UNIVERSITI PUTRA MALAYSIA

VERIFICATION OF H5 GENE EXPRESSION FROM H5-RECOMBINANT FOWLPOX VIRUSES CO-EXPRESSING HOST CYTOKINE USING INDIRECT IMMUNOFLUORESCENCE ANTIBODY TEST (IFAT)

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By

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

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

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

Fowlpox virus (FWPV) has been used in vaccine development against avian influenza virus (AIV) by the expression of the AIV haemagglutinin (HA) gene. Recombinant FWPV (rFWPV) vaccines can be improved by co-expression of interleukins (IL) that can act as immunostimulatory molecule. Prior to any field applications, rFWPV ability to stably express HA and IL genes in cells and its efficacy to induce immune response needs to be verified. Thus, the objective of this study is to verify the H5 gene expression from rFWPV-H5 and rFWPV-H5-IL-15 via indirect immunofluorescence antibody test (IFAT) method. Firstly, freshly prepared chicken embryonic fibroblast (CEF) cells were infected with recombinant viruses and wild type fowlpox virus served as a negative control. After overnight incubation, the cells were overlaid with H5 primary antibodies raised in rabbits for 2 hours. Counter-staining was done using fluorescein (FITC)-conjugated anti-rabbit secondary antibody raised in goats. Phase-contrast fluorescence microscopy showed faint green fluorescence signals in rFWPV-H5 and false positive in rFWPV-H5-IL-15 when ab21292 H5 primary antibody was used. Bright green fluorescence signals were exhibited in both recombinants, but not negative controls, when ab62587 H5 primary antibody was used, indicative of successful H5 recombinant protein expression.

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

PENGESAHAN PENZAHIRAN GEN H5 DARI VIRUS CACAR AYAM REKOMBINAN H5 YANG MENGZAHIRKAN BERSAMA SITOKIN PERUMAH MENGGUNAKAN UJIAN ANTIBODI IMUNOKALIMANTANG TIDAK LANGSUNG (IFAT)

Oleh

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Jun 2015

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

Virus cacar ayam telah digunakan untuk pembuatan vaksin terhadap virus selsema burung (AIV) dengan pengekspresan gen hemaglutinin (HA) virus selsema burung tersebut. Namun, vaksin virus cacar ayam rekombinan (rFWPV) boleh ditambah baik dengan pengekspresan interleukin (IL) yang boleh berfungsi sebagai molekul perangsangan imun. Sebelum penggunaan vaksin di lapangan, kemampuan virus rekombinan cacar ayam untuk mengekspresikan gen HA dan IL dalam sel dan keberkesanan vaksin tersebut untuk merangsang gerak balas imun perlu disahkan. Objektif kajian ini adalah untuk menentusahkan pengekspresan protein H5 daripada rFWPV-H5 dan rFWPV-H5-IL-15 mengunakan ujian antibodi imunokalimantang tidak langsung (IFAT). Sel fibroblast embrio ayam (CEF) daripada embrio ayam yang disediakan segar telah dijangkitkan dengan virus rekombinan dan virus cacar ayam jenis liar sebagai kawalan negatif. Selepas dieramkan semalaman, sel dilapiskan dengan antibodi primer H5 selama dua jam. Pewarnaan balas dibuat mengunakan antibody sekunder berkonjugat fluoresein. Hasil pengamatan dengan mikroskop pendarfluor fasa berbeza menunjukkan isyarat malap pendarfluor hijau untuk rFWPV-H5 dan positif palsu untuk rFWPV-H5-IL-15 dengan pengunaan antibodi primer H5, ab21292. Isyarat terang pendarfluor hijau telah dipamerkan oleh kedua-dua rekombinan, tetapi tidak pada kawalan negatif, apabila antibodi primer H5 ab62587 digunakan, indikatif kepada penzahiran protein rekombinan H5 yang berjaya.

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APPROVAL

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

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Date:

DECLARATION

Declaration by undergraduate student

I hereby confirm that:

- this thesis is my original work;
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Declaration by Supervisor

This is to confirm that:

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

Signature: _____ Date: _____

Chairman of Supervisory Committee: Dr Mariatulqabtiah binti Abdul Razak

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LIST OF ABBREVIATIONS

AI	Avian Influenza
AIV	Avian Influenza Virus
CEF	Chicken Embryo Fibroblast
DMEM	Dulbecco's Modified Eagle's medium
dsDNA	Double Stranded Deoxyribonucleic Acid
FWPV	Fowlpox Virus
H5	Haemagglutinin - 5
HA	Haemagglutinin
HPAI	High Pathogenicity Avian Influenza.
IFAT	Indirect Immunofluorescence Antibody Test
IL-15	Interleukin - 15
IL	Interleukin
LPAI	Low Pathogenicity Avian Influenza
NA	Neuraminidase
NBBS	Newborn Bovine Serum
PBS	Phosphate Buffered Saline
rFWPV	Recombinant Fowlpox Virus
RNAs	Ribonucleic Acid
ssRNA	Single Stranded Ribonucleic Acid

CHAPTER 1
INTRODUCTION

Influenza epidemics caused by highly pathogenic avian influenza virus (AIV) of subtype H5 has affected poultry and human for decades (Cheng *et al*., 2012). AIV can cause mild to highly lethal diseases in hosts and vaccines are developed to provide protections against AIV. Attenuated fowlpox virus (FWPV) was used for recombinant vaccines since 1980s to express antigens of AIV. Recombinant FWPV expressing H5 of AIV was marketed in 1995 due to the rise in highly pathogenic avian influenza (HPAI) in Mexico. This lead to improvements of the vaccines against AIV which involved the co-expression of cytokines to increase the immunogenicity of the recombinant vaccines expressing haemagglutinin (HA) of AIV. Prior to field application of any candidate vaccines, verification of gene expression *in vitro* and *in vivo* is crucial to determine its stability of expression and efficacy for protection. The problem of this study is that recombinant fowlpox virus carrying avian influenza haemagglutinin gene H5, and H5 with interleukin-15 co-expression have been constructed but there is a lack of study on the expression of H5 in cells. Therefore, H5 expressions in cells need to be verified. The verification will allow successful indicator of H5 expression thus the vaccine can be injected into chickens for immune response analysis with H5 protein as the antigen. The hypothesis of this study is recombinant fowlpox virus carrying avian influenza haemagglutinin gene H5, and H5 with interleukin-15 co-expression may express the H5 gene in chicken embryo fibroblast cells. In order to prove the hypothesis, a specific objective is outlined, which is to verify the expression of recombinant fowlpox virus expressing avian influenza haemagglutinin gene by using immunofluorescence antibody test (IFAT).

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