Abstract

In order to identify the vegetative insecticidal protein (Vip) that could be effective against sap-sucking insects belonging to order hemiptera, a screening analysis was performed on the basis of toxicity assays against cotton aphids using the proteins obtained from the culture supernatants of several *Bacillus thuringiensis* (Bt) isolates available in our laboratory. One (Bt #BREF24) of the them showed promising results and upon purification the aphidicidal protein turned out to be a binary toxin. One of the two proteins present in the toxic component was identified by peptide sequencing as a homologue of Vip2A from other *Bacillus spp* and belongs to the reported binary toxin Vip1-Vip2 group, where the Vip2 is responsible for the enzymatic activity and the Vip1 is the translocation and receptor binding protein to the target insect. The two genes corresponding to the individual proteins of the binary toxin, designated as *vip2Ae* and *vip1Ae*, from the Bt #BREF24 were isolated, cloned and sequenced. The coding DNA sequences corresponding to the mature polypeptides for both Vip2Ae and Vip1Ae, designated as dVip2Ae and dVip1Ae respectively, were expressed in *E. coli* and the recombinant proteins after purification were used for toxicity assay against cotton aphids. The assay results confirmed that these proteins are indeed the two constituents of the binary toxins and for their activity the presence of both partners are essential. A deletion analysis of the Vip1Ae protein was performed to identify the minimal peptide region necessary for the functional activity of the binary toxin through Vip1A-Vip2A interaction. From this analysis it might be concluded that besides the C-terminal (receptor binding) domain, some part of the N-terminal region of the mature Vip1Ae is also necessary to cause insect toxicity. To investigate into the aspect of the target insect specificity, a ligand blotting experiment was performed using the brush border membrane vesicles isolated from midguts of cotton aphids (hemipteran), cotton bollworm (lepidopteran) and mice (mammalian). The results showed that the Vip1Ae, the binding component of the binary toxin immuno-detected a ~ 50kDa receptor protein of the aphid midgut only but not in the other two samples. Taken together, the findings of this study open up the possible application of the aphidicidal toxin as a biopesticide in the

integrated pest management program and hold a promise of its use in future as a candidate gene for developing transgenic crop plants tolerant against sap-sucking insects.

Key words

ADP-ribosyltransferase; aphids; *Aphis gossypii*; *Bacillus thuringiensis*; binary toxin; hemiptera; heterologous expression; integrated pest management; ligand blotting; sap-sucking insect pest; truncated proteins; vegetative insecticidal protein.