THESIS

POTENTIAL APPLICATION OF PCR-BASED METHOD FOR EARLY DETECTION OF GRASSERIE DISEASE OF SILKWORM, *Bombyx mori*

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Grasserie disease caused by *Bombyx mori* nucleopolyhedrovirus (BmNPV) is the most destructive disease of silkworm, *Bombyx mori* in Thailand and other sericultural practising countries. Restriction profile of Thai BmNPV genomic DNA was studied by cleaving the DNA with *Bam*HI, *Bgl*II, *Hin*dIII, *Nco*I and *Pst*I. The Thai BmNPV genome was estimated to be in the range of 92.3-125.8 kb with the average of 109.8 kb. The full length of the polyhedrin gene (*polh*) of Thai BmNPV was cloned and sequenced. 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. 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 BmNPV *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.

PCR-based method was developed for BmNPV detection in silkworm. Specific primers were designed from nucleotide sequence of Thai BmNPV *polh.* BmNPV DNA extraction was modified from the alkaline lysis method. Grasserie disease was detected in artificially inoculated and naturally infected silkworm samples. The developed PCR method could be used to detect BmNPV in every stage of silkworm's development. The method was sensitive enough to detect BmNPV using only one infected individual egg and larva. It could also be used to detect BmNPV in both sexes of silkworm pupa and moth. The whole process from DNA extraction to BmNPV detection could be done within 5 hours. It was considered suitable to be practiced as a routine measure for grasserie disease control by the government and private sectors in Thai sericulture. This study indicated that PCR method based on the *polh* sequence of BmNPV was efficient, specific and highly sensitive in detecting graserie disease in any stage of silkworm development.

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Mallika Kaewwises

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