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Isolation and Molecular Characterization of Some Common *Bt* Cry Protein Receptor Genes from Certain Living Organisms.

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عزل وتوصيف جزيئى لبعض جينات مستقبلات بروتينات الـ *Bt* من كائنات حيه معينة.

رسالة مقدمة من

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ABSTRACT

Bacillus thuringiensis (*Bt*) crystal proteins are effective in controlling agriculturally harmful insects. However, the mechanism of *Bt* pesticidal action is fairly understood. It is assumed that the pesticidal protein has affinity for specific receptors in the midgut of the susceptible larvae and binds irreversibly to create holes in the gut leading to eventual death of the target larvae.

The main aim of the research is to identify and characterize *Bt* δ-endotoxin receptor in an important pest, Egyptian Cotton leafworm (*Spodoptera littoralis*).

Biological activities of crystal protoxins of *Btk*HD1 strain and Cry1C were determined by *in vitro* mortality assay on neonate larvae of *S. littoralis*. Also, Brush border membrane vesicles (BBMVs) were prepared from the 5^{th} instar larvae of the same insect. Subsequently, immunoblotting experiments were performed using Cry1A *Bt* δ-endotoxins to detect the presence of specific toxin binding proteins on BBMVs. The toxin binds to proteins of 108.58 kDa on brush border membrane vesicles. It is therefore a strong candidate for *in vitro S. littoralis* Cry1 toxin receptor.

Specific primers for *S. littoralis* APN (*Sl*APN) were designed according to the nucleotide sequence of *Spodoptera litura* APN; GenBank accession number (AF320764). The full length of APN gene from that insect was obtained and it was cloned in pGEMT-easy vector and pET-30a and pPICZ A expression vectors.

The cloned gene was characterized through sequencing and studying expression in *E. coli* and *Pichia*

pastoris. The expressed protein was analyzed by Western blotting with anti- Cry toxin antibody. The Western blotting showed that the expression of target protein was detected by toxin. This analysis proved that APN protein exhibited binding properties towards Cry1A toxin. The sequence of *Sl*APN gene was deposited in GenBank under accession number JF509138.

The predicted amino acid sequence of *Sl*APN cloned gene encoded a putative 952 amino acid residues. The sequence possesses three conserved regions which are zinc-binding/gluzincin motif $HEX_2HX_{18}E$, the third zinc-binding ligand and gluzincin aminopeptidase motif GAMEN. Furthermore, five potential N-linked glycosylation sites (NXS/T) beside four highly conserved Cys residues are also observed. Finally, a highly conserved 64 amino acid residues from Leu 129 to Pro 193 was also detected.

Based on computational alignment, a theoretical 3-D model structure of *Sl*APN was obtained. Whereas, about 814 aa residues of whole suggested 3-D model were corresponded to residues 58-871 of the primary structure. The present *Sl*APN model contained four structural domains, which spread from N-terminal domain I to C-terminal domain IV over the regions Asn^{58}-Ile^{266}; Ser^{267}-Gly^{506}; Asn^{507}-Leu^{581} and Ser^{582}-Ala^{871}, respectively. The recorded overall dimensions of this model were 91Aº x 55 Aº x 65Aº forming together a hook like structure.

Key words:

Bacillus thuringiensis; δ-Endotoxin; Aminopeptidase N receptor; *Spodoptera littoralis*; 3-D homology modeling; *Pichia pastoris*.

CONTENT

LIST OF ABBREVIATIONS

A260	Absorbance at 260 nanometer
A280	Absorbance at 280 nanometer
AlP	Alkaline Phosphatase
Amp	Ampicillin
APN	Aminopeptidase-N
APS	Ammonium peroxosulphate
ATP	Adenosine triphosphate
BBMV	Brush Border Membrane Vesicle
BCIP	5-bromo 4-chloro 3-indolyl phosphate
BLAST	Basic local alignment search tool
BLASTn	Nucleotide blast (Search a nucleotide database using a nucleotide query)
BLASTx	Search protein database using translated nucleotide query
Bm	*Bombyx mori*
bp	Base pair
***Bm*APN**	*Bombyx mori* Aminopeptidase-N
BSA	Bovine Serum Albumin
Bt	*Bacillus thuringiensis*
Btk	*Bt* subsp. *krustaki*
cDNA	Complementary DNA
Cry toxin	Crystal toxin
C- terminal	Carboxy terminal

DTT	Dithiothreitol
DEPC	Diethylpyrocarbonate
dH_2O	Distilled Water
DNA	Deoxyribonucleic Acid
E. Coli	*Escherichia coli*
F1 *Sl*APN	A segment of *Sl*APN (from bp 1 to 2185) was excised from recombinant vector pGEM T-Easy/*Sl*fl with *Eco*R1
GPI	glycosylphosphatidyl-inositol
IPTG	Isopropyl-β-D-thiogalactopyranoside
Kb	Kilobase(s)
KDa	Kilodalton(s)
LB broth	Luria-Bertani broth
LC50	50% lethal concentration
µg	Microgram(s)
min	Minute(s)
MW	Molecular Weight
NBT	Nitro blue tetrazolium chloride
OD	Optical density
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PEG	Polyethylene glycol
PFT	Pore-Forming Toxins
P. pastoris	*Pichia pastoris*
PM	Peritrophic Membrane
PMSF	Phenylmethylsulfonyl fluoride
Ppm	Part per million

PVDF	Polyvinylidene difluoride
RE	Restriction Endonucleases
RNA	Ribonucleic Acid
rpm	Revolution per minutes
RT-PCR	Reverse transcription- PCR
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
Sl	*Spodoptera littoralis*
***Sl*APN**	*Spodoptera littoralis* APN
***Sl*fl**	Full-length cDNA of *Sl*APN
Sl7	5'-truncated 1601 bp fragment of *Sl*APN
Sl8	3'- truncated 1359 bp fragment of *Sl*APN
TEMED	Tetramethylethylenediamine
U	Unit
UV	Ultraviolet
W/V	Weight Per Volume
X-gal	5-bromo 4-chloro 3-indolyl β- D-galactopyrannoside

LIST OF FIGURES