IMPROVEMENT OF THE SOLUBILITY OF THE VP2 HYPERVARIABLE REGION OF INFECTIOUS BURSAL DISEASE VIRUS BY FUSION TO THE NP PROTEIN OF NEWCASTLE DISEASE VIRUS

By

RAFIDAH SAADUN

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

December 2005

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

IMPROVEMENT OF THE SOLUBILITY OF THE VP2 HYPERVARIABLE REGION OF INFECTIOUS BURSAL DISEASE VIRUS BY FUSION TO THE NP PROTEIN OF NEWCASTLE DISEASE VIRUS

By

RAFIDAH SAADUN

December 2005

Chairman: Professor Datin Khatijah Mohd. Yusoff, PhD

Faculty: Biotechnology and Biomolecular Sciences

Infectious bursal disease virus (IBDV) has five proteins, VP1, VP2, VP3, VP4 and VP5. The VP2 protein is the major host-protective immunogen of IBDV. The hypervariable region (HVR) of the VP2 protein [VP2(HVR)] elicits neutralising antibodies. The coding sequence of the VP2(HVR) was amplified by polymerase chain reaction (PCR) and ligated to the 3' end of the NP gene of Newcastle disease virus (NDV). The NP-VP2(HVR) fusion protein was produced under the control of *trc* promoter in the bacterial expression vector, pTrcHis2, for intracellular expression in *Escherichia coli* TOP 10 cells. The NP-VP2(HVR) was expressed as a 75 kDa fusion protein containing the *myc* epitope and His-tag at its C-terminal end. However, most of the fusion proteins produced in the cell existed as insoluble inclusion bodies. Thus , the NP-VP2(HVR) region was then sub-cloned into

pRSETA and pET-43.1(a) expression vectors which contain the T7 promoters. The recombinant plasmids were then introduced into *E. coli* BL 21 (DE3), BL 21 (SI) dan Origami B cells respectively. The yields of the NP-VP2(HVR) fusion protein produced by the different vectors in the respective *E. coli* strains were compared. Although the levels of protein expression still remained the same in the various *E. coli* strains, the solubility of the NP-VP2(HVR) fusion proteins improved significantly in BL 21 (DE3), BL 21 (SI) and Origami B cells from 80% to 97%. The development of these fusion systems will be useful as an alternative strategy in protein solubilization.

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

PENINGKATAN DALAM KETERLARUTAN BAHAGIAN HIPERVARIASI VP2 VIRUS PENYAKIT BURSA BERJANGKIT DENGAN MENGGABUNGKANNYA KEPADA PROTEIN NP VIRUS PENYAKIT SAMPAR AYAM

Oleh

RAFIDAH SAADUN

Disember 2005

Pengerusi: **Profesor Datin Khatijah Mohd. Yusoff, PhD**

Fakulti: **Bioteknologi dan Sains Biomolekul**

Virus penyakit bursa berjangkit (IBDV) mengandungi lima protein iaitu VP1, VP2, VP3, VP4 dan VP5. Protein VP2 pada IBDV ini berfungsi sebagai gen hos-pertahanan yang utama. Ini disebabkan oleh di dalam protein VP2 tersebut terdapat satu bahagian yang di kenali sebagai bahagian hipervariasi (HVR) [VP2(HVR)] yang mampu mencetuskan antibodi peneutralan. Dalam kajian ini, jujukan berkod VP2(HVR) telah digandakan menggunakan teknik tindak balas rantaian polymerase (PCR) dan kemudiannya dicantumkan di hujung 3' pada gen NP virus penyakit sampar ayam (NDV) yang terletak dalam vektor penghasilan bakteria, pTrcHis2 di bawah kawalan promoter *trc*. Hasil gabungan DNA NP-VP2(HVR) seterusnya dimasukkan ke dalam sel *Escherichia coli* TOP 10 untuk penghasilan protein. Protein gabungan NP-VP2(HVR) yang mengandungi epitop *myc* dan His-tag di hujung C-terminal telah dihasilkan pada saiz 75 kDa. Walau bagaimanapun, kebanyakkan protein gabungan yang

terhasil di dapati wujud sebagai gumpalan protein tidak larut. Oleh yang demikian, bahagian NP-VP2(HVR) kemudiannya disubklonkan ke dalam vektor pengekspresan, pRSETA dan pET-43.1(a) di bawah kawalan promoter T7. Seterusnya plasmid rekombinan dimasukkan ke dalam sel-sel BL 21 (DE3), BL 21 (SI) dan Origami B. Perbandingan hasil protein gabungan NP-VP2(HVR) dalam setiap jenis *E. coli* dilakukan. Walaupun terdapat persamaan pada paras protein yang terhasil dalam pelbagai jenis *E. coli* yang digunakan, namun peningkatan secara berturutan sebanyak 80% hingga 97% dalam keterlarutannya dapat dilihat dalam sel BL 21 (DE3), BL 21 (SI) dan Origami B. Dengan kejayaan sistem penyatuan tersebut, diharap ianya berfaedah sebagai strategi alternatif yang boleh diaplikasikan dalam penghasilan protein larut.

ACKNOWLEDGEMENTS

I deeply appreciated to all the wonderful people who have contributed significantly throughout the whole course of my study. I also indebted to all of them for their help and support.

First and foremost, I would like to sincerely acknowledge my supervisors: **Prof. Datin Dr. Khatijah Mohd Yusoff**, for her valuable guidance, suggestions, helpful advice and never-ending patience throughout the entire of this study; **Assoc. Prof. Dr. Tan Wen Siang**, for his stimulating discussions and helpful hints; **Assoc. Prof. Dr. Abdul Rahman Omar** and **Assoc. Prof. Dr. Mohd Hair Bejo**, for their trust, endless encouragement and thoughtful comments.

I am also grateful to all the colleagues in the Virology Laboratory: Amir Rabu, Kho Chiew Ling, Ong Swee Tin, Lee Thong Chuan, Suhana, Lalitha, Tan Geok Hun, Salwa and Taznim for making my time in the laboratory joyable and pleasant. Not forgetting, I also express my thanks to Dr. Majid Eshagi and Wong Sing King for their invaluable help up to the completion of my experiments.

Last but not least, my deepest appreciation will always go to my family, my parent: En. Saadun and Pn. Wan Bi, my beloved husband Adily for their support and sacrifices.

I certify that an Examination Committee has met on 9th of Disember 2005 to conduct the final examination of Rafidah Saadun on her Master of Science thesis entitled "Improvement of the Solubility of the VP2 Hypervariable Region of Infectious Bursal Disease Virus by Fusion to the NP Protein of Newcastle Disease Virus" in accordance with Universiti Pertanian Malaysia (High Degree) Act 1980 and Universiti Pertanian Malaysia (High Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Aini Ideris, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Raha Abdul Rahim, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Raja Noor Zaliha Raja Abdul Rahman, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Sharifah Syed Hassan, PhD
Managing Director
Department of Veterinary Services
Bukit Damansara
Kuala Lumpur
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Khatijah Mohd. Yusoff, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairperson)

Tan Wen Siang, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Abdul Rahman Omar, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mohd. Hair Bejo, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

_____
AINI IDERIS, PhD
Professor/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 UPM or other institutions.

RAFIDAH SAADUN

Date:

TABLE OF CONTENTS