UNIVERSITI PUTRA MALAYSIA

CLONING AND EXPRESSION OF A THERMOSTABLE _-GLUCOSIDASE

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FBSB 2010 24

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2010

CLONING AND EXPRESSION OF A THERMOSTABLE α-GLUCOSIDASE

By

RAUDA A. MOHAMED

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

December 2010

DEDICATION

This thesis is dedicated to my late father, A. Mohamed Sheikh Ahmad, my late brother, Farai A. Mohamed my mother Noor Salleh, my sisters, Nahidah A. Mohamed, Suhaila A. Mohamed and Arfah A. Mohamed and also my brother, Hafiz A. Mohamed. Not forgetting my husband Mohd. Taqiuddin Basiron, who have been a great source of motivation and support during my studies.

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

CLONING AND EXPRESSION OF A THERMOSTABLE α-GLUCOSIDASE

By

RAUDA BINTI A. MOHAMED

December 2010

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

Faculty: **Biotechnology and Biomolecular Sciences**

Yeast is considered as a good host for large scale production of enzymes. This is the first report of α-glucosidase obtained from bacterial source to be expressed in yeast. Seven bacterial isolates were successfully obtained from water sample of Telaga Air Hangat, Langkawi. The optimum growth temperature for these bacterial isolates (L2, L3, L4, GBB1, SR 38, SR 40 and SR 96) was at 55^{o}C. Screening using an α-MUG plate overlay method indicated that 4 out of 7 isolates gave positive α-glucosidase activity (L2, L3, L4 and GBB1). The highest activity was 1.47 U/mL at 55°C from sample L3. This isolate was identified using 16S rRNA as a universal primer and from the BLAST result, the isolate showed 99% similarity to *Geobacillus stearothermophilus.* The gene encoding α-glucosidase was isolated from this identified bacterium using degenerate primers. A complete gene sequence encoding α-glucosidase (~1.7 kb) was obtained by a DNA walking approach. This gene fragment was successfully cloned and expressed into *Escherichia coli* Top10 cells using pBAD and pTrcHis2@TOPO TA expression vectors. The intracellular α-

glucosidase production by recombinant *E. coli* was increased 3.4-fold and 2-fold in pBAD and pTrcHis2 compared to the wild type isolate, respectively. The restriction enzymes (RE) based primers were designed to clone the gene into a yeast expression vector pPICZαA and to allow transformation into *P. pastoris*. Transformation was successfully achieved with the α-glucosidase expression level at 3.3 U/mL before optimization. After optimization, the highest activity obtained was ~10 U/mL. This is about 2-fold higher than the expression by *E. coli* and 6-fold higher than the wild type isolate. *P. pastoris* expression system was shown to be effective in increasing the expression yield of the heterologous protein.

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

CLONING AND EXPRESSION OF A THERMOSTABLE α-GLUCOSIDASE

Oleh

RAUDA BINTI A. MOHAMED

Disember 2010

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

Fakulti: **Bioteknologi dan Sains Biomolekul**

Yeast adalah dianggap sebagai perumah yang bagus untuk penghasilan enzim berskala besar. Ini adalah laporan kali pertama bagi α-glukosidase yang diperolehi daripada sumber bakteria dan diekspresikan dalam sistem yis. Tujuh pencilan bakteria telah berjaya dijumpai daripada sampel air daripada Telaga Air Hangat, Langkawi. Suhu optimum bagi semua pencilan bakteria ini (L2, L3, L4, GBB1, SR 38, SR 40 dan SR 96) adalah pada suhu 55^{o}C. Analisis menggunakan kaedah piring tindanan α-MUG menunjukkan bahawa 4 daripada 7 pencilan (L2, L3, L4 dan GBB1) ini menunjukkan aktiviti positif bagi α-glukosidase. Aktiviti tertinggi adalah daripada pencilan L3 sebanyak 1.47 U/mL pada 55°C. Pencilan ini dikenalpasti menggunakan 16S rRNA sebagai jujukan universal dan daripada keputusan BLAST, pencilan menunjukkan 99% persamaan dengan *Geobacillus stearothermophilus.* Gen yang mengekodkan α-glukosidase telah dipencilkan daripada bakteria yang telah dikenalpasti ini menggunakan jujukan degenerat. Gen lengkap yang mengekodkan α-glukosidase (~1.7 kb) telah diperolehi melalui pendekatan 'DNA Walking'. Gen ini

telah berjaya diklon dan diekspreskan di dalam *Escherichia coli* Top10 menggunakan vector pengekspresan pBAD dan pTrcHis2@TOPO TA. Penghasilan α-glukosidase secara intrasel oleh rekombinan *E. coli* telah meningkat sebanyak 3.4 kali ganda dan 2 kali ganda masing-masing dalam pBAD dan pTrcHis2 berbanding dalam pencilan asal. Pencetus dengan tapak pemotongan enzim penyekatan telah direka bentuk untuk mengklonkan gen di dalam vektor pengekspresan yis pPICZαA dan membolehkan transformasi ke dalam *P. pastoris*. Transformasi ini berjaya dengan tahap pengekspresan α-glukosidase pada 3.3 U/mL sebelum pengoptimuman. Setelah dioptimumkan, aktiviti tertinggi yang diperoleh adalah sebanyak ~10 U/mL. Ini adalah peningkatan sebanyak 2 kali ganda daripada pengekspresan oleh *E. coli* dan 6 kali ganda oleh pencilan asal. Sistem pengekspresan *P. pastoris* telah menunjukkan ianya berkesan dalam meningkatkan tahap pengekspresan protein asing.

ACKNOWLEDGEMENT

Alhamdulillah, praise be to Allah, the most gracious, the most merciful, for His blessings for me to finish this study and writing this thesis. I wish to express my foremost appreciation to my supervisors Professor Dato' Dr. Abu Bakar Salleh, Professor Dr. Raja Noor Zaliha Raja Abdul Rahman and Professor Dr. Mahiran Basri for their invaluable guidance, encouragement, help and patience through the course of this thesis by enlightening me scientifically and resolving many technical problems until the completion of this project.

My appreciation is also dedicated to Dr Adam Leow Thean Chor, Dr. Mohd. Syukuri and Mr. Rofandi Sulong. Gratitude is extended to my seniors, Elyas, Syuhada, Suriana, Wani, Tengku, Afshin, Elly, Jalimah, Fairol, Randa and colleagues, Baya, Hisham, Zarir, Salsabil, Dina, Jijah, Marha, Akmal, Hidayah, Naem, Aishah, Hafizal, Sangkary, Che', Din, Zul and Eddie for their cheerful discussion and occasions. I never forget the care and support from my friends from the Enzyme Technology Lab, IBS and 140 Lab, Faculty of Biotechnology and Biomolecular Sciences, UPM.

Last but not least, I am eternally grateful to my husband and family for their love, care and support throughout this project, without which I would never be here and have succeeded in my academic endeavors. May Allah bless all of you.

I certify that a Thesis Examination Committee has met on 14 December 2010 to conduct the final examination of Rauda binti A. Mohamed on her thesis entitled "Cloning and Expression of a Thermostable α-Glucosidase" 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

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Date: 22 February 2011

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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Date:

DECLARATION

I declare that the thesis is my original work except for equations 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.

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RAUDA BINTI A. MOHAMED

Date: 14 December 2010

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