CONCLUSION

Restriction pattern and the polyhedrin gene (*polh*) of the Thai *Bombyx mori* nucleopolyhedrovirus (BmNPV) were studied. PCR-based method was developed for early detection of BmNPV in all stages of silkworm development.

Restriction pattern of Thai BmNPV was analyzed by digestion with *Bam*HI, *Bgl*II, *Hin*dIII, *Nco*I and *Pst*I and compared with BmNPV isolates from Japan (T3 and D1 isolates) and from India (N isolate). This study showed that the restriction pattern of Thai BmNPV was different from other isolates of BmNPV isolates and size of Thai BmNPV genome was smaller than other isolates.

The full length of the polyhedrin gene (*polh*) of Thai *Bombyx mori* nucleopolyhedrovirus (BmNPV) was cloned and sequenced. The nucleotides sequence of Thai BmNPV *polh* and its flanking region was submitted to GenBank (accession number AY779044). The *polh* sequence contained a 735 bp open reading frame (ORF) encoding a protein of 245 amino acids with a predicted molecular mass of 28.8 kDa. The nucleotide sequence of Thai BmNPV *polh* showed greater than 98% identity to the five different sequences of BmNPV *polh* previously characterized which were submitted to GenBank (Japanese 1-T3 (L33180); Japanese 2 (M30925); Korean-K1 (U75359); Chinease (X63614) and Canadian (M100430). The high degree of sequence identity with the *polh* sequences of other BmNPVs suggested that ORF sequence reported in this study is the Thai BmNPV *polh* gene. Comparison of Thai *Bm*NPV *polh* sequence with other *polhs* of Lepidoteran NPVs (*Autographa californica*, *Helicoverpa armigera*, *Spodoptera litura* and *S. exigua*) indicated that the nucleotide and amino acid sequence identities were greater than 65% and 78%, respectively.

To develop PCR-based method for BmNPV detection in silkworm, specific primers were designed from nucleotide sequence of Thai BmNPV *polh.* *Bm*NPV DNA extraction was modified from the alkaline lysis method. PCR-based method was first evaluated for its potential for grasserie disease detection using samples of silkworm which were artifiacially inoculated with

BmNPV. It was found that by PCR method, BmNPV could be detected in all stages of silkworm. The method was sensitive enough to detect BmNPV in only one infected individual egg. In larval stage, BmNPV was detected in all instars and only one individual infected larva provided adequate BmNPV DNA to be used as PCR template. In pupal and adult stages, BmNPV was detected in both infected male and female.

Application of PCR-based method for grasserie disease detection in silkworm was demonstrated. Naturally infected samples were collected from silkworm rearing houses of the governmental sericultural stations, private company, contracted farms and local farmer. BmNPV could be detected in all stages of silkworm. The whole process of BmNPV genomic extraction and detection could be done within 5 hours. PCR technique has proved to be an effective and reliable method for early detection of grasserie disease of silkworm and can be routinely used to monitor and protect the spread of the disease in sericulture industry in Thailand.