B3

Identification and Molecular Characterization of Insecticidal *cry1*-type Genes from *Bacillus thuringiensis* 2385-1

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Objectives

A *Bacillus thuringiensis* isolate, Bt 2385-1, which showed toxicity to lepidopteran, was isolated from Korean soil sample and characterized. PCR-RFLP showed that this isolate contains two novel *cry1*-type crystal protein genes. In this study, we designed *cry1*-type specific primer set (ATG1-F and N400-R) to clone the toxic domain of the all *cry1*-type genes. The two novel *cry1*-type toxin genes in addition to *cry1Ja1* gene were cloned and sequenced.

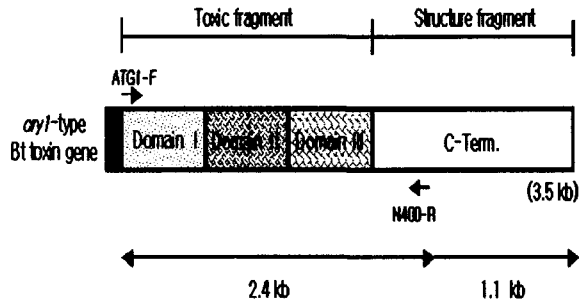
Materials and Methods

Materials - Bt 2385-1, ATG1-F and N400-R primers, pGemT-easy vector

Method - PCR amplification, transformation, sequence analysis

Results and Discussion

About 2.4 kb PCR fragments from the Bt 2385-1 were amplified with *cry1*-type specific primer set, and cloned into pGemT-easy vector. The cloned three *cry1*-type genes were named *cry1-5, cry1-12* and *cry1-15*, respectively. The *cry1-5* was showed 97.9% of maximum nucleotide similarity with *cry1Ab*, and *cry1-12* and *cry1-15* were showed 89.0% and 100% with *cry1Ja1*, respectively. For further characterization of these novel genes, their expression using the baculovirus expression systems and bioassay will be performed.



ATG1-F (33 mer) ATGCAATGCGTACCTTACAATTGTTTAAGTAAT

N400-R (26 mer) CATCGATTCGGTTCACCGCACCTTCC

Fig. 1. General structure of *B. thuringiensis cry*1-type gene and nucleotide sequences of the *cry1*-type specific primer set, ATG1-F and N400-R.

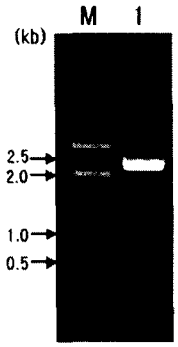


Fig. 2. PCR products amplified with the *cryI*-type specific primer set from Bt 2385-1.

References

Hafted, H. and H. R. Whiteley. 1989. Insecticidal crystal protein of *Bacillus thringiensis*. Microbiol. Rev. 53: 242-255.

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