UNIVERSITI PUTRA MALAYSIA

PROBIOTIC CHARACTERISTICS OF BACTERIOCINOGENIC *LACTOBACILLUS PLANTARUM* STRAINS ISOLATED FROM MALAYSIAN FOODS

LIM YIN SZE
FBSB 2010 1

PROBIOTIC CHARACTERISTICS OF BACTERIOCINOGENIC *LACTOBACILLUS PLANTARUM* STRAINS ISOLATED FROM MALAYSIAN FOODS

By

LIM YIN SZE

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

February 2010

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Doctor of Philosophy

PROBIOTIC CHARACTERISTICS OF BACTERIOCINOGENIC *LACTOBACILLUS PLANTARUM* STRAINS ISOLATED FROM MALAYSIAN FOODS

By

LIM YIN SZE

February 2010

Chair : Foo Hooi Ling, PhD

Faculty : Biotechnology and Biomolecular Sciences

The usage of antimicrobial drugs, such as antibiotics, as growth enhancer in livestock farming is prohibited in many countries. In recent years, antimicrobial resistance associated with the use of antimicrobial drugs in animals has triggered vast public attention. Thus, recent approach to enhance the growth performance of livestock is through supplementation of probiotic and digestive enzymes. Probiotic are live microorganisms, when consumed in adequate amounts will confer health benefit on the host.

Generally recognised as safe status of lactic acid bacteria (LAB) and the increasing demand of probiotic in food and feed industry have driven current attempt to determine the probiotic potential of six bacteriocinogenic *Lactobacillus plantarum* strains (UL4, TL1, RS5, RI11, RG11 and RG14) isolated from Malaysian foods, through *in vitro* probiotic characterisation study and *in vivo* animal trials.

In the *in vitro* study, the antimicrobial potential of *Lb. plantarum* strains and bacteriocins was carried out, followed by the study to assess the ability of these strains to overcome simulated gastric and small intestinal tracts, deconjugate bile salts (sodium taurocholate, sodium glycocholate, sodium taurodeoxycholate and sodium glycodeoxycholate), assimilate cholesterol and adhere to intestinal epithelial Caco-2 cell line. *In vivo* animal trials were then carried out to evaluate the effectiveness of *Lb. plantarum* strains and bacteriocins produced on growth performance, intestinal microflora and histomorphological changes, and alteration of short chain fatty acids and serum cholesterol level of rats.

In the *in vitro* study, *Lb. plantarum* strains and their bacteriocins had exhibited strong bacteriocidal and bacteriostatic antimicrobial action against Gram-positive and –negative bacteria. *Lb. plantarum* strains had strongly inhibited the growth of *Bacillus cereus* B43 and *Listeria monocytogenes* L55, whereas bacteriocins had inhibited the *Pediococcus acidilactici* 4-46, *Enterococcus faecalis* N-103, *Enterococcus faecium* ATCC 19434, *Listeria monocytogenes* L55, *Bacillus cereus* B43, *Streptococcus pneumoniae* S972 and *Staphylococcus aureus* S942. *Lb. plantarum* strains were highly tolerance to the exposure of simulated gastric and small intestinal juices. They were able to deconjugate bile salts and assimilated 56.7 – 100.0 % of cholesterol. *Lb. plantarum* strains adhered strongly to the Caco-2 epithelial cell line and reduced the adherence of *L. monocytogenes* L55 substantially.

In *in vivo* animal trial, rats were force-fed with six *Lb. plantarum* strains (10^8 CFU/mL daily) for 28 days. The high LAB population found in faecal and intestinal samples indicated that the fed *Lb. plantarum* strains could survive and colonise in

gastrointestinal tract. The small intestinal villous height and crypt depth were significantly ($P < 0.05$) greater than Control group. Furthermore, *Lb. plantarum* RI11 and RG11 had significantly reduced the serum cholesterol level of rats by 24.4 % ($P < 0.05$) and 19.3 % ($P < 0.01$), respectively.

In the subsequent animal trial, rats were force-fed with six bacteriocins (800 AU/mL daily) produced *by Lb. plantarum* strains for 28 days. Bacteriocins had reduced the colonisation of Enterobacteriaceae in the intestine, while maintaining the intestinal LAB population. All bacteriocins had significantly increased ($P < 0.05$) the crypt depth of small intestine and caecum. Bacteriocins RS5, RI11 and RG11 had significantly reduced the serum cholesterol level of rats by 21.9 % ($P < 0.05$), 15.6 % ($P < 0.05$) and 15.2 % ($P < 0.01$) respectively. Current study showed that the studied *Lb. plantarum* strains and bacteriocins produced had demonstrated certain extent of probiotic effects in *in vitro* and *in vivo*. These probiotic effects are specific or limited to the *Lb. plantarum* strains or bacteriocins tested.

Abstrak ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN PROBIOTIK BAGI STRAIN-STRAIN BAKTERIOSINOGENIK *LAKTOBASILUS PLANTARUM* YANG DIASING DARIPADA MAKANAN MALAYSIA

Oleh

LIM YIN SZE

Februari 2010

Pengerusi : Foo Hooi Ling, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Penggunaan ubat antimikrob seperti antibiotik sebagai penggalak pertumbuhan dalam bidang ternakan telah dilarangkan oleh kebanyakan negara. Kebelakangan ini, kewujudan resistant antimikrob berikutan dengan pengunaan ubat antimukrob dalam binatang ternakan telah menarik perhatian umum. Oleh itu, pendekatan terkini untuk meningkatkan prestasi pertumbuhan ternakan adalah dengan penggunaan probiotik dan enzim-enzim penghadaman. Probiotik adalah mikroorganisma hidup yang apabila dimakan dalam jumlah secukupnya, dapat memanfaatkan kesihatan penggunanya.

Status ‘dikenali sebagai selamat secara umumnya’ untuk bakteria asid laktik (LAB) dan peningkatan permintaan untuk probiotik dalam industri makanan dan makanan ternakan telah mendorong pelaksanaan kajian ini untuk menentukan potensi probiotic bagi enam strain-strain bakteriosinogenik *Laktobasilus plantarum* (UL4,

TL1, RS5, RI11, RG11 dan RG14) yang diasingkan daripada makanan Malaysia, melalui kajian pencirian probiotik secara *in vitro* dan kajian *in vivo* haiwan.

Dalam kajian *in vitro*, potensi antimikrob bagi enam strain *Lb. plantarum* dan bakteriosin yang dihasilkan telah dijalankan, diikuti dengan kajian untuk menilai keupayaan strain ini untuk mengatasi keadaan simulasi saluran gastrik dan usus kecil, dekonjugasi garam hempedu (sodium taurokolat, sodium glikokolat, sodium taurodeoksikolat dan sodium glikodeoksikolat), asimilasi kolesterol dan pelekatan ke Caco-2 sel epithelium usus. Kajian *in vivo* haiwan seterusnya dilaksanakan untuk menilai keberkesanan strain *Lb. plantarum* dan bakteriosin yang dihasilkan ke atas prestasi pertumbuhan, mikroflora usus dan perubahan-perubahan histomofologikal, perubahan rantaian pendek asid lemak dan paras kolesterol serum tikus.

Dalam kajian *in vitro*, strain *Lb. plantarum* dan bakteriosinnya telah mempamerkan tindakan antimikrob bakteriosidal dan bakteriostatik yang kuat terhadap patogen-patogen. Strain *Lb. plantarum* telah menyinkirkan pertumbuhan *Basilus cereus* B43 dan *Listeria monocytogenes* L55, manakala bakteriosinnya telah merencatkan bacteria *Pediococcus acidilactici* 4-46, *Enterococcus faecalis* N-103, *Enterococcus faecium* ATCC 19434 *Listeria monocytogenes* L55, *Bacillus cereus* B43, *Streptococcus pneumoniae* S972 dan *Staphylococcus aureus* S942. Strain *Lb. plantarum* telah mempamerkan tolerasi yang tinggi dalam simulasi saluran gastrik dan usus kecil. Mereka ini boleh mendekonjugasikan garam hempedu dan mengasimilasikan 56.7 – 100.0 % kolesterol. Strain *Lb. plantarum* juga melekat dengan kuat pada Caco-2 sel epithelium usus, dan telah mengurangkan pelekatan *L. monocytogenes* L55 dengan nyatanya.

Dalam kajian *in vivo* haiwan, tikus telah diberi enam strain *Lb. plantarum* (10^8 CFU/mL harian) selama 28 hari. Populasi LAB yang tinggi telah dijumpai dalam sampel tahi dan usus. Kajian ini telah menunjukkan bahawa strain *Lb. plantarum* dapat hidup dan mengkolonisasikan saluran usus. Ketinggian vilus dan kedalaman krypt telah meningkat dengan lebih nyatanya ($P < 0.05$) daripada kumpulan kontrol. Tambahan pula, *Lb. plantarum* RI11 dan RG11 telah mengurangkan paras kolesterol serum tikus sebanyak 24.4 % ($P < 0.05$) dan 19.3 % ($P < 0.01$) masing-masing.

Dalam kajian haiwan seterusnya, tikus telah diberi enam bakteriosin (800 AU/mL harian) yang dihasilkan oleh *Lb. plantarum* selama 28 hari. Bakteriosin telah mengurangkan populasi *Enterobacteriaceae* dalam usus sementara mengekalkan populasi LAB dalam usus. Secara umunya, semua bakteriosin telah meningkatkan kedalaman krypt usus kecil dan sekum dengan nyatanya ($P < 0.05$). Sementara, bakteriosin RS5, RI11 dan RG11 telah mengurangkan paras kolesterol serum tikus dengan nyatanya sebanyak 21.9 % ($P < 0.05$), 15.6 % ($P < 0.05$) dan 15.2 % ($P < 0.01$) masing-masing. Kajian terkini ini telah menunjukkan bahawa strain-strain *Lb. plantarum* dan bakteriosin yang dihasilkan telah mempamerkan kesan probiotik dalam kajian *in vitro* dan *in vivo*. Walaubagaimanapun, kesan probiotik in adalah spesifik atau terhad kepada strain-strain *Lb. plantarum* dan bakteriosin yang dikaji.

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude and appreciation to my supervisor, Assoc. Prof. Dr. Foo Hooi Ling for her invaluable guidance, patience and constant encouragement throughout my study. I am also immensely grateful for the advice and support given by the members of my supervisory committee, Assoc. Prof. Dr. Loh Teck Chwen, Prof. Dr. Raha Abdul Rahim, Prof. Dr. Mohd. Hair Bejo and Prof. Dr. Gulam Rusul Rahmat Ali.

I owe my deepest gratitude to Assoc. Prof. Dr. Abdul Rahman Omar, who have provided me the facility for carried out cell tissue culture work and I am also indebted to Mr. Kufli C. N., Mr. Saparin Demin, Mr. Zakaria Md. Shah, Mr. Saipuzaman Ali, Mr. Rosli Aslim and Mrs. Aluyah Marzuki for their excellent technical assistance.

I am heartily thankful to friends and staff in UPM who have supported me in various aspects during the completion of this study. Last but not least, this thesis would not have been possible without the love and supports from Boon Chin and my family.

Thanks God for everything!

I certify that a Thesis Examination Committee has met on 24th February, 2010 to conduct the final examination of Lim Yin Sze on her thesis entitled "**Probiotic characteristics of bacteriocinogenic *Lactobacillus plantarum* strains isolated from Malaysian foods**" 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 degree of Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Raja Noor Zaliha Raja Abd. Rahman, PhD
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)

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

Rickey Yoshio Yada, PhD
Professor
Department of Food Science
University of Guelph
Canada
(External Examiner)

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

Date:

The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Foo Hooi Ling, PhD
Associate Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Chairman)

Raha Abdul Rahim, PhD
Professor
Faculty of Biotechnology and Molecular Sciences
Universiti Putra Malaysia
(Member)

Mohd. Hair Bejo, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Gulam Rusul Rahmat Ali, PhD
Professor
School of Industrial Technology
Universiti Sains Malaysia
(Member)

HASANAH MOHD. GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13th May 2010

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.

_____

LIM YIN SZE

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATION

AU	Arbitrary unit
CFS	Cell free supernatant
cm	Centimetre
CFU	Colony forming unit
CE	Competitive exclusion
Da	Dalton
d	Day
^{o}C	Degree Celsius
ETB	Enterobacteriaceae bacteria
EMB	Eosin methylene blue medium
g	G – force
GIT	Gastrointestinal tract
GT	Generation time
h	Hour
HCl	Hydrochloric acid
kg	Kilogram
K_2HPO_4	di-Potassium hydrogen phosphate buffer
LAB	Lactic acid bacteria
L	Litre
LB	Lubria medium
MRS	De Man, Rogosa and Sharpe medium
mM	Millimolar
mg	Milligram
mL	Millilitre

mm	Millimetre
min	Minute
mol	Mole
µg	Microgram
µL	Microlitre
NaCl	Sodium chloride
NaOH	Sodium hydroxide
nm	Nanometre
OD	Optical density
PBS	Potassium buffered saline
%	Percent
s	Second
sp.	*Species*
subsp.	Subspecies
SCFA	Short chain fatty acid
TS	Tryptic soy medium
v/v	Volume/volume
w/v	Weight/volume