UNIVERSITI PUTRA MALAYSIA

EXPRESSION OF HUMAN EPIDERMAL GROWTH FACTOR IN *PICHIA PASTORIS* AND OPTIMIZATION OF FERMENTATION CONDITIONS

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By

SAMIRA EISSAZADEH

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

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Speciallv Dedicated:

*To my beloved **Dad** and **Mum** for all their endless love, invaluable care, unfaltering support, patience, and believe in me; they are the strongest inspiration in my life;*

*To my dear brother, **Mehrdad** and my lovely sister, **Safoora** for their endless love, encouragement and understanding;*

*And to my dear grandpapa, **Shah Reza**, who left me soon but always lives in my mind and heart,*

I am so blessed to have you in my life.

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

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Chairman: Associate Professor Mohd Puad Abdullah, PhD

Faculty: Biotechnology and Biomolecular Sciences

Human epidermal growth factor (hEGF) is a 6.2 kD polypeptide secreted by human tissues into the body fluid. It carries multiple biological functions including induction of tissue proliferation and differentiation, a process that is important in tissue repair. Because of this function, hEGF is widely used in various medical and industrial applications such as for wound healing, corneal transplant and treatment of gastric ulcers. As a result, the demand for hEGF supply for local consumption is always high, and there is a need for enough and cheap local supply of this protein. However, local production of hEGF especially in large commercial scales has not been successful due to various technical challenges.

This study was carried out to express the *hEGF* gene in the yeast, *Pichia pastoris* GS115 using a commercially available expression vector equipped with a specific signal peptide that directs the transportation of the hEGF protein into the culture

medium. The performance of the expression system for hEGF production was enhanced by optimizing the fermentation conditions of the yeast. The *hEGF* gene was ligated into the pPIC9K expression vector, and then integrated into the yeast's genome by electroporation. The hEGF was assayed by using an ELISA-based system and the recombinant cell line that produced the highest hEGF was then used in the optimization of the fermentation conditions. In addition, the amount of methanol, an inducer for the expression system was also optimised.

The highest amount of hEGF secreted into the medium was obtained from clone B1 of the recombinant yeast with an average yield of 2.27 µg/ml. This was obtained through an induction of the yeast culture with 0.5% (v/v) methanol for 60 hours. The best medium for the optimal hEGF production was BMMY buffered at a pH range of 6.0 and 7.0. Within this pH range, the difference in hEGF production was not significant. Two fermentation conditions commonly known to affect heterologous protein production (pH and temperature) were analyzed by using the artificial neural network (ANN). Changes in both pH and temperature significantly affected the hEGF production with the pH change had slightly higher impact on hEGF production than variations in the temperature. As anticipated from the model, the production of hEGF was decreased when the pH of the culture medium was lower than 6.0, or when the temperature was lower than 30°C. As a conclusion, hEGF was correctly secreted into the culture media of the recombinant *P. pastoris* as anticipated from the properties of the expression vector used. The amount of hEGF secreted into the medium was affected by the fermentation conditions

especially the types of culture media used, the pH of the culture media and incubation temperature.

Abstrak thesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGEKSPRESAN FAKTOR PERTUMBUHAN EPIDERMA DALAM *PICHIA PASTORIS* DAN PENGOPTIMUMAN KEADAAN FERMENTASI

Oleh

SAMIRA EISSAZADEH

Julai 2012

Pengerusi: Profesor Madya Mohd Puad Abdullah, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Faktor pertumbuhan epiderma manusia (hEGF) adalah sejenis polipeptida bersaiz 6.2 kD yang dirembeskan keluar oleh tisu manusia ke dalam cecair badan. Ia mempunyai pelbagai fungsi biologi termasuk pengaruhan proses penggandaan dan pembahagian tisu, satu proses penting yang terlibat dalam pemulihan tisu. Disebabkan fungsi berkenaan, hEGF telah digunakan dengan meluas dalam pelbagai aplikasi berasaskan perubatan dan industri seperti penyembuhan luka, transplan kornea, dan dalam rawatan ulser gastrik. Hal ini telah meningkatkan permintaan terhadap hEGF sejak beberapa tahun kebelakangan ini, dan memerlukan penghasilan hEGF tempatan yang mencukupi dan murah. Bagaimanapun, penghasilan hEGF tempatan terutamanya dalam skala besar menghadapi pelbagai halangan teknikal.

Kajian ini telah dijalankan untuk mengekspres gen *hEGF* dalam yis, *Pichia pastoris* GS115 dengan meggunakan sejenis vektor pengekpresan protein komersial yang dilengkapi dengan isyarat penghantaran peptida khusus, yang mengarahkan pengangkutan protein hEGF ke dalam kultur media. Prestasi sistem pengekspresan tersebut telah dipertingkatkan melalui proses pengoptimuman keadaan pertumbuhan fermentasi yis tersebut. Gen *hEGF* telah dicantumkan dengan vektor pengekpresan pPIC9K, dan seterusnya diintegrasikan ke dalam genom yis berkenaan secara elektroporasi. Penghasilan hEGF telah ditentukan dengan kaedah pencerakinan berasaskan ELISA, dan sel rekombinan yang menghasilkan hEGF tertinggi telah digunakan dalam proses pengoptimuman keadaan fermentasi cultur tersebut. Selain itu, kepekatan metanol yang merupakan kimia pengaruh untuk sistem pengekpresan tersebut juga telah dioptimakan.

Jumlah tertinggi hEGF telah dihasilkan oleh klon B1 daripada yis tersebut dengan purata hasil 2.27 µg/ml. Hal ini telah dicapai melalui proses pengaruhan kultur sel tersebut dengan menggunakan 0.5% (v/v) metanol untuk jangkamasa 60 jam. Media yang memberi penghasilan hEGF optima pula adalah BMMY berpenimbal dengan julat pH 6 dan 7. Dalam julat pH ini, perbezaan dalam penghasilan hEGF adalah tidak ketara. Dua keadaan fermentasi kultur (suhu dan pH) yang diketahui umum mempengaruhi penghasilan protein heterologous, telah dianalisis lebih lanjut secara kombinasi dengan menggunakan jaringan neural tiruan (artificial neural network, ANN). Kedua-dua pH dan suhu didapati telah mempengaruhi penghasilan hEGF dengan ketara di mana perubahan nilai pH telah memberi impak yang lebih

besar dalam penghasilan hEGF berbanding dengan perubahan suhu. Berdasarkan kepada model tersebut, penghasilan hEGF menurun apabila pH media kultur tersebut berada pada di bawah paras 6, atau pada suhu dibawah paras 30^0C. Kesimpulannya, hEGF telah berjaya dirembeskan dengan sempurna ke dalam media pertumbuhan kultur *P. pastoris* rekombinan sebagaimana yang dijangkakan berasaskan kepada ciri vektor pengekspresan yang digunakan dalam kajian ini. Jumlah hEGF yang dirembeskan ke dalam media tersebut pula dipengaruhi oleh keadaan pertumbuhan kultur terutamanya jenis media yang digunakan, pH kultur dan suhu pengeraman.

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I certify that a Thesis Examination Committee has met on 27th July 2012 to conduct the final examination of Samira Eissazadeh on her thesis entitled "Expression of Human Epidermal Growth Factor (hEGF) in *Pichia pastoris* and Optimization of Fermentation Conditions" in accordance with the Universities and university colleges Act 1971 and the constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Date: 21 March 2013

This thesis was submitted to the Senate of Universiti 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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DECLARATION

I declare that the thesis is my original work except for quotations 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 at other institusion.

SAMIRA EISSAZADEH

Date:

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