UNIVERSITI PUTRA MALAYSIA

METABOLIC REGULATION ANALYSIS OF RECOMBINANT *Lactococcus lactis* BASED ON GENE EXPRESSION AND ENZYME ACTIVITY

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By

FARZANEH HEIDARNIA

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

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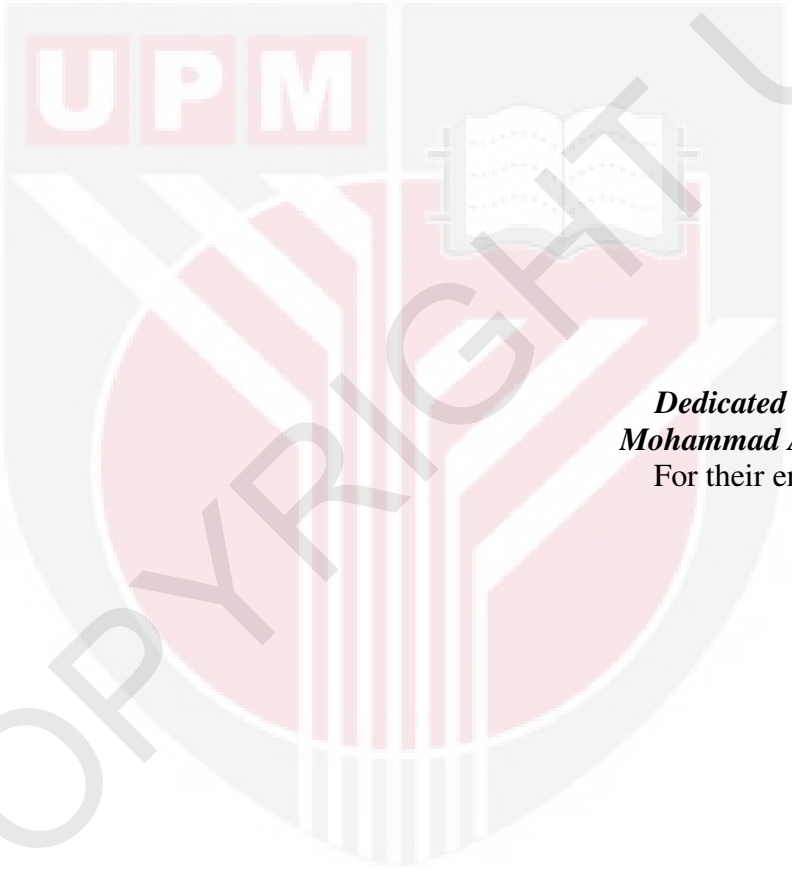

Dedicated to my parents:
Mohammad Ali and Ashraf
For their endless supports

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

METABOLIC REGULATION ANALYSIS OF RECOMBINANT *Lactococcus lactis* BASED ON GENE EXPRESSION AND ENZYME ACTIVITY

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July 2011

Chairman: Nor'Aini Abdul Rahman, PhD

Faculty: Biotechnology and Biomolecular Sciences

Lactic acid bacteria (LAB) are industrially important microorganisms that are widely used in industrial food fermentations for dairy production. However, there is a growing interest in their application in genetic modification and biotechnology processes. *Lactococcus lactis* is a non-pathogenic bacterium whose genome has been completely sequenced and its metabolic pathways are well studied. These reasons make *L. lactis* an attractive target for those approaches including creating live vector vaccine.

The present study was conducted to evaluate the effect of aerolysin on the metabolic regulation and fermentation characteristics of the recombinant *L. lactis*. Both *L. lactis* NZ9000 and Recombinant *L. lactis* carrying D1 of aerolysin gene (Lac-D1ae) were cultivated in M17 medium supplemented with 0.5% (w/v) glucose incubated at 30°C with an agitation of 150 rpm. Chloramphenichol (7.5 μg/mL) was added to maintain the plasmid. Samples for gene expression and enzyme activity assays were taken during late-exponential growth phase.

The alteration of expression of 10 genes (*glk*, *pfk*, *pyk*, *ackA, mdh*, *ldh*, *pgi*, *zwf*, *gnd*) responsible for enzymes at the main metabolic pathways i.e. glycolysis, Triarboxylic Acid (TCA) cycle, fermentation and pentose phosphate pathway (PP pathway) were examined by using semi-quantitative RT-PCR and Real-Time PCR. The activity of these enzymes including glucokinase (GLK), phosphofructokinase (PFK), pyruvate kinase (PYK), acetate kinase (ACK), Malate dehydrogenase (MDH), Lactate dehydrogenase (LDH), Phosphoglucose isomerase (PGI), Glucose-6-phosphate dehydrogenase (G6PDH) and 6-Phosphogluconate dehydrogenase (6PGDH) were also measured to understand the metabolic regulation in Lac-D1ae.

According to the fermentation results obtained, cell growth, lactate production rate and acetate production rate in the Lac-D1ae were 0.77 g/L, 0.82 mg/L/h and 0.16 mg/L/h, respectively. The values were slightly lower compared to the parental strain (0.8 g/L, 0.84 mg/L/h and 0.18 mg/L/h). Glucose consumption rate also showed a considerable decrease in the recombinant strain (3.48 g/L/h) in comparison with *L.lactis* NZ9000 (4.27 g/L/h). HPLC results showed the production of lactate (8.20 g/L) and acetate (1.58 g/L) were reduced in Lac-D1ae in contrast with parental strain (8.35 g/L and 1.83 g/L).

In conclusion, the fermentation characteristics of the recombinant *L. lactis* showed that the presence of aerolysin gene has no inhibitory effect on the growth. Furthermore, integrating methods based on gene expression and enzyme activities showed up-regulation of glycolysis and TCA cycle and down-regulation of Pentose Phosphate pathway in the recombinant *L. lactis* carrying aerolysin gene (Lac-D1ae).

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

ANALISIS PENGAWALAN METABOLIK REKOMBINAN *Lactococcus lactis* BERDASARKAN KEPADA EKSPRESI GEN DAN AKTIVITI ENZIM

Oleh

FARZANEH HEIDARNIA

Julai 2011

Pengerusi: Nor 'Aini Abdul Rahman, PhD

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Bakteria asid laktik (LAB) merupakan mikroorganisma yang penting dalam industri dimana ia digunakan secara meluas dalam industri pemakanan untuk produk tenusu. Disamping itu pada masa kini aplikasi modifikasi gen dan proses bioteknologi terhadap bakteria ini semakin meningkat. *Lactococcus lactis* merupakan bakteria bukan patogen dimana genomnya telah dijujukkan sepenuhnya serta tapak jalannya telah banyak dikaji. Oleh itu *L.lactis* menjadi target yang menarik melalui pendekatan tersebut seperti menghasilkan vektor vaksin hidup.

Kajian ini dijalankan untuk menilai kesan aerolysin terhadap pengawalan metabolik dan kriteria fermentasi rekombinan *L.lactis*. Kedua-dua *L.lactis* NZ9000 (strain asal) and rekombinan *L.lactis* pembawa gen aerolysin D1 (Lac-D1ae) telah ditumbuhkan dalam medium M17 yang dibekalkan dengan glukosa 0.5% (w/v) dan diinkubasi pada 30°C dengan goncangan pada 150 rpm. Chloramphenicol (7.5 ug/mL) telah ditambah untuk mengekalkan plasmid. Sampel untuk ekspresi gen dan asai aktiviti enzim telah diambil semasa akhir fasa eksponen.

Perubahan ekspresi 10 gen (*glk*, *pfk*, *pyk*, *ackA,* *mdh*, *ldh*, *pgi*, *zwf*, *gnd)* yang bertanggungjawab untuk enzim yang terdapat pada tapak jalan utama, contohnya Glikolisis, Kitaran TriKarboksilik, Fermentasi, dan Tapak Jalan Pentos Posfat telah ditentukan menggunakan semi-kuantitatif RT-PCR dan Real-Time PCR. Aktiviti untuk enzim-enzim tersebut termasuk glucokinase (GLK), phosphofructokinase (PFK), pyruvate kinase (PYK), acetate kinase (ACK), Malate dehydrogenase (MDH), Lactate dehydrogenase (LDH), Phosphoglucose isomerase (PGI), Glucose-6-phosphate dehydrogenase (G6PDH) and 6-Phosphogluconate dehydrogenase (6PGDH) juga diukur untuk memahami pengawalan metabolik dalam Lac-D1ae.

Berdasarkan kepada keputusan fermentasi pertumbuhan sel, kadar penghasilan asid laktik, dan asid asetik oleh Lac-D1ae masing-masing adalah 0.17 g/L, 0.82 mg/L/h dan 0.16 mg/L/h. Nilai-nilai tersebut adalah kurang sedikit berbanding dengan strain asal (0.8 g/L, 0.84 mg/L/h dan 0.18 mg/L/h). Kadar penggunaan glukosa menunjukkan pengurangan yang ketara pada strain rekombinan (3.48 g/L/h) berbanding dengan strain asal *L.lactis* NZ9000 (4.27 g/L/h). Keputusan HPLC menunjukkan penghasilan asid laktik (8.20 g/L) dan asid asetik (1.58 g/L) adalah kurang dalam Lac-D1ae dibandingkan dengan strain asal (8.35 g/L dan 1.83 g/L). Secara umumnya kriteria fermentasi Lac-D1ae menunjukkan kehadiran aerolysin tidak merencatkan pertumbuhan strain tersebut.

Sebagai kesimpulan, kriteria fermentasi rekombinan *L.lactis* menunjukkan kehadiran gen aerolysin tidak merencatkan pertumbuhan *L.lactis*. Melalui kaedah integrasi berasaskan ekspresi gen dan aktiviti enzim, pengawalan metabolik rekombinan *L.lactis* pembawa gen aerolysin (Lac-D1ae) menunjukkan peningkatan pengawalan Glikolisis dan Kitaran TCA dan penurunan pengawalan tapak jalan Pentos Posfat.

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I certify that a Thesis Examination Committee has met on …… to conduct the final examination of Farzaneh Heidarnia on her thesis entitled "Metabolic Regulation Analysis of Recombinant *Lactococcus lactis*, Based on Gene Expression and Enzyme Activity" in accordance with the Universities and University Colleges Act 1971 and Construction of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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The thesis was submitted to the senate of Universiti Putra Malaysia has been accepted as the fulfillment requirement for the degree of Master of Science. The members of Supervisor Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for the quotation and citation which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at Universiti Putra Malaysia or at any other institution.

FARZANEH HEIDARNIA

Date: 18 July 2011

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