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Short communication

Leucine Rich Repeat Sequence of the δ Endotoxin Family of *Bacillus thuringiensis*

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In this investigation we report our search for the presence of Leucine Rich Repeats (LRRs) in various *Bacillus thuringiensis* (*Bt*) sub species. Leucine rich repeats are short sequence motifs present in some proteins. The consensus sequence corresponding to the LRR was present in Crystal proteins of *Bacillus thuringiensis* sub species. This LRR sequence has been predicted to be involved in protein-protein interactions or receptor binding functions, hence the importance of this study.

Keywords: *Bacillus thuringiensis*, Crytal protein, Leucine rich repeats.

Introduction

During our structural studies on *Bacillus thuringiensis var israelensis* (*Bti*) 130, 72 and 28 kDa proteins, we observed the presence of a leucine rich repeat (LRR) like structure in 130 kDa protein (Suvarchala, 1998). We also searched for the presence of LRRs in other *Bt* sub species. The objective of the present investigation was to look for LRR like structures in sub species of δ-endotoxin family of *Bacillus thuringiensis*. These structures may play an important role in protein-protein interactions.

Leucine rich repeats (LRRs) are short sequence motifs present in a number of proteins with diverse functions and cellular locations. All proteins containing these repeats are thought to be involved in interaction with membrance proteins (Kobe and Deisenhofer, 1994).

Methods

The consensus sequences were compiled from all known LRR containing proteins; they were found to contain leucines or other aliphatic residues at position 2, 5, 7, 12, 16, 21, 24 and asparagine, cysteine or threonine at position 10. The consensus sequence is generally denoted by xLxxLxLxxNx. Most proteins were found to contain exclusively asparagine at position 10 but some had exclusively cysteine in this position (Kobe & Deisenhofer, 1994).

However the functional and evolutionary significance of these residues at position 10 is not known. We observed Asparagine at position 10 in case of *Bt israelensis*, threonine in *Bt entomocidus and Bt sotto* respectively. The hydrophobic consensus residues in the carboxy terminal parts of the repeats were generally spaced by 3, 4 or 7 residues (Table 1).

The consensus residues of the repeats play structural roles. The side chains of leucines and of other aliphatic residues (Positions 2, 5, 7, 12, 17, 20, 23 and 24) and of asparagines or threonine at position 10 form the core of the protein. The crystal structure of ribonuclease inhibitor protein has revealed that leucine rich repeats (LRRs) correspond to β-α structural units (Kobe and Deisenhofer, 1993). These units are arranged so that they form a parallel β-sheet with one surface exposed to solvent, so that the protein acquires an unusual nonglobular shape. These features may be responsible for the protein binding functions of proteins containing leucine rich repeats (Takahashi *et al.*, 1985). The spacing of leucines is responsible for proper packing of the β-strands and α-helices.

Results and Discussion

The δ-endotoxins of *Bacillus thuringiensis* appears to contain a conserved sequence like LRRs. The use of gene specific probes led to the discovery that various sub species of *Bacillus thuringiensis* contained one, two or three closely related genes. The comparison of the deduced amino acid sequence revealed a number of sequence elements conserved for most crystal proteins. These conserved amino acid sequences might have originated through the same gene which later diversified to give various functions and specificity. LRRs were found in a functionally and evolutionary diverse set of proteins (Table 2). The main function is involvement in protein-protein interactions, signal transduction and also in receptor binding.

Since *Bacillus thuringiensis* δ-endotoxin proteins are

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Table 1. Amino Acid Sequences Of *Bacillus thuringiensis* SubSpecies Showing LRR Like Structures

Strain	crystal protein	Consensus sequence															
israelensis	IV A	**409**	2			5					10					15	
		K	L	K	S	L	G	L	A	T	N	I	Y	I	F	L	
						20					25		**434**				
		L	N	V	I	S	L	D	N	K	Y	L					
																	(Ward and Ellar, 1987)
entomocidus	I(A) a	**351**	2			5					10					15	
		S	L	T	G	L	G	I	F	R	T	L	S	S	P	L	
						20					25					30	**380**
		Y	R	R	I	I	L	G	S	G	P	N	N	Q	E	L	
																	(Masson *et al.*, 1989)
sotto	I(A)a	**351**	2			5					10					15	
		S	L	T	G	L	G	I	F	R	T	L	S	S	P	L	
						20					25					30	**380**
		Y	R	R	I	I	L	G	S	G	P	N	N	Q	E	L	
																	(Shibano *et al.*, 1985)

Table 2. Amino acid sequences of *Bacillus thuringiensis* subspecies showing LRRs containing 'L' at position 10.

Strain	crystal protein	Consensus sequence															
sandiego	III A	**281**	2			5					10					15	
		T	L	T	V	L	D	L	I	A	L	F	P	L	Y	D	
						20					25					30	**310**
		V	R	L	Y	P	K	E	V	K	T	E	L	T	R	D	
																	(Herrnstadt *et al.*, 1987)
entomocidus	I C	**237**	2			5					10					15	
		T	L	T	V	L	D	I	V	A	L	F	S	N	Y	D	
						20					25					30	**266**
		S	R	R	Y	P	I	R	T	V	S	Q	L	T	R	E	
																	(Masson *et al.*, 1989)
sotto	I(A)a	**237**	2			5					10					15	
		T	L	T	V	L	D	I	V	A	L	F	S	N	Y	D	
						20					25					30	**266**
		S	R	R	Y	P	I	R	T	V	S	Q	L	T	R	E	
																	(Shibano *et al.*, 1985)
kurstaki	I(A) b	**237**	2			5					10					15	
		T	L	T	V	L	D	I	V	S	L	F	P	N	Y	D	
						20					25					30	**266**
		S	T	R	Y	P	I	R	T	V	S	Q	L	T	R	E	
																	(Hefford *et al.*, 1987)
aizawai	I(C)	**237**	2			5					10					15	
		T	L	T	V	L	D	I	V	S	L	F	P	N	Y	D	
						20					25					30	**266**
		S	T	R	Y	P	I	R	T	V	S	Q	L	T	R	E	
																	(Hofte *et al.*, 1990)

glycoproteins, these LRR structures might help in binding to the receptor on the midguts of susceptible larvae. It is also known that the α-helix which represents the sequence YESWVNFNRYPREMTLTVLDLIVSLFX of Cry III A δ-endotoxin serves as a binding sensor that initiates the binding of the pore domain to the membrane (Gazit and shaw, 1994). Similarly the sequence from δ-endotoxin of *Bacillus thuringiensis* var *israelensis* contains consensus sequence of leucine rich repeat which might be involved in membrane - protein interaction, protein dimerization or receptor binding

functions. It is therefore evident that the δ-endotoxin family of Bt sub species appear to posses the LRR sequence which were not reported earlier.

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