



UNIVERSITI PUTRA MALAYSIA

***PERIPLASMIC PROTEIN CHANGES IN RESPONSE TO OVER-
EXPRESSION OF RECOMBINANT PROTEIN IN Escherichia Coli***

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EXPRESSION OF RECOMBINANT PROTEIN IN *Escherichia Coli***

By

SOMAYYEH HEIDARY

**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia
in fulfillment of the Requirements for the Degree of Master of Science**

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A Specially dedication

To my parent, Nematollah and Shahla for all their love, care, support and believe

in me; they are the strongest inspiration in my life;

To my sisters Hoda and Pegah for encouragement and understanding

To my dear Navid for his love and support



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

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Because of its many beneficial features, *Escherichia coli* is widely used as host for recombinant protein production. However, one major disadvantage of using *E. coli* is the formation of inclusion body especially when the transgene is over-expressed. Therefore, an understanding of biochemical mechanism that triggers the formation of recombinant protein as inclusion bodies is important before the problem can be solved. To study the biochemical changes following an over-expression of a transgene in *E. coli* at protein level, differential periplasmic proteome was analyzed by using a two-dimensional gel electrophoresis technique.

The recombinant *E. coli* RG 2(DE3) carrying the plasmid pET-26b that encodes a human interferon- α 2b was used as a model organism. Crude protein extracts were prepared from the periplasmic space of the *E. coli* cells by using an osmotic shock

method. The protein samples were then separated on a 2D gel. High resolution of protein spots were successfully obtained from the protein samples after some optimizations were done on the rehydration buffer components. Optimization of CHAPS, ampholyte and DTT concentration and isoelectric focusing procedure had most effects on 2D result.

Based on the software analysis of the protein spots obtained, some potential unique, up- and down-regulated protein spots were observed. Most of the up and down regulated identified proteins were shown to be involved in ABC-transporter protein family such as phosphate ABC transporter, glutathione ABC transporter and oligopeptide ABC transporter. Knowing the types of protein family responded to the transgene over-expression may provide an important clue to what triggers *E. coli* to produce inclusion bodies.

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

**PERUBAHAN PROTEIN PERIPLASMA SEBAGAI RESPONS TERHADAP
EKSPRESI LEBIHAN PROTEIN REKOMBINAN DALAM *Escherichia Coli***

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Escherichia coli banyak digunakan sebagai hos untuk penghasilan protein rekombinan disebabkan oleh kelebihan ciri-cirinya. Walaubagaimanapun, satu keburukan utama dalam penggunaan *E. coli* adalah pembentukan jasad rangkuman, terutamanya apabila satu transgen adalah diekspresi secara lebihan. Maka, pemahaman terhadap mekanisme biokimia yang mencetuskan pembentukan jasad rangkuman daripada protein rekombinan adalah penting untuk penyelesaian masalah ini. Untuk mengkaji perubahan biokimia selepas ekspresi lebihan satu transgen *E. coli* di paras protein, perbezaan proteome periplasma telah dianalisis menggunakan teknik elektroforesis gel dua-dimensi.

E. coli RG 2(DE3) rekombinan yang membawa plasmid pET-26b yang mengkod interferon- α 2b manusia digunakan sebagai organisma model. Ekstrak protein

mentah telah disediakan daripada kawasan periplasma sel-sel *E. coli* dengan menggunakan teknik kejutan osmosis. Kemudiannya, sampel protein ini dipisahkan dengan menggunakan gel 2D. Selepas beberapa perubahan optimis terhadap komponen penimbal penghidratan semula, tempok protein beresolusi tinggi telah berjaya diperolehi daripada sampel protein. Perubahan optimis dalam kepekatan CHAPS, amfolit dan DTT serta prosedur penumpuan isoelektrik memberi kesan yang paling banyak terhadap keputusan 2D.

Berdasarkan analisis perisian tempok-tempok protein yang diperolehi, beberapa tempok protein yang berpotensi sebagai unik, diregulasi secara menaik serta diregulasi secara menurun telah diperhatikan. Kebanyakan protein yang diregulasi secara menaik dan menurun didapati berperanan dalam keluarga protein pengangkut ABC, seperti pengangkut ABC fosfat, pengangkut ABC glutation dan pengangkut ABC oligopeptida. Pengetahuan jenis-jenis keluarga protein yang telah menunjukkan respons terhadap ekspresi lebih transgen berkemungkinan memberikan klu penting terhadap pencetus pembentukan jasad rangkuman oleh *E. coli*.

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that, it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



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Date: 22 January 2013

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