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Isolation and Molecular Characterization of *Bacillus thuringiensis* K1 Harboring Novel cry1-type Genes

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To identify novel *cry1*-type crystal protein genes, 56 *Bacillus thuringiensis* (Bt) isolates having a high toxicity against lepidopteran insects were selected from 300 of them. These isolates were classified into seven distinct groups by PCR-RFLP analysis and seven standard isolates were selected from the groups. Through cloning and sequence analysis of the PCR-amplified fragments, 7 novel *cry1*-type genes were identified. A strain that containing 3 novel *cry1* genes was selected from the 7 isolates and named Bt K1 for further analysis.

Bt K1 belonged to *B. thuringiensis* subsp. *kurstaki* (3a3b3c) producingthe typical bipyramidal-shaped crystals with 130 kDa and 65 kDa polypeptides similar to those of Bt *kurstaki* HD-1. But Bt K1 had some what different characteristics in plasmid DNA pattern. In the toxicity assay, Bt K1 was also more toxic against the diamondback moth (*Plutella xylostella*) and the beet armyworm (*Spodoptera exigua*) than Bt *kurstaki* HD-1 or Bt *aizawai* HD133.

To clone novel *cry1*-type genes from Bt K1, about 2.4 kb-long PCR fragments from Bt K1 were amplified with the degenerated primer set designed from the most conserved active regions of all known *cry1*-type genes. By this PCR-RFLP strategy, five *cry1*-type genes (*cry1-1, cry1-2, cry1-7, cry1-31* and *cry1-44*) were obtained and sequenced. Furthermore, structural and flank regions of four *cry1*-type genes (*cry1-2, cry1-7, cry1-31* and *cry1-44*) were obtained by inverse PCR and their complete open reading frames were detected. In the alignment of the deduced amino acid sequence with the known *cry* genes, the *cry1-2* and *cry1-31* genes were identified to be the same as the *cry1Ea* and *cry1Aa* genes, respectively. The *cry1-7* gene showed 99.4% homology to the *cry1Be1*

(GenBank Accession No. AF077326) gene and the *cry1-44* gene 91.2% homology to the *cry1Ac2* (GenBank Accession No. M35524) gene in nucleotide sequence. For investigation of the *cry1-1* full length gene, Southern hybridization was performed, and a 8.0 kb fragment containing the full gene was cloned into the pBluescript II SK. The *cry1-1* full length gene showed 77.6% homology to the *cry1Ha1* (GenBank Accession No. Z22513) gene in nucleotide sequence.

In order to evaluate the insecticidal activity of the Cry1-1, the *cry1-1* gene was expressed in *B. thuringiensis* Cry-B strain. The Cry1-1 exhibited no toxicity against *P. xylostella*, the silk worm (*Bombyx mori*), *S. exigua*, the cluster caterpillar (*Spodoptera litura*) and the northern house mosquito (*Culex pipiens*). In a solubilization assay, the Cry1-1 was only partially soluble at pH value of = 12. Furthermore, Cry1-1 was not activated by *B. mori* gut juice.

The *cry1*-type genes were also expressed in baculovirus-infected insect cells. All five recombinant viruses (Ap1, AP2, Ap7, Ap31 and Ap44) showed high activities against *P. xylostella* larvae, and Ap31 and Ap44 had higher activities than ApAc which containing Cry1Ac protein. In addition, two recombinant viruses, Ap1 and Ap2 exhibited similar toxicities against both P. *xylostella* and *S. exigua* larvae.

These results indicate that Bt K1 could be used as a novel effective microbial insecticidal agent. Also, the five recombinant viruses containing these Cry1-type proteins and showing high insecticidal activity could be directly used as novel effective viral insecticides. Moreover, expression of *B. thuringiensis* cry genes in baculorvirus-mediated insect cells should be a useful method compared with the other expression method.