

Kriangkrai Witoonsatian 2007: Cloning and Expression of H5 Gene of Avian Influenza Virus and Its Biological Activity. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Theera Rukkwamsuk, Ph.D. 144 pages.

This research aimed to clone and express H5 gene of avian influenza virus (AIV) in insect cell cultures using a baculovirus expression vector system and to study the biological activity of the expressed H5 protein.

Viral samples collected by tracheal swab technique were collected from the H5N1 AIV infected chickens. These samples were preserved in viral transport media and then inoculated in 9 to 11 days old chicken embryonic eggs. The infected allantoic fluids were harvested and subsequently extracted for viral RNA using phenol-chloroform extraction method. The extracted viral RNA was used as template for H5 gene synthesis using two steps RT-PCR.

The synthesized H5 gene products, approximately 1,700 bp, were constructed into pFastBac HT plasmids. These recombinant plasmids were transformed into *E. coli* strain DH10 Bac to produce the recombinant H5 baculovirus bacmids. Thereafter these bacmids were transfected and expressed in insect cell cultures. The expressed H5 protein was primarily determined by using IPMA and dot blotting. Subsequently, the protein was determined for approximately 65 kDa protein fraction by using SDS-PAGE assay and western blotting underling the goat anti-H5N1 AIV polyclonal antibody and the mouse anti-histidine monoclonal antibody. According to biological activity, the expressed H5 protein could aggregate chicken red blood cells to form rosette structures. Additionally, IPMA was not only determined the H5 protein expression but also showed that this protein was transported and anchored at the cell membrane of infected insect cell cultures.

The results indicated that the H5 gene was successfully cloned and the H5 protein could be expressed in insect cell cultures using a baculovirus expression vector system. This protein showed biological activities characteristics as the authentic hemagglutinin protein. Therefore, this H5 protein could be further developed and applied as a candidate H5 AIV subunit vaccine.

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Thesis Advisor's signature

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