UNIVERSITI PUTRA MALAYSIA

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

SOMAYYEH HEIDARY

FBSB 2013 6

PERIPLASMIC PROTEIN CHANGES IN RESPONSE TO OVER-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

January 2013

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

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

By

SOMAYYEH HEIDARY

January 2013

Chairman: Associate Professor Mohd Puad Bin Abdullah, PhD

Faculty: Biotechnology and Biomolecular Science

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-α2b 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*

Oleh

SOMAYYEH HEIDARY

Januari 2013

Pengerusi: Associate Professor Mohd Puad Bin Abdullah, PhD

Fakulti: Bioteknologi dan Sains Biomolekul

Escherichia coli banyak digunakan sebagai hos untuk penghasilam 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, perbezaaan proteome periplasma telah dianalisis menggunakan teknik elektroforesis gel dua-dimensi.

E. coli RG 2(DE3) rekombinan yang membawa plasmid pET-26b yang mengekod interferon-α2b 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, tompok 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 tompok-tompok protein yang diperolehi, beberapa tompok 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 terdahap ekspresi lebihan transgen berkemungkinan memberikan klu penting terhadap pencetusan pembentukan jasad rangkuman oleh *E. coli*.

ACKNOWLEDGEMENTS

Firstly, my praise to Allah for giving me the strength and wisdom to fulfill this challenging task. I would like to express my gratitude to my supervisor, Professor Dr. Mohd Puad Abdullah for his guidance, encouragement and constructive criticisms, which brought me to the completion of this thesis. I would like to thank my co-supervisors, Professor. Dr. Raha Abdul Rahim and Dr Adam Leow Thean Chor, for their valuable comments and critics during my study.

Special appreciations are also extended to my labmates, Thwda, Fadhil, Samira, Wong and Sigit for their supports and encouragements.

I would like to thank all staff especially Mr. Karim Hashem at the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences for their numerous help and support during my study.

Last but not the least, extremely huge thanks to my family for their endless love and encouragement, without which nothing would have been possible for me to come along the way.

This thesis was submitted to the Senate 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**:**

Mohd Puad bin Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Raha binti Abdul Rahim, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Adam Leow Thean Chor, PhD
Senior lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT ,PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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.

SOMAYYEH HEYDARI

Date: 22 January 2013

TABLE OF CONTENTS