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OPTIMIZATION OF qPCR ASSAY OF CHICKEN CYTOKINE GENES USING SYBR GREEN METHOD

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Abstract of thesis presented to the Department of Cell and Molecular Biology
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Chair: Dr. Mariatulqabtiah binti Abdul Razak, PhD.

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Quantitative PCR (qPCR) has been widely utilized in the study of cytokine expression profile upon vaccination of recombinant fowlpox virus and challenge of avian influenza virus. Although Taqman qPCR method offers specific detection of amplified products, SYBR green qPCR is more cost effective and relatively reliable to be used in expression profiling. In this study, total RNA samples were first harvested from spleens of two-week-old control chickens before reverse-transcribed to cDNA using iScript Supermix, with minor modifications. Sensitive qPCR assays based on real-time analysis of amplified products, intercalated with SYBR Green I dyes were designed and optimized using specific pairs of primers, to quantify chicken IL-15, IL-12, IL-18 and IFN-γ cytokine expressions. Amplification efficiency and coefficient of determination (R^2) were calculated by Bio Rad CFX Manager Software after a linear standard curve was generated. From the findings, qPCR assays of two chicken cytokine genes, IL-15 and IL-18, and two housekeeping genes, GAPDH and β-actin, were successfully optimized. Optimal annealing

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temperature of IL-15, IL-18, and β-actin were 59˚C, while GAPDH was 64˚C. Amplification efficiencies of those genes were within the ideal range, which is 95%-105%. R^2 values of those genes were also higher than 0.98 (>0.98). Melt-curve analysis of those genes showed that there was only one significant peak which corresponded to single specific amplified product. However, no result was obtained for cytokines IL-12 and IFN-γ. With the optimized amplification condition and annealing temperature of qPCR assays of each cytokine, differences of cytokine expression between control and vaccinated chicken can be studied in future work.

Keywords : qPCR, chicken cytokine, SYBR green.

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

PENGOPTIMUMAN ASAI qPCR UNTUK GEN SITOKIN AYAM DENGAN KAEDAH SYBR GREEN

Oleh

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Pengerusi: Mariatulqabtiah binti Abdul Razak, PhD.

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Kuantitatif PCR telah banyak digunakan dalam kajian profil pengekspresan sitokin setelah divaksinasi oleh virus cacar ayam rekombinan dan dicabar oleh virus selsema burung. Walaupun kaedah Taqman qPCR menyediakan pengecaman tepat untuk produk terganda, SYBR green qPCR adalah lebih murah dan boleh dipercayai, secara relatif, untuk digunakan dalam ujian pengekspresan. Dalam kajian ini, pada mulanya semua sampel RNA diperolehi daripada limpa ayam berumur dua minggu sebelum ditranskripsi songsang kepada cDNA menggunakan iScript Supermix, dengan modifikasi yang minor. Asai qPCR yang sensitif berdasarkan analisis masa nyata produk terganda, interkalari dengan pewarna SYBR green telah direka dan dioptimumkan menggunakan pasangan primer yang sesuai, untuk mengukur pengekspresan sitokin ayam IL-15, IL-12, IL-18 dan IFN-γ. Suhu sepuh lindap IL-15, IL-18 dan β-actin ialah 59˚C, manakala GAPDH ialah 64˚C. Kecekapan penggandaan dan pekali penentuan (R^2) telah dikira menggunakan perisian Bio Rad

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CFX Manager setelah lengkung piawai linear dihasilkan. Daripada penemuan-penemuan ini, asai qPCR untuk gen sitokin ayam IL-15 dan IL-18, juga gen penyelenggara, GAPDH dan β-actin, telah berjaya dioptimumkan. Kecekapan penggandaan untuk kesemua gen adalah dalam lingkungan julat yang sesuai, iaitu 95%-105%, nilai R^2 untuk kesemua gen adalah lebih tinggi daripada 0.98 (>0.98). Analisis lengkung-lebur untuk kesemua gen menunjukkan hanya satu puncak bermakna yang mewakili satu produk tunggal yang spesifik. Walau bagaimanapun, tiada keputusan didapati untuk sitokin IL-12 dan IFN-γ. Dengan kondisi penggandaan dan suhu sepuh lindap asai qPCR yang telah dioptimumkan, perbezaan pengekspresan sitokin antara ayam kawalan and ayam yang divaksinasi boleh dikaji pada masa depan.

Kata kunci : qPCR, sitokin ayam, SYBR green.

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APPROVAL LETTER

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 degree of Bachelor of Science (HONS.). The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate student

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Figure 11: Standard curve analysis of β-actin with C_q plotted against the log of starting quantity of template for each dilution. **47**

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LIST OF ABBREVIATIONS

AI	Avian influenza
AIV	Avian influenza virus
F	Fusion protein
FWPV	Fowlpox virus
HA	Haemagglutinin
HPAI	Highly pathogenic avian influenza
IBDV	Infectious bursal disease virus
IFN-γ	Interferon-gamma
IL	Interleukin
ILTV	Infectious laryngotracheitis virus
LP	Low pathogenic
LPAI	Low pathogenic avian influenza
MDV	Marek's disease virus
NA	Neuraminidase
NDV	Newcastle disease virus
PBS	Phosphate buffered saline
rFWPV	Recombinant fowlpox virus
qPCR	Quantitative real-time polymerase chain reaction
TK	Thymidine kinase

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CHAPTER 1

INTRODUCTION

In this era, agriculture is still an important sector to economic development of Malaysia. It contributes a high percentage to the national gross domestic product. Agriculture can be defined as practices of farming, crops plantation and cultivation, and raising livestock. In order to maintain the development of agriculture sector, scientific research and their application are essential. For example, marker-assisted selection in plant breeding program, genetic engineering in improving the quality and productivity of crops, and development of vaccine against virus infection in livestock. In sector of animal husbandry, virus infection in poultry has possibility in leading to serious economic losses. One of the popular virus infections or diseases in poultry is avian influenza.

Avian influenza (AI), is a disease syndrome attributed by influenza A virus, a type of virus from Orthomyxoviridae family (Lee et al., 2004; Qiao et al., 2003). This virus is not only accountable for major disease in avian, but also in mammals (Lee et al., 2004). However, first case of secondary spreading which is associated with human involvement, transmission of H5N1 virus from chicken to humans has occurred in 1997 (Subbarao, 1998). Thus, a major worry has emerged and shown the necessary to develop efficacious vaccines that give protection for poultry especially chickens and prevent distribution of virus. In the last 20 years, various types of vaccine against avian influenza virus has been developed and studied extensively. Wide varieties of vaccines were invented and have shown their effectiveness to protect chickens against avian virus.

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However, in a recent research, in neonatal chickens, immunization is fail after vaccination with recombinant fowlpox virus that containing H5 hemagglutinin and certain cytokine gene inserts. Problem statement of this project is that after the injection of recombinant fowlpox virus into those neonatal chickens, signs of illness were observed instead of developing protection against challenge of virus. Therefore the researchers hypothesized that chicken will be able to express IL-12, IL-15, IL-18, and IFN-γ at different level. Reason of doing this is because it is predicted that immunosuppression happened in neonatal chicken due to the overexpression of cytokine. Thus, in order to understand the immune response and cytokine gene expression level, investigation of their different expression level and its composition in neonatal chicken before and after vaccination is one of the ways. Prior of studying the expression level quantitatively, qPCR assay of the cytokine gene of chicken has to be optimized.

In order to address the general aim, specific objectives are outlined:

(i) to isolate pure and good quality RNA from chicken spleen and
(ii) to optimize the qPCR assay of four chicken cytokine genes and two housekeeping genes.

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