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

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A new *cryI*-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 *cryIHa1*. 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 cry*$^{-}$*B*. 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.