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Expression of *Bacillus thuringiensis* Cry1Ac Protein Fused with Coat Protein of Potato Leafroll Virus

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Objectives

Aphidicidal activity of *Bacillus thurigiensis* crystal proteins was recently reported. However, relatively higher dose of crystal protein was needed to kill aphids. In this study, we intended to improve the aphidicidal activity of crystal protein by fusion with coat protein (CP) of potato leafroll virus (PLRV) which is transmitted by aphids.

Materials and Methods

Materials - Vector: pProAc, pPCP2, pBacPAK8

Virus: bApGOZA

Methods - recombinant baculovirus construction, fusion protein expression

Results and Discussion

Toxic fragment and domain I of the *cry1Ac* gene of *B. thuringiensis* were amplified from pProAc using specific primers. CP gene of the PLRV was amplified from pPCP2 using specific primer sets. These PCR amplified fragments were serially cloned into pBacPAK8 to generate transfer vectors, pBac8-1Ac, pBac8-1AcCP, pBac8-1AcD1 and pBac8-1AcD1CP. Recombinant viruses, Ap1AcTF, Ap1AcCP, Ap1AcD1 and Ap1AcD1CP were constructed by co-transfection of each transfer vector with bApGOZA genomic DNA into Sf9 cells. As a result, fusion proteins of Cry1Ac-CP (91 kDa) and Cry1Ac domain I -CP (56 kDa) were expressed from High Five cells infected with Ap1AcCP and Ap1AcD1CP, respectively. Also, Cry1Ac toxic region (68 kDa) and domain I region (33 kDa) were expressed from High Five cells infected with Ap1AcTF and Ap1AcD1, respectively.

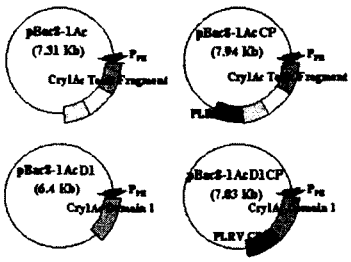

Fig. 1. Schematic diagram of transfer vectors for construction of recombinant viruses.

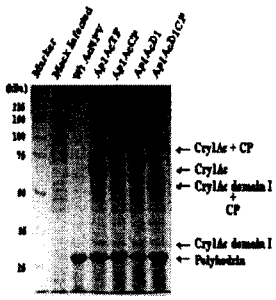

Fig. 2. SDS-PAGE of fusion proteins from High Five cells infected with recombinant viruses.

References

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