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Characterization of Gut Proteases of Cry1Ac Susceptible and Resistant Strains of *Helicoverpa armigera* (Hubner)

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SUMMARY

Gut enzymes of various concentrations (1.49 to 1530 ppm) of susceptible as well as resistant strains of *Helicoverpa armigera* to Bt toxin were incubated with fixed quantity of Cry1Ac toxin. The gut enzymes from the susceptible strain were found more efficient in conversion of protoxins (130 kDa) to active toxin (60 kDa) at lower concentrations also compared to the resistant strain. Active toxin, which is considered to be resistant to further proteolysis, was degraded in resistant strain. Active toxin degradation was not observed in susceptible strain with very high concentration of gut enzyme. Eight isozymes of susceptible strain with relative mobility 0.07, 0.15, 0.26, 0.37, 0.51, 0.59, 0.66 and 0.81 and nine isozymes with relative mobility 0.07,0.15, 0.29, 0.37, 0.52, 0.59, 0.67, 0.74 and 0.81 were detected in resistant strain. Incubation of each of the isozyme with Cry1Ac toxin revealed that all isozymes could activate protoxin into core toxin in both the strains. However core toxin degradation was found in case of isozyme with relative mobility 0.29 in resistant strain. The corresponding isozyme of the susceptible strain lacked this ability to degrade core toxin.

Key words: *Bacillus thuringiensis*, *Helicoverpa armigera*, Cry1Ac resistant, Cry1Ac susceptible, Gut Proteases.

Bacillus thuringiensis (Bt) is a gram-positive bacterium,which produces crystalline parasporal inclusions containing insecticidal crystal proteins (ICPs), called ä-endotoxins, during sporulation. Upon ingestion by susceptible insects, the crystals are solubilized into protoxins with molecular weigh around 130 kDa in the alkaline midgut. The protoxins are cleaved to 60 kDa around C-terminal by the proteolysis mediated by midgut proteases. The active toxin then binds to the receptors on the brush border membrane vesicles (BBMV) of the epithelial cells causing cell lysis, ionic imbalance and death of insect due to starvation Gill *et al*., (1992).

The initial successes in insect control achieved with these transgenic plants will lead to expanded use of Bt crops. The extensive planting of these crops will cause insects to encounter increased exposure to toxins, which could lead to develop selection pressure for resistance Oppert *et al.*, (1997).

Several mechanisms of insect resistance to *Bacillus thuringiensis* toxins have been proposed. One involves changes in the binding of toxins to gut receptors. In a *Bacillus thuringiensis* subspecies *kurstaki*-resistant strain of the Indianmeal moth, *Plodia interpuctella*, reduced binding of Cry1Ab toxin to larval brush border membrane vesicles was associated with increased resistance to the toxin Van Rie *et al*., (1990a).

Another mechanism of resistance may involve gut proteases that interact with *Bacillus thuringiensis* toxins. Proteases from a strain of *Heliothis virescens* resistant to *B. thuringiensis* var. *kurstaki* HD-73 were reported to activate the protoxin more slowly and degrade toxin faster than enzymes from susceptible strain Forcada *et al*., (1996). *Helicoverpa armigera* is one of the major pests of cotton and has shown resistance to most of the insecticides. Transgenic Bt cotton is expected to be useful to combat the bollworm complex (*Helicoverpa armigera, Pectinophera gossypiella and Earias vitella*). The aim of present study is to identify the differences in proteolysis of Cry1Ac protoxins by gut proteases of of Cry1Ac susceptible and resistant strains of *Helicoverpa armigera* and to identify unique, gut protease isozyme/s (if any) mediating resistance to Cry1Ac in *H. armigera*.

MATERIALS AND METHODS

Insects :

Field collected strains of *Helicoverpa armigera* were maintained in the laboratory without selection pressure. A laboratory selected Cry1Ac resistant strain was generated through repeated selection on a diet of Cry1Ac toxin. The Rothamsted Experimental station, UK, kindly provided world susceptible strain of *H.armigera*.

Chemicals and Bacillus thuringiensis toxins :

The chemicals and reagents for for Sodium dodecyl sulfate-Ployacrylamide gel electrophoresis (SDS-PAGE), Azocasein were purchased from Sigma chemicals. Tris buffer, EDTA, casein, bromophenol blue were purchaes from SRL. Monsanto, kindly provided MVP (Mycogen Vegetative Protein) cells containing about 19.5% Cry1Ac toxin.

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