UNIVERSITI PUTRA MALAYSIA

VERIFICATION OF H5 AND IL-15 GENE EXPRESSIONS FROM H5-RECOMBINANT FOWLPOX VIRUSES COEXPRESSING HOST CYTOKINE BY USING SDS-PAGE AND WESTERN BLOTTING

NURUL FARHANA ZULKIFLI

FBSB 2015 164

VERIFICATION OF H5 AND IL-15 GENE EXPRESSIONS FROM H5-RECOMBINANT FOWLPOX VIRUSES COEXPRESSING HOST CYTOKINE BY USING SDS-PAGE AND WESTERN BLOTTING

By

NURUL FARHANA BINTI ZULKIFLI

Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences Universiti Putra Malaysia, in Fulfilment of the Requirements for the Bachelor of Science (Honours) Cell and Molecular Biology

June, 2015

Abstract of thesis presented to the Department of Cell and Molecular Biology in fulfilment of the requirement for the Bachelor of Science (Honours) Cell and Molecular Biology

VERIFICATION OF H5 AND IL-15 GENE EXPRESSIONS FROM H5-RECOMBINANT FOWLPOX VIRUSES COEXPRESSING HOST CYTOKINE BY USING SDS-PAGE AND WESTERN BLOTTING

By

NURUL FARHANA BINTI ZULKIFLI

June, 2015

Chair: Dr. Mariatulqabtiah Abdul Razak, PhD
Faculty: Faculty of Biotechnology and Biomolecular Sciences

A development of effective vaccine as a treatment against avian influenza virus (AIV) has been introduced since the 1980s by using inactivated vaccines. A recombinant fowlpox virus expressing AIV haemagglutinin H5 gene, co-expressed with chicken interleukin (IL)-15 cytokine has been constructed previously. In this study, the recombinant fowlpox virus (rFWPV) were analysed by using SDS-PAGE and Western blotting to verify the expression of H5 and IL-15 genes. The protein samples were separated by SDS-PAGE and transferred onto the surface of nitrocellulose membrane electrophoretically, by semi-dry Western blotting technique. Mouse β-actin, anti-IL-15, ab62587, and ab21292 primary antibodies were used to probe the membrane after blocking process. The antibody-antigen complexes were conjugated with alkaline phosphatase coupled to a secondary anti-IgG antibody which were either anti-mouse, anti-goat, or anti-rabbit, depending on the specific primary antibodies. The membranes were then developed by using commercially available WesternBreeze® Chromogenic Immunodetection System. Early findings revealed that false positive expressions were detected in all of the gel wells including negative controls, based on the presence of non-specific bands of 56 kD and 42 kD. Later results showed a 42 kD protein band size for β-actin expression, however the expressions of H5 and IL-15 proteins in the rFWPV were unsuccessful.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk Ijazah Sains (Kepujian) Biologi Sel dan Molekul

PENGESAHAN PENZAHIRAN GEN H5 DAN IL-15 DARI VIRUS CACAR AYAM REKOMBINAN H5 YANG MENZAHIRKAN BERSAMA SITOKIN PERUMAH MENGGUNAKAN UJIAN SDS-PAGE DAN PEMEDAPAN WESTERN

Oleh

NURUL FARHANA BINTI ZULKIFLI

Jun, 2015

Pengerusi: Dr. Mariatulqabtiah Abdul Razak, PhD
Fakulti: Fakulti Bioteknologi dan Sains Biomolekul

Perkembangan vaksin yang berkesan sebagai rawatan terhadap virus selsema burung telah diperkenalkan sejak tahun 1980-an di Amerika Syarikat dengan menggunakan vaksin tidak aktif. Virus cacar ayam rekombinan yang menzahirkan hemaglutinin H5 gen, bersama dengan sitokin ayam, interlukin (IL)-15, telah dibina sebelum ini. Dalam kajian ini, virus cacar ayam rekombinan (rFWPV) telah dianalisis dengan menggunakan SDS-PAGE dan pemedapan Western untuk mengesahkan penzahiran gen H5 dan IL-15. Sampel protein dipisahkan oleh SDS-PAGE dan dipindahkan ke atas permukaan membran nitroselulosa secara elektroforetik, dengan menggunakan teknik pemedapan Western separa kering. Antibodi primer β-aktin tikus, anti-IL-15, ab62587, dan ab21292 digunakan untuk menyiasat membran selepas proses penyekatan. Kompleks antibodi-antigen telah dikonjugasi dengan fosfat alkali dan ditambah kepada antibodi sekunder anti-IgG, sama ada anti-tikus, anti-kambing, atau anti-arnab, bergantung kepada antibodi primer tertentu. Membran kemudiannya dibangunkan dengan menggunakan bahan yang boleh didapati secara komersial iaitu Sistem Pengecaman Imun Kromogenik WesternBreeze®. Penemuan awal menunjukkan bahawa penzahiran positif palsu dikesan di semua telaga gel termasuk kawalan negatif, berdasarkan kehadiran jalur tidak spesifik bagi 56 kD dan 42 kD berat molekul. Keputusan selepas itu menunjukkan penzahiran saiz jalur protein 42 kD bagi penzahiran β-aktin, namun penzahiran bagi protein H5 dan IL-15 daripada rekombinan virus cacar ayam tidak berjaya.

ACKNOWLEDGEMENTS

First and foremost I would like to express my sincere gratitude to my supervisor, Dr. Mariatulqabtiah Abdul Razak for the continuous support throughout my project and research with her knowledge, motivation and patience that allow me to finally completed my report for the final year project. Her encouragement helped me in all time of research especially for laboratory works.

Besides my supervisor, I owe a great thanks to Dr. Nurhidayah Roslan for her generosity to assist me for some parts of my project and offered much advice throughout my project. She also helped to provide and shared some materials and equipment which is needed for my research. I am also indebted to Dr. Maria's master students who have contributed to my project with their experience in laboratory works, suggestions, and opinions which has lead me to complete my project.

I finally would also like to thank my family for their endless moral support, my fellow lab mates and all my friends who are also worked very hard to complete their final year project. We did a lots of discussion together and went through all the hardship in order to finish the project before the deadlines. Not to forget the fun and happy moments that we had during the process of completing our final year project and also for the last four years as the cell and molecular biology's students which have enlightened me throughout those years.

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfilment of the requirement for the Bachelor of Science (Honours) Cell and Molecular Biology. The member of the Supervisory Committee was as follows:

Dr. Mariatulqabtiah Abdul Razak, PhD
Senior Lecturer
Department of Cell and Molecular Biology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

_____

(Assoc. Prof. Dr. Janna Ong Abdullah, PhD)
Head of Department
Cell and Molecular Biology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
Date:

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by the Department of Cell and Molecular Biology;
- written permission must be obtained from supervisor before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials;
- there is no plagiarism or data falsification/ fabrication in the thesis. The thesis has undergone plagiarism detection software (TURNITIN).

Signature: _____ Date: _____

Name and Matric No.: Nurul Farhana binti Zulkifli, 161977

Declaration by Supervisor

This is to confirm that:

- the research conducted and the writing of this thesis was under supervision

Signature: _____

Name: Dr. Mariatulqabtiah Abdul Razak
Department of Cell and Molecular Biology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

IL	Interleukin
SDS	Sodium Dodecyl Sulphate
FWPV	Fowlpox virus
rFWPV	Recombinant fowlpox virus
AIV	Avian influenza virus
CEF	Chicken embryo fibroblast
NA	Neuraminidase
HA	Haemagglutinin

CHAPTER 1

INTRODUCTION

An effective vaccine has been developed as a treatment against avian influenza virus (AIV) since 1980s to hinder the spread or outbreak of the disease. The avian influenza is a well-known disease infecting poultry and other avian species. Responding to this phenomenon, a fowlpox virus (FWPV) has been used as a recombinant vaccine to activate the specific immune systems leading to effective protection to generate both cell-mediated and humoral immune response. This recombinant fowlpox virus (rFWPV) vaccine is used against AIV which expressed H5 gene together with the co-expression of chicken cytokines (IL-15) to improve the efficacy of the vaccine. The effectiveness and stability of the gene expressed from the newly developed vaccine need to be verified earlier before it is officially introduced as a treatment against AIV. The vaccine based on recombinant fowlpox virus is expected to protect poultry and other birds from the infection from which the inserted genes are derived (Boyle and Coupar, 1988).

Based on the previous study of vaccines development against avian influenza virus (AIV) by Mingxiao and colleagues (2006), the recombinant fowlpox has been constructed, but the protein expressions are yet to be confirmed. Once the expression is confirmed, it can be assumed that H5 and IL-15 antigens can be expressed *in vivo*. Therefore, this study will emphasize on the verification of H5 and IL-15 gene expressions by the recombinant fowlpox virus that will ensure the effectiveness of the vaccine. We

hypothesized that the H5 and IL-15 genes will be expressed in Chicken Embryo Fibroblast (CEF) cells and can be confirmed by SDS-PAGE and Western blotting method. The main objective of this particular project is to verify the expression of H5 and IL-15 genes from recombinant fowlpox viruses (rFWPV) by using SDS-PAGE and Western blotting method.

REFERENCES

Afonso C. L., Tulman E. R., Lu Z., Zsak L., Kutish G. F., and Rock D. L. (2000). The genome of fowlpox virus. *Journal of Virology* 74(8): 3815-3831.

Boyle D. B., and Coupar B. E. H. (1988). Construction of recombinant fowlpox viruses as vectors for poultry vaccines. *Virus Research* 10: 343-356.

Boyle D. B., and Heine H. G. (1993). Recombinant fowlpox virus vaccines for poultry. *Immunology and Cell Biology* 71: 391-397.

Fouchier R. A. M., Munster V., Wallensten A., Bestebroer T. M., Herfst S., Smith D., Rimmelzwaan G. F., Olsen B., and Osterhaus A. D. M. E. (2005). Characterization of a novel Influenza A Virus Hemagglutinin subtype (H16) obtained from black-headed gulls. *Journal of Virology* 79(5): 2814-2822.

Hartawan R., Robinson K., Mahony T., and Meers J. (2011). *In vitro* expression of native H5 and N1 genes of avian influenza virus by using green fluorescent protein as reporter. *JITV* 16(3): 234-241.

Jalilian B., Omar A. R., Bejo M. H., Alitheen N. B., Rasoli M., and Matsumoto S. (2010). Development of avian influenza virus H5 DNA vaccine and MDP-1 gene of *Mycobacterium bovis* as genetic adjuvant. *Genetic Vaccines and Therapy* 8:4.

Kodihalli S., Kobasa D. L., and Webster R. G. (2000). Strategies for inducing protection against avian influenza A virus subtypes with DNA vaccines. *Vaccine* 18(23): 2592-2599.

Kramer, D. K. (2013). Western blotting (immunoblot): Gel electrophoresis for proteins. http://www.antibodies-online.com/resources/ [accessed 28 May 2015].

Lee C. W., Saif Y. M. (2009). Avian Influenza virus. *Comperative Immunology, Microbiology and Infectious Diseases* 32: 301-310.

Leong K. H., Ramsay A. J., Boyle D. B., and Ramshaw I. A. (1994). Selective induction of immune responses by cytokines coexpressed in recombinant fowlpox virus. *Journal of Virology* 68(12): 8125-8130.

Lillehoj H. S., Min W., Choi K. D., Babu U. S., Burnside J., Miyamoto T., Rosenthal M., and Lillehoj E. P. (2001). Molecular, cellular, and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-12. *Veterinary Immunology and Immunopathology* 82: 229-244.

Lim K. L., Jazayeri S. D., Yeap S. K., Alitheen N. B., Bejo M. H., Ideris A., and Omar A. R. (2012). Co-administration of avian influenza virus H5 plasmid DNA with chicken IL-15 and IL-18 enhanced chickens immune responses. *BMC Veterinary Research* 8:132.

Mingxiao M., Ningyi J., Zhenguo W., Ruilin W., Dongliang F., Min Z., Gefen Y., Chang L., Leili J., Kuoshi J., and Yingjiu Z. (2006). Construction and immunogenicity of recombinant fowlpox vaccines coexpressing HA of AIV H5N1 and chicken IL 18. *Vaccine* 24: 4304-4311.

Paoletti E. (1996). Applications of pox virus vectors to vaccination: An update. *Proceedings of the National Academy of Sciences of the United States of America* 93: 11349-11353.

Pavlova S. P., Veits J., Keil G. M., Mettenleiter T. C., and Fuchs W. (2009). Protection of chickens against H5N1 highly pathogenic avian influenza virus infection by live vaccination with infectious laryngotracheitis virus recombinants expressing H5 hemagglutinin and N1 neuraminidase. *Vaccine* 27: 773-785.

Qiao C., Jiang Y., Tian G., Wang X., Li C., Xin X., Chen H., and Yu K. (2009). Recombinant fowlpox virus vector based vaccine completely protects chickens from H5N1 avian influenza virus. *Antiviral research* 81: 234-238.

Qiao C. L., Yu K. Z., Jiang Y. P., Jia Y. Q., Tian G. B., Liu M., Deng G. H., Wang X. R., Meng Q. W., and Tang X. Y. (2010). Protection of chickens against highly lethal H5N1 and H7N1 avian influenza viruses with recombinant fowlpox virus co-expressing H5 hemagglutinin and N1 neuraminidase genes. *Avian Pathology* 32: 25-31.

Shaw I., and Davison T. F. (2000). Protection from IBDV-induces bursal damage by a recombinant fowlpox vaccine, fpIBD1, is dependent on the titre of challenge virus and chicken genotype. *Vaccine* 18: 3230-3241.

Shen G., Jin N., Ma M., Jin K., Zheng M., Zhuang T., Lu H., Zhu G., Jin H., Jin M., Huo X., Qin X., Yin R., Li C., Li H., Li Y., Han Z., Chen Y., and Jin M. (2007). Immune responses of pigs inoculated with recombinant fowlpox virus coexpressing GP5/GP3 of porcine reproductive and respiratory syndrome virus and swine IL-18. *Vaccine* 25: 4193-4202.

Souza A. P. D., Haut L., Reyes-Sandoval A., and Pinto A. R. (2005). Recombinant viruses as vaccines against viral disease. *Brazilian Journal of Medical and Biological Research* 38: 509-522.

Suarez D. L., and Schultz-Cherry S. (2000). Immunology of avian influenza virus: a review. *Developmental and Comparative Immunology* 24: 269-283.

Swayne D. E., Garcia M., Beck J. R., Kinney N., and Suarez D. L. (2000). Protection against diverse highly pathogenic H5 avian influenza viruses in chickens immunized with a recombinant fowlpox vaccine containing an H5 avian influenza haemagglutinin gene insert. *Vaccine* 18: 1088-1095.

Taylor J., Weinberg R., Kawaoka Y., Webster R. G., and Paoletti E. (1988). Protective immunity against avian influenza induced by fowlpox virus recombinant. *Vaccine* 6: 504-508.

Veits J., Wiesner D., Fuchs W., Hoffmann B., Granzow H., Starick E., Mundt E., Schirrmeier H., Mebatsion T., Mettenleiter T. C., and Romer-Oberdorfer A. (2006). Newcastle disease virus expressing H5 hemagglutinin gene protects chickens against Newcastle disease and avian influenza. *PNAS* 103(21): 8197-8202.