



UNIVERSITI PUTRA MALAYSIA

***BIOMANUFACTURING OF AN ORGANIC SOLVENT TOLERANT
AND THERMOSTABLE LIPASE BY RECOMBINANT E. COLI***

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By

RUBINA NELOFER

**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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of the requirement for the degree of Doctor of Philosophy

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Chairman: Arbakariya B. Ariff, PhD

Faculty: Biotechnology and Biomolecular Sciences

Lipases are important industrial enzymes due to their versatile properties, especially the thermostable and organic solvent stable lipases. Natural isolates usually produce lipases in small amounts. Therefore, genes from these microbes are cloned into easily cultivating microorganisms like *Escherichia coli* for hyper production of the target lipase. This study was designed to develop an efficient large scale bioprocess for a thermostable and organic solvent tolerant lipase (Lip 42) from recombinant *E. coli* BL21. Different production media were first screened for lipase production by *E. coli* BL21 in shake flask fermentations. Response surface methodology (RSM) and artificial neural network (ANN) were used to optimize the medium composition and culture conditions. The kinetics of Lip 42 production by *E. coli* BL21 was evaluated using Monod and Luedeking-Piret equations. The effect of dissolved oxygen tension (DOT) level on growth of *E. coli* BL21 and Lip 42 production was investigated in batch fermentation using 1 L stirred tank bioreactor. Exponential fed-batch fermentation for Lip 42 production was first developed in 1 L stirred tank

bioreactor and then scaled up to 10 L and 50 L. Purification of Lip 42 from the culture broth was carried out by step II affinity chromatography using different scales of AKTA explorer.

The highest Lip 42 production was obtained ($28. \pm 4.1$ IU/mL) in LB broth with the addition of 1% (w/v) glucose. Using Plackett-Burman design, the most significant variables that influenced Lip 42 production by *E. coli* BL21 were glucose, NaCl, temperature and induction time. The R^2 value calculated by RSM showed a good fit, but higher values of absolute average deviation (AAD) and root mean square error (RMSE) were obtained. ANN predicted with better R^2 , AAD and RMSE values than RSM. The proposed models for Lip 42 production by *E. coli* BL21 were sufficient to describe the process using a wide range of initial glucose and yeast extract concentrations, where Lip 42 production was found to be growth associated processes. Lip 42 production (73.85 IU/mL) at optimal DOT level (30% saturation) was about 1.5 times higher than that obtained in fermentation with DOT controlled at low level (10% saturation). Antibiotics should be supplied continuously to the culture to maintain the percentage of plasmid bearing cells at higher levels during exponential fed-batch fermentation of Lip 42 by *E. coli*. Exponential fed-batch fermentation, where the specific growth rate was controlled at 0.1 h^{-1} by feeding with glucose, was found optimal for growth of *E. coli* BL21 (30.32 g/L) and Lip 42 production (130.5 IU/mL).

Lip 42 from the culture broth of *E. coli* was successfully purified with optimal DBC conditions using one step step II tag affinity chromatography at laboratory scale (2 mL column) with almost 65% recovery with comparable results for purification

using larger columns (8 mL and 40 mL). Lip 42 can be economically eluted using 0.1 M NaOH instead of using expensive desthiobiotin. The fermentation employing *E. coli* for Lip 42 production was successfully scaled-up to 50 L stirred tank bioreactor using a constant DOT approach, where DOT level was controlled at 30% saturation.

The information and findings obtained from this study are very useful in the designing and the preparation of standard operating procedure (SOP) of Lip 42 production by recombinant *E. coli* BL21 at industrial scale.

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

**BIOPENGHASILAN LIPASE TERMOSTABIL YANG TOLERAN
KEPADA PELARUT ORGANIK OLEH REKOMBINAN *E. COLI***

Oleh

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Lipase adalah enzim industri yang penting disebabkan oleh sifat serba bolehnya terutamanya lipase yang termostabil dan bertoleransi terhadap pelarut organik. Mikroorganisma semulajadi biasanya menghasilkan lipase dalam kuantiti yang kecil. Oleh itu, gen daripada mikroorganisma semulajadi diklon ke dalam mikroorganisma yang mudah dikultur seperti *E. coli* untuk penghasilan lipase sasaran dalam kuantiti yang besar. Kajian ini telah direka untuk membangunkan bioproses skala besar untuk penghasilan rekombinan lipase (Lip 42) yang termostabil dan bertoleransi terhadap pelarut dari *E. coli* BL21 rekombinan. Pelbagai media pengeluaran telah disaring untuk pengeluaran lipase oleh *E. coli* BL21 melalui fermentasi menggunakan kelalang bergoncang. Kaedah respon permukaan (RSM) dan rangkaian neural tiruan (ANN) telah digunakan untuk pengoptimuman komposisi medium dan keadaan pengkulturan. Kinetik penghasilan Lip 42 oleh *E. coli* BL21 telah dianalisa menggunakan persamaan Monod dan Luedeking-Piret. Kesan kepekatan oksigen terlarut (DOT) kepada pertumbuhan *E. coli* BL21

dan penghasilan Lip 42 dikaji melalui fermentasi sekelompok menggunakan bioreaktor tangki berpengaduk yang mempunyai isipadu 1 L. Fermentasi sekelompok suapan eksponen untuk penghasilan Lip 42 pada peringkat awal dibangunkan dalam bioreaktor tangki berpengaduk 1 L dan kemudian ditingkatkan kepada isipadu 10 L dan 50 L. Penulenan Lip 42 dari kaldu fermentasi telah dilakukan oleh kromatografi afiniti strep II menggunakan AKTA explorer pada skala yang berbeza.

Penghasilan Lip 42 tertinggi (28 ± 4.1 IU / mL) telah diperoleh menggunakan medium LB dengan penambahan 1% (w/v) glukosa. Dengan menggunakan reka bentuk Plakett-Burman, pemboleh ubah yang sangat mempengaruhi penghasilan Lip 42 oleh *E. coli* BL21 ialah glukosa, NaCl, suhu dan masa induksi. Nilai R^2 yang dikira oleh RSM menunjukkan nilai yang sesuai, tetapi nilai-nilai sisihan purata mutlak (AAD) yang lebih tinggi dan min punca kuasa kesilapan telah (RMSE) diperoleh. ANN meramalkan nilai R^2 , AAD dan nilai-nilai RMSE yang lebih baik daripada RSM. Model yang dicadangkan untuk penghasilan Lip 42 oleh *E. coli* BL21 telah didapati bersesuaian untuk menerangkan proses fermentasi menggunakan pelbagai kepekatan awal glukosa dan ekstrak yis, dimana penghasilan Lip 42 telah didapati sebagai proses yang berkait dengan pertumbuhan. Penghasilan Lip 42 (73.85 IU / mL) pada DOT optima (30% ketepuan) adalah 1.5 kali lebih tinggi daripada yang diperoleh dalam fermentasi dengan DOT dikawal pada tahap yang rendah (10% ketepuan). Antibiotik perlu dibekalkan secara selanjut kepada kultur untuk mengekalkan peratusan sel yang mengandungi plasmid pada aras yang tinggi semasa fermentasi sekelompok suapan untuk pengeluaran Lip 42 oleh *E. coli*. Dalam fermentasi sekelompok suapan eksponen yang kadar pertumbuhan spesifik

telah dikawal pada 0.1 h^{-1} dengan glukosa sebagai subtrat, telah didapati optima bagi pertumbuhan *E. coli* BL21 (30.32 g / L) dan penghasilan Lip 42 (130.5 IU / mL).

Lip 42 dari kalsdu fermentasi *E. coli* telah berjaya ditulinkan pada keadaan DBC yang optima menggunakan kromatografi afiniti, satu langkah strep II pada skala makmal (2 mL lajur) dengan hasil viiyophi mencapai pemulihan 65% dan keputusan yang bersepadanan juga diperoleh menggunakan turus yang lebih besar (8 mL dan 40 mL). Lip 42 boleh dielusikan secara lebih ekonomi menggunakan 0.1 M NaOH untuk menggantikan desthiobiotin yang mahal. Proses fermentasi untuk penghasilan Lip 42 oleh *E. Coli* telah viiyophil ditingkatkan kepada skala 50 L viiyophilisa tangki berpengaduk menggunakan pendekatan DOT yang malar, di mana aras DOT dikawal pada ketepuan 30%.

Maklumat dan penemuan yang diperoleh daripada kajian ini adalah amat berguna dalam reka bentuk dan penyediaan prosedur operasi piawai (SOP) untuk penghasilan Lip 42 oleh *E. coli* rekombinan BL21 pada skala viiyophili.

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I certify that an Examination Committee has met on **15th May** to conduct the final examination of Rubina Nelofer on her PhD thesis entitled “Biomanufacturing of an organic solvent tolerant and thermostable lipase by recombinant *Escherichia coli*” 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 Doctor of Philosophy.

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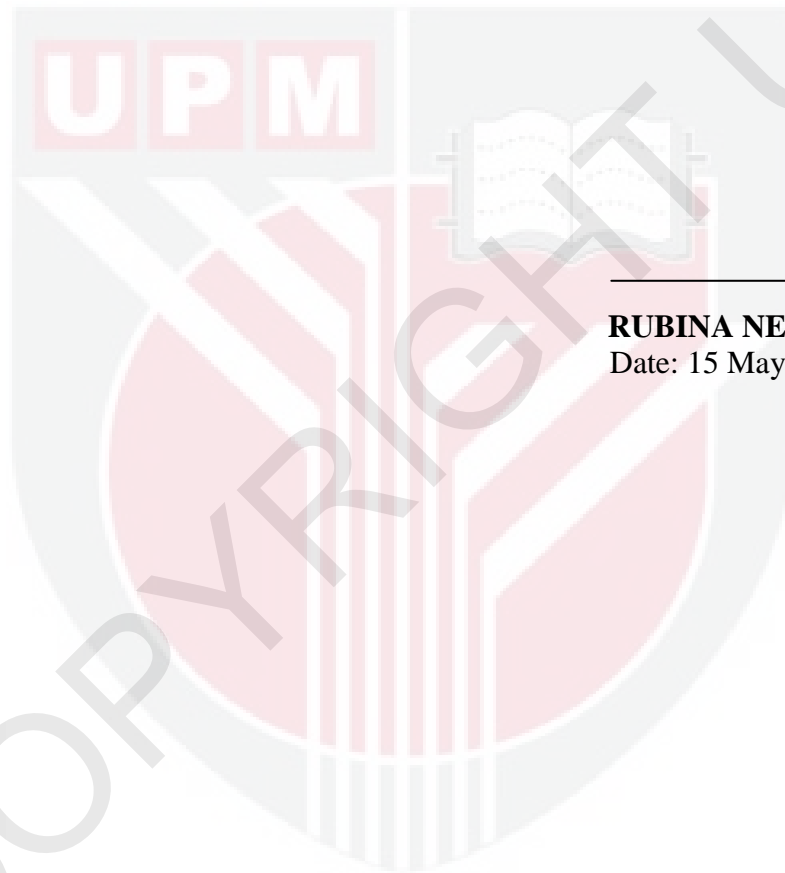
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DECLARATION

I declare that the thesis is my original work except for quotation 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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Date: 15 May 2012

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