SUPPRESSION OF CHITIN SYNTHASE GENE EXPRESSION IN *ECTROPIS OBLIQUA* LARVA BY DSRNAI METHOD

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Summary

Biological control of pests is an important measure to control pesticide residues in tea products. The pests can be controlled by disfunction of chitin synthase. A conserved cDNA fragment (192bp) of chitin synthase gene from *Ectropis oblique* larva was isolated by PCR. Sense and antisense strand of the gene were incorporated into pFGC5941 vector and a double strand RNA (dsRNA) recombinant named cs-intron-csr was formed, and the latter was then incorporated into a shuttle vector (pFastBac 1). The shuttle vector carried the recombined fragment into bacmid DNA, which could transfect insect cells in which a recombinant baculovirus was formed. The dsRNA could be reproduced by recombinant baculovirus, and it interfered the expression of chitin synthase gene in the infected sf9 cells and *Ectropis oblique* larva, resulting growth suppression of the infected pest.

Key words: chitin; chitin synthase; baculoviruse; dsRNAi.

Introduction

Chitin, a polymer of *N*-acetyl- β -D-glucosamine, is one of the most important compositions in insect. Chitin synthase (CS) which catalyzes the chitin formation plays an important role in insect growth and development.

Baculoviruses (Nuclear polyhedrosis virus; NPV) consists of a large circular double stranded DNA and enveloped rod shaped virions are arthropod specific viruses. As NPVs have a limited host and exclusively infect a few species closely related to it, they can be used as an insect biological control agents without disrupting the balance of beneficial insects and environment.

RNAi (RNA interference) is exclusively specific for target genes and leads posttranscriptional gene silencing.

As chitin synthesis occurs in insects but not in vertebrates, chitin synthase was confirmed to be a target for biological control of insect pests. In this paper we constructed a recombinant baculoviruse producing the chitin synthase dsRNA that interfered the expression of the chitin synthase gene *Ectropis obliqua* larva.

Materials and Methods

The *Ectropis obliqua* larvae were fed with tea leaves under conditions of 26°C, 16 h illumination and 8 h dark. The recombinant baculovirus was constructed according to a patented method [1]. Chitin sythase gene expression profiles were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) method.

Results and Discussions

The recombinant baculovirus had great effect on the growth and development of *Ectropis oblique.* The mortality of *Ectropis oblique* infected by the recombinant baculovirus was 83.3%, being the highest among the three treatments, followed by the group infected by wild type baculovirus. The control group which was not infected by baculovirus grew well. The results suggest that the baculovirus suppresses the growth and development of pest *Ectropis oblique* and the recombinant baculovirus is more effective for control the *Ectropis oblique.*

The profiles of expression of chitin synthase (CS) gene showed a contrary trend as the effects of baculovirus on growth of *Ectropis oblique.* CS expression was strongly suppressed in *Ectropis oblique* infected by the recombinant baculovirus, followed by the control group infected by wild type baculovirus. CS had strong expression in the control group of *Ectropis oblique.* The study suggests that the chitin synthase dsRNA produced by the transcription of baculoviruse interfered the expression of the chitin synthase gene, resulting in the growth suppression of *Ectropis obliqua* larva.

The baculoviruses containing insect-specific toxin genes were used to control pests because they suppressed pests eating and growth. The recombining baculovirus producing dsRNAi has an enhanced effectiveness and will be a novel method for biological control of *Ectropis oblique* in tea field.

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Reference

[1] Lu JL, Liang YR, Zhang GH, Lin C, Du YY, Pan SS. The technology of construction of high toxic value recombinant nuclear polyhedrosis virus. *State intellectual propertyl office of P.R.C,* Patent NO.CN1804030.