

#### 4-6. Molecular Cloning of a New Non-toxic *cry1*-Type Crystal Protein Gene from *Bacillus thuringiensis* subsp. *kurstaki* Strain

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A new *cry1*-type gene (named *cryX*) was cloned from *Bacillus thuringiensis* K1 strain. The full *cryX* gene was composed of 3,513 bp and encoded 1,171 amino acids. Through the comparisons of nucleotide and deduced amino acid sequences between the *cryX* and the known *cry* genes, the *cryX* showed 77.6% and 73% homology to those of the *cry1Ha1*. The *cryX* under the control of the native promoter was cloned in *B. thuringiensis*-*E. coli* shuttle vector, pHT3101, and transformed into the *B. thuringiensis cryB*. The expressed CryX protein showed 132.2 kDa and formed relatively small bipyramidal inclusion body with 300 nm~700 nm in size. In the toxicity assay, CryX exhibited non-toxicity against *Bombyx mori*, *Plutella xylostella*, *Spodoptera exigua* and *Culex pipiens*. In the solubilization assays using CAPs buffers, CryX was soluble only at pH 12 and 13 whereas it was not soluble at pH values of < 12 and > 13. Furthermore, CryX was not solubilized by *B. mori* gut juice. Accordingly, the current results suggest that the principal reason why CryX is non-insecticidal is its lack of solubility at pH 9.5 to 10.5 in the lepidopteran or dipteran midguts.