UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF SECRETORY LACTOCOCCUS LACTIS VECTORS WITH CHARACTERIZED HETEROLOGOUS SIGNAL PEPTIDE FROM PEDIOCOCCUS PENTOSACEUS

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BY

ALI BARADARAN

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

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This thesis is dedicated to my wife, parents, parents-in-law, my brothers and sister who have been stressing the importance of academic excellence and always been the fountain of my strength.

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

DEVELOPMENT OF SECRETORY *LACTOCOCCUS LACTIS* VECTORS WITH CHARACTERIZED HETEROLOGOUS SIGNAL PEPTIDE FROM *PEDIOCOCCUS PENTOSACEUS*

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December 2010

Chairperson: Raha Abdul Rahim, PhD

Faculty: Biotechnology and Biomolecular Sciences

Lactococcus lactis, the model of lactic acid bacteria (LAB), is a generally regarded as safe (GRAS) organism and one of the most widely used LAB in the food industry. The potential application of *Lactococcus lactis* as a live vehicle for the production and delivery of heterologous protein for industrial and medical applications are on the rise. Investigation of heterologous protein production in different location of *L. lactis* revealed that secretion is preferable to cytoplasmic production. Although considerable attentions have been given to the development of efficient gene expression and protein secretion systems, however, there is still an acute lack of system to secrete heterologous proteins in *L. lactis*. The Gram-positive low GC content bacterium, *Pediococcus pentosaceus* was isolated from a local herbal plant *Polygonum minus* and identified by biochemical and 16S rRNA sequencing. The nucleotide sequence of the

cell wall binding protein from *P. pentosaceus* was amplified by polymerase chain reaction (PCR), cloned into Zero Blunt® TOPO® plasmid and transformed into *Escherichia coli*. The coding region of signal peptides (SP) SPK1 and SPK3 were amplified from the cell wall binding proteins of *P. pentosaceus* and studied by *in silico* analysis. The *in silico* analysis of signal peptide revealed that SPK1 has higher hydrophobicity, GRAVY index, aliphatic index and more stability compared to SPK3 and USP45. The gene coding region of green fluorescent protein (GFP) and *L. lactis* signal peptide USP45 were then amplified by using *Pfu* DNA polymerase. Secretion cassettes were constructed using GFP as the reporter protein and USP45 as the control. Then, the SP-GFP cassette was cloned into *L. lactis* expression vectors pNZ8084 and pMG36e (inducible and constitutive) resulting in pNZK801, pNZK803, pNZU801 and pMGK36e1, pMGK36e3, pMGU36e1, respectively. The constructed plasmids were electro-transformed into *L. lactis* strain MG1363 and NZ9000 as host. Recombinant plasmids were identified by restriction enzyme digestion and sequence analyses. Western blot and ELISA analysis of transformants indicated the potential of the signal peptides SPK1 and SPK3 from *P. pentosaceus* to be used as a secretory signal for heterologous protein secretion in *L. lactis*.

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

PEMBANGUNAN VECTOR REMBESAN *LACTOCOCCUS LACTIS* DENGAN PEPTIDA ISYARAT HETEROLOG DARIPADA *PEDIOCOCCUS PENTOSACEUS*

Oleh

ALI BARADARAN

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Pengerusi: Raha Abdul Rahim, PhD

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Lactococcus lactis iaitu model bakteria asid laktik (LAB) merupakan organisma yang dianggap selamat secara umumnya (GRAS), dan salah satu LAB yang digunakan dengan meluas dalam industri makanan. Potensi *Lactococcus lactis* untuk digunakan sebagai "kenderaan hidup" bagi penghasilan dan penghantaran protein heterolog dalam aplikasi-aplikasi industri dan perubatan sedang meningkat. Kajian penghasilan protein heterolog di lokasi yang berbeza dalam *L. lactis* mendedahkan rembesan cenderung ke arah sitoplasmik. Walaupun perhatian yang secukupnya telah digunakan bagi membina sistem penzahiran gen dan sistem rembesan protein yang cekap, tetapi masih ada kekurangan dalam sistem berkenaan untuk merembeskan protein heterolog dalam *L. lactis*. Gram-positif bakteria yang rendah kandungan GC, *Pediococcus pentosaceus* telah dipencilkan daripada tumbuhan herba tempatan *Polygonum minus* dan dikenalpasti dengan menggunakan kaedah biokimia dan 16S rRNA. Jujukan nukleotida

daripada protein dinding sel bakteria *Pediococcus pentosaceus* telah digandakan dengan menggunakan tindakbalas berantai polimerase (PCR), diklonkan ke dalam plasmid Zero Blunt®TOPO® dan ditransformasikan ke dalam *Escherichia coli*. Peptida isyarat (SP) iaitu SPK1 dan SPK3 telah digandakan daripada protein dinding sel *Pediococcus pentosaceus* dan diuji dengan analisis *in silico*. Analisis *in silico* pada peptida isyarat mendedahkan bahawa SPK1 mempunyai sifat hidrofobik, indeks GRAVY, indeks alifatik yang tinggi dan lebih stabil berbanding dengan SPK3 dan USP45. Protein pendaflour hijau (GFP) dan peptida isyarat USP45 *L. lactis* kemudiannya digandakan menggunakan *Pfu* polimerase DNA. Kaset rembesan telah dibina menggunakan GFP sebagai gen pelapor dan USP45 sebagai kawalan. Kemudian, kaset-kaset SP-GFP itu telah diklonkan ke dalam vektor penzahiran *L. lactis* pNZ8084 dan pMG36e masing-masing menghasilkan pNZK801, pNZK803, pNZU801 dan pMGK36e1, pMGK36e3, pMGU36e1. Plasmid yang dibina telah di elektrotransformasikan ke dalam *L. lactis* MG1363 dan NZ9000. Transforman positif telah dikenalpasti dengan menggunakan cernaan enzim pembatas dan analisis-analisis jujukan. Analisis ELISA dan blot Western terhadap transforman menunjukkan peptida isyarat SPK1 dan SPk3 dari *Pediococcus pentosaceus* berpotensi untuk digunakan sebagai isyarat perembes bagi rembesan protein heterolog dalam *L. lactis*.

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A special thanks to my wife, parents, parents-in-law, my brothers and sister for their endless support and encouragement**.**

APPROVAL

I certify that an Examination Committee has met on date of viva voce to conduct the final examination of Ali Baradaran on his degree of Master of Science thesis entitled "Development OF Secretory *Lactococcus lactis* Vectors with Characterized Heterologous Signal Peptide From *Pediococcus pentosaceus"* in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the degree of Master of Science.

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citation 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 other institutions.

ALI BARADARAN

Date: 24 December 2010

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