UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF PHOSPHOLIPASE A2 FROM RECOMBINANT YARROWIA LIPOLYTICA FOR BIOPHARMACEUTICAL APPLICATION

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PRODUCTION OF PHOSPHOLIPASE A2 FROM RECOMBINANT *YARROWIA LIPOLYTICA* FOR BIOPHARMACEUTICAL APPLICATION

By

NUR 'AINUN MOKHTAR

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

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

PRODUCTION OF PHOSPHOLIPASE A2 FROM RECOMBINANT *YARROWIA LIPOLYTICA* FOR BIOPHARMACEUTICAL APPLICATION

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October 2013

Chair : Huzwah Khaza'ai, PhD

Faculty : Faculty of Medicine and Health Sciences

Phospholipase A2 (PLA2) is an enzyme that catalyzes the hydrolyisis of glycerophospholipids at the *sn*-2 position to yield the corresponding lysophospholpids and the free fatty acids. It catalytic properties which act as powerful emulsifier make it a widely used enzyme in various industrial application including laboratories, cosmeticeuticals, food industry as well as in pharmaceutical. However, in most industries, the PLA2 used are mainly isolated from mammalian pancreas (bovine and porcine). On the contrary, it had come to an issue regarding the origin of this animal based product which are rejected due to religious concern and the risk of viral infections to the consumers. To prevail the issue, an alternative PLA2 to replace the commercially available PLA2 has been initiated. Optimization of production parameters such as temperature, initial pH, inoculums size, inducer concentration and agitation speed are investigated using Two-Level Factorial Design and Central Composite Design by

Design-Expert®. From this study, the optimal conditions PLA2 production are 6% (v/v) inoculums size; agitation speed, 225 rpm; pH 5.8; temperature of 34.5^{o}C; inducer concentration, 0.03% (v/v) in basal salt medium. A verification run and scale up of PLA2 production yield 26.22 mg/L and 19.07 mg/L respectively compared to 27.15 mg/L predicted by the model. Purification of this enzyme through freeze drying and ultrafiltration and have shown a satisfactory purification factor of 1.15 and 1.35, respectively. The enzymatic properties (optimum activity at 37^{o}C, pH 8.0) of the recombinant produced PLA2 from *Y. lipolytica* in this study shows similar properties to that of commercially available PLA2 in market which indicate that this recombinant PLA2 is a good and remarkable alternative of PLA2 sources for biopharmaceutical usage especially for HALAL applications.

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

PENGHASILAN PHOSPHOLIPASE A2 DARIPADA REKOMBINAN *YARROWIA LIPOLYTICA* UNTUK APLIKASI BIOFARMASEUTIKAL

Oleh

NUR 'AINUN MOKHTAR

Oktober 2013

Pengerusi : Huzwah Khaza'ai, PhD

Fakulti : Perubatan dan Sains Kesihatan

Phospholipase A2 (PLA2) merupakan enzim yang memangkin hidrolisis gliserofosfolipid pada kedudukan sn-2 untuk menghasilkan lysophospholpid dan asid lemak bebas. Atas sifat ini, enzim ini dijadikan sebagai pengemulsi berkuasa dan sering digunakan secara meluas dalam pelbagai aplikasi industri termasuk makmal, komeseutical, industri makanan serta farmaseutikal. Walau bagaimanapun, kebiasaannya, PLA2 yang digunakan adalah berasal dari sumber pankreas mamalia (lembu dan babi). Justeru, isu mengenai asal-usul produk yang berasaskan haiwan ini kebiasaannya ditolak atas sebab tuntutan agama dan risiko jangkitan virus kepada pengguna. Bagi mengatasi isu ini, satu alternatif untuk menggantikan PLA2 komersial telah dijalankan. Pengoptimuman parameter penghasilan seperti suhu, pH awal, saiz

inokulasi, kepekatan pencetus dan kelajuan pergolakan telah dikaji menggunakan rekabentuk Dua-Aras Faktoran dan Rekabentuk Komposit Tengah oleh DesignExpert ®. Hasil kajian ini mendapati keadaan yang optimum bagi penghasilan PLA2 adalah 6% (v/v) saiz inokulum; kelajuan pergolakan, 225 rpm; pH 5.8; suhu, 34.5^{o}C; kepekatan pencetus, 0.03% (v/v) dalam medium garam basal. Ujikaji pengesahan dan ujikaji skala besar mendapati hasil pengeluaran enzim PLA2 adalah masing-masing sebanyak 26.22 mg/L dan 19.07 mg/L berbanding dengan 27.15 mg/L yang diramalkan oleh model. Penyaringan melalui kaedah pengeringan beku dan ultrapenurasan pula telah menunjukkan faktor penyaringan yang memuaskan iaitu masing-masing sebanyak 1.15 dan 1.35. Ciri-ciri enzim PLA2 (aktiviti optimum pada 37^{o}C, pH 8.0) yang dihasilkan melalui kajian ini menunjukkan ciri yang serupa dengan PLA2 boleh didapati secara komersial di pasaran. Ini menunjukkan bahawa enzim PLA2 yang dihasilkan secara rekombinan ini boleh dianggap sebagai satu alternatif yang baik bagi menggantikan PLA2 yang komersial sedia ada untuk kegunaan biofarmaseutikal terutamanya untuk aplikasi HALAL.

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I certify that an Examination Committee has met on 10th October 2013 to conduct the final examination of Nur 'Ainun Mokhtar on her Master of Science thesis entitled "Production of Phospholipase A2 Enzyme from Recombinant *Yarrowia lipolytica* for Biopharmaceutical Application" 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 Master of Science degree. Members of the Examination Chairperson Committee were as follows:

Members of the Examination Committee were as follows:

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Universiti Putra Malaysia
(Chairman)

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfillment 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 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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NUR 'AINUN MOKHTAR

Date:

TABLE OF CONTENTS