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NeuroBactrus, a Novel, Highly Effective, and Environmentally Friendly Recombinant Baculovirus Insecticide

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Abstract : A novel recombinant baculovirus, NeuroBactrus, was constructed to develop an improved baculovirus insecticide with additional beneficial properties, such as a higher insecticidal activity and improved recovery, compared to wild-type baculovirus. For the construction of NeuroBactrus, the Bacillus thuringiensis crystal protein gene (here termed cry1-5) was introduced into the Autographa californica nucleopolyhedrovirus (AcMNPV) genome by fusion of the polyhedrin–cry1-5–polyhedrin genes under the control of the polyhedrin promoter. In the opposite direction, an insect-specific neurotoxin gene, AaIT, from Androctonus australis was introduced under the control of an early promoter from Cotesia plutellae bracovirus by fusion of a partial fragment of orf603. The polyhedrin–Cry1-5–polyhedrin fusion protein expressed by the NeuroBactrus was not only occluded into the polyhedra, but it was also activated by treatment with trypsin, resulting in an_65-kDa active toxin. In addition, quantitative PCR revealed that the neurotoxin was expressed from the early phase of infection. NeuroBactrus showed a high level of insecticidal activity against Plutella xylostella larvae and a significant reduction in the median lethal time against Spodoptera exigua larvae compared to those of wild-type AcMNPV. Rerecombinant mutants derived from NeuroBactrus in which AaIT and/or cry1-5 were deleted were generated by serial passages in vitro. Expression of the foreign proteins (B. thuringiensis toxin and AaIT) was continuously reduced during the serial passage of the NeuroBactrus. Moreover, polyhedra collected from S. exigua larvae infected with the serially passaged NeuroBactrus showed insecticidal activity similar to that of wild-type AcMNPV. These results suggested that NeuroBactrus could be recovered to wild-type AcMNPV through serial passaging.

Keywords : baculovirus, insecticide, neurotoxin, neurobactrus
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