UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF FERMENTATION AND FREEZE-DRYING PROCESSES TO ENHANCE THE PRODUCTIVITY AND STABILITY OF A PROBIOTIC, *LACTOBACILLUS SALIVARIUS* I 24

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By

LIM CHIN MING

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

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

OPTIMIZATION OF FERMENTATION AND FREEZE-DRYING PROCESSES TO ENHANCE THE PRODUCTIVITY AND STABILITY OF A PROBIOTIC, *LACTOBACILLUS SALIVARIUS* I 24

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November 2006

Chairman: **Professor Arbakariya Ariff, PhD**

Institute: **Bioscience**

Production of *Lactobacillus salivarius* I 24, a probiotic strain for chicken; was studied in batch and fed-batch fermentations using shake flasks and a 2-L stirred tank fermenter. In addition, preservation of *L. salivarius* I 24 using freeze-drying technique was also carried out. From a preliminary study, glucose and yeast extract were found to be the best carbon and nitrogen sources, respectively. Response surface method (RSM) was then used to optimize the culture medium for the growth of *L. salivarius* I 24. The factors investigated were yeast extract, glucose and initial culture pH. A polynomial regression model with cubic and quartic terms was used for the analysis of the experimental data. Estimated optimal conditions of the factors for the growth of *L. salivarius* I 24 were: 3.32% (w/v) of glucose, 4.31% (w/v) of yeast extract and an initial pH of 6.10.

Further improvement of cell production was made by using an optimization approach in the process condition. Aeration, pH, mixing and inoculum size were investigated. Cell production and viability were greatly influenced by the culture pH compared to other parameters. The optimum culture conditions for the cultivation of *L. salivarius* I 24 in the 2-L stirred tank fermenter were as follows: impeller tip speed, 0.42 m/s; pH, 6.10, and inoculum size of 10% (v/v) in facultative condition. Under these conditions, the final cell viability was 14.1 x 10^9 cfu/mL; viable cell yield was 4.37 x 10^{11} cfu/$g_{Glucose}$ and productivity was 17.59 x 10^8 cfu/mL.h.

A model employing the logistic and Leudeking-Piret equation for mixed-growth associated product formation was found to be sufficient to describe growth of *L. salivarius* I 24 and lactic acid production. The general kinetic parameters were calculated from the analysis of a large number of experimental data from batch fermentations. The calculate value of μ_{max} was 0.69 h^{-1}. Fed-batch cultivation was used in an attempt to further improve biomass production of *L. salivarius* I 24 by enhancing carbon flux to cell built up and reduce the flux to lactic acid production. Stepwise fed-batch cultivation (SFBC) gave better result than constant fed-batch cultivation (CFBC) when operated at a μ of 0.3 h^{-1}, which gave 528% improvement in viable cell counts when compared to CFBC. Results obtained form SFBC at a μ of 0.3 h^{-1} indicated that this cultivation mode might be a good alternative for *L. salivarius* I 24 production as higher cell concentration and lower lactic acid production could be achieved compared to batch cultivation.

Prior to the freeze-drying process, addition of protective agents could effectively improve the viability of the freeze-dried *L. salivarius* I 24 cultures. Among the protective agents investigated, 9.85% (w/v) of skim milk and 10.65% (w/v) of sucrose demonstrated the best survival rate of *L. salivarius* I 24. Better survival of *L. salivarius* I 24 during freeze-drying was also observed when the pH and temperature were controlled during cultivation, and when the cultures were frozen at –80^{o}C for 5 h before the freeze-drying process. Under these conditions, the highest survival rate, which was 65.2%, was achieved, and the viable counts of *L. salivarius* I 24 remained almost stable after 6 months of storage at –30^{o}C.

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

PENGOPTIMUMAN DALAM PROSES FERMENTASI DAN PENGERINGAN SEJUK-BEKU DEMI MEMPERTINGKATKAN PRODUKTIVITI SERTA KESTABILAN SATU PROBIOTIK, *LACTOBACILLUS SALIVARIUS* I 24

Oleh

LIM CHIN MING

November 2006

Pengerusi: **Profesor Arbakariya Ariff, PhD**

Institut: **Biosains**

Penghasilan *Lactobacillus salivarius* I 24, satu probiotik untuk industri penternakan ayam telah dikaji secara fermentasi sesekelompok dan fermentasi suapan sesekelompok dengan penggunaan kelalang kon dan fermenter berpengaduk 2 L. Tambahan pula, pengawetan *L. salivarius* I 24 dengan cara pengeringan sejuk-beku juga dijalankan. Glukosa dan ekstrak yis merupakan unsur karbon dan nitrogen yang terbaik dalam kajian awal. Kaedah Respons Permukaan (KRP) telah digunakan untuk megoptimumkan medium kultur bagi pertumbuhan *L. salivarius* I 24. Faktor media yang dikaji ialah kepekatan glukosa, ekstrak yis dan pH permulaan media kultur. Model regresi polinomial dengan sebutan kubik dan kuartik digunakan umtuk analisis data eksperimen. Keadaan optimum factor-faktor berkenaan bagi

pertumbuhan *L. salivarius* I 24 adalah seperti berikut: 3.32% (w/v) bagi glukosa, 4.31% (w/v) bagi ekstrak yis dan pH permulaan adalah 6.10.

Penghasilan sel seterusnya dipertingkatkan dengan menggunaan pendekatan pengoptimuman proses. Keadaan pengudaraan, pH, pengadukan dan saiz inokulum merupakan parameter fermentasi yang dikaji. pH memberi pengaruhan yang lebih kuat ke atas penghasilan sel berbanding dengan parameter yang lain. Keadaan optimum factor-faktor berkenaan bagi penhasilan *L. salivarius* I 24 dalam fermenter berpengaduk 2 L adalah seperti berikut: pengadukan, 0.42 m/s; pH, 6.10 and saiz inokulum, 10 % (v/v) dalam keadaan fakultatif. Di bawah keadaan tersebut, bilangan sel yang terhasil adalah 14.1 x 10^9 cfu/mL; penghasilan sel hidup adalah 4.37 x 10^{11} cfu/$g_{Glucose}$ dan produktiviti adalah 17.59 x 10^8 cfu/mL.h.

Satu model yang berdasarkan persamaan-persamaan logistik dan Leudeking-Piret bagi penghasilan produk secara proses bercampur (pertumbuhan berkait dan pertumbuhan tidak berkait) telah dikemukakan untuk menerangkan pertumbuhan *L. salivarius* I 24 dan penghasilan asid laktik. Unsur-unsur kinetic yang biasa telah ditentu selepas menganalisis sejumlah besar data eksperimen dari fermentasi sesekelompok. Nilai μ_{max} yang dikirakan adalah 0.69 h^{-1}. Fermentasi suapan sesekelompok digunakan untuk meningkatkan lagi penghasilan sel *L. salivarius* I 24 dengan merangasangkan aliran karbon ke arah pembinaan sel. Di sebaliknya, mengurangkan aliran ini dalam penghasilan asid laktik. Fermentasi suapan sesekelompok secara peringkat (FSSP) menunjukkan keputusan yang lebih baik berbanding dengan fermentasi suapan sesekelompok secara konstan (FSSK) bila μ

ditetapkan pada 0.3 h^{-1}, peningkatan sebanyak 528 % bagi bilangan sel hidup. Keputusan yang diperolehi dari FSSP mencadangkan ia merupakan satu pilihan baik bagi penghasilan *L. salivarius* I 24 jika μ ditetapkan pada 0.3 h^{-1} kerana bukan sahaja bilangan sel yang lebih tinggi tetapi kepekatan asid laktik yang lebih rendah boleh diperolehi jika berbanding dengan fermentasi sesekelompok.

Sebelum menuju ke proses pengeringan sejuk beku, penambahan agen perlindungan ke atas sel didapati boleh meningkatkan keupayaan hidup *L. salivarius* I 24 yang telah dikering sejuk-bekukan. 9.85 % (w/v) susu skim dan 10.65 % (w/v) sukrosa memamerkan kadar hidupan yang paling tinggi di kalangan agen perlindungan yang dikaji. Bila pH dan suhu dikawal semasa penghasilan serta sejuk-beku pada –80^{o}C selama 5 jam sebelum proses pengeringan sejuk-beku juga menunjukkan peningkatan dari segi kestabilan sel. Dalam keadaan sedemikian, kadar kehidupan yang tertinggi telah dicapai iaitu sebanyak 65.2 %. Sementara, bilangan sel hidup *L. salivarius* I 24 berada dalam keadaan yang agak stabil selepas penyimpanan pada suhu –30^{o}C selama 6 bulan.

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I certify that an Examination Committee has met on 17th November 2006 to conduct the final examination of Lim Chin Ming on her Doctor of Philosophy thesis entitled "Optimization of Fermentation and Freeze-drying Processes to Enhance The Productivity and Stability of A Probiotic, *Lactobacillus salivarius* I 24" 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:

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

Foo Hooi Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

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

Mohammad Roji Sarmidi, PhD
Professor
Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia
(External Examiner)

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

Date:

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

Arbakariya Ariff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Ho Yin Wan, PhD
Professor
Institute of Bioscience
Universiti Putra Malaysia
(Member)

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

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 8 MARCH 2007

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.

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LIM CHIN MING

Date: 10 JANUARY 2007

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