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CLONING AND EXPRESSION OF *STAPHYLOCOCCUS EPIDERMIDIS* AT2 LIPASE GENE IN *YARROWIA LIPOLYTICA*

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By

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

CLONING AND EXPRESSION OF *Staphylococcus epidermidis* AT2 LIPASE GENE IN *Yarrowia lipolytica*

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Faculty : Biotechnology and Biomolecular Sciences

Proteolytic degradation and the production of protein that accumulates as misfolded form always occur in bacterial expression system. In view of that, *Yarrowia lipolytica* is chosen as a host to express heterologous protein. The gene encoding sequence of *Staphylococcus epidermidis* AT2 lipase (1.2kb) was cloned into *Y. lipolytica* expression vector (pYLEX1) and placed under the regulation of the strong hybrid promoter (hp4d) carrying four tandem copies of an upstream activator sequence (UAS1B) from *pXPR2* and a minimal *pLEU2* fragment. Previously, primers were designed on the basis of *S. epidermidis* lipase precursor (geh1) gene (AF053006). PCR (Polymerase chain reaction) was used to amplify the gene and cloned into pJET 1.2/blunt-end vector (Fermentas) transformed

into *E. coli* DH5α competent cell. After the gene was propagated in *E. coli*, the gene were purified and ligated into pYLEX1 vector (Yeastern Biotech). The recombinant plasmid was extracted and linearized before it was transformed into *Y. lipolytica* host strain Po1g. The recombinant *Y. lipolytica* was grown on YNB selection medium. Five positive transformants harboured the expected size of AT2 lipase gene were obtained and one of the transformants showed the highest expression. The expression of AT2 lipase enzyme was optimized at of 28 ^{0}C with the agitation speed of 200 rpm in optimized YNB medium. Process of breaking the cells or sonication profile was optimized at 7.5 min and the highest activity obtained was 14 U/mL. The crude proteins were electrophoresed on 12% (w/v) of SDS-PAGE and estimated protein band of 43 kDa was detected when stained with Coomassie brilliant blue. The expressed enzyme retained 100% of its activity after 30 min incubation (37 ^{0}C) in n-hexane, p-xylene and dimethyl sulfoxide.

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

PENGKLONAN DAN PENGEKSPRESAN GEN AT2 LIPASE *Staphylococcus epidermidis* DALAM *Yarrowia lipolytica*

Oleh

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Degradasi proteolitik dan pengeluaran protein yang tersalah bentuk seperti tersalah-lipatan selalu berlaku dalam sistem ekspresan bakteria. Sehubungan itu, *Yarrowia lipolytica* dipilih sebagai hos untuk mengekspreskan protein heterologous. Jujukan gen pengekodan *Staphylococcus epidermidis* AT2 lipase (1.2kb) diklonkan pada *Y. lipolytica* vektor (pYLEX1) dan diletakkan di bawah pengaruh promoter hibrid (hp4d) membawa empat salinan sejajar urutan (UAS1B) pXPR2 dan serpihan minimum pLEU2. Sebelum ini, primer telah direka berdasarkan *S. epidermidis* lipase (geh1) gen (AF053006). PCR (Tindak Balas Berantai Polymerase) digunakan untuk mengandakan gen dan diklon pada pJET 1.2/blunt-end vektor (Fermentas) yang dimasukkan di dalam *E. coli*

DH5α sel kompeten. Selepas gen itu digandakan dalam *E. coli*, gen yang telah diasingkan dan diligat pada pYLEX1 vektor (Yeastern Biotech). Plasmid rekombinan diekstrak dan diluruskan sebelum ia dimasukkan kedalam hos *Y. lipolytica* starin Po1g. Y. *lipolytica* rekombinan telah disaring pada medium pemilihan YNB. Lima transformants positif menunjukkan saiz yang dijangka pada AT2 lipase gen telah diperolehi dan salah satu yang transformants menunjukkan ekspresan yang paling tinggi. Ekspresan enzim AT2 lipase dioptimumkan pada suhu 28 ^{0}C dengan kelajuan perkocakan 200 rpm dalam medium YNB dioptimumkan. Proses pemecahan sel-sel atau profil sonikasi dioptimumkan pada kadar 7.5 min dan aktiviti tertinggi yang diperolehi adalah 14.2 U / mL. Protein mentah elektrophorisiskan di dalam 12% (w / v) SDS-PAGE dan jalur protein yang dianggarkan pada 43 kDa, dikesan apabila direndam dengan Coomassie Brilliant Blue. Enzim tersebut telah mengekalkan 100% aktiviti selepas 30 min diinkubasi (37 ^{0}C) dalam n-heksana, p-xylene dan dimetil sulfoxide.

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I certify that an Examination Committee has met on **date of viva voce** to conduct the final examination of **name of student** on his (or her) **degree** thesis entitled " **CLONING AND EXPRESSION OF *Staphylococcus epidermidis* AT2 LIPASE GENE IN *Yarrowia lipolytica***" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (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 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 at any other institution.

HISHAM BIN MOHD NOOH

Date: 17 October 2011

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