DEVELOPMENT OF A MONOCLONAL ANTIBODY ASSAY FOR INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROIS VIRUS (IHHNV) OF SHRIMP

BUI THI BICH HANG

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BUI THI BICH HANG  4837387 SCBT/M

M.Sc. (BIOTECHNOLOGY)

THESIS ADVISORS: TIMOTHY W. FLEGEL, Ph.D., PAISARN SITHIGORNGUL, Ph.D., SAENGCHAN SENAPIN, Ph.D.

ABSTRACT

The IHHN virus has been recognized as a disease of penaeid shrimp since 1981 in the imported blue shrimp *Penaeus stylirostris* in Hawaii. It then was found to have spread widely to Tahiti, Florida, Texas, Cayman Islands, Israel, Panama, Costa Rica, Belize, Ecuador, Philippines, Singapore, Guam, Brazil, Honduras, France, Jamaica, and recently in Thailand. Consequences of IHHNV infection in shrimp cultures were assessed to cause serious problems for shrimp production and its values. Several methods including histological examination, *in situ* hybridization and PCR are used to detect IHHNV infection, but these methods are time consuming and expensive. This study, therefore, developed monoclonal antibodies as one of the most rapid, simple, accurate, and low cost methods to test the virus infection. Gene encoding GP3 capsid protein of IHHNV was amplified and constructed into pET15b plasmid and transformed into *Escherichia coli* BL21. Recombinant GP3 capsid protein of 37 kDa was then expressed and extracted for immunization of mice (5 female BALB/C mice). The polyclonal antibody was collected after 4 injections of antigen into the mice and tested by western blot and immunohistochemistry. The mouse IV gave a strong signal with IHHNV inclusion and was chosen for producing the monoclonal antibody (MAb). The MAb was tested by dot blot ELISA, western blot as well as immunohistochemistry. By dot blot ELISA, the monoclonal antibody detected GP3 capsid protein with different sensitivities ranging from 0.1 – 0.5 x 10^-3 µg/µl. By western blot, MAb-1 could detect a 37 kDa protein in the lysate of *E. coli* containing GP3-pET15b at a dilution of 1: 100 in skimmed milk. The result of immunohistochemistry showed that MAb strongly bound to IHHNV inclusions in the gill, nerve and epithelial cells of *P. vannamei* infected with IHHNV. MAb were also tested against other shrimp viral pathogens (HPV, WSSV, YHV and MBV) using immunohistochemistry, and the results showed no cross-reactivity of the antibodies to tested viruses.

KEY WORDS: IHHNV/Dot blot ELISA/ Western blot/ Immunohistochemistry

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