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A NOVEL DNA SEQUENCE OF *BACILLUS THURINGIENSIS* δ-ENDOTOXIN RECEPTOR IN *HELICOVERPA ARMIGERA*

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Abstract

The pesticidal bacterium *Bacillus thuringiensis* has been the subject of intensive research. *Bacillus thuringiensis* (Bt) crystal proteins are effective in controlling agriculturally and biomedically harmful insects. However, the mechanism of Bt protein pesticidal action is not well understood. It is assumed that the pesticidal protein has affinity for specific receptors in the midgut of the susceptible larvae and binds irreversibly to create holes in the gut leading to eventual death of the target larvae. The study is endeavored to characterization of *Bt* delta endotoxin receptor in agronomically important pest, American bollworm (*Helicoverpa armigera*). Presence of a novel Protein is reported in the extract of the larval midgut membrane of *Helicoverpa armigera* as putative receptor for *Bt* Cry1A delta-endotoxins. The gene sequence has novelty because it has no significant homology to already existing sequences of Bt receptor.

Introduction

Bacillus thuringiensis (Bt) is a valuable source of insecticidal proteins for use in conventional sprayable formulations and in transgenic crops. It is the most promising alternative to synthetic insecticides. However, evolution of resistance in insect populations is a serious threat to this technology. Several important crops have been engineered to express toxins of *Bacillus thuringiensis* (Bt) for insect control. In principle, the mechanism of insect resistance to Bt could be located at each of the various steps (solubilization, proteolytic processing, passage through the peritrophic membrane, receptor binding, membrane insertion, pore formation, and osmotic lysis of midgut cells) in the mode of action of Bt Cry proteins. Conclusively, three different biochemical mechanisms of resistance to Bt have been observed so far, proteolytic processing of protoxin, improved repair of damaged midgut cells and modification of a Cry protein-binding site.

Binding site modification is thought to be the major mechanism of resistance to Cry1A toxins in *Pectinophora interpunctella* (Van Rie *et al*., 1990), *H. virescens* (Lee, *et al*., 1995), and the diamondback moth (Sayyed *et al*., 2000). This has also been proposed to be responsible for Cry1F resistance in the diamondback moth (Ballester *et al*., 1999). Central to learning to curb resistance to Bt is understanding the mechanism by which an insect resists the toxins.

Aminopeptidase N has been reported to be a *Bacillus thuringiensis* (Bt) Cry1A toxin-binding protein in several lepidopteran insects. It is believed that insect resistance build up emanates from mutational changes in the receptor protein (Aronson, and Shai. 2001).

The cotton bollworm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is one of the most serious insect pests in many cotton-producing countries, including Australia, India, China and Pakistan. The larva causes substantial economical losses to

legume, fibre, cereal oilseed and vegetable crops. This pest has proven to be difficult to control by conventional means, mainly due to the development of pesticide resistance (Estebanez *et al*., 2001; Krathi *et al*., 2001). The main crops affected are cotton, chickpea, maize, wheat, sorghum, sunflowers, tomatoes, potato and variety of vegetables and fruits.

Toxicity of insecticidal endotoxins produced by *Bacillus thuringiensis* correlates with the presence of specific proteins in the midgut of susceptible larvae. So characterization of the novel Bt receptor present in the midgut of the American bollworm *Helicoverpa armigera*, was done and cDNA sequencing of Bt receptor gene was performed. The present study relates to a novel DNA sequence of the gene of Bt receptor protein in the brush border membrane vesicles of *Helicoverpa armigera*.

Materials and Methods

Purification of insecticidal crystal proteins, preparation of brush border membrane vesicles, purification of the receptor protein, analysis of its affinity for various *Bt* crystal proteins and associated aminopeptidase, alkaline phosphatase activities, N-terminal amino acid sequencing, characterization of receptor protein by cloning and expression of the receptor protein, were done according to the procedures described in Malik *et al*., 2006.

Sequencing of Cry1A-receptor gene: Partial sequencing of the confirmed clone of receptor gene, by automated sequencing system (ABI) was performed.

Automated DNA sequencing system (ABI 3100) from applied Biosystems was used along with ABI PRISM Ready Reaction DyeDeoxy Terminator cycle sequencing Kit according to manufacturer's instructions. This method is based on dye terminator chemistry, in which each of the four dideoxynucleotides is labeled with a different fluorochrome (Prober *et al*., 1987; Lee *et al*., 1992). The ABI 3100 can simultaneously detect fluorescence at four different wavelengths, set to coincide with the emission of four different fluorescent dyes. The reaction mixture was run in a single capillary so that color of each band passing the detector represents the DNA sequences.

Homology studies of sequenced nucleotides: Homology studies of the nucleotide sequence of clone with known nucleotide sequences present in gene data bank was done through standard nucleotide-nucleotide and protein (swiss prot) BLAST (Basic Local Alignment Search Tool) software (Altschul *et al*., 1997) available at NCBI web site.

www.ncbi.nlm.nih.gov/home/BLAST <http://www.ncbi.nlm.nih.gov/home/BLAST>

Results

Nucleotide sequence: Partial nucleotide sequence of cDNA from *Helicoverpa armigera*, midgut`s BBMVs is given below:

GATTCATATGGCACCCGCCCGGTCACCCAGCCCCAGCACGCAGCCCTGGGAG
CATGTGAATGCCATCCAGGAGGCCCGGCGGCTCCTGAACCTGAGTAGAGACA
CTGCTGCTGAGATGAATGAAACAGTAGAAGTGATATCAGAAATGTTTGACCT
CCAGGAGCCGACTTGCCTACAGACCCGGCCTGGAGCTGTACAAGCAGGGCCT
GCGGGGCAGCCTCACCAAGCTCAAGGGCCCCTTGACCATGATGGCCAGCCAC

TACAAGCAGCACTGCCCTCCAACCCCGGAAACTTCCTGTGCAACCCAGATTA
TCACCTTTGAAAGTTTCAAAGAGAACCTGAAGGACTTCCTGCTTGTCATCCCC
TTTGACTGCTGGGAGCCAGTCCAGGAGTGAGGATCCAATCACTAGTGAATTC
GCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCA
TAGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCAT
AGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACATTTCCACACAACATACGA
GCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCA
CATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGC
CAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTG
GGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTG
CGGCGA

Homology studies: Homology studies of the nucleotide sequences of clone with known nucleotide sequences present in gene data bank was done. The results showed no significant homology with existing sequences so this is a novel Bt receptor gene.

Discussion

Toxicity of insecticidal endotoxins produced by *Bacillus thuringiensis* correlates with the presence of specific proteins in the midgut of susceptible larvae. In susceptible lepidopteran insects, aminopeptidase N and cadherin-like proteins are the putative receptors for *Bacillus thuringiensis* (Bt) toxins.

The Cry1A toxin-binding proteins that have been characterized are primarily leucine aminopeptidases (Denolf *et al*., 1997; Gill *et al*., 1995; Knight *et al*., 1995; Yaoi *et al*., 1999), cadherinlike proteins (Nagamastsu *et al*., 1998; Vadlamudi *et al*., 1995), and, in one study, biotin-containing proteins (Du *et al*., 1996). Aminopeptidase cDNAs have been cloned from *H. virescens* (Gill *et al*., 1995; Luo *et al*., 1997a; Jurat *et al*., 2001; Oltean *et al*., 1999), *Manduca sexta* (Denolf *et al*., 1997; Knight *et al*., 1995), *Plutella xylostella* (Denolf *et al*., 1997; Luo *et al*., 1997b; Nakanishi *et al*., 1999; Chang, *et al*., 1999), *Bombyx mori* (Yaoi *et al*., 1999), *L. dispar* (Valaitis *et al*., 1995; Lee and Dean, 1996; Garner *et al*., 1999) *Helicoverpa punctigera* (Emmerling *et al*., 2001) *Helicoverpa armigera* (Rajagopal *et al*., 2003) and *Plodia interpunctella*, while cadherinlike Cry1A-binding proteins have been cloned from *M. sexta* (Vadlamudi *et al*., 1995) and *B. mori* (Nagamastsu *et al*., 1998).

Homology studies of the DNA sequence of this invention with known nucleotide sequences present in gene data bank was done. The results showed no significant homology with existing sequences of Bt receptor gene.

The purpose of present study was molecular characterization of Bt receptor protein in the brush border membrane vesicles of *Helicoverpa armigera* The results of this study and a comparison between susceptible and resistant insects receptor gene sequences will help in the elucidation of mode of action of Bt pesticidal protein, specificity and insect resistance build up.

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