Identification and purification of a novel high-affinity receptor BTR_{250} from Pink Bollworm (*Pectinophora gossypiella*) for *Bacillus thuringiensis* Cry1A toxins

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ABSTRACT

The pink bollworm Pectinophora gossypiella Saunders (PBW) is a devastating cotton pest worldwide. Bacillus thuringiensis Cry1A toxins are lethal to pink bollworm (P. gossypiella) larvae. Cry1Ac binds to ~250, 200, 120, and 115 kDa proteins in the brush border membrane vesicles. The toxin binds specifically and with high affinity (K_d ~10 nM) to the receptors binding sites. A competition ligand blot using unlabeled Cry1Ac to compete with ^{125}I-Cry1Ac shows that binding is abolished by Cry1Ac. Consequently, Cry1Ac binding to the mentioned proteins is specific and more likely to be of a mediating toxicity in P. gossypiella. The Cry1Ac immuno-precipitation gives rise to three major bands of ~200 and 120/115 kDa. Interestingly, a novel 250 kDa band was observed to bind with 125I-Cry1Ac. The work was focused on the purification of the 250 kDa protein using ion exchange chromatography and 2D gel electrophoresis. Collectively, these results demonstrate that BTR250 in junction with the 200 kDa Cadherin-like receptor may play an important role in Cry1A toxicity to P. gossypiella. Protein sequencing and DNA cloning of this new receptor will help in identification of the novel receptor and deciphering its nature. Understanding the molecules involved in toxin binding will help in developing strategies for insect resistance management and development of better bio-pesticides.

Keywords: *Pectinophora gossypiella, Bacillus thuringiensis, Cry toxin, PBW-BTR_{250}.*

INTRODUCTION

Control of the PBW using *Bacillus thuringiensis* (Bt) formulations has shown very promising results during the last few decades (Perlak *et al.*, 1990). *Bacillus thuringiensis* is a gram-positive, spore-forming bacterium that forms a parasporal crystal which contains insecticidal toxins (Bulla *et al.*, 1980; Höfte and Whiteley, 1989). *Bacillus thuringiensis* (Bt), as a biopesticide, is a viable alternative for the control of insect pests in agriculture. Bt use is also compatible with sustainable and environmentally friendly agricultural practices. Bt produces insecticidal proteins (Cry toxins) during sporulation as parasporal crystals. These crystals are predominantly composed of one or more proteins, called endotoxins. These toxins are highly specific to their target insect; are safe to humans, vertebrates and plants; and are completely biodegradable. The mode of action of Cry toxins is a multistage process. Crystal toxins ingested by susceptible larvae