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

INTRODUCTION

The silkworm, *Bombyx mori* has been domesticated for silk production for over 4,000 years. Silk is "the queen of fibers" because it is a smooth, shining, fabulous and unique natural fiber produced by silkworm. Nowadays, silkworm is an insect of economic importance to Asian countries like China, India, Thailand, Vietnam and many other developing countries. In Thailand, sericulture is an important environmentally sustainable agro-industry activity practiced for centuries. Thailand is the most international reputation as a producer of high quality silk and silk products. Presently, the Thai silk has become world-famous for its quality and unique character. The popularity of Thai silk among customers in Thailand and in other countries all over the world stems from its distinctive beauty, consummate craftsmanship, soft and smooth texture, iridescent sheen, attractive colours and artistic designs which set it apart from silk elsewhere (Ministry of Agriculture and Cooperative, 2002). It provides substantial contributions to the national economy and serves as source of income for many farmers especially in the northeastern part of Thailand. In the past, sericulture was practiced only at family level as cottage industry. As a result in reign of King RamaV, he conserved the keep up and contributed the supportive to sericulture (Sikkhamondhol and Tengratanaprasert, 1993) and now it has become the nation-wide industry. The major obstacle in sericultural industry in Thailand is diseases of silkworm. Silkworm diseases that have been found in Thailand are aspergillus disease and muscadine disease caused by fungi, sotto disease caused by bacteria, pebrine disease caused by protozoa, flacerie disease caused by cytoplasmic polyhedrosis virus and grasserie disease caused by nucleopolyhedrovirus (United Nation, 1998). However, grasserie disease caused by *Bombyx mori* nucleopolyhedrovirus (BmNPV), is the most destructive disease of silkworm.

BmNPV belongs to genus Nucleopolyhedrovirus, family Baculoviridae (Van Regenmortel and Fauquet, 2000). The virus particle is composed of deoxyribonucleic acid (DNA) surrounded by capsid protein to form the nucleocapsid. The nucleocapsids are then

enveloped and are called virions (Tanada and Kaya, 1993). The two morphological subgroups within the NPVs are the single nucleocapsid NPV in which only one nucleocapsid is present per envelope, and the multinucleocapsid NPV in which several nucleocapsids are packed per envelope (Hunter-Fujita *et al*., 1998). The virions are occluded within polyhedral-shapes occlusion bodies called polyhedra that surround and protect the infectious virion (Blissard and Rohrmann, 1990). Polyhedra are mainly composed of a single polypeptide known as polyhedrin (Polh). Polh, which constitutes the crystalline matrix of baculovirus occlusion bodies, plays a significant role in the replication cycle of baculovirus. The Polh is encoded by an orthologous gene highly conserved among baculoviruses, and it is the gene from which most data from different isolates are available, so it is the most comprehensive option available for estimating the relationship among baculoviruses (Zanotto *et al.*, 1993).

Grasserie disease is difficult to cure or overcome as the life cycle of silkworm is short. The best way to control grasserie disease is to prevent disease infection. The infected silkworm expresses disease symptom during the final stage of larval development and die without cocoon production resulting in the waste of expense, time and labour work. The damage of this disease in Thailand is about 30-100% (Kaewwises and Niyomvit, 1995). In Thailand, silkworm can be reared throughout the year and grasserie disease is most severe in rainy season and least severe in the winter (Kaewwises and Sananvong, 1999). Recommendation for disease prevention from the Department of Agriculture is sanitation the rearing houses and equipments with formalin before rearing the next generation of silkworm. Farmers can use lime or chlorine in substitution to formalin (Sithisongkram and Ruksong, 1987). However, poor hygienic conditions and ineffective disease control as practiced by most of the farmers have often been followed by unstable sanitation results. Nowadays, sericulturists try to breed multivoltine and bivoltine silkworm varieties resistant to BmNPV. However, the presumable most effective solution for the control of grasserie disease is to detect viral infection as early as possible in order to stop spread of the disease in rearing house. Lack of rapid and accurate disease detection technique causes severe spread of grasserie disease annually. Many techniques have been developed to provide an early and accurate diagnosis method for this viral disease such as the enzyme-link immunosorbent assay (ELISA) (Vanapruk *et al.*, 1992; Shamim *et al.*, 1994), DNA hybridization (Attathom *et al.*,

1994), colloidal textile dye-based dipstick immunoassay (Nataraju *et al.*, 1994), monoclonal antibody (Nagamine and Kobayashi, 1991) and western blot analysis (Chaeychomsri *et al.*, 1995). Most of those detection methods were complicate and difficult to interpret the results.

The polymerase chain reaction (PCR) is a highly sensitive technique which amplifies target DNA sequences and PCR amplification of conserved fragment enabled the detection of low level of viral DNA. It has been employed for the detection of viral DNA such as human virus (Umlauft *et al.*, 1996), aminal virus (Peng *et al.*, 1998) and plant virus (Levesque, 2001). For NPV, the preferable gene employed for PCR detection was the polyhedrin gene (*polh*). This gene had been used to detect the NPVs of *Spodoptera literalis* (Chou *et al.*, 1996), *Autographa californica*, *Anticarsia gemmatalis*, *Bombyx mori*, *Orygia pseudotsugata*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Anagrapha falcifera*, *Heliothis zea* (Moraes and Maruniak, 1997), *Perina nuda* (Wang *et al.*, 2000) and *Helicoverpa armigera* (Christian *et al.*, 2001). Moreover, PCR technique was employed to detect baculovirus DNA sequences from viral occlusion bodies contaminating the surface of gypsy moth (*Lymantria dispar*) eggs (Burand *et al.*, 1992). Many researchers brought advantage of PCR for baculovirus DNA detection in environment, for example detection of NPV DNA of *Anticarsia gemmatalis* and *Helicoverpa armigera* in soil (Moraes *et al.*, 1999; Christian *et al.*, 2001) and detection of baculovirus DNA in lake water (England *et al.*, 2001).

Detection of this viral disease at the early stage of infection will help to improve sanitary management, prevent the spread of the disease, and finally to eliminate this viral disease within silkworm population. Hence, farmers can obtain more silk productivity with high quality. In addition, the invention of the practical detection protocol will significantly eases the disease preventive strategy in sericultural industry. This study aims to explore the potential application of PCR-based methods for early detection of grasserie disease in silkworm and to develop PCR method for practical use in sericultural industry.

Objectives

1. To study BmNPV polyhedrin gene (*polh*) of the Thai isolate and determine its relatedness to other *polh*s of nucleopolyhedrovirus of some economic important insects.

2. To investigate the use of PCR-based method for early detection of grasserie disease of silkworm.

3. To evaluate the sensitivity and specificity of the developed PCR-based method.

4. To develop a simple protocol for practical application of PCR-based method for early detection of grasserie disease of silkworm.