UNIVERSITI PUTRA MALAYSIA

EFFECT OF PHOSPHOGLUCOISOMERASE (*has*E) AND HYALURONAN SYNTHASE (*has*A) CO-EXPRESSION ON HYALURONIC ACID PRODUCTION IN *ESCHERICHIA COLI* ROSETTA (DE3)

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

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Chairman: Raha Abdul Rahim, Ph.D

Faculty: Biotechnology and Biomolecular Sciences

Hyaluronic acid (HA) is one of nature's most versatile and fascinating materials due to its unique behavior and characteristic. This substance has a wide usage in clinical sector and plays a very important role in physiological and cell biological functions. Previously, the main source of this polysaccharide was from animal, but due to ethical issues and viral contamination, bacteria has emerged as an alternative resource for HA. The utilization of bacteria to produce HA is further improved by using heterologous recombinant host such as *Escherichia coli*. This study was aimed to enhance the production and molecular weight of HA in a new recombinant *E. coli* host that posed

useful industrial characteristic. Productions of hyaluronic acid were performed by expression of hyaluronan synthase (*has*A) and phosphoglucoisomerase (*has*E) genes in selected expression vectors into *E. coli* Rosetta (DE3) expression host. In this study, genomic DNA was isolated from *Streptococcus zooepidemicus* ATCC 39920. The *has*A and *has*E genes were succesfully PCR amplified using primers derived from NCBI GeneBank database. The fragments were then cloned into TOP 10 *E.coli* cloning vector. Sequencing results showed that both genes cloned were 100% identical to the published sequences. The inserts from the TOP 10 clones were sub-cloned into two different expression vectors, pRSF-DUET and pCDF. The *has*A was cloned into pRSF-DUET, while *has*E gene was cloned into both pRSF-DUET and pCDF producing pRSF-DUET-A, pRSF-DUET-AE and pCDF-E. All clones were transformed into *E. coli* Rosetta (DE3).

Four recombinant clones, *E. coli* Clone A, AE, A-E and AE-E were produced by transforming the recombinant plasmids into the expression host. All clones successfully expressed the recombinant proteins corresponding to the expected sizes of ~42 kDa and ~48 kDa. Both recombinant proteins were confirmed by western blotting using monoclonal anti-His and anti-S antibody. Batch cultivation of all clones in shake-flask showed a rapid decreased in cell growth after induction with Isopropyl β-D-1-thiogalactopyranoside (IPTG). The highest concentration and molecular weight of HA obtained were from clone AE-E at 30°C with the concentration of 0.072 g/L and a molecular weight of $1.1X10^5$ Da.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KESAN KO-EKSPRESAN GEN PHOSPHOGLUCOISOMERASE (*has*E) DAN HYALURONAN SYNTHASE (*has*A) KE ATAS PRODUKSI ASID HIALURONIK DI DALAM *ESCHERICHIA COLI* ROSETTA (DE3)

Oleh

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Asid hialuronik (HA) adalah merupakan bahan semula jadi yang serba guna dan menarik disebabkan oleh sifat dan ciri-cirinya yang istimewa. Bahan ini mempunyai pelbagai kegunaan dalam bidang klinikal dan mempunyai peranan penting dalam fungsi fisiologikal dan biologikal sel. Sebelum ini, sumber utama bagi polisakarida ini adalah dari haiwan. Walau bagaimanpun, isu-isu etika dan pencemaran virus menyebabkan bakteria kini menjadi pilihan sumber alternatif HA. Kegunaan bakteria sebagai sumber kepada HA ditambah baik lagi dengan penggunaan perumah rekombinan heterologos seperti *Escherichia coli*. Kajian ini bertujuan untuk meningkatkan penghasilan dan berat molekular asid hialuronik di dalam *E. coli* rekombinan baru yang berpotensi. Penghasilan HA dilakukan dengan menzahirkan gen hyaluronan synthase (*has*A) dan gen phosphoglucoisomerase (*has*E) melalui vektor plasmid zahiran yang sesuai di dalam perumah *E.coli* Rosetta (DE3). Di dalam penyelidikan ini, DNA genomik bakteria

Streptococcus zooepidemicus ATCC 39920 telah dipencilkan. Gen *has*A dan *has*E telah berjaya diamplifikasi menggunakan primer yang direka menggunakan jujukan nukleotida di GeneBank, NCBI.

Fragmen-fragmen tersebut kemudian diklonkan di dalam vektor pengklonan. Keputusan penjujukan menunjukkan kedua-dua gen mempunyai 99% kesamaan dengan rekod jujukan di dalam GeneBank NCBI. Gen-gen yang telah diklonkan didalam TOP 10 kemudian diklonkan ke dalam dua vektor ekpresi, pRSF-DUET dan pCDF. Gen *hasA* diklonkan didalam pRSF-DUET manakala *has*E diklonkan dalam pRSF-DUET dan pCDF menghasilkan pRSF-DUET-A, pRSF-DUET-AE dan pCDF-E. Kesemua klon tersebut ditransformasi ke dalam *E.coli* Rosetta (DE3).

Empat jenis klon, *E. coli* klon A, AE, A-E dan AE-E dihasilkan dengan mentransformasikan klon-klon plasmid ke dalam perumah. Kesemua protin klon telah berjaya dirembeskan dengan saiz protin yang dijangka iaitu ~42 kDa dan ~48 kDa. Kedua-dua protin tersebut dikenalpasti melalui teknik western blotting dengan menggunakan antibodi monoclonal anti-His dan monoclonal anti-S. Keputusan fermentasi menunjukkan pengurangan pertumbuhan sel yang cepat untuk semua klon selepas proses induksi. Kepekatan asid hialuronik dan berat molekul tertinggi diperolehi daripada klon AE-E di suhu 30°C di mana nilai kepekatan adalah 0.072 g/L dengan berat molekul $1.1X10^5$ Da.

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In the name of ALLAH The Beneficent, The Merciful

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Lastly, I would like to remind myself and everyone that we all could do worse, as writers, as readers and as human beings in general because every human being is not perfect and full perfection is ALLAH S.W.T alone.

"He who leaves home in search of knowledge, walk in the path of God"
Muhammad Bin Abdullah (570 – 632)

I certify that an Examination committee has met on 18 October 2013 to conduct the final examination of Muhammad Azmi B. Samsudin on his thesis entitled "Effect of phosphoglucoisomerase (*has*E) and hyaluronan synthase (*has*A) co-expression on hyaluronic acid production in *Escherichia coli* Rosetta (DE3)" 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 degree of Master of Science.

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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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DECLARATION

I hereby confirm that:

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This to confirm that:

- the research conducted and the writing of this thesis was under our supervison;
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