UNIVERSITI PUTRA MALAYSIA

ENGINEERING INTEGRATIVE VECTORS BASED ON BACTERIOPHAGE SITE SPECIFIC RECOMBINATION MECHANISM FOR HETEROLOGOUS EXPRESSION OF SECRETED AND SURFACE-ANCHORED PROTEIN IN *Lactococcus lactis*

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FBSB 2019 29

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By

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

July 2019

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

ENGINEERING INTEGRATIVE VECTORS BASED ON BACTERIOPHAGE SITE SPECIFIC RECOMBINATION MECHANISM FOR HETEROLOGOUS EXPRESSION OF SECRETED AND SURFACE-ANCHORED PROTEIN IN *Lactococcus lactis*

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Bacterial integrating system allows integration of foreign DNA into bacterial host chromosome enabling stable expression of foreign gene for recombinant protein production. In this study, integrative expression vectors for secretion and surface display of heterologous protein in *Lactococcus lactis* have been successfully constructed based on the site-specific recombination mechanism, of temperate lactococcal phage TP901-1. Two variations of integrative vectors were constructed, denoted pS1-4, and pSD1-4, which allows the heterologous protein to be secreted into the extracellular environment or surface displayed on *L. lactis,* respectively. The integrative vectors composed of (i) P_{170} auto-inducible promoter or P_{nisA} inducible promoter, (ii) multiple cloning sites (MCS), (iii) TP901-1 bacteriophage attachment site, attP, (iv) signal peptide-encoding sequence USP45 fused with LEISSTCDA propeptide or SPK1 signal peptide for extracellular targeting, and (v) 344 amino acids of proteinase anchor domain ($PrtP_{344}$) for surface display application. A helper plasmid harbouring *int* gene, pNZint was also constructed to facilitate plasmid integration into the genome. A staphylococcal nuclease reporter gene was cloned into each of the constructed integrative vectors which were then successfully integrated into the *L. lactis* genome. Toluidine blue O-DNA assay and immunofluorescence microscopy data proved that the expressed nuclease was able to be secreted or anchored on the *L. lactis* cell wall. From the findings, signal peptide SPK1 was shown to be superior over USP45 in the secretion of Nuc, even though the USP45 was fused with LEISSTCDA propeptide which reportedly could enhance protein secretion. Meanwhile, the expression of Nuc showed that integrative vectors driven by P_{170} promoter have comparable strength to P_{nisA} promoter. However, the combination of P_{170} with USP45-LEISSTCDA fusion performed significantly worse than the other constructs for surface display of Nuc. Therefore, these newly constructed synthetic integrative vectors can be applied for the secretion and surface display of heterologous protein in *L. lactis*

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for research or industrial purposes where strong and weak stable expression vectors are required.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana (Sains)

PEMBINAAN VEKTOR INTEGRATIF BERDASARKAN MEKANISMA REKOMBINASI KHUSUS BAKTERIOFAJ UNTUK EKSPRESI PROTEIN HETEROLOG SECARA REMBESAN DAN PAPARAN PERMUKAAN DI DALAM *Lactococcus lactis*

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Sistem integrasi bakteria membolehkan pengintegrasian DNA asing ke dalam kromosom hos bakteria yang membolehkan ekspresi gen asing dengan stabil untuk pembikinan protein rekombinan. Dalam kajian ini, vektor ungkapan terintegrasi untuk rembesan dan paparan permukaan protein heterolog dalam *Lactococcus lactis* telah berjaya dihasilkan berdasarkan mekanisma rekombinasi khusus bakteriofaj lactococcal TP901-1. Dua variasi vektor integratif telah dibina, dengan nama pS1-4, dan pSD1-4, yang membolehkan protein heterolog dirembes ke persekitaran ekstraselular atau diparparkan ke permukaan *L. lactis*. Vektor integratif yang dibina terdiri daripada (i) promoter teraruhkan auto, P_{170} atau promoter teraruh, P_{nisA}, (ii) tapak pengklonan berbilang, (MCS), (iii) tapak pelekat bakteriofaj TP901-1, attP, (iv) peptida isyarat dengan propeptida LEISSTCDA atau peptida isyarat SPK1 untuk pentargetan ekstraselular, dan (v) 344 asid amino pelekat domain protein, ($PrtP_{344}$) untuk aplikasi paparan permukaan sel. Plasmid pembantu yang membawa gen *int*, pNZint juga dibina untuk membantu integrasi plasmid ke dalam genom. Satu gen pelapor telah diklon ke dalam setiap vektor integratif yang dibina yang kemudiannya berjaya digabungkan ke dalam genom *L. Lactis.* Data yang diperoleh daripada asai *toluidine blue* O-DNA dan mikroskopi imunopendarfluor membuktikan bahawa Nuc yang terekspresi dapat dirembes atau berlabuh pada dinding sel *L. lactis*. Daripada hasil kajian ini, isyarat peptida SPK1 menunjukkan perembesan protein nuklease lebih unggul berbanding isyarat peptida USP45, walaupun USP45 telah digabungkan bersama propeptida LEISSTCDA yang dilaporkan dapat meningkatkan rembesan protein. Sementara itu, ungkapan Nuc menunjukkan bahawa vektor integratif yang didorong oleh promoter P_{170} mempunyai kekuatan setanding dengan promoter P_{nisA}. Walaubagaimanapun, Nuc yang diekspresikan oleh kombinasi P_{170} dengan gabungan USP45-LEISSTCDA jauh lebih buruk

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daripada vektor-vektor lain yang telah dihasilkan untuk paparan permukaan Nuc. Oleh itu, vektor integratif sintetik yang baru dibina ini boleh digunakan untuk rembesan dan paparan permukaan protein heterolog bagi *L. lactis* untuk tujuan penyelidikan atau kegunaan industri di mana vektor ungkapan kuat dan lemah yang stabil adalah diperlukan.

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ACKNOWLEDGEMENTS

Alhamdulillah, in the name of Allah, The Most Gracious, The Most Merciful, all the praises and thanks be to Allah who has graciously bestowed me the strength and guided me throughout this journey.I would like to take this opportunity to express my deepest gratitude to my supervisors, Prof. Dr. Raha Abdul Rahim and Dr. Adelene Song Ai Lian. They have been very supportive throughout my project by giving the guideline, encouragement and helpful advice in order to help me finish my lab work and this thesis preparation. My sincere appreciation also goes to Dr. Mas Jaffri Masarudin who served as my supervisory committee member and kindly provided valuable advice and suggestions for this work.

My sincere thanks and appreciation to my labmates in Bacterial Molecular Biology laboratory, Jeevan Nathan, Lee Chai Yan, Sarah Safwah, Nur Elina, Abdullah Munir, Aishah and Aqlili Riana for their helps and great cooperation. I would also like to express my gratitude to my family for their advice, encouragement, love, and understanding in all my life throughout these years.Last but not least, I would like to thank Ministry of Education and University Putra Malaysia for awarding me with MyMasters scholarship and Graduate Research Fellowship. A million thanks to all persons involved throughout this project and the thesis submission. Thanks to all, and only Allah will repay all of your kindness.

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

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