UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *GANODERMA LUCIDUM* CRUDE POLYSACCHARIDES ON THE GROWTH OF BIFIDOBACTERIA AND OTHER SELECTED INTESTINAL BACTERIA

MOHD HAMIM BIN HAMDAN MUSTAFA

FBSB 2009 13

EFFECTS OF *GANODERMA LUCIDUM* CRUDE POLYSACCHARIDES ON THE GROWTH OF BIFIDOBACTERIA AND OTHER SELECTED INTESTINAL BACTERIA

By

MOHD HAMIM BIN HAMDAN MUSTAFA

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

July 2009

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

EFFECTS OF *GANODERMA LUCIDUM* CRUDE POLYSACCHARIDES ON THE GROWTH OF BIFIDOBACTERIA AND OTHER SELECTED INTESTINAL BACTERIA

By

MOHD HAMIM BIN HAMDAN MUSTAFA

July 2009

Chairman: Associate Professor Shuhaimi Bin Mustafa, Ph.D

Faculty: Biotechnology and Biomolecular Sciences

Ganoderma lucidum (G. lucidum) is a fungus commonly used as a traditional Chinese medicine. The high value of *G. lucidum* is related to its polysaccharides content. The polysaccharides are all glucan that are closely related to scleroglucan, but vary in their water solubility and in their degree and nature of their side chains. *G. lucidum* crude polysaccharides from (GLCP) were obtained by hot water extraction. There was about 0.56 g of GLCP in 1 g *G. lucidum*. Fractionation of GLCP lead to the isolation of four polysaccharides sub fractions, PF-1, PF-2, PF-3 and PF-4 with molecular weight of 2.3 x 10^4, 4.6 x 10^4, 1 x 10^5 and 3 x 10^5 Da respectively.

The growth of probiotic bacteria (*Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4, *Lactobacillus acidophilus* and *Lactobacillus casei* shirota) and other selected intestinal bacteria (*Enterococcus faecalis* JCM5803 and *Salmonella choleraesuis* JCM6977) were studied in 10 mL Trypticase Phytone Yeast (TPY) medium containing various concentrations of GLCP (0.5%, 1.0%, 1.5% and 2.0%) (TPYgl). The growth was compared to the growth in TPY medium supplemented with glucose and inulin. The growth in a basal TPY medium (TPYp), the medium with glucose (TPYglu)

and a TPY medium containing both GLCP and glucose (TPYglu+gl) were also studied. Viable cell counts of the bacteria and the pH of the media were determined during anaerobic incubation of 0 h, 12 h, 24 h and 48 h at 37°C. In the absence of any carbohydrate source, all bacteria tested showed growth with mean log cfu/mL ranging from 6.87 to 7.99 after 24 hours incubation. However, in the presence of carbohydrate sources, cultures showed various degree of growth relative to the growth in TPYp. The bacteria tested showed highest growth in TPYglu+gl, followed by growth in TPYglu, TPYinu, TPYgl and TPYp sequentially. Highest viability of bacteria was observed in the media with the highest concentration of each carbohydrate source. Maximum population of bacteria was achieved after 24 hours and decreased significantly ($p<0.05$) after 48 hours in all media tested.

B. longum BB536 showed the highest growth in TPYgl medium. At 24 hours incubation, highest population 10.53 log cfu/mL of *B. longum* BB536 was observed in medium with 2.0% GLCP and the lowest 9.37 log cfu/mL was in 0.5% concentration. *B. pseudocatenulatum* G4 was the second highest growth with 10.40 log cfu/mL. *E. faecalis* was the least growth (10.15 log cfu/mL) in this medium.

Growth of *B. pseudocatenulatum* G4, *E. faecalis* JCM5803 and *S. choleraesuis* JCM6977 in GLCP fractions (TPYF1, TPYF2, TPYF3 and TPYF4) were compared to their growth in TPYinu 250 µg/mL (control). Results show that, all four fractions could support the growth of bacteria tested. The highest growth was the *B. pseudocatenulatum* G4 in TPYF2 with 1000 µg PF-2/mL concentrations. The growth was increased from 6.82 to 8.91 log cfu/mL after 24 hours and decreased to 7.92 log cfu/mL after 48 hours. In medium with 250 µg/mL PF-2, there was no significant ($p<0.05$) growth difference

compared to control. This suggested that, all the four fractions and inulin are supporting the growth of this bacterium.

E. faecalis JCM5803 and *S. choleraesuis* JCM6977 showed similar growth pattern. Comparing among the different media, these bacteria showed a very high growth in TPYF1. This was followed by growth in TPYF2, TPYF3 and TPYF4. High concentration of each fraction gave high viability of the bacteria. Highest population (8.89 and 8.72 log cfu/mL) was in the TPYF1 with 1000 µg/mL PF-1 after 24 hours incubation.

This study shows that, GLCP could support the growth of bacteria tested. GLCP shows high potential of growth effect to bifidobacteria compared to other bacteria. Moreover, *B. pseudocatenulatum* G4 showed highest population in TPYF2 fraction compared to other bacteria tested. This suggested that PF2 of GLCP exhibited growth promoting effect on bifidobacteria. However, more study should be done to support this finding.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Master Sains

KESAN POLISAKARIDA KASAR DARIPADA *GANODERMA LUCIDUM* KE ATAS PERTUMBUHAN BIFIDOBAKTERIA DAN BAKTERIA USUS TERPILIH

Oleh

MOHD HAMIM BIN HAMDAN MUSTAFA

Julai 2009

Pengerusi: Profesor Madya Shuhaimi Bin Mustafa, Ph.D

Fakulti: Bioteknologi dan Sains Biomolekul

Ganoderma lucidum (*G. lucidum*) ialah sejenis kulat yang sering digunakan dalam perubatan tradisional masyarakat Cina. Nilai *G. lucidum* yang tinggi terletak pada kandungan polisakaridanya. Kesemua polisakaridanya adalah glukan yang berkait rapat dengan skleroglukan tetapi berbeza dari segi keterlarutan dalam air and darjah serta sifat rantai-rantai sisinya. Polisakarida mentah *G. lucidum* diperolehi melalui pengekstrakan air panas. Sebanyak 1 g *G. lucidum* menghasilkan 0.56 g GLCP. Pemfraksian GLCP boleh menghasilkan empat sub fraksi polisakarida iaitu PF-1, PF-2, PF-3 dan PF-4 dengan berat molekul masing-masing 2.3 x 10^4, 4.6 x 10^4, 1 x 10^5 and 3 x 10^5 Da.

Pertumbuhan bakteria probiotik (*Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4, *Lactobacillus acidophilus* dan *Lactobacillus casei* shirota) dan juga bakteria usus (*Enterococcus faecalis* JCM5803 dan *Salmonella choleraesuis* JCM6977) telah dikaji dalam 10 mL medium Trypticase Phytone Yeast (TPY) yang mengandungi pelbagai kepekatan GLCP (0.5%, 1.0%, 1.5% and 2.0%) (TPYgl). Perbandingan pertumbuhan bakteria tersebut di dalam medium TPY yang ditambah glukosa dan inulin juga dijalankan. Medium yang mengandungi TPY sahaja (TPYp),

medium TPY yang mengandungi glukosa (TPYglu) dan medium TPY yang mengandungi kedua-kedua GLCP dan glukosa (TPYglu+gl) adalah antara medium lain yang turut dikaji peranannya dalam menampung pertumbuhan bakteria tersebut. Pengiraan sel hidup bakteria dan pengukuran pH medium dibuat ketika pertumbuhan anaerobik selama 0, 12, 24 dan 48 jam pengeraman pada suhu 37°C. Semua bakteria menunjukkan pertumbuhan daripada 6.87 kepada 7.99 log cfu/mL selepas 24 jam eraman tanpa sebarang sumber karbohidrat. Walaubagaimanapun, bakteria menunjukkan pelbagai darjah pertumbuhan apabila disertakan sumber karbohidrat. Bakteria yang dikaji menunjukkan pertumbuhan paling baik di dalam medium TPYglu+gl, diikuti dengan TPYglu, TPYinu, TPYgl dan TPYp. Jumlah sel hidup bakteria yang paling tinggi didapati pada kepekatan paling tinggi bagi setiap sumber karbohidrat. Pertumbuhan mencapai maksimum selepas 24 jam dan mula berkurang selepas 48 jam bagi semua medium yang dikaji.

B. longum BB536 menunjukkan pertumbuhan paling tinggi berbanding bakteria lain di dalam medium ini. Pada 24 jam inkubasi, populasi paling tinggi *B. longum* iaitu 10.53 log cfu/mL dapat dilihat pada kepekatan 2.0% GLCP dan populasi paling rendah iaitu 9.37 log cfu/mL pada kepekatan 0.5% GLCP. Pertumbuhan *B. pseudocatenulatum* G4 adalah yang kedua tertinggi selepas *B. longum* BB536 dengan 10.40 log cfu/mL manakala *E. faecalis* pula menunjukkan populasi terendah dalam medium ini berbanding semua bakteria yang dikaji dengan hanya 10.15 log cfu/mL pada kepekatan 2.0% GLCP.

Pertumbuhan *B. pseudocatenulatum* G4, *E. faecalis* JCM5803 and *S. choleraesuis* JCM6977 dikaji di dalam medium yang mengandungi fraksi GLCP (TPYF1, TPYF2, TPYF3 dan TPYF4) dan perbandingan dilakukan dengan pertumbuhan mereka di dalam TPYinu 250 µg/mL (kawalan). Berdasarkan keputusan yang diperolehi, kesemua empat

fraksi GLCP mampu menyokong pertumbuhan bakteria yang dikaji. Pertumbuhan terbaik bagi *B. pseudocatenulatum* G4 adalah di dalam medium TPYF2 pada kepekatan 1000 µg/mL. Pertumbuhan tersebut dapat dilihat meningkat daripada 6.82 ke 8.91 log cfu/mL selepas 24 jam dan kemudian menurun ke 7.92 log cfu/mL selepas 48 jam. Pada kepekatan 250 µg/mL, tiada perbezaan ketara pertumbuhan berbanding kawalan. Ini menunjukkan bahawa kesemua empat fraksi dan inulin mampu menyokong pertumbuhan bakteria ini.

E. faecalis JCM5803 dan *S. choleraesuis* JCM6977 menunjukkan corak pertumbuhan yang serupa. Kedua-dua bakteria menunjukkan pertumbuhan yang paling tinggi di dalam medium TPYF1 diikuti dengan TPYF2, TPYF3 dan TPYF4. Kepekatan tertinggi setiap fraksi memberi jumlah sel hidup bakteria yang tertinggi. Populasi paling tinggi bagi kedua-dua bakteria ialah pada kepekatan 1000 µg/mL TPYF1 iaitu masing-masing 8.89 dan 8.72 log cfu/mL selepas 24 jam.

Kajian ini menunjukkan bahawa GLCP mampu menyokong pertumbuhan bakteria yang telah dikaji. GLCP juga memperlihatkan potensi yang tinggi dan berkesan untuk menggalakkan pertumbuhan bifidobacteria berbanding bakteria lain. Populasi paling tinggi *B. pseudocatenulatum* G4 di dalam medium TPYF2 yang jelas berbanding bakteria lain mencadangkan bahawa PF2 daripada GLCP menggalakkan pertumbuhan bifidobakteria. Namun demikian, kajian selanjutnya perlulah dijalankan untuk menyokong penemuan ini.

AKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisor Associate Professor Dr. Shuhaimi Mustafa for his guidance, advices, valuable discussions, encouragement, assistance and support throughout the period of my study at Universiti Putra Malaysia. My sincere appreciation to my co-supervisors Associate Professor Dr. Mohd Noor Abd Wahab, Associate Professor Dr. Mohd Yunus Abd Shukor and Professor Dr. Mohd Yazid Abd Manap for their valuable comments and advices.

My special thanks to my lovely wife Asilah, my mother Abarizah, my parents in law, Ahmad Tajudin and Zabidah, and all my family members for their support. Their love, forbearance and support throughout the period of this study have made this a truly memorable journey. I am appreciative of the valuable assistance and moral support of the Lab 202 members Mr. Arifen, Mr, Khalid, Mr. Karim, Sis Putri, Sis Ruzaina, Anas, Yamin, Sis Atun, Sis Huda and Sis Khalilah. I wish to thank members of Probiotic Lab (Department of Management and Food Service, UPM) Barka and Arezou for their co-operation. I am grateful to the Ministry of Science, Technology and Innovation, Government of Malaysia, for providing research fund under the IRPA scheme that financed this study.

I certify that a Thesis Examination Committee has met on **15 July 2009** to conduct the final examination of **Mohd Hamim bin Hamdan Mustafa** on his degree thesis entitled **"Growth of bifidobacteria and other selected intestinal bacteria in medium supplemented with *Ganoderma lucidum* crude polysaccharides"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Member of the Thesis Examination Committee were as follows:

Suraini Abd. Aziz, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Norhani Abdullah, Phd
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Rosfarizan Mohamad, Phd
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Khan Ayob, Phd
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

_____
BUJANG BIN KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

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:

Shuhaimi Mustafa, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd Noor Abd Wahab, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd Yunus Abd Shukor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd Yazid Abd Manap, PhD
Professor
Faculty of Food Science & Technology
Universiti Putra Malaysia
(Member)

_____
HASANAH MOHD GHAZALI, Phd
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 16 November 2009

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.

_____

MOHD HAMIM BIN HAMDAN MUSTAFA

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

µg	microgram
µL	microliter
mL	mililitre
L	liter
cm	centimetre
mm	milimetre
nm	nanometre
°C	degree centrigrade
rpm	route per minute
g	gram
mg	milligram
min	minute(s)
%	percent
Cfu	colony forming unit
M	molar
mM	milimolar
EDTA	ethylenedinitrilotetraacetic acid
v/v	volume/volume
w/v	weight/volume
Da	Dalton
h	Hour
NaOH	natrium hydroxide
HCl	acid hydrochloride

CHAPTER 1

INTRODUCTION

Ganoderma is a basidiomycete which originated from China and Japan. It is known as 'Ling Zhi' in China and 'Reishi' or 'Mannentake' in Japan. It has been used in China for thousands of years as a miraculous remedy for a variety of diseases ranging from traumatic wounds, inflammation and various ulcers to the treatment of cancer (Kisida *et al.,* 1988). In Chinese folk fore, the fruiting body of *G. lucidum* has always been regarded to be a high quality medicine, the so called 'longevity drug', which can improve one's constitution, increases the body's healing ability and helps maintain good health (Tong *et al*., 1994).

There are several species of *Ganoderma* of which some common ones are known as *G. lucidum, G. tsugae, G. tropicum, G. applanatum, G. japonicum*, and *G. boninense*. According to Jong *et al*. (1991), these mushrooms differ from the ordinary mushrooms, which belong to the order Agaricales in that they have pores rather than gills on the surface of the underside of the fruiting bodies.

Polysaccharides are the main compounds that have attracted considerable attention because of their high medicinal values. Polysaccharides are a structurally diverse group of biological macromolecules of widespread occurrence in nature. They are composed of repetitive structural features that are polymers of monosaccharide residues joined to each other by glycosidic linkages. Polysaccharides present the highest capacity for carrying biological information since they have the greatest

potential for structural variability. This characteristic suggesting that bacteria can utilize the polysaccharides as a carbohydrate source and might have commercial application as a food or feed additive to stimulate probiotic bacteria in the gut.

Gut health is currently getting much attention among researchers and the public. This mainly because many of the physical health problems are found to be closely connected to the improper functionality of the gut. The functions of the human gut include absorption of water and certain minerals, and the storage and excretion of waste materials. However, because of the resident microbiota, it is clear that the colon has an important role in human nutrition and possibly health (Gibson *et al.,* 1995). Over the years, study of the microbiological system in the gut uncovered the general effect brought to the host. Evolved from birth, this group of microorganism has adapted and changed according to the maturity of the gut, which make their present as one entity in the so-called "symbiosis" relationship. These organisms and their metabolic activities are not inert to the human host and have both positive and negative impacts on health. The balance of this ecosystem is dynamic and maybe altered by several factors. The maintenance of a community of bacteria which contains a predominance of beneficial species and minimal putrefactive processes is believed to be important for maintaining intestinal health.

Among more than 400 species of bacteria present in the intestine of adult human being, bifidobacteria is considered to be the most beneficial to human health (Gibson, 1998). Members of this genus are thought to contribute to digestion, absorption of nutrients, prevention of colonization by pathogens and stimulation of immune responses in humans (Yaeshima, 1996). An increase in the number and