UNIVERSITI PUTRA MALAYSIA

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FSMB 2003 35

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MASTER OF SCIENCE
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2003

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By

LIM YIN SZE

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

September 2003

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

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September 2003

Chairperson : Foo Hooi Ling, Ph.D.

Faculty : Food Science and Biotechnology

A variety of bacteriocins have been discovered, however there is limited information on their physico-chemical, biochemical and genetic characteristics. This study was carried out to isolate bacteriocinogenic lactic acid bacteria (LAB) from local fermented foods (*Tempeh, Tapai Ubi* and *Tapai Pulut*) and food condiment (*Chili Bo*). Selected bacteriocinogenic isolates and the bacteriocins produced were then characterized. The bacteriocins were purified using Fast Protein Liquid Chromatography (FPLC). Among 55 isolates isolated from the fermented foods, 20 of the isolates were able to produce bacteriocins in the range of 200-800 AU/ml inhibitory activity. Bacteriocins produced by UL4, UB6 and GB5 were tested against selected gram-positive and –negative pathogens. Isolate UL4, which produced the

highest antagonistic activity against *Pediococcus acidilactici*, *Enterococcus faecalis*, *Enterococcus faecium* and *Listeria monocytogenes* was selected for further characterization. The isolate UL4 was identified as *Lactobacillus plantarum* I using API 50 CHL test kit. *Lb. plantarum* I-UL4 is a gram-positive cocco-bacilli facultative anaerobe. The maximum bacteriocin production of 800 AU/ml was achieved after 12 h incubation at 30 °C in neutralized MRS medium. The bacteriocin UL4 was characterized physico-chemically and classified as Class II, heat stable bacteriocin, since it was able to maintain 200 AU/ml bacteriocin activity after being autoclaved at 121 °C for 15 min. Bacteriocin UL4 was also able to tolerate a broad pH range, from acidic pH 2-5 to basic pH 7-8. Bacteriocin UL4 was suitable to be applied in refrigerated foods due to its stability at temperature below 15 °C for 45 days. However, bacteriocin UL4 was inactivated by proteolytic enzymes such as trypsin, α- and β-chymotrypsin, proteinase K and papain, inferring the proteinaceous nature of bacteriocin UL4. A four-step purification procedure, involving precipitation with 40-80 % ammonium sulphate, Mono-S cation-exchange chromatography, Superose-12 packed and prepacked gel-filtration chromatography, successfully purified the bacteriocin UL4 to apparent homogeneity, with a yield of 0.10 %. Tricine-SDS-PAGE was conducted to determine the molecular mass of the purified bacteriocin. The estimated molecular mass of unbound bacteriocin fraction of Mono-S cation-exchange chromatography was 7.0 kDa. However, the estimated molecular mass has to be confirmed by other techniques, such as MALDI-TOF spectrometry. Two groups of inhibitory compounds with different pI values, ranging from 6.55-7.35 and 3.5-5.2, were separated by IEF-PAGE. The results of IEF-PAGE analysis further confirmed the result of pH stability, inferring that the bacteriocin might not only consists of

cationic compound, but also consists of anionic compound. Further study needs to be carried out to increase the yield of bacteriocin prior to application by the food industry.

Abstrak ini dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PEMENCILAN BAKTERIA LAKTIK ASID YANG BAKTERIOSINOGENIK DAN PENULENAN BAKTERIOSIN-BAKTERIOSIN YANG TERPILIH DARIPADA MAKANAN TRADISIONAL TEMPATAN

Oleh

LIM YIN SZE

September 2003

Pengerusi : Foo Hooi Ling, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Pelbagai jenis bakteriosin telah ditemui, walaubagaimanapun keterangan berkaitan dengan ciri-ciri kimia-fizik, bio-kimia dan genetik adalah amat terhad. Dengan demikian, kajian ini telah dijalankan untuk memencilkan bacteria asid laktik (BAL) yang bakteriosinogenik daripada sumber makanan tempatan seperti *tempeh, tapai ubi* dan *tapat pulut* serta daripada perisa makanan seperti *Chili Bo.* Kajian pencirian telah dijalankan ke atas pencilan-pencilan BAL yang terpilih dan bakteriosin-bakteriosin yang dihasilkan. Proses penulenan bakteriosin diteruskan dengan menggunakan kromatografi cecair khas untuk protein (FPLC). Dalam proses pemencilan BAL yang bakteriosinogenik, 50 BAL telah dipencilkan, dimana 20 BAL diantaranya telah menunjukkan aktiviti perencatan sebanyak 200-800 AU/ml. Kajian anti-mikrob ke

atas bacteria gram-positif dan gram-negatif telah dijalankan untuk bakteriosin-bakteriosin yang dihasilkan oleh pencilan-pencilan UL4, UB6 dan GB5. Pencilan UL4 yang menghasilkan activiti perencatan optima ke atas *Pediococcus acidilactici*, *Enterococcus faecium*, *Enterococcus faecalis* dan *Listeria monocytogenes* telah dipilih untuk kajian berikutnya. Pencilan UL4 telah dikenalpastikan sebagai *Lactobacillus plantarum* I melalui ujian identifikasi yang telah dijanlankan dan juga dengan menggunakan unit identifikasi API 50 CHL. *Lactobacillus plantarum* I-UL4 merupakan bacteria Gram-positif yang bersifat anaerobik dan mempunyai sel berbentuk cocco-bacilli. Bakteria ini dapat menghasilkan bakteriosin yang optima, iaitu 800 AU/ml, selepas incubasi selama 12 jam pada suhu 30 °C dalam media MRS (pH 7-7.5). Bakteriosin UL4 yang terhasil telah dikategorikan sebagai Kelas II, bakteriosin yeng tahan haba, dimana setelah diautoklafkan pada suhu 121 °C, selama 15 minit, aktiviti bakteriosin UL4 masih dikekalkan pada 200 AU/ml. Bakteriosin UL4 juga dapat mengekalkan aktivitinya dalam julat pH yang besar, iaitu dari pH 2-5 yang berasid sehingga pH 7-8 yang berbes. Bakteriosin UL4 adalah stabil pada suhu dibawah 15 °C untuk penyimpanan selama 45 hari, maka ia amat berpotensi untuk penggunaan dalam makanan bersuhu rendah. Dalam kajian tindakbalas enzim terhadap kestabilan bakteriosin, bakteriosin UL4 telah direncatkan oleh enzim proteolitik, seperti trypsin, α- dan β-chymotrypsin, proteinase-K dan papain. Keputusan ini juga menunjukkan bahawa bakteriosin bersifat protein. Satu proses penulenan yang mengandungi empat langkah, iaitu pemendakan dengan menggunakan 40-80 % ammonium sulfat, kromatografi penukar kation Mono-S dan 2 jenis kromatografi penurasan gel Superose-12 (jenis kolum *prepacked* dan *packed*). Proses penulenan ini telah berjaya menulenkan bakteriosin UL4, walaupun dengan

hasil yang rendah, iaitu 0.10 %. TRICINE-SDS-PAGE telah dijalankan untuk mengenalpastikan berat molekul bagi bakteriosin yang telah ditulenkan. Berat molekul sebanyak 7 kDa telah dianggarkan bagi bakteriosin tulen (jenis bakteriosin yang tidak terikat dengan kolum) dari kromatografi penukar kation Mono-S. Anggaran berat molekul ini memerlukan pengesahan lanjutan dengan meggunakan teknik-teknik yang lain, misalnya MALDI-TOF spektrometri. 2 komponen yang mempunyai julat nilai pI yand berbeza, iaitu dari 6.55-7.35 dan dari 3.5-5.2 telah dipisahkan oleh IEF-PAGE. Keputusan yang diperolehi ini juga seiras dengan keputusan yang diperolehi dalam kajian pengaruh pH terhadap kestabilan bakteriosin yang telah dijalankan. Keputusan ini juga sahkan bahawa bakteriosin UL4 bukan sahaja mengandungi komponen bersifat kationik, bahkan ia juga mengandungi komponen yang bersifat anionic. Kajian lanjutan perlu dijalankan untuk meningkatkan penghasilan bakteriosin demi applikasi dalam industri makanan.

ACKNOWLEDGEMENTS

I would like to express my most sincere gratitude and appreciation to my supervisor, 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, Prof. Dr. Gulam Rusul Rahmat Ali and Assoc. Prof. Dr. Norihan Mohd. Saleh.

I would like to convey my warm thanks to Kwan Kit, Yock Ann, Sia Yen, Apinya, Wanna, Manichanh and friends in the Faculty of Food Science and Biotechnology, UPM. Thanks for struggle through the good and bad times with me. I also wish to express enormous gratitude to Mr. Halim, Mr. Rosli and Mrs. Aluyah for their technical assistance.

Last but not least, love and appreciation to my family.

Thanks God for everything!

I certify that an Examination Committee met on 17th September 2003 to conduct the final examination of Lim Yin Sze on her Master of Science thesis entitled "Isolation of Bacteriocinogenic Lactic Acid Bacteria and Purification of Selected Bacteriocins from Traditional Fermented Foods" 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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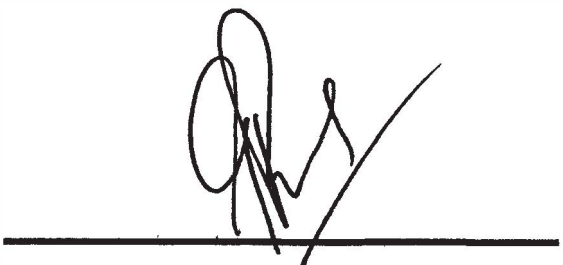

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Date: 29 JAN 2004

The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the Degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIM YIN SZE

Date: 28/01/04

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