

# Construction of an Improved Recombinant Baculovirus Producing Polyhedra that Contain *Bacillus thuringiensis* Cry1Ac Crystal Protein

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For improvement of insecticidal activity and reduction of side effect on environment, a novel recombinant baculovirus, Bactrus was constructed by the insertion of *Bacillus thuringiensis cry1Ac* gene between two polyhedrin genes of *Autographa californica* nucleopolyhedrovirus (AcNPV) under the control of polyhedrin gene promoter. The Bactrus stably produced recombinant polyhedra, which were nearly similar to those of AcNPV. The insecticidal activity of recombinant polyhedra against *Plutella xylostella* larvae showed a significantly high pathogenicity in comparison with that of AcNPV and it decreased while the passage number of Bactrus increased.

For selection of an optimal insect cell line to produce recombinant polyhedra *in vitro*, the productivity of recombinant polyhedra and fusion protein was investigated with 5 kinds of insect cell lines using two culture system such as monolayer and suspension culture system. In monolayer culture, the largest amount of fusion protein was expressed in Se301 cells, but in suspension culture, the largest expression of fusion protein was observed in High-Five cells. When High-Five cells at  $3.0 \times 10^5$  cells per ml infected with 5 MOI in suspension culture, the largest amount of fusion protein and polyhedra were produced.

In conclusion, recombinant baculovirus, Bactrus, had improved insecticidal activity and returned to wild-type AcNPV in several passages. For the large scale production of Bactrus *in vitro*, High-Five cells were suitable in this experiment.