UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF THERMOSTABLE L2 LIPASE USING RECOMBINANT Pichia pastoris

NOOR HIDAYAH SHAHIDAN

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

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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

May 2012

This thesis is especially dedicated to:

Fauzi Hanif Hamzah

Thank you for being my source of inspirations

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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

PRODUCTION OF THERMOSTABLE L2 LIPASE USING RECOMBINANT *Pichia pastoris*

By

NOOR HIDAYAH SHAHIDAN

May 2012

Chairman: Professor Raja Noor Zaliha Raja Abd Rahman, D. Eng

Institute: Institute of Bioscience

Thermostable lipases are widely used for various industrial applications, such as in the production of detergents, oleo chemical industry, food-processing as well in dairy and milk productions. Recombinant *Pichia pastoris* strain GS115 harbouring L2 lipase gene under constitutive expression of glyceraldehyde-3-phosphate (GAP) was subjected to a series of batch cultivation studies at shake-flask scale (100 mL) and in 7.5 L bioreactor. Batch cultivation studies at shake-flask scale were conducted to investigate the effect of different carbon sources on the expression level of L2 lipase and growth of *P. pastoris*. Growth of *P. pastoris* at 2% (w/v) glucose concentration gave the highest lipase activity. Other carbon sources were also tested for growth; oleic acid, trehalose, glycerol, lactose, methanol and sorbitol in which it was observed that 1% (v/v) glycerol produced the highest L2 lipase activity of 34 U/mL. In addition, study on fifth grade molasses as potential carbon source revealed that the highest activity of thermostable L2 lipase of 40 U/mL occurred when the concentration of fifth grade molasses was set at 2% (w/v).

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Growth of the recombinant on the remnant of sugar refinery process, mud cake produced little or zero expression level of L2 lipase and poor growth of *P. pastoris*, showing that it was not an effective carbon source. Study conducted on different sugar components of the molasses which were sucrose, maltose, glucose, and fructose showed that the cultivation on fructose led to the highest expression of L2 lipase of 50 U/mL among growth on other carbon sources, followed by growth of *P. pastoris* cells on glucose.

A preliminary study of batch cultivation of recombinant *P. pastoris* was performed using medium containing 2% (w/v) glucose as carbon source in 7.5 L bioreactor with the following parameters: controlled dissolved oxygen tension (DOT) 35%, controlled pH 6.0, 1 vvm, temperature 30°C, 30 h inoculum age, and resulted in 60 U/mL of lipase activity at 24 h of cultivation time. Batch cultivation studies were also conducted using different carbon sources namely glycerol, fifth grade molasses and mixture of glycerol and the fifth grade molasses, which produced 56, 120, and 60 U/mL of lipase activity, respectively. Further optimization studies were conducted for the selected parameters with cultivation medium containing fifth grade molasses as main carbon source: controlled pH at 6.0 and uncontrolled pH (with initial pH at 6.0), inoculum age, controlled DOT, and molasses concentration. Optimized condition with fifth grade molasses as main carbon source produced 120 U/mL when cultivation conditions were set at 35% air saturation, initial pH 6.0, 30 h of inoculum age, and 2% (w/v) of fifth grade molasses concentration. This study showed that the constitutive pGAP expression system of *P. pastoris* is inducible by various carbon sources; therefore, it offers flexibility for diverse expressions of heterologous proteins.

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

PENGHASILAN LIPASE L2 TERMOSTABIL MENGGUNAKAN REKOMBINAN *Pichia pastoris*

Oleh

NOOR HIDAYAH SHAHIDAN

Mei 2012

Pengerusi: Profesor Raja Noor Zaliha Raja Abd Rahman, D. Eng

Institut: Institut Biosains

Lipase termostabil telah digunakan secara meluas untuk pelbagai kegunaan industri seperti pembuatan detergen, industri oleo kimia, pemproseses makanan serta untuk penghasilan tenusu dan susu. Rekombinan *Pichia pastoris* strain GS115 yang mengandungi gen L2 lipase di bawah kawalan promoter konstitutif gliseraldehida-3-fosfat dehydrogenase (GAP) adalah tertakluk kepada satu siri kajian pertumbuhan sesekelompok pada skala kelalang goncang (100 mL) dan di dalam 7.5 L bioreaktor. Kajian pembiakan berkumpulan pada skala kelalang goncang telah dijalankan untuk mengkaji kesan pelbagai sumber karbon ke atas penghasilan L2 lipase dan pertumbuhan *P. pastoris*. Pertumbuhan pada kepekatan glukosa 2% (w/v) menghasilkan aktiviti lipase tertinggi. Sumber karbon lain yang dikaji termasuk asid oleik, trehalose, gliserol, laktosa, metanol dan sorbitol, di mana bagi sumber karbon lain, gliserol menghasilkan aktiviti L2 lipase tertinggi iaitu 34 U/mL bagi rekombinan *P. pastoris*. Di samping itu, kajian mengenai sirap pekat (*molasses*) gred kelima sebagai potensi sumber karbon

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menunjukkan bahawa aktiviti tertinggi L2 lipase iaitu 40 U/mL berlaku pada kepekatan sirap pekat 2% (b/i) gred kelima. Pertumbuhan rekombinan menggunakan sisa proses kilang penapisan gula, kek lumpur (*mud cake*) menghasilkan aktiviti L2 lipase yang rendah serta kadar pertumbuhan *P. pastoris* yang rendah, menunjukkan ia bukan sumber karbon yang efektif. Kajian yang dijalankan ke atas komponen gula yang berbeza terkandung dalam sirap pekat iaitu sukrosa, maltosa, glukosa, dan fruktosa menunjukkan bahawa pembiakan yis di dalam medium mengandungi fruktosa menghasilkan kadar tertinggi L2 lipase iaitu 50 U/mL berbanding sumber-sumber karbon yang lain, diikuti oleh pertumbuhan sel-sel *P. pastoris* di dalam glukosa.

Kajian awal pembiakan berkumpulan rekombinan *P. pastoris* dijalankan menggunakan medium yang mengandungi 2% (b/i) glukosa sebagai sumber karbon dengan parameter berikut: ketegangan terkawal oksigen terlarut (DOT) 35%, pH terkawal 6.0, 1 vvm, suhu 30°C, 30 jam umur inokulum didapati menghasilkan kadar aktiviti L2 lipase sebanyak 60 U/mL pada 24 jam masa pertumbuhan. Kajian pertumbuhan sesekelompok juga dijalankan ke atas sumber karbon yang berbeza iaitu gliserol, sirap pekat gred kelima dan campuran gliserol dan sirap pekat gred kelima, yang masing-masing menghasilkan 56, 120 dan 60 U/mL lipase aktiviti. Kajian pengoptimuman seterusnya adalah menggunakan sirap pekat gred kelima sebagai sumber karbon utama: pH 6.0 terkawal dan pH yang tidak terkawal (dengan pH awal pada kadar 6.0), umur inoculum, DOT, dan kepekatan sirap pekat. Keadaan optimum bagi sirap pekat gred kelima sebagai sumber karbon utama menghasilkan 120 U/mL apabila parameter telah ditetapkan pada 35% keterlarutan oksigen, pH awal 6.0, 30 jam umur inoculum dan 2% (b/i) kepekatan sirap pekat gred kelima. Kajian ini telah menunjukkan bahawa sistem expresi pGAP *P.*

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pastoris boleh diaruh oleh pelbagai sumber karbon, menjadikannya fleksibel untuk ekpresi protein asing dengan lebih meluas.

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I certify that a Thesis Examination Committee has met on 18 May 2012 to conduct the final examination of Noor Hidayah Shahidan on her thesis entitled "**Production of Thermostable L2 Lipase Using Recombinant *Pichia pastoris***" in accordance with the Universities and University Colleges 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:

Janna Ong Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Sieo Chin Chin, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Suraini Abd. Aziz, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Fahrul Zaman Huyop, PhD
Associate Professor
Faculty of Bioscience and Bioengineering
Universiti Teknologi Malaysia
(External Examiner)

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

Date: 27 May 2012

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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:

Raja Noor Zaliha Raja Abd Rahman, D. Eng
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

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

Rosfarizan Mohamed, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Adam Leow Thean Chor, PhD
Senior Lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

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

Date:

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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.

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