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**PRODUCTION OF INFLUENZA H1N1 VIRUS LIKE PARTICLE
(VLP) BY BACULOVIRUS EXPRESSION VECTOR SYSTEM**

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**A THESIS SUBMITTED IN PARTIAL PULFILLMENT OF
THE REQUIREMENTS FOR
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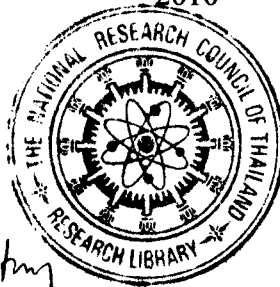
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by Baculovirus Expression Vector System

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Abstract

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Influenza infection is one of the most devastating diseases for human and animals, which is best prevented through vaccination. Currently, licensed influenza vaccines are produced in embryonic chicken egg and these have some drawbacks including contra-indication in people with egg allergies, long duration for vaccine production, limitation of specific egg supply, biosafety concern in handling infectious virus, etc. To address the need for safer influenza vaccines and more effective production process, we have engineered influenza virus like particle (VLP) as an alternative influenza vaccine. VLP consists of structural proteins of influenza virus with structure and morphology mimics the virus itself but does not contain any genetic materials therefore it is non-infectious and production processes are much simpler. Furthermore, it has been reported that VLP based vaccines have a broader immunogenic efficiency and better protection than the egg based vaccine. In this study, the influenza H1N1 VLP was generated by expressing three structural genes of influenza A virus i.e. hemagglutinin (HA), neuraminidase (NA) and matrix M1 genes using Baculovirus Expression Vector System (BEVS). Those three genes were inserted into the baculovirus genome as confirmed by PCR analysis and then the recombinant baculovirus was produced and taken to infect into Sf-9 insect cells. Upon infection of Sf-9 cells, the three genes were co-expressed as observed by RT-PCR analysis. The

presence of recombinant proteins produced by the infected insect cell in the culture supernatant that were expected to form VLP then analyzed. Western blot analysis using monoclonal antibody specific to HA protein revealed a band of approximately 70 kDa which is correspondent to hemagglutinin protein. The recombinant HA protein size is a little higher than the expected size from the amino acid sequences analysis. This possibly due to the glycosylation of the recombinant HA protein as post-translational modification performed by infected insect cells. Furthermore, hemagglutination activity was also observed. The infected cell culture supernatant was found to be able to agglutinate the goose red blood cells. In addition, neuraminidase (NA) activity based on the ability of neuraminidase enzyme in cleaving the sialic acid binding of MUNANA (4-methylumbelliferyl-N-acetyl- α -D-neuraminic acid) synthetic substrate was 0.12 ± 0.04 U/ml. These results demonstrate that the BEVS can be used to produce recombinant proteins of H1N1 influenza virus with two main important activities of hemagglutination and neuraminidase. Whether or not the VLP is formed must be further investigated by visualization using electron microscope.

Keywords: Influenza vaccine/ Virus Like Particle (VLP)/ H1N1/ Baculovirus Expression Vector System (BEVS)

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LIST OF ABBREVIATION

°C	= degree centigrade or Celcius
%	= percentage
Ab	= antibody
AcMNPV	= <i>Autographa californica</i> MNPV
Amp	= ampicillin
β-Gal	= β-galactosidase
bp	= base pair
BV	= budded virus
BEVS	= baculovirus expression vector system
CIAP	= calf intestinal alkaline phosphatase
C-terminal	= carboxy terminal
DMSO	= dimethyl sulfoxide
DNA	= deoxyribonucleic acid
EDTA	= ethylene diamine tetraacetic acid
EtBr	= ethidium bromide
FBS	= fetal bovine serum
g	= gram
GV	= granulovirus
h	= hours
kb	= kilobase of 1,000 bp
kDa	= kiloDalton
L	= liter
LB	= Luria-Bertani broth
M	= Molar (mole/ liter)
MCS	= multiple cloning site
mg	= milligram
min	= minutes
ml	= milliliter
mM	= millimolar
MNPV	= multiple nucleopolyhedrovirus

MOI	= multiplicity of infection
mRNA	= messenger ribonucleic acid
NA	= neuraminidase
NC	= nucleocapsid
NP	= nucleoprotein
ng	= nanogram
nm	= nanometer
NPV	= nucleopolyhedrovirus
nt	= nucleotide
OB	= occlusion body
ODV	= occlusion derived virus
ORF	= open reading frame
PAGE	= polyacrylamide gel electrophoresis
PBS	= phosphate buffer saline
PCR	= polymerase chain reaction
pfu	= plaque forming unit
RNA	= ribonucleic acid
rpm	= resolution per minute
SDS-PAGE	= sodium dodecyl sulfate polyacrilamide gel electrophoresis
Sf-9	= <i>Spodoptera frugiperda</i> -9
X-Gal	= 5-bromo-4-chloro-3-indolyl- β -D-galactopyranose
μ g	= microgram
μ l	= microliter