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Cloning and Expression of Cadherin Fragment from *Helicoverpa armigera*

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Abstract

Helicoverpa armigera is an important pest of agricultural crops which causes severe damage to the fruits and pods of crop plants, larval stage which causes this kind of damage. Helicoverpa armigera cadherin fragment contains its toxin binding region which enhance cry1Ac activity against Helicoverpa armigera larvae because this kind of interaction between HaCad1 receptors and cry1Ac induces toxin oligomerization. The aim of current investigation was to clone and expression the cadherin fragment. Cadherin gene was isolated from mid gut portion of Helicoverpa armigera and cloned into protein expression vector pMALc2x for expression study. The recombinant clone was transformed into protein expression host and protein was isolated and analysed the expected kDa by SDS- PAGE and Western blotting. The protein band was 70 kDa *that confirmed 26.9 kDa cadherin protein was fused with 42.5 kDa Maltose binding protein. Present study successfully cloned and expressed cadherin fragment HaCad1 and Cry1Ac protein in Helicoverpa armigera. Our result provided a tactic for cadherin gene cloning and transformation into cry1Ac transgenic plants that can significantly enhance the insecticidal activity of Cry1Ac against Helicoverpa armigera.*

Key words: *Helicoverpa armigera, Cry1Ac insecticidal protein and cadherin*

I. Introduction

Pest management has always been an important challenge to agricultural community. Crop yields are substantially reduced by insect pests that attack them. Bt crystal proteins are the first insecticidal protein for introduction into plants because of their environmental safety. A variety of genes encoding for different classes of insecticidal proteins such as protease inhibitors, lectins, amylase inhibitors, scorpion-venom toxins, cytokinin biosynthesis enzymes, cholesterol oxidase, chitinase and δ-endotoxins of *Bacillus thuringiensis* are being tested for insect control. Among these, the insecticidal crystal proteins of *Bt* assume major significance due to their potency, insect specificity and lack of activity in mammals and other organisms (Kumar *et al*., 1996). *Bacillus thuringiensis* is a ubiquitous gram-positive, spore forming bacterium. It produces parasporal insecticidal crystal proteins (ICPs) also known as Cry proteins which exhibit toxicity to certain insect species. These insecticidal crystal proteins (ICPs) display insect specific toxicity and are harmless to humans and non-target animals. Consequently, they have been widely used in biopesticides and transgenic plants to control agricultural pests (Schnepf *et al*., 1998; Pigott and Ellar, 2007). *Bacillus thuringiensis* is already a useful alternative or supplement to synthetic chemical pesticide. Cadherin belongs to one of the families of animal glycoproteins responsible for calcium-dependent cell-cell adhesion, Cadherin (named for calcium dependent adhesion). The interaction between *Bacillus thuringiensis* insecticidal crystal protein Cry1A and Cadherin receptors in lepidopteran insects induces toxin oligomerization, which is essential for membrane insertion and mediates Cry1A toxicity. They are dependent on

calcium (Ca^{2+}) ions to function, hence their name. Recent literature showed that the Cadherin-like protein in midgut of several insects served as the receptor of *Bt* toxin Cry1A and variation of Cadherin-like protein is related to insect's resistance to *cry1A*. cDNA sequence of a *cadherin* gene in *Helicoverpa armigera* (*HaCad*) and the *cry1Ac* toxin binding region (TBR) to residues 1217-1461 (Wang *et al.*, 2005). Now a days cloning of the cadherin fragment is a novel strategy to enhance insecticidal activity or to overcome the resistance of insects. As *Helicoverpa armigera* is a major crop pest, cloning of *cadherin* gene fragment into the protein expression vector can help to manage this pest. The view of the present study was to clone the receptor region of *HaCad1* into *E .coli* expression vector pMALc2X and analyze the protein by SDS- PAGE and Western blotting.

II. Material and Methods

An experiment was conducted in National Research Center on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India with a view for designing of primers for amplification of *HaCad1* from *Helicoverpa armigera*: Based on published *HaCad1* (Genebank no.DQ973295 - Peng *et al.*, 2009) gene sequences, a set of specific primers were used to amplify the receptor binding portion of cadherin from the cDNA sample of *Helicoverpa armigera* midgut portion.

Total RNA isolation from *H. armigera* mid gut: Total RNA was isolated from the mid gut portion of the 4th instar larvae of the *Helicoverpa armigera*. RNA isolation was done by using TRizol reagent (Invitrogen). Quality and quantity of the total RNA was checked by gel electrophoresis and Nanodrop 1000 spectrophotometer respectively (Figure 01).

cDNA synthesis from the total RNA: Single stranded cDNA was prepared by using AffinityScript QPCR cDNA Synthesis Kit. (Stratagene) (Figure 02). The following reaction components were added in microcentrifuge tube in order:

RNase-free H_2O	5.0 µl
Total RNA (1 µg)	1.0 µl
First strand master mix (2×)	10.0 µl
Oligo (dT) primer (0.1 µg/µl)	3.0 µl
AffinityScript RT/RNase Block enzyme mixture	1.0 µl

The above reaction was kept in thermal cycler and followed the condition according to the manufacturer's instruction. After cDNA synthesis the quality and quantity of the cDNA was checked on 1.2% agarose gel and Nanodrop respectively.

Amplification of the *cadherin* gene fragment: Gradient PCR was performed to find out the annealing temperature for amplification of *cadherin* receptor region at 50-60ºC by using Taq DNA polymerase enzyme (Bangalore Genei). Pfu DNA polymerase was used to amplify the final product and avoid the mismatch of the nucleotide sequences. Amplification was performed in an eppendorf thermal cycler (Eppendorf AG, Germany) (Figure 03).

Analysis and Purification of PCR products: After amplification of cDNA, it was checked in 1% agarose gel electrophoresis and purified the cDNA using QIAquick PCR purification kit (QIAGEN, Germany).

Cloning of *cadherin* gene (HaCad1) fragment into pMALc2x expression vector: For the expression of *cadherin* gene, 732 bp of PCR purified *cadherin* gene was restricted with *Bam*HI and *Sac*I site and cloned into correspondent site of pET29 (a+) vector. The cadherin fragment was further cloned into pMALc2X expression vector by *Bam*HI – *Hind*III restriction sites for improving the

protein expression. Routine lab protocols were followed for ligation, transformation and screening of transformed colonies (Figure 04).

Overexpression of cadherin protein: The clone was transformed into the TB1 host cells and the transformants were used for screening. Protein was isolated according to manufacture instruction (New England BioLabs) from single colony and induced by 1mM IPTG. The overexpressed protein was checked by SDS-PAGE and Western blotting.

Screening for overexpression colony by SDS-PAGE analysis and Western Blotting: Isolated protein was checked on 12% SDS-PAGE (BIO-RAD Mini-Protein-3 Electrophoresis system). Because of the small fragment of the *cadherin* gene the expected band size was not clearly seen under the SDS-PAGE. For further confirmation, western blotting was carried out. SDS-PAGE separated proteins were transferred to nitro cellular membrane using mini trans-blot electrophoretic transfer cell apparatus (BIO-RAD, USA). After adding primary and secondary antibody to the membrane, it was further washed with PBST for three times and air dried on what man paper and immediately photograph was taken (Figure 05a and 05b).

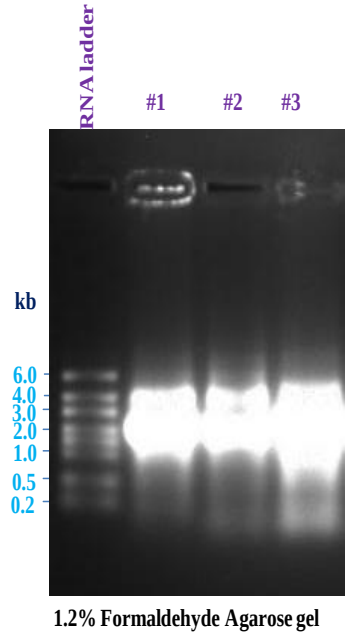
III. Results

Experimental setup and cloning of cadherin gene

Based on published HaCad1 (Gene Bank no. DQ973295-Peng *et al*., 2009) gene sequence, set of primers were designed for amplification of receptor binding portion of cadherin fragment. Total RNA was isolated from the larval mid gut (Figure 01) and converted into single stranded cDNA (Figure 02) and used as template for PCR amplification (Figure 03). 732bp PCR amplified cDNA fragment (toxin binding region) was cloned into the protein expression vector. PCR amplified 732bp gene fragment was restricted with *BamH1* and *SacI* and cloned into the corresponding sites of protein expression vector pET29a (+). Cadherin fragment was inserting out from pET29a (+) vector using BamHI-HindIII sites and further cloned into pMALc2x expression vector (Figure 04) for improving the expression. The clone was further confirmed by multiple restriction digestion analysis.

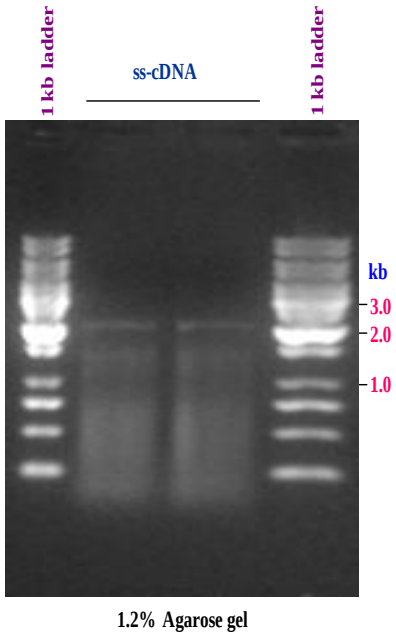
Analysis of protein expression by SDS-PAGE and Western Blotting

PMALc2X-cadherin vector were transformed into TB1 *E. coli* host to express the protein. Since the gene fragment was extremely small consisting of 732bp and the proteins molecular weight being only 26.9 kDa it which was giving a very faint band on the SDS-PAGE so it was decided that this protein would be cloned into pMALC2X vector which contains a maltose binding fusion protein which is 42.5kDa. After cloning both proteins fuse together and yielded 70kDa fusion protein was found in SDS PAGE (Figure 05a). For further confirmation western blotting was carried out and a 70kDa fusion protein was obtained which contains 26.9kDa of cadherin protein along with 42.5kDa of maltose binding protein (Figure 05b).



1.2% Formaldehyde Agarose gel

Figure 01. Analysis of total RNA from mid gut portion of *H. armigera*



1.2% Agarose gel

Figure 02. Synthesis of cDNA from midgut total RNA of H. armigera

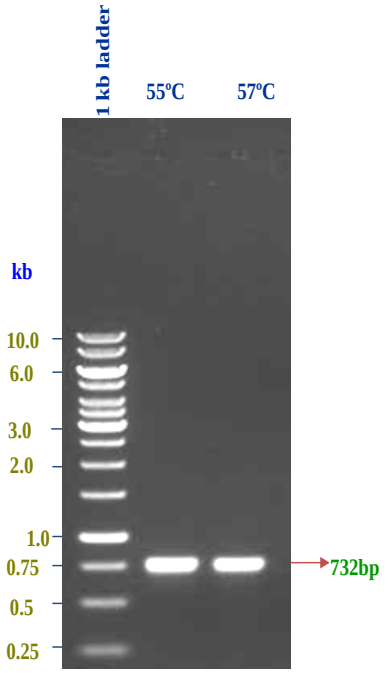
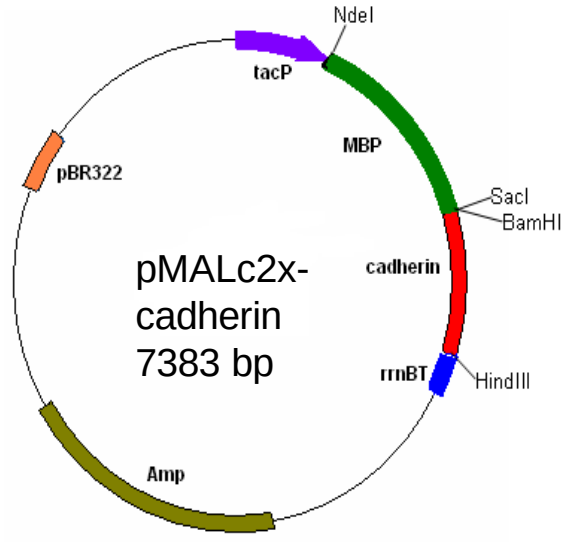


Figure 03. PCR amplification of *cadherin* gene fragment using cDNA sample



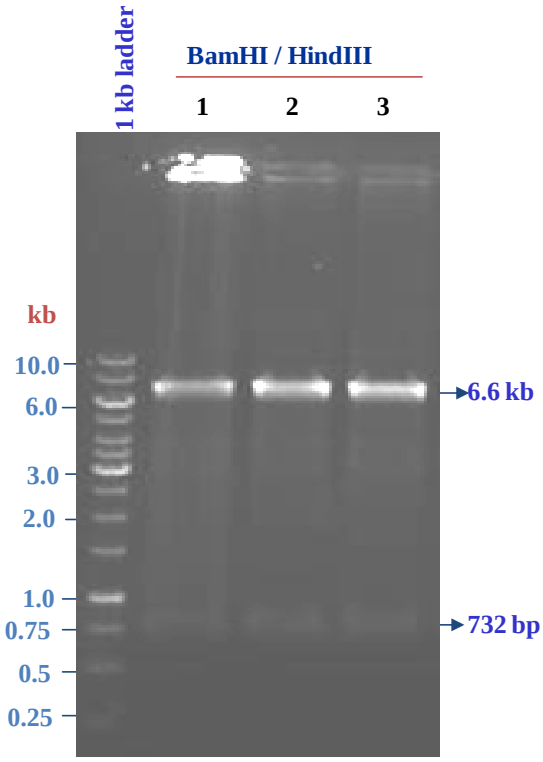


Figure 04. Construction and restriction digestion analysis of pMALc2x protein expression vector carrying *cadherin* gene

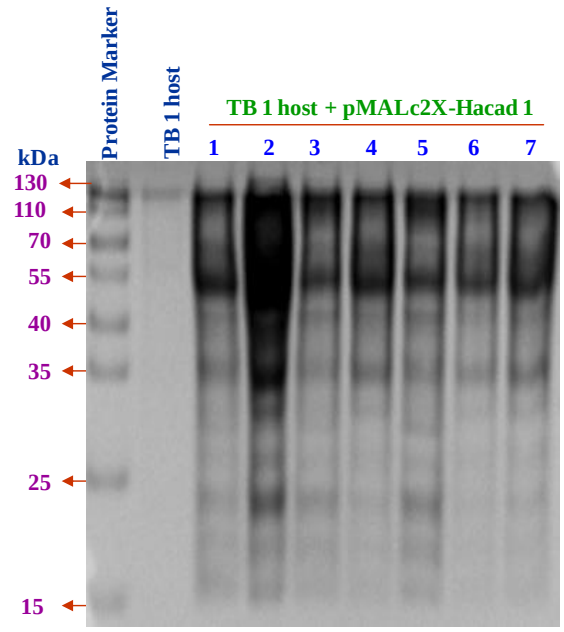


Figure 05a. SDS-PAGE confirmation of cadherin protein

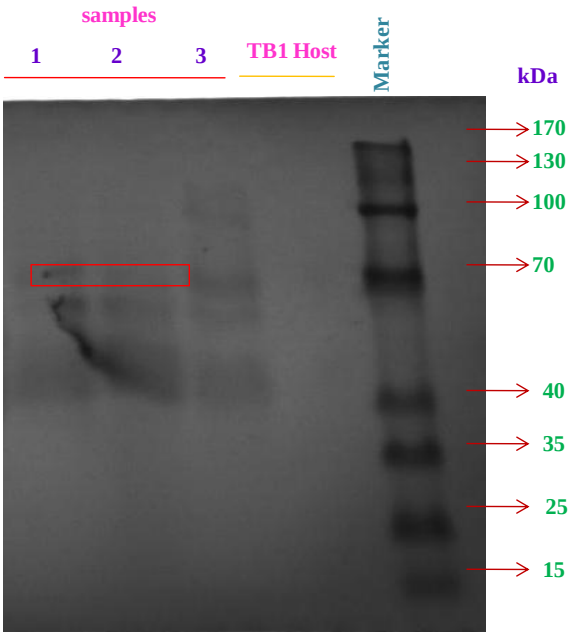


Figure 05b. Western blotting confirmation of cadherin protein

IV. Discussion

Genetically engineered crops to produce Bt toxins for insect control have reduced the use of conventional insecticides. *Bacillus thuringiensis* produces insecticidal crystal protein. Previously, toxin binding region of *Helicoverpa armigera* cadherin fragment (*HaCad1*) cloning into binary vector and retransformation into the *cry1Ac* plants through *Agrobacterium* mediated plant transformation was done by Momena *et al.* (2014). The cry1Ac toxin binding sites of HaCad have been mapped to residues 1217 to 1461 by Wang *et al.* (2005) and found that HaCad1 enhanced the insecticidal activity of *Bacillus thuringiensis* cry1Ac toxins to *Helicoverpa armigera.* A critical step in the mode of action of cry proteins is binding of toxins to mid guts membrane receptors (Schnepf *et al.*, 1998; Whalon and Wingerd, 2003). It was proposed that midgut epithelium receptors were involved in insect toxicity by interacting sequentially with the cry1A toxin (Bravo *et al.*, 2004 and 2007; Jurat- Fuentes and Adang, 2006; Gomez *et al.*, 2007). Cadherin belongs to one of the families of animal glycoproteins responsible for calcium-dependent cell-cell adhesion hence, named cadherin. The interaction between *Bacillus thuringiensis* insecticidal crystal protein Cry1A and cadherin receptors in lepidopteran insects induces toxin oligomerization, which is essential for membrane insertion and mediates Cry1A toxicity. Based on these previous reports the present study has done to demonstrate the amplification of receptor binding portion of cadherin fragment from *H. armigera*, cloning to protein expression vector and over express the protein, analysed by SDS-PAGE and western blotting.

In SDS-PAGE analysis it shown very faint band due to low molecular weight of protein, so that it was further confirmed by western blot analysis. The result shows that 70 kDa protein band on the SDS-PAGE and western blot, which indicates 26.9 kDa cadherin proteins, was fused with 42.5 kDa Maltose binding protein.

V. Conclusion

The present study showed that 732 bps *cadherin* gene fragment was successfully cloned into the protein expression vector pMALc2X. Over expressed protein was analysed and confirmed by SDS-PAGE and western blot, it shows 70 kDa protein band which confirmed by western blot, which indicates 26.9 kDa cadherin protein was fused with 42.5 kDa Maltose binding protein. To study the toxin enhancement of the cry1Ac, cadherin gene can be cloned into plant transformation vector and retransformed into cry1Ac transgenic plants.

VI. References

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