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DEVELOPMENT OF YEAST EXPRESSION SYSTEMS FOR PRODUCING THERMOSTABLE T1 LIPASE

SITI NURBAYA OSLAN

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DEVELOPMENT OF YEAST EXPRESSION SYSTEMS FOR PRODUCING THERMOSTABLE T1 LIPASE

By

SITI NURBAYA OSLAN

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

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A special dedication to:

My beloved family who inspired me the most, my father, Oslan Abdul Ghani, my mother, Noor Hayati Mohd Zain, my brothers Mohd Lukhman, Muhammad Faris Firdaus, Muhammad Bazli Fahmi, Muhammad Yatimi Hakim, and my sister, Siti Nur Hazwani. My lovely nephew, Mohammad Norman Danial, my grandmother, Sapinah Jusoh and my favorite auntie Norizan Mohd Zain, who light up my life and always be with me. Abang, Ma, Abah for their supports and the unforgettable moments shared with me. Thank you for being my source of inspirations.

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Chairman: **Professor Dato' Abu Bakar Salleh, PhD**

Faculty: **Biotechnology and Biomolecular Sciences**

Escherichia coli is known to be a good system to express heterologous protein. However, proteins always form as inclusion bodies and low level of active protein could be achieved through this system. In addition, the proteins were expressed intracellularly, thus required tedious purification steps. In order to overcome the problems, yeast expression system was developed. This study highlights on the development of yeast expression systems to express thermostable T1 lipase from *Geobacillus zalihae*. This study has three chapters which cover on isolation of local yeasts, expression of T1 lipase in commercial and local yeast system. Finally, the new yeast-vector system was constructed to express the protein.

Eight yeast isolates were isolated from different sources (isolate: WB, SO, S4, S5, R1, R2, RT, and RG). The isolates were identified through PCR amplification and sequencing of the ribosomal DNA. Among the isolates, isolate WB was determined as new species in order Saccharomycetales. Isolate SO, RT and RG were identified as *Pichia* sp. While isolates S4 and S5 were determined as *Issatchenkia* sp. Isolates R1 and R2 were grouped in *Hanseniaspora* sp. Geneticin 50 µg/mL was determined to be the selection marker for all isolates except for isolates RT and SO. Some thermostable lipases were observed in isolates RT and R1 with 0.61 U/mg and 0.1 U/mg, respectively. All *Pichia* sp. strains possessed formaldehyde dehydrogenase (FLD) promoter. However, only isolate SO has the alcohol oxidase (AOX) promoter.

Commercial yeast expression system, *Pichia pastoris* strains (GS115, X-33 and KM71H) was used to express T1 lipase under regulation of methanol inducible AOX promoter by using pPICZαB. Recombinants X-33/pPICZαB/T1-2 (XPB2), GS115/pPICZαB/T1-5 (GPB5) and KM71H/pPICZαB/T1-7 (KPB7) were chosen for optimization of T1 lipase expression in shake flask. 2% (v/v) methanol was used to induce GPB5 and XPB2 optimally, while 3% (v/v) methanol for KPB7. The highest expression level was attained at the optimum time with GPB5 (88 U/mL – 192 h), XPB2 (81 U/mL – 144 h) and KPB7 (26 U/mL – 144 h). Western blot analysis confirmed that the molecular mass of recombinant T1 lipase was 45 kDa without glycosylation. Initial study proved that thermostable T1 lipase was successfully

expressed by using the secretory *P. pastoris* expression system at 2-fold higher than *E. coli*.

Isolate SO was chosen to be used as a host for T1 lipase expression under regulation of commercial plasmid (pPICZαB). Recombinant isolate SO/pPICZαB/T1-2 (SO2) was chosen for T1 lipase expression in shake flask. YPTG and YPTM media were used to grown and induce the SO2 expression. Methanol concentration of 1.5% (v/v) was shown to be the best inducer for recombinant SO2. The optimum T1 lipase expression was achieved after 30 h. The optimum T1 lipase expression in SO2 was 14 U/mL with 83% lower methanol and the time taken was 84% faster than commercial system.

A new series of host-vector system was developed by using isolate WB and *P. pastoris*. Plasmid pUC19 was used as the backbone for the new episomal and integrated plasmids. Replicon from *P. pastoris* (PARS1) was used to maintain episomal form of plasmid (pTBR5ECT1) while 25S rDNA from isolate WB was used as integration site (pTBR5ECT1-25S and pTBR5ECT1-25S WB). *TEF1* promoter from *Saccharomyces cerevisiae* and isolate WB were used to regulate the T1 lipase expression in new yeast system. In conclusion, locally isolated yeasts (isolate SO and WB) and commercial system (*P. pastoris)* were developed to be used to express recombinant thermostable T1 lipase from *Geobacillus zalihae*. Newly developed systems could be used to express other protein of interest.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN SISTEM PENGEKSPRESAN YIS UNTUK MENGHASILKAN LIPASE T1 TERMOSTABIL

Oleh

SITI NURBAYA OSLAN

Disember 2012

Pengerusi: **Professor Dato' Abu Bakar Salleh, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Escherichia coli dikenali sebagai satu sistem yang baik untuk mengekspres protein heterolog. Walau bagaimanapun, kebanyakan protein yang diekspres daripada sistem ini adalah sedikit dan membentuk jasad inklusi. Di samping itu, protein yang diekspres secara intrasel memerlukan proses penulenan yang banyak. Dalam usaha untuk mengatasi masalah ini, sistem pengekspresan yis telah dibangunkan. Kajian ini merangkumi pembangunan sistem pengekspresan yis untuk mengekpres lipase termostabil T1 dari *Geobacillus zalihae*. Kajian ini meliputi pemencilan yis tempatan, pengekspresan lipase T1 dalam sistem yis komersial dan pencilan tempatan serta pembangunan sistem yis-vektor yang baru untuk mengekspreskan protein.

Lapan pencilan yis telah dipencil daripada sumber-sumber yang berbeza (Pencilan: WB, PP, S4, S5, R1, R2, RT, dan RG). Pencilan-pencilan ini telah dikenal pasti melalui teknik PCR dan penjujukan DNA ribosomal. Di antara pencilan-pencilan, pencilan WB telah dikenal pasti sebagai species baru dalam order Saccharomycetales. Pencilan SO, RT dan RG telah dikenal pasti sebagai *Pichia* sp. Pencilan S4 dan S5 telah ditentukan sebagai *Issatchenkia* sp. Manakala pencilan R1 dan R2 telah dikumpulkan dalam *Hanseniaspora* sp. Geneticin 50 μg/mL telah menjadi penanda kepada semua pencilan kecuali pencilan RT dan SO. Terdapat beberapa lipase termostabil dalam pencilan RT dan R1 dengan masing-masing 0.61 U/mg dan 0.1 U/mg. Semua pencilan strain *Pichia* sp. memiliki promoter formaldehid dehidrogenase (FLD). Walau bagaimanapun, hanya pencilan SO mempunyai promoter oxidase alkohol (AOX).

Sistem yis pengekspres yang komersial iaitu *Pichia pastoris* strains (GS115, X-33 dan KM71H) telah digunakan untuk mengekspres lipase T1 di bawah promoter teraruh metanol (AOX) dengan menggunakan plasmid pPICZαB. Rekombinan X-33/pPICZαB/T1-2 (XPB2), GS115/pPICZαB/T1-5 (GPB5) dan KM71H/pPICZαB/T1-7 (KPB7) telah dipilih untuk pengoptimumaan lipase T1. 2% (v/v) metanol telah digunakan untuk mengaruh GPB5 dan XPB2, manakala 3% (v/v) metanol untuk KPB7. Produk tertinggi lipase T1 yang telah dicapai pada masa yang optimum melalaui GPB5 (88 U / mL - 192 h), XPB2 (81 U / mL - 144 h) dan KPB7 (26 U / mL - 144 h). Analisa 'Western blot' mengesahkan bahawa jisim molekul rekombinan lipase T1 adalah 45

kDa tanpa glikosilasi. Kajian awal membuktikan bahawa lipase termostabil T1 telah berjaya diekspres dalam *P. pastoris* sebanyak 2-kali ganda lebih tinggi daripada *E. coli*.

Pencilan SO (*Pichia guilliermondii*) telah dipilih untuk digunakan sebagai hos untuk mengekspres lipase T1 dengan menggunakan plasmid komersial (pPICZαB). Rekombinan SO/pPICZαB/T1-2 (SO2) telah dipilih untuk pengoptimumaan. Media YPTG dan YPTM telah digunakan untuk mengekspreskan lipase T1 dalam SO2 dengan aruhan metanol sebanyak 1.5% (v/v). Ekspresi lipase T1 yang optimum telah dicapai selepas 30 h. Kepekatan metanol adalah 83% lebih rendah manakala masa yang diambil adalah 84% lebih cepat daripada sistem komersial dengan 14 U/mL.

Beberapa sistem perumah-vektor baru telah dibangunkan dengan menggunakan pencilan WB dan *P. pastoris*. Plasmid pUC19 telah digunakan sebagai tulang belakang bagi plasmid 'episomal' dan integrasi. 'Replicon' dari *P. pastoris* (PARS1) telah digunakan untuk mengekalkan bentuk 'episomal' plasmid (pTBR5ECT1) manakala 25S rDNA daripada pencilan WB telah digunakan sebagai tapak integrasi (pTBR5ECT1-25S dan pTBR5ECT1-25S WB). Promoter *TEF1* dari *Saccharomyces cerevisiae* dan pencilan WB telah digunakan untuk mengekspres lipase T1. Kesimpulannya, yis pencilan tempatan (SO dan WB) dan sistem komersial (*P. pastoris*) telah dibangunkan untuk mengekspreskan rekombinan lipase T1 daripada *Geobacillus zalihae*. Sistem yang dibangunkan ini boleh digunakan untuk mengekspreskan protein lain.

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I certify that a Thesis Examination Committee has met on **27th December 2012** to conduct the final examination of Siti Nurbaya binti Oslan on her thesis entitled "DEVELOPMENT OF YEAST EXPRESSION SYSTEMS FOR PRODUCING THERMOSTABLE T1 LIPASE" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

PROF. DR. Norhani Abdullah, PhD
Department of Biochemistry
Faculty of Biotechnology and Biomolecular Sciences
(Chairman)

PROF. DR. Raha Abdul Rahim, PhD
Department of Cell and Molecular Biology
Faculty of Biotechnology and Biomolecular Sciences
(Internal Examiner)

ASSOC. PROF. DR. Chong Pei Pei, PhD
Department of Biomedical Sciences
Faculty of Medicine and Health Sciences
(Internal Examiner)

ASSOC. PROF. DR. Chuenchit Boonchird, PhD
Department of Biotechnology
Faculty of Science, Mahidol University
Rama VI Road, Ratchathewi, Bangkok 10400, Thailand
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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

Abu Bakar Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Raja Noor Zaliha Raja Abd. Rahman, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mahiran Basri, PhD
Professor
Faculty of Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

SITI NURBAYA BINTI OSLAN

Date: 27 December 2012

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