## THESIS

**Tran thi Bich Trinh**. 2007. Stability of *Helicovera armigera* nucleopolyhedroviruses through serial passaging in insect cell culture. MSc. Thesis on Biotechnology. School of Molecular and Microbial Science, University of Queensland, Australia. 62 p.

## ABSTRACT

Baculoviruses are found as arthropod insect pathogen and they have been studied and used as insecticide in agriculture. The nucleopolyhedroviruses (NPVs)( are extensively studied because of their potential to be used as bioinsecticide to control a number of crop pests. Baculoviruses have been produced *in vivo*, but *in vivo* production of baculoviruses at large-scale encounters a number of disadvantages such as difficulty in quality control over the purity of products, being labour intensive and costly. In contrast, *in vivo* production of baculoviruses can overcome disadvantages relating to *in vivo* production. Therefore, the large scale production of baculoviruses using insect cell culture techniques is of increasing interest. However, there are challenges for *in vivo* production including accumulation of defective interfering particles (DIPs) and few polyhedre (FP) mutants resulting from the serial passage of virus in insect cells in culture. Therefore, a study of FP mutants and DIPs is necessary to help solve the problem associated with *in vivo* production of baculoviruses.
The *Helicoverpa zea* cell line was cultured in VPM3L medium and used for virus infection. The source of virus used for infection was extracted from OB which was harvested from later stage of HaSNPV infected caterpillars, H25EA1-B31. HaSNPV was serially passaged from passage 1 to passage 10 using *H. zea* cell and VPM3L medium in 250ml shaker flask. Passage 1 was harvested at 4 dpi. Cell densities and OB yield were estimated by triplicate counts using a haemocytometer and light microscopy. Polyhedra was counted after lysing infected cells with 0.5% sodium dodecyl sulfate at 28$^{o}$C for 30 min. Virus genomic DNA from either BV samples of virus passages 1 to 10 or caterpilla OB's was purified using Genomic-tip columns (QIAGEN). The genomic DNA of wild type HaSNPV from both larval OB and BV samples harvested were separated using PFGE. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used to analyze the expression of total protein from HaSNPV-infected zea cells. The expression of FP25K protein in HaSNPV-infected zea cells during serial passaging was analyzed by Western blot with a 1:5000 dilution of 25K rabbit antibody.

The result in section 3 showed that as FP mutants accumulate, FP25K protein levels drop during serial passage, and the OB yield (OB per cell) decreased with increasing passage number. In addition to FP mutants, DIPs are thought to be the cause of the decline in OB yield. Based on PFGE analysis, DIPs are present at least by P6, and they may appear earlier. However, it is difficult to clarify exactly when, (which passage number), DIPs first appear and predominant because viral genomic DNA was sheared during processing such as during the DNA purification and concentration steps used in this work.