Construction of spider venom peptide expressing baculovirus and its potential application as bioinsecticide

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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Abstract：

Insect pests cause a great loss of agricultural crop production which ultimately affects global food security. Therefore to improve global food security, management of crop yield loss is an essential part in a limited source of expanding agricultural land on earth. Since 1940s insect pest management solely depends on chemical pesticides. Needless to say that these agrochemicals increase staple food crop yield and leading to improve food security from local to global. However, use of these chemical pesticides causes a tremendous negative impacts on environment, biodiversity and human as well as animal health risks. Most of the developed chemical pesticides have negative effects on non-target organisms which also affect food web ecosystem. In order to avoid those negative effects and keep food security biological control methods such as using natural enemies of a specific insect pest (predators, parasites, parasitoids), microbial agents which infect host specific insect pest including baculovirus, fungus, bacteria provide an alternative option for safe plant protection. Furthermore, recent scientific advancement discovers another tool such as insecticidal toxins that present in many venomous organisms might be possible to use pest management program. “Baculovirus”, among the possible alternative other than chemical pesticides, could be a reasonable option for replacing several widely used agrochemical pest management system. Baculoviruses have a long history of safe use as specific, environmentally friendly insecticides. However, their wide use has been limited by several factors, especially their slow pathogenicity. When baculovirus contaminated foliage is ingested by insect, baculovirus polyhedrins are dissolved within midgut and released occlusion derived-virions which start to replicate. Replication in tissues causes extensive damage and thereafter death. Thus from initial infection to death of infected insect requires 4–5 days in laboratory, but more than a week in field, allowing the insects (larva) to feed for longer and thereby damaging the host crop. To make shorten the time from infection to death, insect-specific toxin could resolve this problem. In this dissertation two kinds of recombinant baculoviruses which expressed an insect-specific spider venom toxin gene were constructed. This toxin gene is identified from venom of the ant spider *Lachesana tarabaevi,* which has two distinct properties; insecticide and antimicrobial effects. In addition, the spider venom peptide was expressed using baculovirus expression system, purified and evaluated its

functional activity against several bacterial strains.

Spiders deploy their venoms to feed prey and protect themselves from other natural enemies and their venom contains wide range of toxins including reurotoxins and cytotoxins. Many of these toxins are insecticidal, particularly some spider venom peptide toxins are already registered as biopesticide. One of these toxins, a spider venom peptide called cyto-insectotoxin1a (*cit1a*) derived from *Lachesana tarabaevi* was used in this study. The full length sequence of cit1a was amplified by PCR and subsequently used for construction of recombinant bnaculovirus. In part 1, *cit1a* was fused with *egfp* and expressed in silkworm larvae using baculovirus expression system. The EGFP-Cit1a fusion protein was expressed in silkworm larval fat body as well as silkworm pupa. The fusion protein was purified both from larva and pupa and analyzed the antimicrobial activity against several bacterial strains using disk diffusion method. The purified EGFP-Cit1a fusion protein remained active and showed growth inhibitory effect against *Bacillus subtilis* (NBRC13719) as Gram-positive and *Pseudomonas aeruginosa* (NBRC12689, NITE) and *Escherichia coli* W3110 (NBRC12713) as Gram-negative. The minimum inhibitory concentration (MIC) value of EGFP-Cit1a protein was also calculated using microdilution technique. The purified EGFP-Cit1a fusion protein showed growth inhibitory effect at micro molar concentration level. This study highlight that spider venom peptide (*cit1a*) might be used to increase the pathogenicity of baculovirus. In addition this study developed a new strategy for the expression and production of *cit1a* using silkworm fused with the *egfp* which ensures low cost, ease of treatment and high biohazard safety. The *cit1a* could be a promising candidate as a insecticidal as well as therapeutic.

In part 2, the *cit1a* was fused with polyhedrin (polh) and cloned into *Bombyx mori* nucleopolyhedrovirus (BmNPV) and *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) using recombinant DNA technology. Thus the recombinant baculoviruses, BmNPV/Polh-Cit1a and AcMNPV/Polh-Cit1a were constructed. The Polh-Cit1a fusion protein was expressed in silkworm larval fat body, Bm5 and Sf9 cell lines which were confirmed by western blot analysis and detected by CBB staining. The BmNPV/Polh-Cit1a showed significant reduction of median lethal time (LT_{50}) when compared to wild-type baculovirus bearing no toxin, which suggest that Polh-Cit1a improved the pathogenicity of BmNPV. The BmNPV/Polh-Cit1a induces early cuticular melanization of silkworm larva compared to wild-type BmNPV. Furthermore, recombinant virus induced early Bm5 cell death. Similar results were found in AcMNPV/Polh-Cit1a infected cell line. The analysis of recombinant virus infected cells showed considerable different from wild-type virus-infected cells. The cells infected with BmNPV/Polh-Cit1a seem to lose its cytoplasm and outline of cells was blurred. This study suggests a new strategy for the biopesticide using recombinant baculovirus that produced insect specific toxin fused polyhedra, which proposes to directly field application.