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DEVELOPMENT OF SECRETORY EXPRESSION SYSTEM USING SYNTHETIC SIGNAL PEPTIDES spA AND spD FOR PROTEIN PRODUCTION IN *Escherichia coli*

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By

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DEVELOPMENT OF SECRETORY EXPRESSION SYSTEM USING SYNTHETIC SIGNAL PEPTIDES spA AND spD FOR PROTEIN PRODUCTION IN *Escherichia coli*

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September 2011

Chairman: Raha Abdul Rahim, PhD

Faculty: Biotechnology and Biomolecular Sciences

Recombinant protein expression is very important in biotechnology. Successful protein expression depends on the expression host, vector and target protein. *Escherichia coli* being a popular expression host, is still plagued by various problems in expression like formation of inclusion bodies, incorrect folding and low soluble protein yield. These problems can be circumvented by using different promoters, different host strains, co-expression of chaperones, reduction of culture temperature and secretion of proteins into periplasm and culture medium. In this study, periplasmic protein secretion was investigated by using novel synthetic signal peptides, spA and spD. The two signal peptides were designed based on signal peptides of *Bacillus* spp. for secretion of heterologous proteins. They were amplified and ligated to genes coding for the green fluorescent protein (GFP) and cyclodextrin glucanotransferase (CGTase) to construct secretion cassettes spA*GFP, spD*GFP and spA*CGT and

spD*CGT. These secretion cassettes were first cloned into pCR®-Blunt II-TOPO cloning vector. The cassettes were then sub-cloned into pET-32b(+) expression vector to construct pAGFP, pDGFP, pACGT and transformed into competent *E. coli* BL21(DE3) and BL21(DE3)pLysS cells. The cloning of secretion cassette spD*CGT into pET-32b(+) was not successful. GFP without the signal peptide was cloned into similar pET-32b(+) as a control and the construct was named pGFP. SDS-PAGE and western blotting results for recombinant GFP clones showed successful expression and secretion into the periplasm. Fluorescence analysis for GFP clones showed that the secreted GFP was not fluorescent while cytoplasmically expressed GFP was fluorescent. Induction temperature also affected the secretion of recombinant GFP as better secretion was attained at 37°C. SDS-PAGE analysis for recombinant clone BL-pACGT showed that CGTase was detected in both cytoplasm and periplasm but in Western blotting only cytoplasmic expression was detected. However, the positive control, (CGTase with native signal peptide) was detected in both cytoplasm and periplasm. Cell growth analysis for recombinant clones GFP and CGTase did not show any adverse effect to the secretion host. These results show that the synthetic signal peptides, spA and spD, could direct recombinant proteins to the periplasmic space.

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

PEMBANGUNAN SISTEM EXPRESI SEKRETORI MENGGUNAKAN PEPTIDA ISYARAT SINTETIK spA dan spD UNTUK PENGHASILAN PROTEIN DI DALAM *Escherichia coli*

By

VITHYA A/P VELAITHAN

September 2011

Pengerusi: Raha Abdul Rahim, PhD

Fakulti: Biotechnologi dan Sains Biomolekul

Penghasilan protein rekombinan adalah penting untuk sektor bioteknologi. Penghasilan protein yang baik bergantung kepada factor-faktor seperti perumah, vektor serta protein yang ingin diekspreskan. Walaupun *E. coli* merupakan strain yang popular, ia menghadapi masalah seperti pembentukkan badan inklusi, penghasilan struktur protein kuartenari yang tidak tepat, dan juga penghasilan protein pada tahap yang rendah. Masalah-masalah tersebut boleh diatasi dengan menggunakan kaedah seperti, penggunaan promoter berbeza, strain perumah berbeza, ko-ekspresi penghantar, pengurangan suhu kultur serta rembesan protein ke periplasma dan media. Dalam kajian ini, rembesan protein dikaji dengan peptida isyarat sintetik spA dan spD. Peptida isyarat sintetik tersebut dihasilkan berdasarkan struktur asid amino peptida isyarat dari *Bacillus* sp. Peptida isyarat spA dan spD digandakan dan kemudiannya diligasikan dengan gen penanda GFP (green fluorescent protein) serta gen penanda

CGTase (cyclodextrin glucanotransferase) untuk menghasilkan kaset rembesan spA*GFP, spD*GFP, spA*CGT dan spD*CGT. Kaset-kaset itu diklonkan ke dalam vektor pengklonan, pCR®-Blunt II-TOPO. Seterusnya, kaset rembesan tersebut diklonkan ke dalam vektor ekspressi pET-32b(+) untuk menghasilkan konstruk pGFP, pAGFP, pDGFP serta pACGT. Konstruk tersebut dimasukkan ke dalam perumah *E. coli* BL21(DE3) dan BL21(DE3)pLysS. Kaset rembesan spD*CGT tidak dapat dimasukkan ke dalam perumah *E. coli* BL21(DE3) serta BL21(DE3)pLysS. Gen GFP tanpa peptide isyarat diklon ke dalam pET-32b(+) sebagai kawalan dan konstruk trsebut dinamakan pGFP. Keputusan kajian SDS-PAGE dan western blotting untuk klon rekombinan GFP menunjukkan bahawa pengekspresan serta rembesan GFP ke periplasma berjaya dilakukan. Analisis pendafluor juga menunjukkan bahawa rembesan GFP ke periplasma tidak menghasilkan pendafluor tetapi GFP di dalam sitoplasma menghasilkan pendafluor. Suhu induksi turut mempengaruhi rembesan GFP dan suhu 37°C menghasilkan rembesan yang baik. Kajian SDS-PAGE bagi klon rekombinan BL-pACGT menunjukkan bahawa CGTase berjaya dikesan di dalam sitoplasma serta periplasma tetapi western blotting cuma dapat mengesan CGTase di dalam sitoplasma. Kawalan positif (CGTase dengan peptide isyaratnya sendiri) dapat dikesan di dalam sitoplasma serta periplasma. Manakala, kajian pertumbuhan sel bagi klon rekombinan GFP dan CGTase tidak menunjukkan sebarang kesan tindakbalas negative terhadap perumah *E. coli*. Keputusan kajian menunjukkan bahawa peptida isyarat spA serta spD berjaya merembeskan protin penanda ke ruang periplasma.

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APPROVAL

I certify that an Examination committee has met on date of viva voce to conduct the final examination of Vithya A/P Velaithan on her degree of Master of Science thesis entitled "Development Of Secretory Expression System Using Synthetic Signal Peptides spA and spD For Protein Production In *Escherichia coli*" in accordance with Universiti Putra 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.

Members of the Examination Committee were as follows:

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

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DECLARATION

I declare that the thesis is my original work except for quotations and citations 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.

VITHYA A/P VELAITHAN

Date: 20 September 2011

TABLE OF CONTENTS