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Isolation, characterization, cloning and bioinformatics analysis of a novel receptor from black cut worm (*Agrotis ipsilon*) of *Bacillus thuringiensis* vip 3Aa toxins

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ABSTRACT

Black cutworm (BCW) is an economically important lepidopteran insect. The control of this insect by a Bt toxin and the understanding of the interaction between the Bt toxin and its receptor molecule were the objectives of this research work. A gene coding for a Vip3A receptor molecule was identified, characterized, and cloned, from the brush border membrane vesicles (BBMV) of the BCW. The nucleotide sequence analysis of the cloned putative Vip3A-receptor gene revealed that the gene was 1.3-kb long and exhibited no homology with any gene in the gene bank. We succeeded in identifying and characterizing most of the Vip3A-receptor gene sequence; and the nucleotide sequence analysis of the cloned putative Vip3A-receptor gene (accession no. KX858809) revealed about 92% of the expected sequence was recovered, which exhibited no homology with any gene in the GenBank.

1. Introduction

The primary action of Bt toxin occurs in the midgut of susceptible insects. The insect larval midgut is composed of outer longitudinal and circular muscle layers, bordered by a basal lamina and a monolayer of epithelial cells overlaid by the peritrophic membrane (Knowles, 1989; Sinu et al., 2010). The epithelial cell layer mainly consists of columnar and goblet cells. The columnar epithelial cells maintain an apical brush border of microvilli exposed to the midgut lumen. Goblet cells do not have microvilli; however, they contain an apical pore through which digestive enzymes are secreted into the midgut lumen (Du et al., 2013). These two epithelial cell types are held together by tight junctions to form a single layer of cells. Both the cell types communicate through electrochemical coupling within the tight junctions (Moffett and Koch, 1992). This coupling helps the epithelial cell layer to maintain a potassium gradient between hemolymph (blood) and the gut lumen. A potassium pump that creates an approximate 180 mV potential across the epithelial cell layer preserves the gradient. This large membrane potential is responsible for the symport of nutrients to the basement membrane using K^+ as the functional cation (Vasilyeva and Forgac, 1998). The insect's digestive tract,

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as stated above, is protected by a peritrophic membrane (Vasilyeva and Forgac, 1998), which is porous and extends through the whole length of the digestive tract, from mouth to anus. This membrane is considered extremely important as it is the first line of defense to protect the underlying cells from the damage caused by insect feeding. The pores in the peritrophic membrane are large enough to prevent any pH gradient between the gut lumen and the environment that surrounds the epithelial cells. Consequently, Bt toxins remain active and accessible even at extreme acidic and alkaline pH conditions. Insect midguts have been characterized either as acidic ($pH \leq 5$), such as in certain beetles, or alkaline ($pH = 10$), such as in various mosquitoes and moths. Certain other beetles such as corn rootworm have neutral midgut pH.

1.1. Toxin binding

Immunoactivation studies with activated Bt toxin suggest that their primary action is on the midgut epithelium of sensitive insects. Using ^{125}I-labeled Bt toxins (Osman et al., 2015) demonstrated their specific binding to the brush border membrane vesicles (BBMV), which are the primary constituents of the apical brush border of midgut epithelial cells (De Maagd, 1996) and, therefore, represent an ideal system for studying toxin interaction *in vitro*. Toxin binding studies have demonstrated a correlation between the toxicity and occurrence of high-affinity binding sites in the membrane of brush border vesicles (Valaitis et al., 1997). Several studies on the interactions between Cry toxins and BBMV have demonstrated that toxin binding is not a simple reversible process; the initial reversible binding is followed by irreversibility, and probably reflects the insertion of the toxin into the membrane (Valaitis et al., 1997). Competition binding assays with various Cry toxins using the brush border membranes of Lepidopteran insects revealed the complex nature of toxin binding sites, i.e., a given toxin may bind more than one class of binding sites in a single insect, and more than one toxin can compete for binding to a single site (Wolfersberger et al., 1987). The affinities of Cry1 toxins for the brush border membranes of lepidopteran insects ranged from low to high, with K_d values in the range of 10^{-7}–10^{-10} nM (Dorsch et al., 2002). Binding assays with CryIII toxins using beetle BBMV suggested that the binding affinity of CryIIIA toxins is lower than that of CryI toxins (Bravo et al., 2004). Toxin binding is the first step, and a very important one, in the mode of action of Bt toxins (Osman, 2012). In several instances, a positive correlation has been observed between toxin binding and toxicity. However, for certain Cry toxins, the toxicity correlates with the number of binding sites rather than binding affinity (Valaitis et al., 1997). In the lepidopteran insect *Lymantria dispar*, negative correlation between toxin affinity and toxicity was reported (Wolfersberger et al., 1987). However, in this particular case, the toxicity correlates with the ability of the toxin to inhibit the K^+-dependent amino acid uptake. Insects belonging to *Spodoptera* sp. are less susceptible to Cry1A toxins, despite their ability to bind the brush border membranes of these insects with high affinity (Midboe et al., 2001).

1.2. Receptor binding

The first step in the mode of action of Bt toxin appears to be its reorganization and binding to the high-affinity sites of specific receptors on the brush border surface in the midgut of the susceptible insect. Several immunological methods have been used to identify the toxin targets that apparently reside in the luminal brush border of the larval midgut (Bravo et al., 2005). Radio-labeled blotting of BBMV from tobacco budworm (TBW) and corn earworm using Cry1Aa, Cry1Ab, and Cry1Ac toxins revealed that Cry1Aa and Cry1Ab bind to 170 kDa proteins, and that Cry1Ac binds to proteins of sizes 120, 140, and 150 kDa. Similar experiments using THW BBMV revealed that Cry1Ac binds to proteins of sizes 120 and 210 kDa (Keeton et al., 1998) whereas Cry1Aa and Cry1Ab recognize only the 210 kDa proteins (Vadlamudi et al., 1995). The aim of the present study was to clone and characterize the Vip3A-receptor gene from black cutworm.

2. Materials and methods

2.1. Preparation of brush border membrane vesicles (BBMV)

Early fourth instar larvae of black cutworm were kept on ice for an hour and were then dissected to remove their midguts. BBMV were prepared from the midgut tissues using differential magnesium precipitation method given by Wolfersberger et al. (1987), in the presence of protease inhibitors [5 mg/mL pepstatin, antipain, aprotonin, and leupeptin; 1 mM PMSF; 5 mM benzamidine]. The midgut tissue was homogenized in 9 vol buffer A [300 mM mannitol; 5 mM EGTA; 17 mM Tris-HCl] using a glass Teflon homogenizer [9 up-and-down strokes at 3000 rpm]. An equal volume of 24 mM $MgCl_2$ solution was added to the homogenate, which was then re-homogenized. The homogenate thus obtained was left on ice for 15 min and then centrifuged at 4 °C, at 4500 rpm for 15 min. The supernatant was decanted into a fresh tube and centrifuged again at 4 °C, at 31,000*g* for 30 min. The supernatant obtained was discarded and the pellet was resuspended in a half volume of buffer A; the procedure was repeated with this suspension. The final pellet was resuspended in buffer A containing protease inhibitors, flash-frozen in liquid nitrogen, and stored at –85 °C.

2.2. Immunoprecipitation of Vip3A-Binding protein

Immunoprecipitation was carried out according to the method given by Vadlamudi et al. (1993). The antiserum Vip3Aa (25 μL) was added to 1 mL protein A-Sepharose CL–4B resin equilibrated in washing buffer [1% Nonidet P-40; 6 mM EDTA; 50 mM Tris-HCl; 250 mM NaCl], and mixed for one hour at 4 °C. After washing the blot three times with washing buffer, 700 μg of Vip3A toxin was added and the mixture was incubated for 1 h at 4 °C, followed by three more washes with the washing buffer. The BCW BBMV proteins (6 mg) were solubilized in 1 mL washing buffer containing 1% NP-40 and protease inhibitors [10 μg/mL pepstatin, antipain, aprotonin, and leupeptin; 5 mM iodoacetamide; 1 mM PMSF]. The insoluble proteins were removed by centrifugation at 15,000 rpm for 30 min. The soluble proteins were filtered through a 0.45-μm filter, added to 1 mL protein A-Sepharose beads linked to Vip3A toxins, and the mixture was stirred gently for 1 h at 4 °C. The sepharose beads were centrifuged and washed four times with washing buffer containing 0.25% NP-40 and 0.02% SDS. The toxin-protein complex was dissociated from the beads by heating the mixture in the sample buffer (El-Menofy et al., 2014). Binding proteins were stained with Coomassie brilliant blue and detected by using ligand blotting with ^{125}I-Vip3A toxin and western blotting using Vip3A antiserum [provided by Microbial Genetics lab (AGERI, Egypt)]. The proteins were resolved by SDS-PAGE and transferred to a PVDF membrane.

2.3. Amino acids sequencing

The putative receptor protein band was pulled out of immunoprecipitation SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membrane using an electric blotter (Fisher Scientific, Pittsburgh, PA) at 0.8 mA/cm^2 for 50 min. The blotted protein was stained with Coomassie brilliant blue for 5 min and destained for 10 min. Both the staining and destaining buffers were prepared

without acetic acid to prevent amino acid modification. The membrane containing the putative receptor protein band was excised and several pieces of the cut membrane containing approximately 100 μg protein were prepared for amino acid sequencing according to the following protocol: The protein was reduced and alkylated using N-isopropyl iodoacetate and then digested with endoproteinase Lys-C. The separation of the resulting peptides was accomplished by High-Pressure Liquid Chromatography (HPLC). A purified fragment obtained from a single highly-resolved major peak was eluted and subjected to sequential Edman degradation using microsequencer (Applied Biosystems Inc.). A seventy-five base oligonucleotide corresponding to the sequence of 25 N-terminal amino acids of the proteolytic fragment was then synthesized.

2.4. Isolation of total RNA and poly (A) RNA

Total RNA was isolated from the BBMV of fourth instar larvae of BCW using TRIzol method. TRIzol reagent was used to maintain the integrity of the total RNA. In order to isolate the total RNA, chloroform was added to the BCW midgut, followed by centrifugation to separate the solution into an aqueous and an organic phase. RNA remained exclusively in the aqueous phase, and the recovery of this RNA was carried out by isopropanol precipitation. The poly (A) RNA was then isolated from this total RNA by using PolyATract™ mRNA isolation system (Promega).

2.5. Isolation of cDNA encoding BCW Vip3A-R

The first strand of cDNA was synthesized using oligo (dT) and random hexamer primers, which were used as a template for amplification. Reverse transcriptase was used to clone the Vip3Aa-R sequence and the degenerate primers, based on the amino acid sequence, were used to clone the fragments of Vip3Aa-R. We adopted the 3′ RACE techniques to accomplish this objective. The 3′ end was amplified using gene-sense primer GSP (G4) and Abridged Universal Amplification Primer (AUAP). The gene primers were annealed to the RNA and PCR reactions were performed as instructed for the 3′ RACE systems (GIBCO BRL). Generally, the PCR was performed in 50 μL reaction volume. The reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 μM of dGTP, dATP, dCTP and dTTP, 2.5 units of *Pfu, rTth* or *Taq* DNA polymerase, 0.2 μM of the above-mentioned primers and the synthesized cDNA. The amplification was carried out for 35 cycles, each consisting of a denaturing step at 94 °C for 30 s, annealing at 45–55 °C for 30 s, and extension at 72 °C for 1–5 min [depending on the primers, T_m, and product length]. The last cycle was followed by a 7-min extension at 72 °C. The products were separated on a 1–1.5% agarose gel run in 1X TBE buffer [89 mM Tris base; 89 mM boric acid; 2 mM EDTA]. The PCR product fragment of the predicted size was isolated and sub-cloned into TA cloning vector pCR2.1 (Invitrogen), and transformed into host *E. coli* INVαF. The cloned gene was sequenced according to the method given by Sambrook et al. (1989).

2.6. Sequence and domain analysis

Two programs were used to detect the open reading frame of the isolated sequence: NCBI's ORF Finder (http://www.ncbi.nlm.nih.gov/orffinder) and CLC Genomics Workbench 3.6.5. NCBI's BLASTx was used to identify this sequence in related organisms. SMART online program (Letunic et al., 2015) was used for protein domain analysis.

3. Results

3.1. Immunoprecipitation of Vip3Aa-Binding protein

Immunoprecipitation experiments were conducted using Vip3A toxin, which has the highest binding affinity for solubilized BBMV prepared from the midgut epithelial cells of BCW larvae. The Vip3A toxin binding protein was precipitated by adding anti-Vip3A serum to protein A-Sepharose. The Vip3A toxin was added and incubated with the solubilized BBMV proteins from BCW. The bound proteins were precipitated by centrifugation and separated on 10% denaturing SDS-PAGE (Fig. 1). This demonstrated the selective precipitation of the ~48 kDa protein from the solubilized BBMV of BCW along with Vip3A toxin. Previously, it has been demonstrated by radio-labeled blotting using ^{125}I-Vip3A that Vip3A toxin binds to the same ~48 kDa protein from the BBMV of BCW (Osman et al., 2017); this suggests that the precipitated protein formed a complex with Vip3A toxin, anti-Vip3A is the only binding protein for Vip3A, and it is the same binding protein that is found on the epithelial cells of BCW (Fig. 1).

3.2. Isolation and sequencing of cDNA encoding BCW Vip3A-receptor

The strategy used to clone the gene that encoded the putative receptor for Vip3A toxin from Bt involved purifying the protein, sequencing the proteolytically-generated peptides, and then synthesizing the gene oligonucleotide primers based on their amino acid sequences. The receptor protein was immunoprecipitated, fractionated in putative receptor band on SDS-PAGE as mentioned earlier, and electrically transferred to a PVDF membrane. The target band (~48 kDa) was excised and the amino acids at its N-terminal end were identified using sequential Edman degradation procedure. The purified protein generated an internal peptide with 17 amino acids, having the following sequence: NH2-ISAGDIVSDPAESLRTC-COOH. On the basis of this information, an oligonucleotide primer G4 (24-mer) was designed and used in the amplification of the specific DNA segment coding for Vip3A toxin receptor. The oligonucleotide primers, G4 and AUAP [the

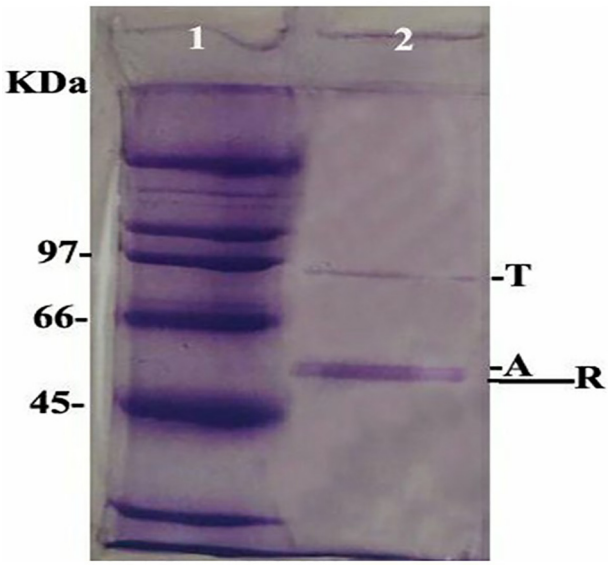

Fig. 1. Immuno-precipitation of the Vip3Aa toxin binding protein. The Vipp3Aa binding protein was precipitated by adding anti Vip3Aa serum to Sepharose protein-A. Vip3Aa was added followed by the incubation with the solubilized BCW BBMV proteins. The bound proteins were separated by 10% SDS-PAGE, as shown in lane 2, while lane 1 contains high molecular weight protein marker.

latter primer was provided in the RACE kit] were used in 3′ rapid amplification of the cDNA ends using RACE technique. The 3′ RACE system is useful for the amplification of rare messages for which little sequence information is available, and for capturing the 3′-end information of mRNA. These two DNA primers were directed to amplify the specific DNA fragment corresponding to Vip3A toxin receptor by using the cDNA prepared from the midgut of BCW larvae as a template. The amplified PCR product (1.37 kb) was cleaned before being cloned into a TA-cloning vector (PCR 2.1, 3.9 kb). The recombinant TA-cloning vector was transformed into competent cells of *E. coli* INVαF strain. The presence and identity of correctly inserted Vip3A toxin-R from BCW was confirmed with PCR and *EcoRI* digestion to release the insert (1.3 kb) for positive transformants (Fig. 2) and DNA sequencing. The complete nucleotide sequence was determined by auto sequencing technology, and the size of the insert was found to be 1372 nucleotides in length. The sequences of the 3′ ends were obtained using forward and reverse M13 sequencing primers, in amounts sufficient to obtain the complete nucleotide sequence of the Vip3A toxin-R. The receptor gene exhibited homology to a family of extracellular glycoproteins, known as Tenascins (Mor et al., 2012). This family of proteins contains EGF-like repeats that are known to interact with multiple ligands and perform a role in cell adhesion. It also exhibited homology to the family of proteins with the so-called death-domain, which is involved in protein interactions (Mor et al., 2012). Open reading frame analysis by two methods (Fig. 3) confirmed that there is a partial coding sequence from nt 1 to 1182; therefore, we suggest that the sequence from nt 1183 to 1312 is a part of mRNA 3′ UTR. There were 393 amino acids; the deduced polypeptide molecular mass was estimated to be 43.7 kDa, and it had a calculated isoelectric point (pI) of 8.5. However, the protein data indicated that the molecular weight of the receptor was 48 kDa. Therefore, we suggest that the obtained nucleotide sequence recovered about 92% of the expected sequence. The domain analysis revealed the localized Knot1 (knottin domain) and RPT (internal repeat 1) (Figs. 4 & 5). In arthropods, proteins containing the knottin domain are found in defensins. They are known as knottins or cystine-knot peptides and consist of small inhibitors, toxins, and

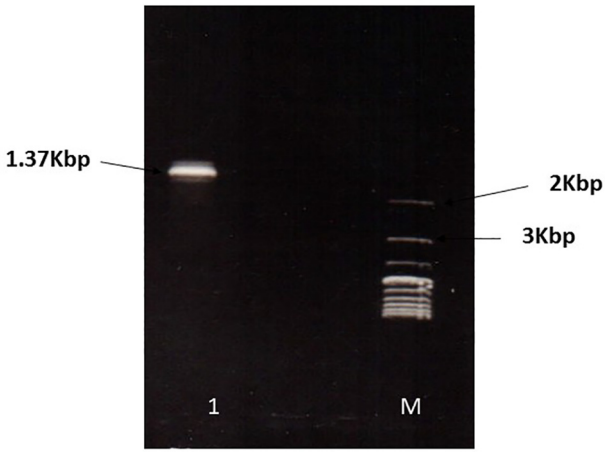

Fig. 2. Ethidium Bromide stained agarose gel resolving the 1.37 Kbp PCR fragment of Vip3 Aa-R gene from BCW cDNA. Lane 1: Step DNA Marker and lane 2: The amplified PCR fragment.

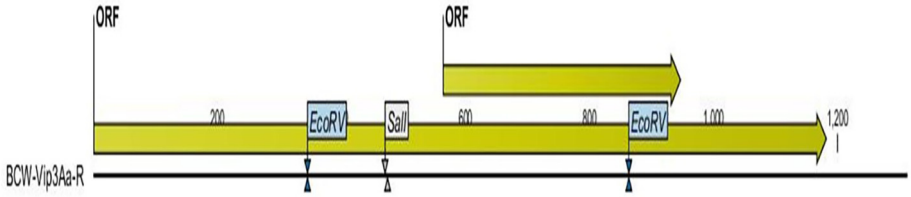

Fig. 3. CLC ORF restriction map for the obtained BCW-Vip3Aa-R sequence (accession No. KX858809). This sequence was characterized as ORF from nt (1) to nt (1182) and the presence of *EcoRV* site (at 345-863 nt) and *SalI* (at 470 nt).

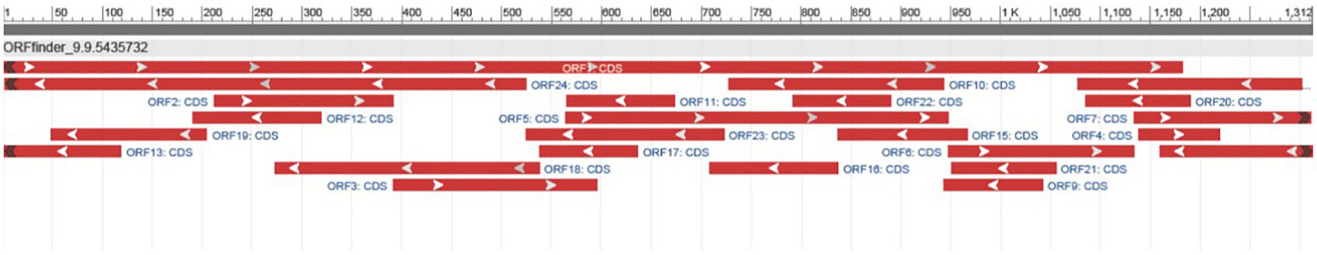

Fig. 4. NCBI ORF Finder for the obtained BCW-Vip3Aa-R sequence (accession No. KX858809). This sequence was characterized as ORF 1 from nt (1) to nt (1182).

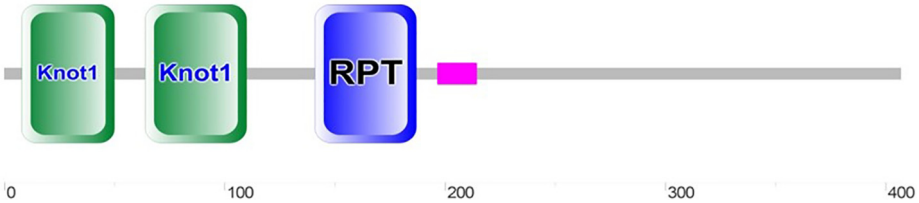

Fig. 5. The SMART diagram predicted domains, repeats, motifs and features of BCW-Vip3Aa-R protein.

Table 1
Accession numbers, description and the calculated e-value of homologous proteins to *Agrotis ipsilon* BCW-Vip3Aa-R amino acids sequence (accession No. KX858809) identified using BLASTx search.

Description	Total score	Query cover	E value	Ident	Accession
Spod-11-tox b protein [Spodoptera frugiperda]	1026	98%	2e−42	40%	AFC87713.1
immune related protein [Spodoptera frugiperda]	1025	98%	2e−42	40%	AAZ94260.1
x-tox [Spodoptera exigua]	645	98%	3e−41	39%	AKJ54496.1
Heli-5-tox protein [Helicoverpa armigera]	501	98%	9e−40	41%	AFC87714.1
PREDICTED: tenascin-like isoform X2 [Papilio xuthus]	929	98%	9e−32	31%	XP_013161775.1
PREDICTED: tenascin-like isoform X3 [Papilio xuthus]	884	98%	2e−16	33%	XP_013161776.1
Heli-7-tox protein [Helicoverpa armigera]	609	98%	8e−29	38%	AFC87715.1
PREDICTED: tenascin-like isoform X4 [Papilio xuthus]	841	98%	2e−28	34%	XP_013161778.1
antibacterial protein [Heliothis virescens]	662	98%	6e−28	42%	ACI02333.1
PREDICTED: tenascin-like isoform X1 [Papilio xuthus]	948	98%	1e−27	34%	XP_013161774.1
immune-related protein 5Tox [Bombyx mori]					

Table 2
Accession numbers, description and the calculated e-value of homologous 47 amino acid of RPT1 domain in *Agrotis ipsilon* BCW-Vip3Aa-R identified using BLASTp search.

Description	Total score	Query cover	E value	Ident	Accession
x-tox [Spodoptera exigua]	212	95%	0.011	42%	AKJ54496.1
Heli-7-tox protein [Helicoverpa armigera]	109	93%	0.052	44%	AFC87715.1
Spod-11-tox b protein [Spodoptera frugiperda]	249	100%	0.071	44%	AFC87713.1
immune related protein [Spodoptera frugiperda]	248	100%	0.072	44%	AAZ94260.1
antibacterial protein [Heliothis virescens]	104	91%	0.45	53%	ACI02333.1
Heli-5-tox protein [Helicoverpa armigera]	71.6	87%	0.67	53%	AFC87714.1
6tox [Galleria mellonella]	35.0	91%	1.3	40%	AAN15783.1
immune-related protein 5Tox [Bombyx mori]	67.4	89%	2.9	33%	AAZ94261.1
possible antimicrobial peptide [Bombyx mori]	33.1	87%	5.5	34%	BAE53370.1
antimicrobial protein 6Tox precursor [Bombyx mori]	66.2	89%	5.7	33%	NP_001037004.1

lectins (Gracy et al., 2008). Also, internal repeat 1 is, in most insects, related to antibacterial toxins (Table 1 & 2).

4. Discussion

The main objectives of this study were identification, isolation, and cloning of a receptor gene from black cutworm (BCW) *Agrotis ipsilon,* corresponding to an insecticidal protein expressed during the vegetative growth of *Bacillus thuringiensis*. The search along these lines is considered one of the avenues to biologically control the insect pests and alleviate the hazardous effects of chemical pesticides on humans and the environment. The characterization of the tissue targeted by Bt toxins and the histopathology developed inside the susceptible insect upon their ingestion are extremely important (Osman et al., 2013). Previous *in vitro* studies on the mechanism of toxicity have revealed a variety of symptoms, including paralysis of insect gut, disruption of the midgut epithelial cell structure and function, and cessation of feeding followed by death (EL-Ghareeb et al., 2012). The inhibition of K^+-dependent amino acid transport was observed when the BBMV were inoculated with activated toxins (Assaeedi et al., 2011; Abulreesh et al., 2012). Pore formation leading to colloid-osmotic lysis of midgut epithelial cells has also been proposed as a possible mechanism of toxin action (Osman et al., 2016). If the toxin forms pores, it is unknown whether the toxin does so by interacting with the proteins present in the BBMV or by insertion into the BBMV (Vadlamudi et al., 1993, 1995). It has also been concluded that several factors may influence the insect host range of the insecticidal proteins produced by Bt subspecies, which include: (i) solubilization of the crystal protein, (ii) processing by midgut proteases, (iii) presence of specific binding sites on the susceptible cells, and (iv) insertion of the bound toxin into the membrane (Vadlamudi et al., 1993, 1995). In a recent study, several proteins of size 48, 40, and 31 kDa from the BBMV of *Agrotis ipsilon* (BCW) were identified and purified (Osman et al., 2017). Immunoprecipitation with the same antiserum and subsequent ligand blotting with Vip3A toxin demonstrated binding of the toxin to the 48-kDa protein from the BBMV of BCW midgut. All the above-presented evidence unequivocally demonstrated that the 48-kDa protein is a specialized binding protein for the Vip3A toxin only. This result strongly suggests that the insect host range specificity of the Bt toxins is dependent on receptor-mediated differences in the midgut of the insect as well as on the nature and type of the toxins (Maaty, 1999). The cloning strategy for the gene encoding the 48-kDa protein from the BBMV of BCW was based on obtaining the amino acid sequence of the N-terminal of this protein. As described in the materials and methods section, the 48-kDa protein was immune-precipitated, and its N-terminal end was sequenced. A G4 primer was designed and used with AUAP primer provided in the RACE kit to amplify the target gene from the cDNA prepared from the BCW midgut tissue. This resulted in the amplification of a 1.37-kb DNA fragment corresponding to the Vip3A receptor molecule. This fragment was cloned into a TA-cloning vector and its identity was confirmed using a variety of techniques including DNA sequencing. This fragment had a single open reading frame of 1182 bases and 394 amino acids, with a pI of 8.5, and the deduced polypeptide molecular mass was estimated to be 43.6 kDa. The Vip3A-R gene exhibited a little homology to a family of extracellular glycoproteins, known as Tenascins (Mor et al., 2012). On the other hand, several researchers have purified toxin-binding proteins from BBMV of insect midgut cells. In *M. sexta*, a 210-kDa cadherin-like protein and a 120-kDa glycosylphosphatidylinositol (GPI)-anchored aminopeptidase N (APN) were identified, which bound Cry1Ab and Cry1Ac, respectively (Knight et al., 1995). Further, a 106-kDa aminopeptidase was identified as a Cry1C receptor in *M. sexta* (Luo et al., 1997). Cry1Ac-binding APN proteins have also been purified from *Lymantria dispar* (Valaitis et al., 1997), *Heliothis virescens* (Bravo et al., 2005), and *Plutella xylostella* (Luo et al., 1997) BBMVs. However, a report on receptors suggests that not all APN surface enzymes act as toxin-binding proteins (Zhou et al., 2016). Interference in the midgut function appears to be the common mechanism through which the most effective insecticidal proteins, including the Vip3A toxin, kill their target insect.

In conclusion, cloning, sequencing, and bioinformatics analysis of partial Vip3A-receptor gene were successfully carried out. However, the real mechanism through which Vip3A toxin affects its target insect BCW was not examined. Nonetheless, the results of the present study narrowed down the spectrum of mechanisms through which Vip3A toxin can kill BCWs to an interaction between the toxin and the receptor molecule, mediated by binding to the specific receptor. Moreover, the strength of binding determines the specificity of the toxin. It was hypothesized that the receptor molecules may aggregate to form a channel that transports nutrients into the insect gut, and Bt toxins probably interfere with this function either during channel formation or during nutrient transport. Vip3A is envisioned to exert its toxicity in this manner, although no evidence for this has been presented in this study.

Conflicts of interest

No conflict of interest exists.

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