECIES AND FAECAL MICROFLORA IN VITRO.**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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## 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 other institutions.



**MOHD YAMIN BIN SHAARI**

**Date:** 15 September 2011

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