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Cloning of a New Truncated *cry*1*Ac* Gene from an Indian Isolate of *Bacillus thuringiensis*

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ABSTRACT

Transgenic Bt crops producing insecticidal crystal proteins from *Bacillus thuringiensis* (Bt), so-called Cry toxins, have proved useful in controlling insect pests. Among the cry toxins, *Cry*1*A* toxins are important because of high toxicity to lepidopteran pests and their widespread distribution among Bt strains. In *Cry*1*A* proteins, toxin fragment is comprised of about 620 amino acids of N-terminal region and C-terminal half is not required for toxicity. Four indigenous isolates of Bt *viz*., T15, T16, T20 and T31 were screened by PCR-RFLP for 3'-truncated *cry*1*A* gene(s) corresponding to toxin fragment. RFLP analysis of *cry*1*A* amplicons obtained from the four isolates of Bt showed presence of *cry*1*Ac*-type gene alone in three isolates. One of the *cry*1*Ac*-postive isolates, T15 which showed 100 percent mortality in *Helicoverpa armigera*, was selected for cloning of DNA fragment of about 2.1 kb containing 3'-truncated *cry*1*Ac* gene. Nucleotide sequence data generated for 3'-truncated *cry*1*Ac* gene of T15 showed 98 to 99 percent homology with 1958 bp of already reported sequences of all *cry*1*Ac* genes (*cry*1*Ac*1 to *cry*1*Ac*24). Deduced amino acid sequence of *cry*1*Ac* of Bt strain, T15 showed one to four percent variation in comparison to all reported *Cry*1*Ac* holotypes (*Cry*1*Ac*1 to *Cry*1*Ac*24) by differing at 5 to 19 positions. This suggests that the *cry*1*Ac* toxin of Bt isolate, T15 is a new kind of its group.

KEYWORDS

***Bacillus thuringiensis*; *cry*1*Ac*; Cloning**

1. Introduction

The Bt has been used as a successful biological insecticide for more than 40 years and is a uniquely specific, safe, and effective tool for the control of a wide variety of insect pests [1]. The advancement in molecular biology led to the cloning of Bt crystal protein (*cry*) gene for the first time in 1981 [2]. Till date more than 412 *cry* genes have been successfully cloned and characterized for their insecticidal potential. Till August 2009, the Cry proteins are classified into 59 families based on their amino acid sequence similarity (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/). Among the Cry toxins, Cry1A toxins are important because of high toxicity to lepidopteran pests and their widespread distribution among Bt strains [3,4].

The *cry*1*Ac* gene is used into first version of insect resistant transgenic Bt-cotton. However, continuous exposure of a single Bt protein in Bt cotton can lead to resistance development in lepidopteran insects [5,6]. Genes developed in one country may not be much more effective against insect pests present in other countries. *Cry*1*Ac* protein is about 30 fold less toxic to *H. armigera* than to *Heliothis virescens*, the original target pest of transgenic cotton in USA [7]. Therefore a large number of Bt strains have been isolated and many types of insecticidal crystal proteins genes have been cloned. The diversity of Bt strains facilitates isolation of new types of *cry* genes. New variants of the already known *cry* gene subgroups could encode crystal proteins with significant difference in the level and spectrum of toxicity due to variation in

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their sequences [8]. Variation of even a single amino acid residue at certain positions of Cry proteins can remarkably influence the level of toxicity [9]. Apart from the full-length genes, truncated genes which produce insecticidally active protein have been expressed in different crops like potato [2] and rice [10]. The present study describes screening of indigenous isolates of Bt for *cry*1*A* genes by PCR-RFLP and cloning of a new truncated *cry*1*Ac* gene from an Indian isolate of Bt.

2. Materials and Methods

2.1. Bacterial Strains, Plasmids

Four indigenous Bt strains (T15, T16, T20 and T31) and reference strain, Bt subsp kurstaki (HD1) are from the collection of Bt strains maintained by the corresponding author in the Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India. The reference strain, HD1 were originally obtained from Bacillus Genetic Stock Centre, Ohio state university, Columbus, Ohio, USA. The T/A cloning vector, pGEM-T Easy used in the present study was purchased from Promega BioScience, INC.

2.2. Amplification of Bt DNA by PCR

Total DNA from Bt strains, T15, T16, T20 and T31 was extracted as described earlier [11] and used as a template for the Polymerase Chain Reaction (PCR) amplification. Based on the published sequence of *cry*1*A* gene subtypes, *cry*1*Aa*1, *cry*1*Ab*1 and *cry*1*Ac*1 (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/), cloning primers (1AF and 1AR) specific for 3'-truncated *cry*1*A* gene(s) were designed and listed in **Table 1**. These primers are specific to upstream and internal region of the following genes: *cry*1*Aa*1, *cry*1*Ab*1 and *cry*1*Ac*1. The PCR was accomplished using an Eppendorf thermal cycler in 25 µl reaction volume containing 50 ng of total genomic DNA of Bt, 2.5 µl of 10X PCR buffer (10 mMTris-HCl; pH: 9.0, 50 mM KCl, 1.5 mM $MgCl_2$), 75 µM each of dNTPs, 50 ng each of forward and reverse primers and 1.5 Units of *Taq* DNA polymerase. The PCR was performed for 30 cycles as follows: 94˚C for 1 min, 58˚C for 45 s, 72˚C for 45 s and the final extension was performed for 7 min at 72˚C.

2.3. RFLP for 3'-Truncated *Cry*1*A* Gene(s) by *Eco*RI

The amplicon (3'-truncated *cry*1*A* gene(s)) obtained by PCR was verified on 1.2 percent agarose gel. The PCR product was column purified with PCR clean up kit as per the manufacturer's instruction provided by Sigma. The final concentration of the purified product was checked by resolving in 1.2 per cent agarose gel. The column purified product of 3'-truncated *cry*1*A* gene(s) was digested by *Eco*RI as per the manufacturer's instruction. Restriction digestion was set up for 20 µl as follows: DNA: 500 ng, Buffer (10X): 2.0 µl, BSA (10X): 2.0 µl, *Eco*RI enzyme (10U): 0.5 µl , sterile distilled water : to 20 µl. The restriction digestion was carried out at 37˚C for 1 h and 30 min. The digested product was analyzed by agarose 1.2 per cent gel electrophoresis.

Table 1. Primers used for amplification of 3'-truncated *cry*1*A* gene(s).

Primer name and sequence[a]	Truncated *cry*1*A* gene(s)	Position from ATG	Product size (kb)
1AF: 5'GC**CCCGGG**CCTGGGTC AAAAATTGATATTTAG 3'	*cry*1*Aa*1	−147 to +1952	~2.1
	*cry*1*Ab*1	−141 to +1955	
1AR: 5'CGG**GTCGAC**TAAATTG GATACTTGATCA 3'	*cry*1*Ac*1	−147 to +1958	

[a]Primer sequences containing restriction endonuclease recognization sites for ***Xma*I (CCCGGG)** and ***Sal*I (GTCGAC)** are in bold faces.

2.4. Cloning of 3'-Truncated *cry1Ac* Gene from Bt Isolate, T15

The column purified PCR product of 3'-truncated *cry*1*Ac* gene (~2.1 kb) from Bt isolate, T15 was ligated into T/A vector (pGEM-T Easy, Promega) as per the manufacturer's instruction. The ligated mixture was transformed into *E. coli* as per the standard procedure [12]. The transformed colonies of *E. coli* were screened by colony PCR with M13F and M13R primers for checking the presence of insert (3'-truncated *cry*1*A* gene of Bt isolate, T15).

2.5. Nucleotide Sequencing of Recombinant Plasmids

The plasmid DNA was isolated from the *E. coli* transformants containing truncated *cry*1*Ac* gene of Bt isolate, T15 and Nucleotide sequence of recombinant plasmids was carried out by automated sequencing (Ist Base, Singapore and Chromous Biotech Pvt. Ltd., Bangalore, India). The sequence data generated for upstream region of about 147 bp and toxin fragment of 1958 bp were subjected to homology search through Basic Local Alignment Search Tool (BLAST) of National Centre for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov/Blast). The deduced amino acid sequence was generated by BioEdit [13].

3. Result

3.1. PCR-RFLP for 3'-Truncated *Cry*1*A* Gene(s)

The four indigenous isolates of Bt *viz*., T15, T16, T20

and T31 which showed 90 to 100 per cent mortality in *H. armigera* (data not shown) were positive for amplification by *cry*1 genes specific primers described by [14]. Hence, these four isolates were selected for amplification of truncated *cry*1*A* gene(s) corresponding to toxin fragment and PCR-RFLP to know the novelty in their sequences. Total genomic DNA isolated from four indigenous isolates of Bt was used as a template for amplification of DNA fragment containing 3'-truncated *cry*1*A* gene(s) sequence. An intact band of DNA fragment of ~2.1 kb corresponding to 3'-truncated *cry*1*A* gene(s) was amplified from four Bt isolates, with 1AF and 1AR primers by PCR, without any nonspecific amplification (**Figure 1**).

The expected restriction fragment sizes of the known *cry*1*A* truncated genes with *Eco*RI were listed in **Table 2**. Restriction analysis of the reference strain of Bt, HD1 was performed for comparison of indigenous isolates of Bt. The column purified PCR products of truncated *cry*1*A* gene(s) from four Bt isolates along with reference strain, HD1 were digested with restriction enzyme, *Eco*RI. Data from agarose gel electrophoresis of *cry*1*A* amplicons digested by *Eco*RI showed that three of four indigenous Bt isolates *viz*., T15, T16 and T20 had a *cry*1*Ac*-type RFLP pattern. Another isolate (T31) showed fragments corresponding to *cry*1*Aa* and/or *cry*1*Ab* along with *cry1Ac* genes as in the case of reference strain, HD1 (**Figure 2** and **Table 3**).

3.2. Cloning and Sequence Analysis of 3'-Truncated *cry*1*Ac* Gene from Bt Isolate, T15

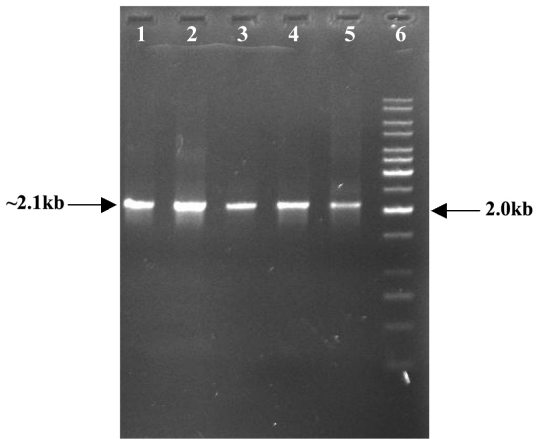
Based on PCR-RFLP data, the Bt isolate, T15 which showed 100 per cent mortality in *H. armigera* and having *cry*1*Ac* gene alone was selected for cloning of DNA fragments of ~2.1 kb corresponding to 3'-truncated *cry*1*A* gene. The column purified PCR product (~2.1 kb) of *cry*1*Ac* truncated gene from Bt isolate, T15 was cloned into pGEM-T easy vector (T/A vector). The recombinant clones (white colonies) were selected on LB agar containing X-gal, IPTG and ampicillin. Presence of insert was confirmed in recombinant *E. coli* colonies, by colony PCR with M13 forward and M13 reverse primers. Agarose gel electrophoresis showed amplification of ex-

Table 2. RFLP for *Eco*RI enzyme in 3'-truncated *cry*1*A* genes of ~2.1 kb.

S. No.	Truncated genes	Fragment sizes (bp)
1)	*cry*1*Aa*1	378, 415, 580, 726
2)	*cry*1*Ab*1	378, 415, 583, 726
3)	*cry*1*Ac*1	415, 726, 958

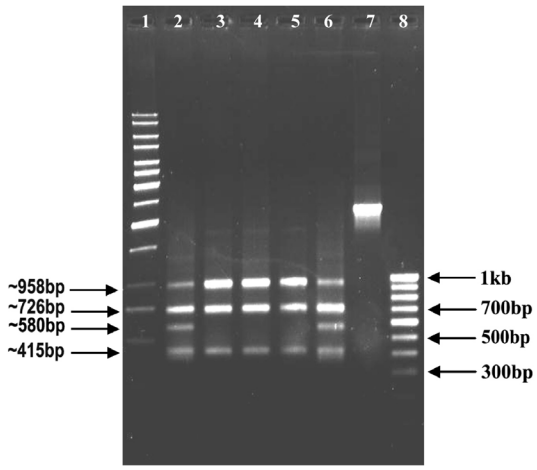
Table 3. Restriction analysis of 3'-truncated *cry*1*A* gene fragments of indigenous isolates of *B. thuringiensis* by *Eco*RI enzyme.

S. No.	Bt isolates	*Eco*RI fragment size(s)	*cry*1*A* gene sub-type(s)
1)	Reference strain, HD1	378, 415, 580, 726, 958	*cry*1*Aa*/*cry*1*Ab* & *cry*1*Ac*
2)	T15	415, 726, 958	*cry*1*Ac*
3)	T16	415, 726, 958	*cry*1*Ac*
4)	T20	415, 726, 958	*cry*1*Ac*
5)	T31	378, 415, 580, 726, 958	*cry*1*Aa*/*cry*1*Ab* & *cry*1*Ac*



Lane 1: positive control, HD1; Lane 2 to 5: Bt isolates, T15, T16, T20 and T31; Lane 6: 1 kb marker.

Figure 1. Amplification of 3'-truncated *cry*1*A* gene(s) from indigenous isolates of Bt.



Lane 1: 1 kb marker; Lane 2: positive control, HD1; Lane 3 to 6: Bt isolates, T15, T16, T20 and T31 Lane 7: undigested product; Lane 8: 100 bp marker.

Figure 2. RFLP analysis of 3'-truncated *cry*1*A* gene(s) from indigenous isolates of Bt.

pected size of ~2.3 kb corresponding to the sum of insert DNA of 2.1 kb and vector sequence of about 200 bp.

Recombinant plasmid isolated from three of the *E. coli* clones were used to determine the nucleotide sequence of the 3'-truncated *cry1Ac* gene of Bt strain, T15 by automated DNA sequencing. The nucleotide sequence revealed the presence of upstream (147 bp) and internal region (1958 bp) corresponding to *cry1Ac* gene. The internal region of 1958 bp starting from ATG encodes 652-amino acids (**Figure 3**). The computer-based homology

```
1    ATGGATAACAATCCGAACATCAATGAATGCATTCCTTATAATTGTTTAAGTAACCCTGAA 60
     M  D  N  N  P  N  I  N  E  C  I  P  Y  N  C  L  S  N  P  E
61   GTAGAAGTATTAGGTGGAGAAAGAATAGAAACTGGTTACACCCCAATCGATATTTCCTTG 120
     V  E  V  L  G  G  E  R  I  E  T  G  Y  T  P  I  D  I  S  L
121  TCGCTAACGCATTTTCTTTTGAGTGAATTTGTTCCCGGTGCTGGATTTGTGTTAGGACTA 180
     S  L  T  H  F  L  L  S  E  F  V  P  G  A  G  F  V  L  G  L
181  GGTGAGATAATATGGGGAATTTTTGGTCCCTCTCAATGGGACGCATTTCTTGTACAAATT 240
     G  E  I  I  W  G  I  F  G  P  S  Q  W  D  A  F  L  V  Q  I
241  GAACAGTTAATTAACCAAAGAATAGAAGAATTCGCTAGGAACCAAGCCATTTCTAGATTA 300
     E  Q  L  I  N  Q  R  I  E  E  F  A  R  N  Q  A  I  S  R  L
301  GAAGGACTAAGCAATCTTTATCAAATTTACGCAGAATCTTTTAGAGAGTGGGAAGCAGAT 360
     E  G  L  S  N  L  Y  Q  I  Y  A  E  S  F  R  E  W  E  A  D
361  CCTACTAATCCAGCATTAAGAGAAGAGATGCGTATTCAATTCAATGACATGAACAGTGCC 420
     P  T  N  P  A  L  R  E  E  M  R  I  Q  F  N  D  M  N  S  A
421  CTTACAACCGCTATTCCTCTTTTTGCAGTTCAAAATTATCAAGTTCCTCTTTTATCAGTA 480
     L  T  T  A  I  P  L  F  A  V  Q  N  Y  Q  V  P  L  L  S  V
481  TATGTTCAAGCTGCAAATTTACATTTATCAGTTTTGAGAGATGTTTCAGTGTTTGGACAA 540
     Y  V  Q  A  A  N  L  H  L  S  V  L  R  D  V  S  V  F  G  Q
541  AGGTGGGGATTTGATGCCGCGACTATCAATAGTCGTTATAATGATTTAACTAGGCTTATT 600
     R  W  G  F  D  A  A  T  I  N  S  R  Y  N  D  L  T  R  L  I
601  GGCAACTATACAGATTATGCTGTACGCTGGTACAATACGGGATTAGAACGTGTATGGGGA 660
     G  N  Y  T  D  Y  A  V  R  W  Y  N  T  G  L  E  R  V  W  G
661  CCGGATTCTAGAGATTGGGTAAGGTATAATCAATTTAGAAGAGAATTAACACTAACTGTA 720
     P  D  S  R  D  W  V  R  Y  N  Q  F  R  R  E  L  T  L  T  V
721  TTAGATATCGTTGCTCTGTTCCCGAATTATGATAGTAGAAGATATCCAATTCGAACAGTT 780
     L  D  I  V  A  L  F  P  N  Y  D  S  R  R  Y  P  I  R  T  V
781  TCCCAATTAACAAGAGAAATTTATACAAACCCAGTATTAGAAAATTTTGGAGGTAGTTTT 840
     S  Q  L  T  R  E  I  Y  T  N  P  V  L  E  N  F  G  G  S  F
841  CGAGGCTCGGCTCAGGGCATAGAAAGAAGTATTAGGACCCCACATTTGATGGATATACTT 900
     R  G  S  A  Q  G  I  E  R  S  I  R  T  P  H  L  M  D  I  L
901  AACAGTATAACCATCTATACGGATGCTCATAGGGGTTATTATTATTGGTCAGGGCATCAA 960
     N  S  I  T  I  Y  T  D  A  H  R  G  Y  Y  Y  W  S  G  H  Q
961  ATAATGGCTTCTCCTGTAGGGTTTTCGGGGCCAGAATTCACTTTTCCGCTATATGGAACT 1020
     I  M  A  S  P  V  G  F  S  G  P  E  F  T  F  P  L  Y  G  T
1021 ATGGGAAATGCAGCTCCACAACAACGTATTGTTGCTCAACTAGGTCAGGGCGTGTATAGA 1080
     M  G  N  A  A  P  Q  Q  R  I  V  A  Q  L  G  Q  G  V  Y  R
1081 ACATTATCGTCCACTTTATATAGAAGACCTTTTAATATAGGGATAAATAATCAACAACTA 1140
     T  L  S  S  T  L  Y  R  R  P  F  N  I  G  I  N  N  Q  Q  L
1141 TCTGTTCTTGACGGGACAGAATTTGCTTATGGAACCTCCTCAAATTTGCCATCCGCTGTA 1200
     S  V  L  D  G  T  E  F  A  Y  G  T  S  S  N  L  P  S  A  V
1201 TACAGAAAAAGCGGAACGGTAGATTCGCTGGATGAAATACCGCCACAGAATAACAACGTG 1260
     Y  R  K  S  G  T  V  D  S  L  D  E  I  P  P  Q  N  N  N  V
1261 CCACCTAGGCAAGGATTTAGTCATCGATTAAGCCATGTTTCAATGTTTCGTTCAGGCTTT 1320
     P  P  R  Q  G  F  S  H  R  L  S  H  V  S  M  F  R  S  G  F
1321 AGTAATAGTAGTGTAAGTATAATAAGAGCTCCTATGTTCTCTTGGATACATCGTAGTGCT 1380
     S  N  S  S  V  S  I  I  R  A  P  M  F  S  W  I  H  R  S  A
1381 GAATTTAATAATATAATTGCATCGGATAGTATTACTCAAATCCCTGCAGTGAAGGGAAAC 1440
     E  F  N  N  I  I  A  S  D  S  I  T  Q  I  P  A  V  K  G  N
1441 TTTCTTTTTAATGGTTCTGTAATTTCAGGACCAGGATTTACTGGTGGGGACTTAGTTAGA 1500
     F  L  F  N  G  S  V  I  S  G  P  G  F  T  G  G  D  L  V  R
1501 TTAAATAGTAGTGGAAATAACATTCAGAATAGAGGGTATATTGAAGTTCCAATTCACTTC 1560
     L  N  S  S  G  N  N  I  Q  N  R  G  Y  I  E  V  P  I  H  F
1561 CCATCGACATCTACCAGATATCGAGTTCGTGTACGGTATGCTTCTGTAACCCCGATTCAC 1620
     P  S  T  S  T  R  Y  R  V  R  V  R  Y  A  S  V  T  P  I  H
1621 CTCAACGTTAATTGGGGTAATTCATCCATTTTTTCCAATACAGTACCAGCTACAGCTACG 1680
     L  N  V  N  W  G  N  S  S  I  F  S  N  T  V  P  A  T  A  T
1681 TCATTAGATAATCTACAATCAAGTGATTTTGGTTATTTTGAAAGTGCCAATGCTTTTACA 1740
     S  L  D  N  L  Q  S  S  D  F  G  Y  F  E  S  A  N  A  F  T
1741 TCTTCATTAGGTAATATAGTAGGTGTTAGAAATTTTAGTGGGACTGCAGGAGTGATAATA 1800
     S  S  L  G  N  I  V  G  V  R  N  F  S  G  T  A  G  V  I  I
1801 GACAGATTTGAATTTATTCCAGTTACTGCAACACTCGAGGCTGAATATAATCTGGAAAGA 1860
     D  R  F  E  F  I  P  V  T  A  T  L  E  A  E  Y  N  L  E  R
1861 GCGCAGAAGGCGGTGAATGCGCTGTTTACGTCTACAAACCAACTAGGGCTAAAAACAAAT 1920
     A  Q  K  A  V  N  A  L  F  T  S  T  N  Q  L  G  L  K  T  N
          1921 GTAACGGATTATCATATTGATCAAGTATCCAATTTAGT 1958
               V  T  D  Y  H  I  D  Q  V  S  N  L
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Figure 3. Nucleotide sequence and deduced amino acid sequence of truncated *cry1Ac* gene of Bt strain, T15.

search program of the National Centre for Biotechnology Information revealed that it is a new *cry*1*Ac* gene. It has 98 to 99 per cent homology with 1958 bp of already reported sequences of all *cry*1*Ac* genes (*cry*1*Ac*1 to *cry*1*Ac*24 till Aug, 2009). Deduced amino acid sequence of truncated *cry*1*Ac* of Bt isolate, T15 showed one to four per cent variation from all the other *Cry*1*Ac* sequences (*Cry*1*Ac*1 to *Cry*1*Ac*24, till Aug, 2009) by differing at 5 to 19 positions. Minimum variation at five positions (44, 61, 62, 276 and 293) was observed with six reported *Cry*1*Ac* sequences (*Cry*1*Ac*1, *Cry*1*Ac*7, *Cry*1*Ac*8, *Cry*1*Ac*9, *Cry*1*Ac*10 and *Cry*1*Ac*16). Maximum variation at 19 positions was observed in truncated *Cry*1*Ac* sequence Bt strain, T15 when compared to *Cry*1*Ac*17 (**Tables 4** and **5**).

4. Discussion

Restriction fragment length polymorphic (RFLP) analysis of *cry* genes amplified from novel Bt isolates could provide preliminary information about the diversity [15]. This is a two-step approach in which PCR amplification with specific primers is followed by restriction analysis of the PCR products. In the earlier studies, a novel *cry*1*A*-type gene and *cry*1*Ie*1 gene were detected in Bt isolates using RFLP of *cry* genes by [16,17], respec-

Table 4. Comparison of deduced amino acid sequence of *cry*1*Ac* gene of Bt isolate T15 with other sequences of *Cry*1*Ac.**

Sequences	Position of amino acid**																		
	11	44	61	62	96	148	156	169	198	199	206	233	248	277	293	316	350	366
*Cry*1*Ac* (T15)	I	H	G	E	Q	F	P	L	R	L	Y	R	P	G	T	W	I	L
*Cry*1*Ac*1		Q	V	D										D	S			
*Cry*1*Ac*2		Q	V	D		L					H			D	S			F
*Cry*1*Ac*3		Q	V	D		L								D	S			F
*Cry*1*Ac*4		Q	V	D		L								D	S			
*Cry*1*Ac*5		Q	V	D		L								D	S			F
*Cry*1*Ac*6		Q	V	D		L								D	S			F
*Cry*1*Ac*7		Q	V	D										D	S			
*Cry*1*Ac*8		Q	V	D										D	S			
*Cry*1*Ac*9		Q	V	D										D	S			
*Cry*1*Ac*10		Q	V	D										D	S			
*Cry*1*Ac*11		Q	V	D		L								D	S			F
*Cry*1*Ac*12		Q	V	D		L								D	S			F
*Cry*1*Ac*13		Q	V	D		L								D	S			F
*Cry*1*Ac*14		Q	V	D		L								D	S			F
*Cry*1*Ac*15		Q	V	D										D	S			F
*Cry*1*Ac*16		Q	V	D										D	S			
*Cry*1*Ac*17			V	D		L				N				D	S			F
*Cry*1*Ac*18		Q	V	D		L				H				D	S			F
*Cry*1*Ac*19		Q	V	D			L							D	S			
*Cry*1*Ac*20		Q	V	D		L								D	S			F
*Cry*1*Ac*21		Q	V	D		L								D	S			F
*Cry*1*Ac*22		Q	V	D								T		D	S			
*Cry*1*Ac*23		Q	V	D	P	L		Y	S	P					S	C	M	Y
*Cry*1*Ac*24	N	Q	V	D		L							S	D	S			F

*upto 366 amino acids; **empty boxes indicates no variation.

Table 5. Comparison of deduced amino acid sequence of *cry1Ac* gene of Bt isolate T15 with other sequences of *Cry1Ac*[*].

Sequences	Position of amino acid[**]																	
	419	440	442	448	449	457	458	465	502	508	526	542	558	559	560	586	634	648
Cry1Ac (T15)	N	F	N	I	R	H	R	I	N	I	R	N	T	A	T	I	Q	Q
Cry1Ac1																		
Cry1Ac2		G	S															
Cry1Ac3			S															
Cry1Ac4		G																
Cry1Ac5			S															
Cry1Ac6			S															
Cry1Ac7																		
Cry1Ac8																		
Cry1Ac9																		
Cry1Ac10																		
Cry1Ac11		S																
Cry1Ac12			S															
Cry1Ac13			S															
Cry1Ac14		G	S															
Cry1Ac15		G	S															
Cry1Ac16																		
Cry1Ac17	S		G			Y	G		Y		T		K	G	A	M	P	P
Cry1Ac18		G	S									Y						
Cry1Ac19																		
Cry1Ac20		G	S															
Cry1Ac21		G	S															
Cry1Ac22				M														
Cry1Ac23					S			T										
Cry1Ac24		G	S							F								

[*]From 367 to 652 amino acids; [**]empty boxes indicates no variation.

tively. In the present study, *Eco*RI digestion of 3'-truncated *cry1A* gene(s) fragments amplified from four indigenous isolates of Bt and the reference strain, HD1 showed the presence of *cry1Ac* gene alone in three of the four Bt isolates. Therefore, the banding patterns of the three indigenous isolates are different from that of the reference strain, HD1.

Lepidopteran active insecticidal crystal proteins are protoxins of molecular weight about 135 kDa. These protoxins are proteolyticallty cleaved into smaller active forms of molecular weight of 60 - 70 kDa derived from the N-terminal half of the protein [18]. The C-terminal half of 135-kDa *Cry*1 protoxins is not required for toxicity, if it could be eliminated and the cellular resources could be redirected to synthesize an equivalent additional amount of the N-terminal half, the specific toxicity—*i.e.*, the toxicity per unit of mass of bacterial insecticides—might be improved [19]. This would in essence convert *Cry*1 proteins by truncation into toxins like *Cry*2*A* or *Cry*3*A*.

In the present study, the 3'-truncated derivative of *cry*1*Ac* of a Bt strain, T15, was amplified and cloned into a T/A vector. Nucleotide sequencing data of the newly cloned (3'-truncated) *cry*1*Ac* gene showed 98 to 99 percent similarity with sequences of already reported *cry*1*Ac* genes. Deduced amino acid sequence (652 residues) of

*cry*1*Ac* of Bt isolate, T15 showed variation from already reported *Cry*1*Ac* sequences at 5 to 19 positions. Even slight variation in amino acid sequence within a *Cry* protein class can dramatically impact insecticidal activity [20]. Lee *et al*. [21] reported that *Cry*1*A*c proteins differing at only two amino acid positions exhibited a 10-fold difference in toxicity towards the gypsy moth, *Lymantria dispar*.

Tertiary structure of *Cry*1*A* toxins are formed of three domains. The domain I is extending from residue 33 to residue 253, containing eight helices. Residues from 265 - 461 and 463 to 609 are domain II and domain III, respectively [22]. Different mutational studies have demonstrated that domain I of *Cry* proteins involved in pore formation and toxicity [23]. In the present study, the amino acid variations were observed in domain I of *Cry*1*Ac* protein too (3 to 8 variations). Because of the variations in the *Cry*1*Ac* amino acid sequence of Bt isolate, T15 may influence the level of toxicity. Further studies on expression of the newly cloned truncated *cry*1*Ac* gene in recombinant bacteria will reveal its insecticidal property prior to its use in the development of indigenous Bt crops.

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