Sukanda Papirom. 2004. Antiserum Production for Detection of *Nosema Bombycis* N.

Causal Agent of Pebrine Disease of Silkworm *Bombyx mori* L. Master of Science

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## Abstract

The surveying and collecting of silkworm (*Bombyx mori*) samples were conducted in the northeastern region from 78 villages, 42 districts of 10 provinces. It was found that *Nosema bombycis* the causal agent of pebrine disease distributed in all examined provinces with pebrine infected samples in the range of 33.33-100.00%. The representative isolate of *Nosema bombycis* was multiplied in representative Thai silkworm variety “Na Dang” in this study. The results revealed that this isolate was able to produce in mass and transmit through eggs continuously not less than 36 generations. Typical symptom of pebrine disease on this variety on larval stage especially at late instar larva was yellowing on hind abdomen than other parts of the body, rather shiny skin, clear and easily visible blood circulation system on the dorsal site. In addition, there was more different symptoms found on larva stage, e.g. small in size, sluggish with white or brown body, irregularity in growth. The preliminary morphological observation of spores by phase contrast microscopy revealed that the spores were oval shape and reflective. The average of spore size was 1.95-2.00x3.82-4.21 μm, which is not significantly different among 36 generations (P=0.05). Purification of the causal agent was undertaken using linear sucrose density gradient centrifugation (SDGC), the spore band was mostly located at 40-50% (w/w). Polyclonal antiserum production of *N. bombycis* was made from two kinds of antigen untreated-whole spore in water (Un-Ag) and alkali-treated spore (KOH-Ag). By employing the indirect-ELISA, antiserum produced from untreated-whole spore in water (Un-As) had titer at 1:1,000 while the antiserum produced by alkali-treated spore (KOH-As) had titer at 1:500. The sensitivity of both antisera, Un-As and KOH-As, using antiserum at 1:250 dilution, secondary antiserum (goat anti-rabbit IgG conjugate) at 1:20,000 dilution showed positive detection at minimum
concentration of 9 and 8 spores/ml., respectively. In addition, the result indicates that these antisera are highly specific to all *N. bombycis* tested isolates with no cross-reactivity with any other tested causal agents of silkworm diseases e.g. *Aspergillus flavus*, *A. tamarii*, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Nucleopolyhedrovirus*, *Serratia marcescens*, *Staphylococcus aureus* and *S. sciuri*

This study is the development of serological method for detection of pebrine disease of silkworm pathogen, which has been firstly succeeded in Thailand.