

**Bio-Safety Evaluation of Cry1C Protein  
Expressed in Bt cotton carrying cry1C gene  
event MLS9124**

*Submitted to*  
**Review Committee on Genetic Modifications**  
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## *1. Executive Summary*

This document presents a consolidation report on the bio-safety analysis of the Cry 1C protein expressed in Bt-cotton plants carrying the event MLS9124, developed by Metahelix Life Sciences, Private Limited. The scientific, two-tiered, weight-of-evidence strategy as recommended by the International Food Biotechnology Committee of the International Life Sciences Institute, Washington DC, USA has been adopted in this analysis. Accordingly, tier I studies included those which identify potential hazards like history of safe use of the protein, bioinformatics analysis, mode of action, lab studies, and protein levels in predicted dietary intake. All these analyses showed that the Cry 1C protein expressed in the Bt cotton plants does not pose any real or potential danger to man, livestock or to the environment. As such, it is argued that further tier II studies, which include acute toxicological study using the purified Cry 1C protein, are not necessary in order to establish the safety of the Bt-cotton hybrids carrying the event MLS9124.

## 2. Introduction

Recently the Department of Biotechnology, Government of India, has come out with new guidelines for pure-protein based studies for determining the potential for toxicity and allergenicity of foreign proteins expressed in transgenic crops plants. Such studies are to be undertaken on a case-to-case basis in order to minimize any potential risks while at the same time, balancing it with practical considerations like feasibility and costs. Recently, the Food Biotechnology Committee of the International Life Sciences Institute, Washington DC, USA has recommended a two-tier approach in order to evaluate protein safety in the context of agricultural biotechnology<sup>1</sup>. Tier-I analyses draw on the prior knowledge and testing methods for evaluating the potential hazards of the expressed protein which include history of safe use, bioinformatics analysis, mode of action, *in vitro* digestibility and stability and the expression levels and dietary intake. If any potential hazard is identified by these analyses, then the hazard is characterized by tier-II studies which include acute toxicology study, repeated-dose toxicology and other hypothesis-based evaluations.

Metahelix Life Sciences has developed Bt-cotton plants carrying the event 9124 expressing the Cry1C protein which confers resistance to *Lepidopteran* pests in cotton. All the biosafety studies using the plant tissues prepared for Bt cotton carrying event 9124 as prescribed earlier have been completed and all those studies show that it is safe<sup>2</sup>. Further, the evaluation of the safety of the expressed Cry1C protein by adopting the two-tiered, weight-of-evidence strategy approach is reported here in this document. All the components of the tier-I analyses tend to suggest that the Cry1C protein expressed in Bt-cotton plants carrying the event 9124 is safe. It is further argued that, for this reason, acute toxicity studies with the purified Cry1C protein are not necessary.

## 3. History of safe use of Cry1C

One of the most widely used *Bacillus thuringiensis* strains in the biological control of insect pests of crops is *aizawai* (Bta strain) which carries the crystal protein Cry1C. The very high insecticidal activity of this Cry protein on the diamondback moth and the army worm (*Plutella xylostella*, and *Spodoptera* spp. respectively) combined with the impeccable safety record of the strains carrying this crystal protein has been primarily responsible for this widespread use. Extensive studies on biosafety of this strain have been carried out, mostly in the US and details

have been published under the joint sponsorship of the UNEP, ILO, WHO and the International Program of Chemical Safety are available<sup>3</sup>. Relevant extract of this report is attached as **Annexure I**. In summary, in a number of studies to assess the acute toxicity and pathogenicity of commercial Bt formulations in birds (young mallards, *Anas platyrhynchos*; bobwhite quail, *Colinus virginianus*) and aquatic vertebrates (frogs, *Hyla regilla* and *Rana temporaria*; gold fish, *Carassius auratus*; mosquito fish, *Gambusia affinis*; newts, *Taricha torosa* and *Triturus vulgaris*; rainwater killifish, *Lucania parva*; toads, *Bufo* spp. and rainbow trout, *Oncorhynchus mykiss*) there was no apparent toxicity or pathogenicity. In the study with birds, it was also observed that the feed consumption and consequent weight gain were similar in Bt-treated and non-treated controls. The US Environmental Protection Agency (EPA) has approved the registration of several formulations, either Bt strains or *Pseudomonas fluorescens* engineered to express Cry1C protein (**Annexure II**) as safe pesticides to be used in all agricultural crops including fruits, vegetables, corn, cotton and nuts; turf; forests; ornamentals; landscape trees; nursery crops. The long history of safe use of these formulations even in crops which are eaten raw like lettuce attest to the high degree of safety of the organism and the Cry1C protein that is expressed in them. Many commercial products by some of the leading companies in the world like Abbot (Xentari, Florbac) and Novartis (Certan) contain the expressed Cry1C protein and are in wide-spread use in many countries<sup>4</sup>.

The gene coding for the Cry1C family of proteins has been extensively studied. A search of the GENBANK database shows many sequences of this family of protein deposited. A non-exhaustive list is presented in Table 1. There are also a number of studies in which Cry1C sequences have also been expressed in bacterial systems (like *Pseudomonas fluorescens*) or in higher plants (like cabbage, cauliflower, tobacco, rice, and several other crops). Strizhov et. al.<sup>5</sup> made transgenic lines of tobacco and alfalfa by introducing a truncated codon-optimized Cry1C gene (630 amino acids from the amino terminal end). The expression of the Cry 1C protein in the transgenic plants was found to afford protection against the Egyptian cotton leaf worm (*Spodoptera littoralis*) and the beet army worm (*Spodoptera exigua*).

**Table 1. Selection of Cry1C gene sequences deposited in public databases.**

<b>Gene designation</b>	<b>Accession No.</b>	<b>Reference</b>
Cry1C a1	X 07518	Honee et al., 1988. NAR 16: 6240
Cry1C a2	X 13620	Sanchis et. al., 1989. Mol. Micro., 3: 229-238
Cry1C a3	M 73251	Payne and Sick, 1993. US Patent 5246852
Cry1C a4	A 27642	Van Malleart et. al., 1990. European Patent 0400246
Cry1C a5	X 96682	Strizhov, et. al., 1996. MGG, 253:11-19.
Cry1C b1	M 97880	Kalwan et. al., 1993. AEM, 59:1131-1137

In a collaborative study between Cornell University, USA and the Max-Planck Institute, Germany, Elizabeth Earle and her co-workers expressed high levels of a synthetic Cry1C protein in transgenic broccoli<sup>6</sup>. They found these transgenic plants resistant to the diamondback moth as well as two other lepidopteran pests of crucifers, the cabbage looper and the imported cabbage worm. Notably, in this study they found that the Cry 1 C protein was effective against diamondback moth larvae which were resistant to the Cry 1A group of Bt toxins. In a more recently reported study from China<sup>7</sup>, Qifa Zhang and his colleagues have developed Bt rice expressing a synthetic, codon-optimized, truncated Cry1C gene and conducted field trials of the hybrid rice, Hy-19-Zh, expressing this gene, in 2004 rice growing season at Wuhan, China. They found that these hybrids expressing the Cry1C protein were highly resistant to both leaf folders and stem borers even under conditions of extreme pest infestations that completely damaged the non-transgenic hybrid Shanyou 63, that was used as a control.

In a very significant recent study<sup>8</sup>, Cry1C expressing broccoli plants were exposed to the laboratory bred resistant strains of diamondback moth, *Plutella xylostella* in order to understand the effect of the Cry1C protein on the parasitoid, *Diadegma insulare*, which is a natural enemy of *Plutella*. The results of this study provide the first clear evidence of the lack of hazard to a parasitoid by a Bt plant, compared to traditional insecticides.

#### **4. Mode of action of Cry1C insecticidal protein**

The general mode of action of the class of insecticidal crystal proteins from *Bacillus thuringiensis* strains, at both physiological and molecular levels, has been well studied and documented over last four decades. Crickmore and his colleagues<sup>9</sup> and more recently the group

led Soberon<sup>10</sup> have written up very comprehensive reviews on the subject. The important role of receptor interaction in determining the specificity of the Bt toxins to groups of insects is brought out in a recent article by Gomez<sup>11</sup> et. al. It has been known for quite some time now that the Cry1C toxin does not share a common binding site with the Cry1 A toxins<sup>12</sup>.

The reason for different insect specificities of the Cry1C protein as compared to other Cry proteins has been studied using binding assays with <sup>125</sup>I – or biotin labeled toxins and larval brush border membrane vesicles (BBMV)<sup>13</sup>. In the same study the pore-forming activity of the Cry proteins on BBMV were determined using voltage-sensitive fluorescent dye DiSC<sub>3</sub><sup>14</sup>. In competition experiments it was observed that while Cry1 Aa, Cry1 Ab and Cry1 Ac proteins share a binding site, Cry1Ca and Cry1 Fa bind to a different site. It was also observed that all the active toxins induced increased membrane permeability which was related to binding activity. Based on their results the authors have recommended pyramiding of the Cry1C gene along with that of a member of the Cry1 A family for effective insect resistance management.

Sakai et. al.<sup>15</sup> have tried to understand the role of the protein domain of Cry1C protein that confers its specificity to *Spodoptera* sp. by domain swapping studies on the protein and then analyzing the cytotoxicity to Sf9 insect cell lines. They concluded that the domain III of the Cry1C protein is not crucial for cytotoxic specificity of the Cry1C against Sf9 cells.

## **5. Bioinformatics Analysis of Cry1C protein**

The protein sequence of Cry1C available in Metahelix was subject to an in silico analysis of pepsin and trypsin digestibility (Tables 2, 3 and 4 below)<sup>16</sup>. The number of cleavage sites in Cry1C for both enzymes is almost identical to the data obtained for Cry1Ac. There is experimental data available for an in vitro gastric digestion study cited in a biosafety assessment report where there is rapid degradation of the protein<sup>17</sup>. The rate of degradation of the Cry1Ac protein was evaluated in a simulated gastric fluid at pH 1.2 (constituted based on recommended levels in US Pharmacopeia, 1995). The degradation was assessed by Western blot analysis and insect bioactivity. The study showed that the Cry1Ac protein degrades in approximately 30 seconds upon exposure to gastric fluid. The acidic conditions of the stomach denature the native conformation of the Cry1Ac protein, facilitating its rapid degradation. The inference from results

obtained for Cry1Ac was extended to Cry1C based on the in silico analysis of pepsin digestibility. It is similar to Cry1Ac, and thereby safe. In vivo, the protein would be exposed to gastric conditions prior to entering the intestinal lumen. The low pH and pepsin in the stomach is expected to either fully digest the protein or render it susceptible to intestinal digestion. Tables 2, 3 & 4 below give the data of predicted cleavages of Metahelix's Cry1C protein by pepsin (pH 1.3 and pH > 2.0) and trypsin.

**Table 2: Pepsin (pH 1.3) – There are 186 cleavages by pepsin at pH 1.3.**

Position of cleavage site	Resulting peptide sequence	Position of cleavage site	Resulting peptide sequence
11	MEENNQNCIP	133	WEEDPNNPETRTRVIDRF
14	YNC	135	RI
15	L	138	LDG
21	SNPEEV	139	L
22	L	140	L
23	L	147	ERDIPSF
38	DGERISTGNSSIDIS	151	RISG
39	L	152	F
40	S	155	EVP
41	L	157	LL
43	VQ	159	SV
44	F	160	Y
45	L	165	AQAAN
48	VSN	166	L
54	FVPGGG	167	H
55	F	168	L
56	L	170	AI
58	VG	171	L
59	L	176	RDSVI
61	ID	177	F
62	F	181	GERW
63	V	183	GL
64	W	191	TTINVNEN
71	GIVGPSQ	192	Y
72	W	195	NRL
74	DA	201	IRHIDE
75	F	202	Y
76	L	209	ADHCANT
81	VQIEQ	210	Y
82	L	213	NRG
89	INERIAE	217	LNNL
90	F	222	PKSTY
98	ARNAAIAN	224	QD
99	L	225	W
101	EG	227	IT
102	L	228	Y
105	GNN	231	NRL
106	F	236	RRDLT
108	NI	237	L



109	Y	239	TV
112	VEA	240	L
113	F	244	DIAA
115	KE	246	FF
249	PNY	449	F
261	DNRRYPIQPVGQ	450	S
262	L	451	W
267	TREVV	457	TDRSAT
278	FNPQL	458	L
284	QSVACL	471	TNTIDPERINQIP
287	PTF	475	LVKG
300	NVMESSRIRNPHL	478	FRV
303	FDI	490	WGGTSVITGPGF
304	L	495	TGGDI
306	NN	496	L
307	L	501	RRNTF
309	TI	503	GD
310	F	504	F
312	TD	506	VS
313	W	507	L
314	F	519	QVNINSPITQRY
319	SVGRN	521	RL
321	FY	523	RF
322	W	525	RY
330	GGHRVISS	535	ASSRDARVIV
331	L	536	L
342	IGGGNITSPIY	553	TGAASTGVGGQVSVNMP
353	GREANQEPPRS	563	LQKTMEIGEN
355	FT	564	L
356	F	568	TSRT
361	NGPVF	571	FRY
363	RT	573	TD
369	LSNPTL	574	F
371	RL	577	SNP
382	LQQPWPAPPFN	579	FS
383	L	580	F
390	RGVEGVE	593	RANPDIIGISEQP
391	F	595	LF
397	STPTNS	604	GAGSISSGE
398	F	605	L
399	T	606	Y
400	Y	613	IDKIEII
408	RGRGTVDS	614	L
409	L	618	ADAT
425	TELPEDNSVPPREGY	619	F
434	SHRLCHATF	624	EAESD
441	VQRSOTP	625	L
443	FL	630	ERAQK
448	TTGVV		

**Table 3: Pepsin (pH >2.0) – There are 145 cleavages for pepsin at pH >2.0.**

Position of cleavage site	Resulting peptide sequence	Position of cleavage site	Resulting peptide sequence
14	MEENNQNQCIPYNC	152	F
15	L	155	EVP
21	SNPEEV	157	LL
22	L	165	SVYAQAAN
23	L	166	L
38	DGERISTGNSSIDIS	167	H
39	L	168	L
40	S	170	AI
41	L	171	L
43	VQ	176	RDSVI
44	F	177	F
45	L	183	GERWGL
48	VSN	195	TTINVNENYNRL
54	FVPGGG	213	IRHIDEYADHCANTYNRG
55	F	217	LNNL
56	L	231	PKSTYQDWITYNRL
58	VG	236	RRDLT
59	L	237	L
61	ID	239	TV
62	F	240	L
74	VWGIVGPSQWDA	244	DIAA
75	F	246	FF
76	L	261	PNYDNRRYPIQPVGQ
81	VQIEQ	262	L
82	L	270	TREVYTD
89	INERIAE	273	LIN
90	F	278	FNPQL
98	ARNAAIAN	284	QSVACL
99	L	287	PTF
101	EG	300	NVMESSRIRNPHL
102	L	303	FDI
105	GNN	304	L
106	F	306	NN
112	NIYVEA	307	L
113	F	309	TI
133	KEWEEDPNNPETRTRVIDRF	310	F
135	RI	313	TDW
138	LDG	314	F
139	L	319	SVGRN
140	L	330	FYWGGHRVISS
147	ERDIPSF	331	L
151	RISG	353	IGGGNITSPIYGREANQEPPRS
		355	FT
356	F	574	F
361	NGPVF	577	SNP
363	RT	579	FS
369	LSNPTL	580	F
371	RL	593	RANPDIIGISEQP
382	LQQPWPAPPFN	595	LF
383	L	604	GAGSISSGE

390	RGVEGVE	605	L
391	F	613	YIDKIEII
397	STPTNS	614	L
398	F	618	ADAT
408	TYRGRGTVDS	619	F
409	L	624	EAESD
434	TELPEDNSVPPREGYSHRLCHATF	625	L
441	VQRSGTP	630	ERAQK
443	FL		
448	TTGVV		
449	F		
457	SWTDRSAT		
458	L		
471	TNTIDPERINQIP		
475	LVKG		
490	FRWGGTSVITGPGF		
495	TGGDI		
496	L		
501	RRNTF		
503	GD		
504	F		
506	VS		
507	L		
521	QVNINSPITQRYRL		
523	RF		
535	RYASSRDARVIV		
536	L		
553	TGAASTGVGGQVSVNMP		
563	LQKTMEIGEN		
564	L		
568	TSRT		

**Table 4: Trypsin – There are 55 cleavage sites when digested with trypsin.**

Position of cleavage site	Resulting peptide sequence
27	MEENNQNQCIPYNCLSNPEEVLLDGER
86	ISTGNSSIDISLSLVQFLVSNFVPGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINER
92	IAEFAR
114	NAAIANLEGLGNNFNIIYVEAFK
126	EWEDPNNPETR
128	TR
132	VIDR
134	FR
142	ILDGLLER
148	DIPSFR
172	ISGFVPLL SVYAQAANLHLAILR
180	DSVIFGER
194	WGLTTINVNENYNR
197	LIR
212	HIDEYADHCANTYNR
219	GLNNLPK

230	STYQDWITYNR
232	LR
233	R
252	DLTLTVLDIAAFFPNYDNR
253	R
264	YPIQPVGQLTR
294	EVYTDPLINFNPQLQSVAQLPTFNVMESSR
296	IR
318	NPHLFDILNNLTIFTDWFSVGR
326	NFYWGGHR
344	VISLIGGGNITSPIYGR
352	EANQEPPR
362	SFTFNGPVFR
370	TLSNPTLR
384	LLQQPWPAPPFNLR
401	GVEGVEFSTPTNSFTYR
403	GR
422	GTVDLSTELPPEDNSVPPR
428	EGYSHR
437	LCHATFVQR
454	SGTPFLTGGVFSWTDR
466	SATLTNTIDPER
474	INQIPLVK
477	GFR
497	VWGGTSVITGPGFTGGDILR
498	R
518	NTFGDFVSLQVNINSPITQR
520	YR
522	LR
524	FR
529	YASSR
532	DAR
556	VIVLTGAASTGVGGQSVNMPLQK
567	TMEIGENLTSR
570	TFR
581	YTDFSNPFSFR
609	ANPDIIGISEQPLFGAGSISSGELYIDK
627	IEIILADATFEAESDLER
630	AQK

## 6. Potential Toxicity of Cry1C Protein vis-à-vis known Bacterial and Plant Toxins

Pairwise sequence alignment analysis was conducted for the Cry1C protein against each protein of a subset of extremely lethal bacterial and plant toxins. The sequences of these toxins were downloaded from the public databases. The results are compiled in Annexure III. None of these known toxins were homologous to the polypeptide sequence of Metahelix's Cry1C toxin and is therefore, considered to be safe for animals and humans.

## 7. Testing Thermal Stability and Pepsin Digestibility of the Cry1C Endotoxin

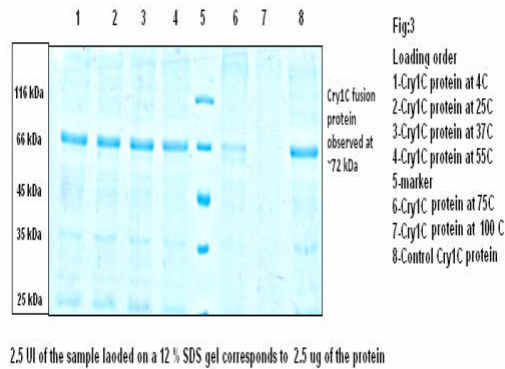
The thermal stability and pepsin digestibility of the Cry1C endotoxin was tested in an effort to augment our understanding biosafety of the Bt cotton event 9124. A recombinant Cry1C

endotoxin was over-expressed in *E. coli* (**Annexure IV**). This protein was subjected to different temperatures as well as digestion with pepsin with the objectives of simulating conditions wherein a particular food is (a) either cooked and thereby the biomolecules are degraded into simpler structures, (b) or subjected to proteolytic degradation in the gut of animals that could possibly graze on them.

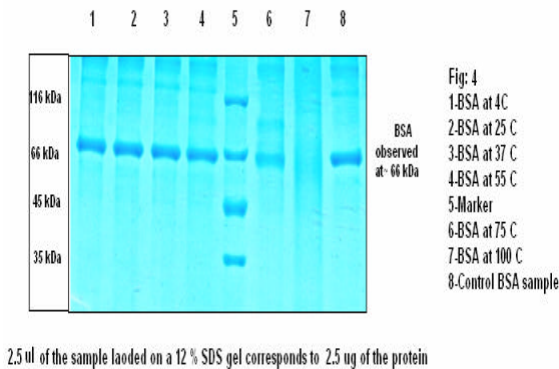
**a. Thermal Stability**

A 1 mg/ml solution of the recombinant Cry1C was prepared in the bioassay buffer, i.e., carbonate buffer pH 9.5. BSA was also prepared in the same concentration in the same buffer. The protein was incubated at the following temperatures, viz., 4°C, 25°C, 37°C, 55°C, 75°C and 100°C. A 2.5 µl aliquot corresponding to 2.5 µg of protein was loaded on a 12% SDS-PAGE gel and stained using Coomassie Brilliant Blue. The experiment was performed in 3 replicates and in all instances, identical results were observed. Slight degradation was seen at 75°C and complete degradation was observed at 100°C, the boiling point of water and the probable temperature of cooking for most food items. The results are shown in Figure 1 below:

**Figure 1 (a) – thermal stability profile of Cry1C recombinant protein**



**Figure 1 (b) – thermal stability profile of control protein (BSA)**



## b. Pepsin Digestibility

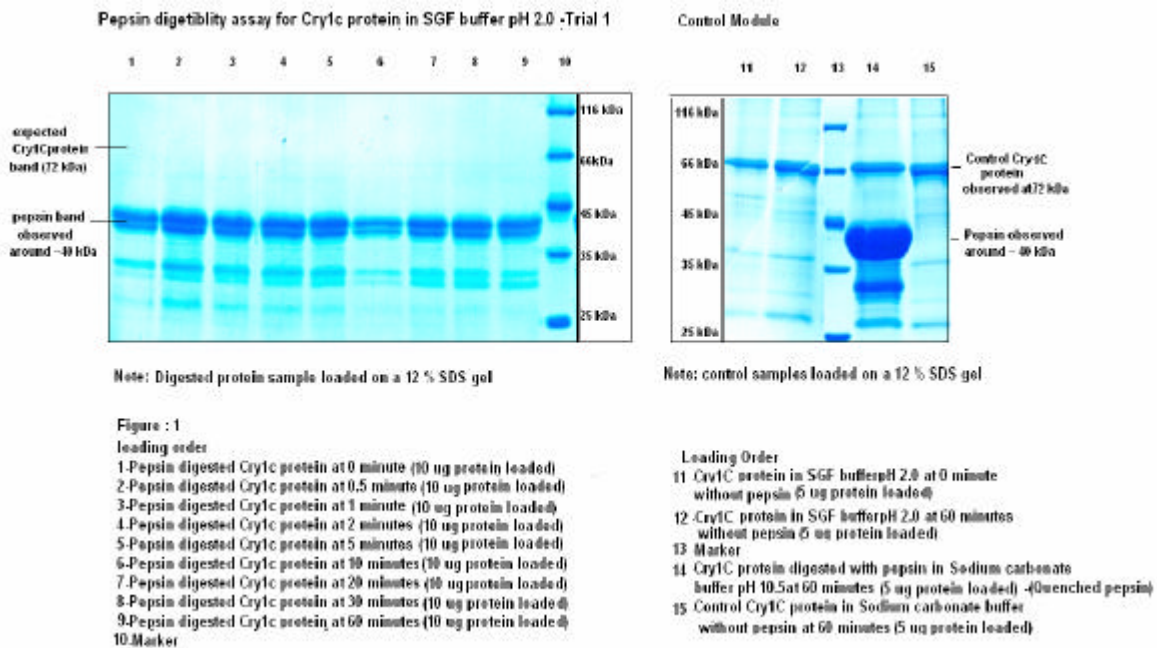
Purified porcine pepsin has been used to evaluate the stability of a number of food allergens and non-allergenic proteins in a multi-laboratory study that demonstrated the rigor and reproducibility in nine laboratories<sup>18</sup>. Porcine pepsin is an aspartic endopeptidase with broad substrate specificity. Pepsin is optimally active between pH 1.2 and 2.0, but inactive at pH 3.5 and irreversibly denatured at pH 7.0. The assay is performed under standard conditions of 10 units of pepsin activity per microgram of test protein. The original assay described by Astwood *et al.* (1996)<sup>19</sup> recommends performing the digestion at pH 1.2. However, the FAO/WHO (2001)<sup>20</sup> recommends using two pH conditions (pH 1.2 and pH 2.0). The assay is performed at 37°C and samples are removed at specific times and the activity of pepsin is quenched by neutralization with carbonate buffer and sodium dodecyl sulfate (SDS-) polyacrylamide gel electrophoresis (PAGE) loading buffer, then heating to more than 70°C for 3 to 5 minutes. The timed digestion samples are separated by SDS-PAGE and stained with Coomassie Brilliant Blue to evaluate the extent of digestion. Assessment of the digestibility assays by Bannon *et al.* (2002)<sup>21</sup> and by Thomas *et al.* (2004)<sup>18</sup> indicate that most of the non-allergenic food proteins that have been tested are digested by approximately 30 seconds, while major food allergens are stable, or produce pepsin-stable fragments that are detectable for from eight to 60 minutes.

The test system is an *in vitro* digestion model using porcine pepsin in simulated gastric fluid (SGF) at pH 1.2. The pepsin activity assay is based on the method described by Sigma for determining the activity of pepsin. At predetermined times (*e.g.*, 0, 0.5, 1, 2, 5, 10, 20, 30, 60 minutes) a fixed volume of the digestion reaction mixture was withdrawn and added to sample tubes containing neutralization and denaturing reagents, which stop the digestion. Samples were then heated to ~ 95°C before analysis by SDS-PAGE, or storage at -20°C for later analysis. All samples from a single digestion experiment were applied to wells of the same SDS-PAGE gel along with molecular weight markers. Control samples included: test protein in SGF reaction mixture without added pepsin, T=0 min; test protein in SGF reaction mixture without added pepsin, T=60 min; SGF with added pepsin but without test protein, T=0; SGF with added pepsin but without test protein, T=60; and a 10% test protein sample and quenched pepsin without SGF reaction mixture (to verify detectability of at least 10% of the original protein concentration).

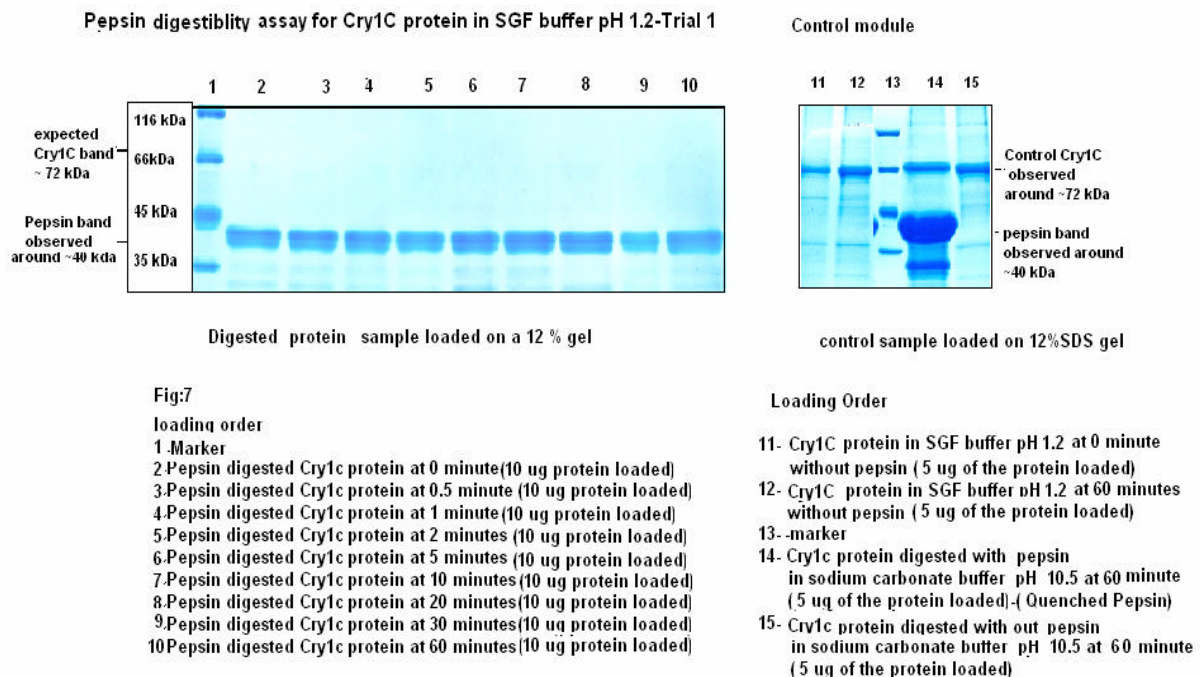
Samples are separated by electrophoresis, fixed, stained with Coomassie Brilliant Blue. It was observed that the Cry1C protein was completely digested by pepsin in less than 0.5 minutes under both the experimental conditions i.e.; at pH 1.2 and pH 2.0 SGF buffer.

The results are highlighted in Figure 2 below.

**Figure 2 (a) – Pepsin digestibility of Cry1C recombinant protein at pH 2.0**



**Figure 2 (b) – Pepsin digestibility of Cry1C recombinant protein at pH 1.2**



## 8. Cry1C protein expression levels and animal dietary intake in goats and cattle

In order to assess the Cry1C protein expression levels in different parts of cotton plant, the Cry1C protein levels were analysed in terminal leaf tissue, squares, and bolls in Bt cotton hybrids expressing cry1C gene event MLS9124 at six locations across India in multi-location trials during kharif 2006<sup>22</sup>. The results are summarized across north, central and south zones, with protein expression levels averaged over two locations in each zone and three replications at each location. Cry1C protein levels were expressed as µg/g of fresh tissue.

**Table 5. Mean protein expression levels of Cry1C (µg/g of fresh tissue) in the tissues of test Bt hybrids in:**

### a. Central Zone, MLT, K06

Plant tissue	Days after sowing							
	30	45	60	75	90	105	120	135
Terminal leaf	2.98	2.97	2.96	2.67	2.63	2.28	1.30	1.04
Square		1.10	1.02	1.05	1.01	0.89	0.79	0.66
Boll			0.66	0.95	0.89	0.84	0.68	0.65

### b. South zone, MLT Kharif 06

Plant tissue	Days after sowing							
	30	45	60	75	90	105	120	135
Terminal leaf	4.18	3.82	3.01	2.42	1.95	1.82	1.51	1.41
Square		1.16	0.87	0.82	0.71	0.45	0.28	0.26
Boll				0.52	0.46	0.36	0.18	0.13

### c. North zone, MLT Kharif 06

Plant tissue	Days after sowing							
	30	45	60	75	90	105	120	135
Terminal leaf	2.15	2.21	2.81	2.42	3.17	2.28	1.25	1.04
Square		0.78	0.93	0.97	1.43	0.91	0.93	0.62
Boll			0.14	0.80	0.86	0.82	0.71	0.64



The protein expression varied considerably between the stage of development and the plant tissue sampled. Leaf tissue expressed the maximum. The highest expression level recorded was 5.17 µg/g in young terminal leaf tissue. The average expression level across all plant parts at all locations was 1.35 µg/g of leaf tissue.

In order to calculate the quantity of protein ingested in a day by two representative animal types, goat and cattle, following calculations were made based on the body weight of the animal and at two levels of dietary intake, average and maximum. Average body weight of goat were assumed at 35 kgs for the Jamnapuri breed<sup>23</sup>, and in case of cattle, at 587 and 418 kgs for Holstein and Jersey breeds<sup>24</sup>. Based on the highest and average expression of Cry1C protein in Bt cotton plants, following Cry1C ingestion levels were arrived at for the breed and daily dietary intake. It is assumed that the animal's ration will consist only of cotton leaves.

**Table 6: Tissue Cry1C level and daily intake calculations for goat and cattle.**

Tissue Cry1C content		Daily intake (g/day of Cry1C protein)					
Expression level	Level recorded	Goat, Jamnapuri breed, 35 kg body weight		Cattle, Holstein breed, 587 kg body weight		Cattle, Jersey breed, 418 kg body weight	
		@ 1 kg	@ 2 kg	@ 17 kg	@ 24 kg	@ 13 kg	@ 20 kg
Maximum	5.17 µg/g	0.517 g	1.034 g	8.789 g	12.408 g	6.721 g	10.340 g
Average	1.35 µg/g	0.135 g	0.270 g	2.295 g	3.240 g	1.755 g	2.700 g
Minimum	0.13 µg/g	0.013 g	0.026 g	0.221 g	0.312 g	0.169 g	0.260 g

It appears from the above, that the intake of Cry1C protein, even if the animal consumes only the terminal cotton leaves of the Bt cotton plants carrying the event 9124, would be a very tiny proportion of the total dietary proteins and other components consumed to be of any significance in terms of its effect on the health of the animal. Further, from the earlier section on the digestibility studies, it is clear that any quantity of the Cry1C protein that may reach the alimentary system of the animal is most likely to be digested completely, almost immediately.

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## PAIRWISE ALIGNMENTS OF DIFFERENT TOXINS TO CRY1C

Aerolysin (*Aeromonas hydrophila*) versus Cry1C - identity 12.2%

```

1                               50
aerolysin ABA28308 (1) -----MQLKLTGLSLTI SGLLMAQAHAHAEPVY
Cry1C 630aa (1) MEENNQNCQIPYNCLSNPEEVL L DGERISTGNSSI DISL SLVQFLVSNFV
Consensus (1)                               L KIS I L

51                               100
aerolysin ABA28308 (29) PDQLR LFS LGQEVCGDKYRPITREEAQS VKSNIVNMMGQWQISGLANGWV
Cry1C 630aa (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAE FARNAAIANLE
Consensus (51) P L L V G I I IA F A

101                               150
aerolysin ABA28308 (79) IMGPGYNGEIKPGSASNTWCYPI NP-----VTGEIPTLSALDIPDGDEVD
Cry1C 630aa (101) GLGNFN--LYVEAFKWEEDPNP ETRTRVIDRFRI LDGLLERDIPDFR
Consensus (101) LG FN I A P NP V L AL D

151                               200
aerolysin ABA28308 (124) VQWRLVHDSANFIKPTS YLAHYLGYAWVGGNHSQYVGEDMDVTRDGDGWV
Cry1C 630aa (149) LSGFEVPLLSVYAQAANLHLAIRD SVIFGERWGLTTINVNENYNRLIRH
Consensus (151) I V A F L A I G M

201                               250
aerolysin ABA28308 (174) IRGNNDGGCEGYRCEK-----TAKVSNFAYN
Cry1C 630aa (199) IDEYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLVLDIAAFPN
Consensus (201) I D Y G T I IA F N

251                               300
aerolysin ABA28308 (202) LDPDSFKHGDVTQSDRQLVKTVG-----WAINSDTPQSG-----
Cry1C 630aa (249) YDNRRYPIQPVGQLTREYITDPLINFNPQLQVAQLPTFNVMESSRIRNP
Consensus (251) D F V Q R L L I S

301                               350
aerolysin ABA28308 (238) --YDVT LRYDTATNWSKTN-----TYGLSEKV
Cry1C 630aa (299) HLF DILNNLTIFTDVFSVGRNFYWGHRVISSLIGGGNITSPIYGREANQ
Consensus (301) FDI T W YG

351                               400
aerolysin ABA28308 (263) TTKN--KFKWPLVGETELSIEIAANQSWASQ-----NGGSTTISL
Cry1C 630aa (349) EPPRSFTFNGE VFR T LSNPTLRLLQQPWAPPFNLRGVEGVEFSTPTNSF
Consensus (351) F PL NQ W A S T S

401                               450
aerolysin ABA28308 (301) S QSVRS TVPARSKIPVKIELYKADISYPYEFKADVSYDLT-----
Cry1C 630aa (399) TYRGRGTVDSLTELPEPENSVPREGYSHRLCHATFVQRS GTPFLTTGVV
Consensus (401) S R TV A S IP Y H S

451                               500
aerolysin ABA28308 (341) -----L S GFLR WGN A WYTH PDNRP-NWNHT
Cry1C 630aa (449) FSWTDRSATLTNTIDPERINQIPLV K GFRV WGGTS VITGFGFTGGDILRR
Consensus (451) L GF WGG A T P

501                               550
aerolysin ABA28308 (366) FVI GPYKDKASS IRYQWDKRYIP--GEVKWWDWNWTLQQNGLSTMQNNLA
Cry1C 630aa (499) NTF GDE VSLQVNI NSPITQRYRLRFYASSRDARVIVLTGASTGVGGQV
Consensus (501) G F I RY D I A ST

551                               600
aerolysin ABA28308 (414) R VLR PVRAGTIGDFSAESQFAGNI R IGAPVPLAADSKVRRTRS--VD GAG
Cry1C 630aa (549) S VNMPLQKIMEIGENLTSRTFRYTD FSNPFSFRANPDLIGISEQPLF GAG
Consensus (551) V PL I S D P A I L GAG

601                               632
aerolysin ABA28308 (462) QGLRLEIPLDAQELSGLGFSNVLSVTPV ANQ
Cry1C 630aa (599) S ISSG ELYIDKIEIILADATFEAESDLERAACK
Consensus (601) EI ID EI S A S AN

```

Alpha lysin (*S. aureus*) versus Cry1C - identity 6%

```

1                                     50
alpha lysin P0C1U7 (1) -----
Cry1C 630aa (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
Consensus (1)
51                                     100
alpha lysin P0C1U7 (1) -----
Cry1C 630aa (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
Consensus (51)
101                                     150
alpha lysin P0C1U7 (1) -----
Cry1C 630aa (101) GLGNFNFIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRIS
Consensus (101)
151                                     200
alpha lysin P0C1U7 (1) -----MOKKVIAAIIGTSAISAVAATQANAATHTV
Cry1C 630aa (151) GFEVPLLSVYAQAANLHLAALLRDSWIFGERWGLTHINVNENYRRLIRHID
Consensus (151) I K I S N N H
201                                     250
alpha lysin P0C1U7 (32) KPGESVWAISNKYGISIAKLSLNNLTSNLIIFPNQVLKVS GSSNSTSNSS
Cry1C 630aa (201) EYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
Consensus (201) AD NK I K IT N I L V A N
251                                     300
alpha lysin P0C1U7 (82) --RP--STN---SGGSSYYTVQAGDSLISLIASKYGTTYQNIMRINGLN
Cry1C 630aa (251) NRRYPIQPVGQLTREVYIDPLINFPQLQSVLAQLPTFNVMESSRIRNPHL
Consensus (251) R S IN L IA RI
301                                     350
alpha lysin P0C1U7 (124) FFLYPGQKIKVSGTASSNAASNSSRPSTNSGGG-S---YTVQAGDSL
Cry1C 630aa (301) EDILNNLTIIFTDWFSVGRNFYWGGRVVISLIGGNGNITSPIYGREANQEP
Consensus (301) F I I A N R S GG Y A
351                                     400
alpha lysin P0C1U7 (169) SLIASKYGTTYQKIMSLNGLNFFIYFGQKLVITGNASTNSGSAITINRG
Cry1C 630aa (351) PRSFTFNGLPVRTLSNPTLRLQLQPPAPPFNLRGVEGVFSTIPINSFTY
Consensus (351) S G F I WPA L G S T S
401                                     450
alpha lysin P0C1U7 (219) YN-----TPVFSHQNLVTWGQCTYHVFNRRAELGKGI STYWWN--ANN
Cry1C 630aa (401) RGRGTVDSLTELPPEDNSVPPREGYSRLCHATFVCRSGTPELTTGVVFS
Consensus (401) T L N H I K S F
451                                     500
alpha lysin P0C1U7 (260) WDNAAAAADGYTIDNRPTVGSIAQTDVGYGHVFMFVERVNNDSIILVSEMN
Cry1C 630aa (451) WTDRSATLNTIDPERINQIPLVKGFRVWG GTSVITGPGFTGGDILRRNT
Consensus (451) W AA TID WG I G IL
501                                     550
alpha lysin P0C1U7 (310) YSAAPGILTYRTVPAYQVNNYRYIH-----
Cry1C 630aa (501) FGDFVSLQVNINSPITQRYRLRERYASSRDARVIVLTGAASTGVGGQVSV
Consensus (501) F I P Q RF H
551                                     600
alpha lysin P0C1U7 (335) -----
Cry1C 630aa (551) NMPLQKTMIEIGENLTSRTFRYTDFSNPFSTRANPDIIGISEQPLFGAGSI
Consensus (551)
601                                     630
alpha lysin P0C1U7 (335) -----
Cry1C 630aa (601) SSGELYIDKIEIILADATFEAESDLERAQK
Consensus (601)

```

Alpha toxin (*S. aureus*) versus Cry1C - identity 5.9%

```

1                                     50
alpha toxin AAR21507 (1) -----
Cry1C 630aa (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
Consensus (1)

51                                     100
alpha toxin AAR21507 (1) -----
Cry1C 630aa (51) PGGGFLVGLIDFVWGVGIPSWDAFLVQIEQLINERIAEFARNAAIANLE
Consensus (51)

101                                    150
alpha toxin AAR21507 (1) -----
Cry1C 630aa (101) GLGNFNFIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRIS
Consensus (101)

151                                    200
alpha toxin AAR21507 (1) -----
Cry1C 630aa (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHID
Consensus (151)

201                                    250
alpha toxin AAR21507 (1) -----
Cry1C 630aa (201) EYADHCANTYNRGLNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
Consensus (201)

251                                    300
alpha toxin AAR21507 (1) -----MKTRIVSSVTTLTLLGSLMNPVANAADSDINIKTGT
Cry1C 630aa (251) NRRYPIQPVGQLTREVYTDPLNFNPNQLQSVAQLPPTFNVMSSTRIRNPHL
Consensus (251) I L S I P N D S

301                                    350
alpha toxin AAR21507 (38) TDIGSNITVVKTGDLVITYDKENGMHKKVVFYSFTDDKNHNKLLLVIRTKG-T
Cry1C 630aa (301) FDILNNTLITFDWFSVGRNFYWGGRVIVSSLIIGGNITSPFIYGREANQEP
Consensus (301) DI N TI T KV S I N I

351                                    400
alpha toxin AAR21507 (87) IAG--Q---YRVYS EEGANKSGLAWPSAFKVQLQLPDNEVAQISDYVPR
Cry1C 630aa (351) PRSFTFNGPVFRRLSNPTLRLQLQWPAPPFNLRGVEGVVFSPTINSEITY
Consensus (351) FR S WPA L E A S F

401                                    450
alpha toxin AAR21507 (131) NSIDTKHYMSTLYGFNGN----VTGDDTGKIGGLIGANVSIHTLKYV
Cry1C 630aa (401) RGRGTVSLTELPPEDNSVPPREGYSHRLCHATFVQRSPTPFLITGVVHS
Consensus (401) T D L S L N S A I L F

451                                    500
alpha toxin AAR21507 (176) QPDFKTIIESPTDKKVGWKKVIFNNMVNQNWGPYDRDSWNPVYGNQLFMKT
Cry1C 630aa (451) WDRSATLNTITDPERINQIPLVKGFRVWGSTSVITGPGFTGCDILRRNT
Consensus (451) D L D I G G L T

501                                    550
alpha toxin AAR21507 (226) RNGSMKAADNFLDENKASSLSSSGFSPDFATVIMDRKASKQQTNIDVIY
Cry1C 630aa (501) FGDFVSLQVNIINSPIQRYRLRFRVASSRDARVIVLTGAASTGVGGQVSV
Consensus (501) M N P L FA I M AA V

551                                    600
alpha toxin AAR21507 (276) ERVRDDYQLHWTSTNWKGTNTKDKWTDRSSERYKIDWEKEEEMTN-----
Cry1C 630aa (551) NMPLQKTMEIGENLTSRTFRYTDFSNPFSSFRANPDIIGISEQPLFGAGSI
Consensus (551) K D S E

601                                    630
alpha toxin AAR21507 (320) -----
Cry1C 630aa (601) SSGELYIDKIEIILADATFEAESDLERAQK
Consensus (601)

```

Cholera toxin (V. cholera) versus Cry1C - identity 1.3%

```

1
cholera toxin ACF35008 (1) -----M I K I K F G V F F T V L
Cry1C 630aa (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFIVSNFV
Consensus (1) I L L L

51
cholera toxin ACF35008 (14) LSSAYAHGTPQNITDLCAEYHNTQIHTLNDKILSYTESLAGKREMAIITF
Cry1C 630aa (51) PGGGFLVGLIDFVWGLVGPSPQWDAFLVQIEQLINERIAEFARNAAIANLE
Consensus (51) AF G I I A D I I A AK

101
cholera toxin ACF35008 (64) KNGATFOVEVPSQHIISQKKALERMKDTIRIAYITEAKVEKLCVWNNKI
Cry1C 630aa (101) GLGNFNLYVEAFKEWLEDPNNEETRTRVIDRFRILDGLIERDIPSFRLS
Consensus (101) G FNI V A D E I I DA LEK S

151
cholera toxin ACF35008 (114) PHAIAAISMAN-----
Cry1C 630aa (151) GFEVPLLSVYQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHID
Consensus (151) I ISM

201
cholera toxin ACF35008 (125) -----
Cry1C 630aa (201) EYADHCANTYNRGLNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
Consensus (201)

251
cholera toxin ACF35008 (125) -----
Cry1C 630aa (251) NRRYPIQPVGQLTREVYTDPLINFNPQLQSVACLPTFNVMESSRIRNPHL
Consensus (251)

301
cholera toxin ACF35008 (125) -----
Cry1C 630aa (301) FDILNLLTIFTDWFVSGRNFYWGGRVIVSSLIGGGNITSPIYGREANQEP
Consensus (301)

351
cholera toxin ACF35008 (125) -----
Cry1C 630aa (351) PRSFTFNGPVFRTLNSNPTLRLQLQPWPAPPFNLRGVGVEFSTPTNSFTY
Consensus (351)

401
cholera toxin ACF35008 (125) -----
Cry1C 630aa (401) RGRGTVDLSLTELPPEDNSVPPREGYSHRLCHATFVQVRSRGTFFLTIGVVFS
Consensus (401)

451
cholera toxin ACF35008 (125) -----
Cry1C 630aa (451) WTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRRNT
Consensus (451)

501
cholera toxin ACF35008 (125) -----
Cry1C 630aa (501) FGDVSLQVNNINSPIQRYRLRFYASSRDARVIVLTGAASTGVGGQVSV
Consensus (501)

551
cholera toxin ACF35008 (125) -----
Cry1C 630aa (551) NMPLQKTMEIGENLTSRTFRYTDVSNPFSFRANPDIIGISEQPLFGAGSI
Consensus (551)

601
cholera toxin ACF35008 (125) -----
Cry1C 630aa (601) SSGELYIDKIEIILADATFEAESDLERAQK
Consensus (601)

```

Cytotoxin (Clostridium difficile) versus Cry1C identity - 6.4%

	1	50
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(1)	MSLVNRKQLEKMANVRFVQDEYVAILDALEEYHNMENTVVEKYLKLK
Consensus	(1)	
	51	100
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(51)	DINSLTDTFYIDTYKKSGRNKALKKKFKEYLVIEILELENSNLTPEKNLHF
Consensus	(51)	
	101	150
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(101)	IWIGGQINDTAINYINQWKDVNSDYNVNVFYDSNAFLINTLKKTTIESAS
Consensus	(101)	
	151	200
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(151)	NDTLESFRENLDPEFNHTAFFRKRMQIIYDKQQNFINYKAQKEENPDL
Consensus	(151)	
	201	250
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(201)	IIDDIVKTYLSNEYSKDIDELNAYIEESLNKVTENSGNDVRNFEEFKTGE
Consensus	(201)	
	251	300
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(251)	VFNLYEQELVERWNLAGASDILRVAILKNIGGVYLDVDMLPGIHPDLFKD
Consensus	(251)	
	301	350
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(301)	INKPDSVKTAVDWEEMQLEAIMKHKEYIPEYTSKHFDLDEEVQSSFESV
Consensus	(301)	
	351	400
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(351)	LASKSDKSEIFLPLGDIEVSPLEVKIAFAKGSIIHQALISAKDSYCSDDL
Consensus	(351)	
	401	450
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(401)	IKQIQNRYKILNDTLGPIISQGNDFNTTMNFGESLGAIANEENISFIAK
Consensus	(401)	
	451	500
Cry1C 630aa	(1)	-----
cytotoxin AAG18011	(451)	IGSYLRVGFYPEANTTVTLSGPTIYAGAYKDLLTFKEMSIDTSILSSELR
Consensus	(451)	
	501	550
Cry1C 630aa	(1)	-----MEENNQ
cytotoxin AAG18011	(501)	NFEFPPKVNISQATEQEKNLSLWQFNEERAKIQFEEYKKNYFEGALGEDDNL
Consensus	(501)	N
	551	600
Cry1C 630aa	(9)	CIPYNCLSNPEEVL DGERISTGN-----S--S- IDISLSLVQFLVSNFV
cytotoxin AAG18011	(551)	DFSQNTVTDK EYLL EKISSSTKSSERYVHYIVQLQGDKISYEAACNLFA
Consensus	(551)	N LS E LL S I F
	601	650
Cry1C 630aa	(51)	PGGGFLVGLIDFVWGIVGPSQDAFLVQIEQINERIAEFARNAAIANLE
cytotoxin AAG18011	(601)	KNPYDSL FQKNIEDSEVAYYINPTDSEIQEIDKYRIPDRISDRPKIKIT
Consensus	(601)	I I W I I R I D L
	651	700
Cry1C 630aa	(101)	GLGN-NFNIYVEAFKEWEE DPNNE TRTRVIDRFRI LDG-LLERDIPSF
cytotoxin AAG18011	(651)	FIHGKAEFNTDIFAGLDVDSLSS E IETA IGLAKEDI SPKSEINLGCN
Consensus	(651)	I G D F D D E T I I I E I
	701	750
Cry1C 630aa	(149)	ISGFVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYRLIR-
cytotoxin AAG18011	(701)	MFSYSVNV EETYPGKLLLRVKDKVSELMPMSQDSIIIVSANQYEVRLINSE
Consensus	(701)	I F V L Y L L LI I N RI
	751	800



Cry1C 630aa	(198)	HID <b>EYADH</b> CANTY <b>N</b> RGLNNLPKST <b>YQDWI</b> TYNRLRRDLTITVLDIAAFP	801	850
cytotoxin AAG18011	(751)	GRR <b>ELLDS</b> GEWIK <b>EESI</b> LKDIS <b>SKEYI</b> SFNPKENKVIIVKSKNLP <b>ELST</b>		
Consensus	(751)	E DH A NK I S DWISFN L L I		
Cry1C 630aa	(248)	NYDNRR <b>YPI</b> Q <b>PV</b> Q <b>L</b> TR <b>E</b> VYTDPLIN <b>F</b> NP <b>Q</b> L <b>S</b> V <b>A</b> Q <b>L</b> PTFN <b>V</b> M <b>E</b> SS <b>R</b> LR <b>N</b>		
cytotoxin AAG18011	(801)	LLQ <b>E</b> I <b>R</b> N <b>N</b> S <b>S</b> DI <b>E</b> L <b>E</b> E <b>K</b> V <b>M</b> L <b>A</b> E <b>C</b> E <b>I</b> N <b>V</b> I <b>S</b> N <b>I</b> E <b>T</b> Q <b>V</b> V <b>E</b> E <b>R</b> I <b>E</b> E <b>A</b> K <b>S</b> L <b>T</b> S		
Consensus	(801)	R N E N N QL I EA I		
Cry1C 630aa	(298)	PH <b>L</b> FD <b>I</b> L <b>N</b> NT <b>I</b> FTD <b>W</b> FS <b>V</b> GR <b>N</b> F <b>Y</b> WG <b>G</b> H <b>R</b> V <b>I</b> S <b>L</b> I <b>G</b> GG <b>N</b> I <b>T</b> S <b>P</b> I <b>Y</b> CR <b>E</b> AN		
cytotoxin AAG18011	(851)	DS <b>I</b> N <b>Y</b> I <b>K</b> N <b>E</b> FK <b>L</b> I <b>E</b> S <b>I</b> S <b>D</b> AL <b>C</b> DL <b>K</b> Q <b>Q</b> NE <b>L</b> DS <b>H</b> F <b>I</b> S <b>F</b> E <b>D</b> I <b>S</b> E <b>T</b> D <b>E</b> G <b>F</b> S <b>I</b> R		
Consensus	(851)	I I N I S I IS G		
Cry1C 630aa	(348)	QE <b>P</b> PR <b>S</b> - <b>F</b> I <b>F</b> NG <b>P</b> V <b>F</b> R <b>T</b> L <b>S</b> NP <b>T</b> LR <b>L</b> L <b>Q</b> Q <b>P</b> W <b>A</b> PP <b>F</b> N <b>L</b> R <b>G</b> VE <b>G</b> VE <b>F</b> ST <b>P</b> T <b>N</b>		950
cytotoxin AAG18011	(901)	F <b>I</b> N <b>K</b> E <b>T</b> G <b>E</b> S <b>I</b> F <b>V</b> E <b>T</b> E <b>K</b> T <b>I</b> F <b>S</b> E <b>Y</b> AN <b>H</b> I <b>T</b> E <b>E</b> I <b>S</b> K <b>I</b> K <b>I</b> G <b>T</b> I <b>F</b> D <b>T</b> V <b>N</b> G <b>K</b> L <b>V</b> K <b>K</b> V <b>N</b>		
Consensus	(901)	S S KTI I I N		
Cry1C 630aa	(397)	S <b>F</b> TY <b>R</b> GR <b>G</b> I <b>V</b> DS <b>L</b> TE <b>L</b> PP <b>E</b> D <b>N</b> S <b>V</b> PP <b>R</b> E <b>G</b> Y <b>S</b> H <b>R</b> I <b>C</b> H <b>A</b> T <b>F</b> V <b>O</b> R <b>S</b> G <b>T</b> P <b>F</b> L <b>T</b> G		1000
cytotoxin AAG18011	(951)	LD <b>T</b> THE <b>V</b> N <b>I</b> L <b>N</b> A <b>A</b> FF <b>I</b> Q <b>S</b> L <b>I</b> E <b>Y</b> N <b>S</b> S <b>K</b> E-S <b>L</b> S <b>N</b> I <b>S</b> V <b>A</b> M <b>K</b> V <b>O</b> V <b>Y</b> A <b>Q</b> L <b>F</b> S <b>I</b> G <b>L</b>		
Consensus	(951)	T TL A I KE L A VQ A F T		
Cry1C 630aa	(447)	V <b>V</b> FS <b>W</b> T <b>D</b> R <b>S</b> A <b>T</b> L <b>N</b> T <b>I</b> D <b>P</b> ER <b>I</b> N <b>Q</b> I <b>P</b> LV <b>K</b> G-----FR <b>V</b> W <b>G</b> G <b>I</b> S <b>V</b> I <b>T</b> G <b>P</b>		1050
cytotoxin AAG18011	(1000)	NT <b>I</b> T <b>D</b> A <b>A</b> R <b>V</b> VE <b>L</b> V <b>S</b> T <b>A</b> LD <b>E</b> T <b>I</b> DL <b>L</b> P <b>L</b> SE <b>G</b> L <b>P</b> I <b>A</b> T <b>I</b> ID <b>G</b> V <b>S</b> L <b>G</b> A <b>I</b> K <b>E</b> L		
Consensus	(1001)	S R L T E I I P L A I		
Cry1C 630aa	(489)	G <b>F</b> T <b>G</b> G <b>D</b> L <b>R</b> R <b>N</b> T <b>F</b> G <b>D</b> F <b>V</b> S <b>L</b> Q <b>V</b> N <b>I</b> N <b>S</b> P <b>I</b> T <b>Q</b> R <b>Y</b> R <b>L</b> R <b>F</b> R <b>Y</b> A <b>S</b> S <b>R</b> D <b>A</b> R <b>V</b> I <b>V</b> L <b>T</b> G		1100
cytotoxin AAG18011	(1050)	SE <b>T</b> S <b>D</b> P <b>L</b> R <b>Q</b> E <b>I</b> E <b>A</b> K <b>I</b> G <b>I</b> M <b>A</b> V <b>N</b> I <b>T</b> A <b>T</b> A <b>I</b> I <b>T</b> S <b>S</b> L <b>G</b> I <b>A</b> S <b>G</b> F <b>S</b> I <b>L</b> L <b>V</b> P <b>L</b> A <b>G</b>		
Consensus	(1051)	T ILR A L VNI S T AS LI L G		
Cry1C 630aa	(539)	AA <b>S</b> T <b>G</b> V <b>G</b> Q <b>V</b> S <b>V</b> N <b>M</b> PL <b>Q</b> K <b>T</b> M <b>E</b> I <b>G</b> N <b>L</b> T <b>S</b> R <b>T</b> F <b>R</b> Y <b>T</b> D <b>--</b> F <b>S</b> N <b>P</b> F <b>S</b> F <b>R</b> A <b>N</b> P <b>D</b> I		1150
cytotoxin AAG18011	(1100)	IS <b>A</b> G <b>I</b> P <b>S</b> L <b>V</b> N <b>N</b> E <b>L</b> V <b>L</b> R <b>D</b> K <b>A</b> T <b>K</b> V <b>D</b> Y <b>F</b> K <b>H</b> V <b>S</b> L <b>V</b> E <b>T</b> E <b>G</b> V <b>F</b> LL <b>D</b> D <b>K</b> V <b>M</b> M <b>P</b> Q <b>D</b>		
Consensus	(1101)	AA M K I D S TD FS P		
Cry1C 630aa	(587)	I <b>G</b> I <b>S</b> E <b>Q</b> P <b>L</b> F <b>G</b> A <b>G</b> S <b>I</b> S <b>S</b> G <b>E</b> L <b>Y</b> I <b>D</b> K <b>I</b> E <b>I</b> I <b>L</b> A <b>D</b> A <b>T</b> F <b>F</b> A <b>E</b> S <b>D</b> L <b>E</b> R <b>A</b> Q <b>K</b>		1194
cytotoxin AAG18011	(1150)	DL <b>V</b> I <b>S</b> E <b>I</b> D <b>F</b> NN <b>S</b> I <b>V</b> L <b>G</b> K <b>C</b> E <b>I</b> W <b>R</b> M <b>E</b> G <b>G</b> S <b>G</b> H <b>T</b> V <b>I</b> D <b>D</b> I <b>D</b> H <b>F</b> F <b>S</b> A <b>P</b> S		
Consensus	(1151)	I F SI G I KIE A D A		

Delta lysin (26 aa long; *S. aureus*) versus Cry1C identity - 14.8%

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                230                256
Cry1C 630aa (230) RLRRDLTLVLDIAAFPNYDNRYPYI
delta lysin ABQ49855 (1) -MAQDIISTIGDLVKMIIDTVNKFSTKK
Consensus (230) L DI TI DI F NK
    
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Enterotoxin A (*Clostridium perfringens*, Type A) versus Cry1C identity - 12.8%

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1                50
Cry1C 630aa (1) MEENNQNCIPYNCLSNPEEVLLDGERISTGNSSDISLSLVQFLVSNFV
enterotoxin A AAG18010 (1) -----NEYYPEIIVLNEP-----TFHKKVINLDSSEYKWKST
Consensus (1) N P L NP T I I L F

51                100
Cry1C 630aa (51) PGGFLVGLIDFVWGVGVPQWDAFLVQIEQLINERIAEFARNAATANLE
enterotoxin A AAG18010 (35) EGS-----DFILVRYLEESNKKILQKIRIKGILSNT
Consensus (51) G D LV N KI R AI

101                150
Cry1C 630aa (101) GLGNFNFTYVEAFKWEEDPNNPETRTRVIDRFRILDGLLERDIPSPFRIS
enterotoxin A AAG18010 (66) KSFNKMSTDFKDIKKLSLG-----
Consensus (101) N I K

151                200
Cry1C 630aa (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHLD
enterotoxin A AAG18010 (85) -----YIMSNPKSFNSENE
Consensus (151) I NF D

201                250
Cry1C 630aa (201) EYADHCANTYNRGLNLLPKSTYQDWITYNRLRRDLTLVLDIAAFPNYD
enterotoxin A AAG18010 (99) LDRDHLG-----FKIID
Consensus (201) DH A F D

251                300
Cry1C 630aa (251) NRRYPIQPVGQLTREVYTDPLINFNPLQSVAPLPTFNVMESSRIRN-PH
enterotoxin A AAG18010 (111) NKTYYDEASKLVHG-----LININNSLFYFDPLESNLVTGWQTIKNGKKY
Consensus (251) NK Y L K LIN N L I S V I H

301                350
Cry1C 630aa (300) LFDILNNTLTFIDWFSVGRNFWGGHRVVISSLIGGNITSPIYGREANQE
enterotoxin A AAG18010 (156) YFDINTGAASTYKIINGKHFFYFNNGVMQLGVFKG-----
Consensus (301) FDI S GK FYF VI I G

351                400
Cry1C 630aa (350) PPRSFITNGPPVFRITLSNPTLRLLLQQPWAPPFNLRGVEGVEFSTPTINSFT
enterotoxin A AAG18010 (192) -PDGFEYFAPANTQNN-----IEGQAIIVYQSKFLT
Consensus (351) P F F AP N IEG S T

401                450
Cry1C 630aa (400) YRGRGTVDLSLTELPEPNDN SVPPREGVSHRLCHATVORSGTFFLITGVVF
enterotoxin A AAG18010 (222) LNGK-----KYFDNDSKAVTGMQITIDGKKYFNLNTAEAAATG---W
Consensus (401) GK DN GW F N T F

451                500
Cry1C 630aa (450) SWTDRSATLNTIDPERINQIPLVKGFRVGGTSVITGPGFTGDI LRN
enterotoxin A AAG18010 (261) QTIDGKKYFNNTNTSIASTGYTLINGKHFFNTDGLMQIGVFKG----PN
Consensus (451) D NT II G W T I G G N

501                550
Cry1C 630aa (500) TFGDFVSLQVNNINSPITORYRLRFRVASSRDARVIVLTGAASTVGGQVS
enterotoxin A AAG18010 (307) GFEYFAPANTDANNIEGQAIRYQNRRLYLHND--IYFGNNSKAVTGWQI
Consensus (501) F F N N Q R RF D I G S AV G S

551                600
Cry1C 630aa (550) VNMPLQKTMIEIGENLTERTRFRYTDFSNPFVFRANPDIIQISEQPLFGAGS
enterotoxin A AAG18010 (355) LINGNVIYFMPDTAMAAAGGLFEIDGVIYFFG-----VDGNKAPGLYG---
Consensus (551) IN L M A D F I GI IFG

601                631
Cry1C 630aa (600) ISSGELYIDKIEIILADATFEAESDLERAQK
enterotoxin A AAG18010 (397) -----
Consensus (601)
    
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Kappa toxin (*C. perfringens* Type A) versus Cry1C identity 6.2%

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1                                     50
Cry1C 630aa (1) MEENNQNCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
kappa toxin ABA63419 (1) -----
Consensus (1)

51                                     100
Cry1C 630aa (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
kappa toxin ABA63419 (1) -----
Consensus (51)

101                                    150
Cry1C 630aa (101) GLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSPFRIS
kappa toxin ABA63419 (1) -----
Consensus (101)

151                                    200
Cry1C 630aa (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVENYNRLIRHID
kappa toxin ABA63419 (1) -----
Consensus (151)

201                                    250
Cry1C 630aa (201) EYADHCANTYNRGLNLPKSTYQDWITYNRLRRDLTLTVLDIAAFPNYD
kappa toxin ABA63419 (1) ----KAQDGVVEALGRLIGNASADPEVINNCIYVLSDFKNDIDKYGSNYD
Consensus (201) AL L D N LS I F NY

251                                    300
Cry1C 630aa (251) NRRYPIQPVGQLTREVYTDPLNPNPQLQSVACLPTFNVMSSRIINPHL
kappa toxin ABA63419 (47) ---KGNVAVFNLMKIDYITNSIINTKGYDAKNTEFYNRIDPYMERLESLL
Consensus (251) L Y I FN N FN ID R L

301                                    350
Cry1C 630aa (301) FDILNNTIFTDWFVSVGRNFYWGHRVISSLIGGGNITSPIYGREANQEP
kappa toxin ABA63419 (94) CTIGDKLNNDNAWLVNNALYTGIRMGKFREDP-----SISQRA
Consensus (301) I L W FY G Q

351                                    400
Cry1C 630aa (351) PRSFTFNGPVFRTLNSPTLRLQQPWPAPPFNLRGVEGVEFSTPTNSFTY
kappa toxin ABA63419 (132) LERAMKEYPYLSYQYIEAANDLDLNFGGKNSSGNDIDFNKIKADAREKYL
Consensus (351) P L F A ID

401                                    450
Cry1C 630aa (401) RGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTGVVFS
kappa toxin ABA63419 (182) PKTYTFDDGKFVVKAGDKVTEK-----IKRLYWASKEVKAQEM
Consensus (401) T D L V I R F

451                                    500
Cry1C 630aa (451) WTDRSATLTNTIDPERINQIPVKGFRVWGGTSVITGPGFTGGDILRRNT
kappa toxin ABA63419 (221) RVVQNDKALEEGNPDILTVMYNSPEEYKLNRIINGFSTDNGGIYIENI
Consensus (451) PD I I I W II G G I N

501                                    550
Cry1C 630aa (501) FGDVSLQVNNINSPIIQRYRLRFVASSRDARVIVLTGAASTGVGGQVSV
kappa toxin ABA63419 (271) GTFFTYERTPEESIYILEELFRHEETHYLQGRYVVPGM-----
Consensus (501) F S T R F AR IV

551                                    600
Cry1C 630aa (551) NMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQPLFGAGSI
kappa toxin ABA63419 (309) -----
Consensus (551)

601                                    630
Cry1C 630aa (601) SSGELYIDKIEIILADATFEAESDLERAQK
kappa toxin ABA63419 (309) -----
Consensus (601)

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Lethal factor (B. anthracis) versus Cry1C identity 10.4%

		1		50
Cry1C 630aa	(1)	-----		-----
lethal factor AAY15237	(1)	MNIKKEFIKVISMSCLVTAITLSGPVFIPLVQAGGGHGDVGMHVKEKEKN		
Consensus	(1)			
		51		100
Cry1C 630aa	(1)	-----MEENNQCIPYNCISNPEEVLLDGERISTGNSSIDISLSLV		
lethal factor AAY15237	(51)	KDENKRKDEERNKTOEEHLKEIMKHIKVKLEKGEAEVAKKEAAEKILEKVP		
Consensus	(51)	E N N L I L GE AA I L		
		101		150
Cry1C 630aa	(43)	QFLVSNFVPGGGFLVGLIDFWGIVGPSQWDAFLVQIEQLINERIAEFAR		
lethal factor AAY15237	(101)	SDVLEMYKAIIGGKIYIDGDTKHSLEALSSEDKKKIKDIYGDALLHEH		
Consensus	(101)	LL F GG I L I I I I		
		151		200
Cry1C 630aa	(93)	NAAIAN-----LEGLGNFNIVYEAFAKWEEDDPNPE--TR		
lethal factor AAY15237	(151)	YVYAKEGYEPLVVIQSSSEYVENTEKALNVVYEIGKILSRDILSKINQPY		
Consensus	(151)	LE NIY E K D		
		201		250
Cry1C 630aa	(127)	TRVLDLDRFRILDGLLERDIPSFRISS---G---FEVPLLSVYAQAANLHL		
lethal factor AAY15237	(201)	QKFLDVLNLTIKNASDSGGDLLFTNQLKEHPTDFSVFLEQNSIEVQEVF		
Consensus	(201)	K ID I D D S F V L AN N		
		251		300
Cry1C 630aa	(169)	AALLRDSVIFGERWGLTINVNENYRILRHIDEYADHCANTYNRG----		
lethal factor AAY15237	(251)	AKAFAYYIEPQHRVLDQIYAPAFNYDKFNEQIEINLSLEELKDKRMLAR		
Consensus	(251)	A I L I E FN L K D		
		301		350
Cry1C 630aa	(214)	--LNNLPKSTYODWITYNRLRRDLTLTVLDIAAFFPNYDNRRYPIQPVGQ		
lethal factor AAY15237	(301)	YEKWEIKQHYQHSDSLSEEGRGLKKLQIPIEPKDDIHSLSQEEKE		
Consensus	(301)	K YQ W L L I D Q		
		351		400
Cry1C 630aa	(262)	LITREYVYTDPLINFNPLQSQVAQLPTFNVMSS---R---IRNPHLFDILNN		
lethal factor AAY15237	(351)	LLKRIQIDSSDFLSTEEKEFLKKLQIDIRDSLSEEEKEILNRIQVDSNP		
Consensus	(351)	L K I D I DS I N D N		
		401		450
Cry1C 630aa	(307)	LTIIFTDWFVSVGRNFYWGGRVIVSSLIGCGNITSPIYGRANQEPFRSFT		
lethal factor AAY15237	(401)	LSEKEKEFLKKLKLDIQPYDINQRLQDTGGLIDSPSINLIVRKQYKRDIQ		
Consensus	(401)	LS F H I L GG I SP D R		
		451		500
Cry1C 630aa	(356)	FNGPVFRTLSNPTLRLLLQQPWPAPPFNLRGVEGVFSTPTN-----		
lethal factor AAY15237	(451)	NIDALLHQSIGSTLYNKIYLEMNMINLTLTLCADLVDSIDNTKINRGI		
Consensus	(451)	L TL W N L A G D T		
		501		550
Cry1C 630aa	(397)	-----S---FTYRGRGTVDSITELPPEDNSVPPREGYSHRLCHATFVQRS		
lethal factor AAY15237	(501)	FNEFKKNFKYSISSNYMVDINERPALDNERLKWRIQLSPDTRAGYLE-N		
Consensus	(501)	FS I I E P DN A FL		
		551		600
Cry1C 630aa	(439)	GTPFLITGVVFSWTDRSATLTNTIDPERINQIPLVKG-----		
lethal factor AAY15237	(550)	GKLLIQRNIGLEIKDVQIIKQSEKEYIRIDAKVVPKSKIDTKIQEAQLNI		
Consensus	(551)	G L I D D RI L K		
		601		650
Cry1C 630aa	(476)	-----FRVWG-GTSSVITGPGFTGGDILRRNTFGDFVS		
lethal factor AAY15237	(600)	NQEWNKALGLPKYTKLITFNVHNRYASNIVESAYLILNEWKNNIQSDLIK		
Consensus	(601)	F V S I AF K N D I		
		651		700
Cry1C 630aa	(507)	LQVNIINSP-----ITQRVRLRFYASSRDARVILVLTGAAST		
lethal factor AAY15237	(650)	KVTNYLVDGNGRFVFTDITLPNIAEQYTHQDEIYEQVHSGKLYIPESRSI		
Consensus	(651)	N I Y AK I L A S		
		701		750
Cry1C 630aa	(543)	GVGC-QVS--VNMPLQKTMEIGENLTSRTFRYITDFSNPFSFRAN-P--DI		
lethal factor AAY15237	(700)	LLHGSPKGVELRNDSEGFITHEFGHAVDDYAGYLLDKNQSDLVTNSKKFID		
Consensus	(701)	L G L I Y N N		
		751		800
Cry1C 630aa	(587)	IG-ISEQPIFGAGSISSELYIDKIEIILADATFEAESDLERAQK----		
lethal factor AAY15237	(750)	LFKEEGSNLTSYGRITNEAEFPAFAFRMLHSTDHAEARLKVKQKNAPKTFQFI		
Consensus	(751)	I L G AE F D I I A E A K		
		801		
Cry1C 630aa	(631)	-----		
lethal factor AAY15237	(800)	NDQIKFIINS		
Consensus	(801)			

Listeriolysin (*Listeria monocytogenes*) versus Cry1C identity 10%

		1		50
Cry1C 630aa	(1)	MEENNQNCIPYNCLSNPEEVLIDGERISTGNSSIDISLSLVQFLVSNFV		
listeriolysin CAA42639	(1)	-----MKKIMLVFITLILVSLPIAQQTEAKDASAFNKENLIS SMA		
Consensus	(1)		L LIL IA A D S LIS	
		51		100
Cry1C 630aa	(51)	PGGFLVGLIDFVWGIQVPSQWDAFLVQIEQLINERTAEFARNAALANLE		
listeriolysin CAA42639	(41)	PPASPPASPKPTLEKHADEIDKYIQGLDYKNNVLYVHGDAVTNMPPRK		
Consensus	(51)		P A I A N N I I	
		101		150
Cry1C 630aa	(101)	GLGNFNIVVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSPFRIS		
listeriolysin CAA42639	(91)	GKDGNEYIVVEKSKKSINQNNADIQVVNAISSLTYPGAVKANSSELVEN		
Consensus	(101)		G V K NN D G L K	
		151		200
Cry1C 630aa	(151)	GFEVPLLSVYAQAANHLAIIRDVSIFGERWGLTTINVNENYNRILIRHID		
listeriolysin CAA42639	(141)	QPDVLPVKRDSLTLSDLPGLTQDNKIVVKNAITKSNVNAVNTLVERWN		
Consensus	(151)		DV L A I L L T NVN N LI	
		201		250
Cry1C 630aa	(201)	EYADHCANTYNRGLNLPKSTVQDWITYNRLRRDLTLTVLDIAAFFPNYD		
listeriolysin CAA42639	(191)	EKYAQAYPNVS-----AKIDVDEEMAYSEQ--LIAKFGTAFKAVNSL		
Consensus	(201)		E K Y D I Y L N	
		251		300
Cry1C 630aa	(251)	NRRPIQPVGQITREYVDPLINFPOLQSVLQPLTFNVMESLRIRNPHL		
listeriolysin CAA42639	(233)	NVNFGAISEGKQEEVIFKQIYYNVNNEPTRPSRFSGKAVIKKQLQAL		
Consensus	(251)		N F G L E V S I FN NLN F SK L	
		301		350
Cry1C 630aa	(301)	FDILNLTITFDWFSVGRNFWGGHRVISSLIGGNITSPYGREANQEP		
listeriolysin CAA42639	(283)	GVNAENPPAYISSVAYGRQVYLLKLTNSHSTKVKAFDAVMSCKSVSGIV		
Consensus	(301)		N F A GRN Y S A A I GK D	
		351		400
Cry1C 630aa	(351)	PRSEFTFNGPVFRTLSNPTLRLQLQPWPAPPFNLRGVEGVESTPTNSFTY		
listeriolysin CAA42639	(333)	ELTNIILK-----SSFKAVIYGGSAKDEYQ		
Consensus	(351)		S AV F	
		401		450
Cry1C 630aa	(401)	RGRGTVDSTELPPEDNSVPPREGYSHRLCHATFVQRSCTPFLTIGVVF		
listeriolysin CAA42639	(358)	IIDGNLGLRDLKKGATFN-----RETPGVFIAYITNPLK		
Consensus	(401)		G L L DI S G P T	
		451		500
Cry1C 630aa	(451)	WIDRSATLNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRRNT		
listeriolysin CAA42639	(394)	DNELAVIKNNSEYIETTSKAYTDGKINLDHSGGYAQFNISWDEINNDPE		
Consensus	(451)		D A NS E I I S DI	
		501		550
Cry1C 630aa	(501)	FGDFVSLQVNINSPIQRYRLRFYASSRDARVIVLITGAASTGVGGQVSV		
listeriolysin CAA42639	(444)	GNEIVQHKNWSENNKSKLAHFTSSIYLPGNARNINNYAKECTGLAWEWWR		
Consensus	(501)		D V S AR I L A TGLA	
		551		600
Cry1C 630aa	(551)	NMPLQKIMEIGENLTSRTFRYIDFSNPFSSFRANPDIIGISEQPLFGAGSI		
listeriolysin CAA42639	(494)	TVIDDENLPLVKNRNISINWGTLLYPKYSNSVDNPIE-----		
Consensus	(551)		M K L I N F T F NP	
		601		630
Cry1C 630aa	(601)	SSGELYIDKIEIILADATFEAESDLERAQK		
listeriolysin CAA42639	(530)	-----		
Consensus	(601)			

Neurotoxin A (Clostridium botulinum Type A) versus Cry1C identity 13%

		2		51
Cry1C 630aa	(1)	-----MEE	NNQNQCIPYNCL	
neurotoxin A5HZZ9	(2)	PFVVKQFNYKDPVNGVDIAYIKIPNAGQMOPVKAFKIH	NKIWVIPERDTF	
Consensus	(2)		N	
		52		101
Cry1C 630aa	(16)	SNPEE-----VLLDGERI	STGNSSSIDISLSLVQFLVSNFVFP	
neurotoxin A5HZZ9	(52)	INPEEGDLNPPPEAKQVPVSYD	STYLSTDNKEDNYLKGVTKLFERIYST	
Consensus	(52)	SNPEE	D IST N L F	
		102		151
Cry1C 630aa	(52)	GGG--FLVGLIDFVWGI	GPSQWDAFLVQIEOLINERIAEFARNAAIANL	
neurotoxin A5HZZ9	(102)	DLGRMLLTSIVRGLIPFWG	STIDTELKVIDTNCINVIQPDGYSRSEELNL	
Consensus	(102)	G L II I G S V N IN D A A NL		
		152		201
Cry1C 630aa	(100)	EGLGNNFNIVVEAFKE	VEEDPNNPETRTRVIDRFRILDGLLERDIPSFRI	
neurotoxin A5HZZ9	(152)	VILGPSADIIQFECK	SGHEVLN-LTRNGYGSTQYIRFSPDFTFGFEESL	
Consensus	(152)	IG I K F D N TR I		I
		202		251
Cry1C 630aa	(150)	SGFEVPLL	SVYAQAANLHAI LRDSVIFGERWGLTTINVENNRLIRHI	
neurotoxin A5HZZ9	(201)	EVDTNPLL	GAGKFAATDPAVTLAHELHAGHRLYGIAINPNRVKVTNAY	
Consensus	(202)	PLL A L I D I G R IN N F		
		252		301
Cry1C 630aa	(200)	DEYADHCANTYNRGLN	NLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNY	
neurotoxin A5HZZ9	(251)	YEMSGLEVSFEELR	TFGGHDAKFIDSLQENEFRLYYNKFKDIASTLNKA	
Consensus	(252)	E A A F D I N R DIAA		
		302		351
Cry1C 630aa	(250)	DNRRYPIQPVG	QLTREVYTDPLINFNPLQSVLQPTFNVMESSRIRNPH	
neurotoxin A5HZZ9	(301)	KSIVGTTASIQY	MKNVFKKEYLLSEDTSGKFSVDKLFDFKLYK-----M	
Consensus	(302)	L L LI F L		
		352		401
Cry1C 630aa	(300)	LFDILNLTIFTD	WFSVGRNFYWGGRVVISLIGGGNLTSPITYGREANQE	
neurotoxin A5HZZ9	(345)	LTEIYTEDNFVK	FEKVLNKKTYLNFDKAVFKINIVPKVNYTYDGFNLRN	
Consensus	(352)	L DI F L R Y K I I I IY		
		402		451
Cry1C 630aa	(350)	PPRSFTFNG	PVVRTLSNPTLRLLQWPWAPPFNLRGVEGVFSTPTNSFT	
neurotoxin A5HZZ9	(395)	TNLAANFNG	QNTTEINNMNFTKLNKFTGLFEFYKLLCVRGITTSKTKSLDK	
Consensus	(402)	A FNG KL N F L V GI S		
		452		501
Cry1C 630aa	(400)	YRGRGTVDSL	TELPPEDNSVPP-REGYSHRLCHATFVQRSGTPFLTGGV	
neurotoxin A5HZZ9	(445)	GYNKALNDLC	IKVNNWDLFFSPSEDNFTNDLNKGEETSDTNIEAAEENI	
Consensus	(452)	KA D L D P D FS L A I I		
		502		551
Cry1C 630aa	(449)	FSWTDRSATL	TNTIDPER-----INOTPLVKGFRVWGGTTSVIT	
neurotoxin A5HZZ9	(495)	SLDLIQYYL	TNFNDEPENISIENLSSDIIGOLELMPNIEREPNGKKYE	
Consensus	(502)	LT D E I QI LM F		
		552		601
Cry1C 630aa	(487)	GPGFTGGDI	LRRNTEGDFVSLQVNI NSPITQRYRLRFRYASSRDARVIVL	
neurotoxin A5HZZ9	(545)	LDKYTMFHY	LRAQEFEHGKSRIALTNSVNEALLNPSRVYTFSSDYVKKV	
Consensus	(552)	FT LR N F S NS Y V L		
		602		651
Cry1C 630aa	(537)	TGAASTGV	GGQVSNMPLQK TMEIGENLTSRTFRYTDFSNPF SFRANPDI	
neurotoxin A5HZZ9	(595)	NKATEAAM	FLGWVEQLVYDF TDETSVSTTDKIADITIIIPYIGPALNIG	
Consensus	(602)	A AM NL T E E TS PF A		
		652		695
Cry1C 630aa	(587)	IGTSEQPLF	GAGSISSEIYIDKIEI LLADATFEAESDLERACK	
neurotoxin A5HZZ9	(645)	NMLYKDDFV	GALIFSGAVLLLEFIPETAIIPVLGTFALVSYIANK	
Consensus	(652)	I GA S A I ID I I ANK		

Neurotoxin B (C. botulinum Type B) versus Cry1C identity 7%

		1		50
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(1)	MPVTINNFNYNDPIDNNNIIMMEPPFARGTGRIYKAFKITDRIWIIPERY		
Consensus	(1)			
		51		100
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(51)	TFGYKPEDFNKSSGIFNRDVCEYYDPDYLNNTNDKKNIFLQTMIKLFNRIK		
Consensus	(51)			
		101		150
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(101)	SKPLGKELLEMIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNPGEVER		
Consensus	(101)			
		151		200
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(151)	KKGIFANLIIFGPGPVLNENETIDIGIQNHFASREGFGGIMQMKFCPEYV		
Consensus	(151)			
		201		250
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(201)	SVFNNVQENKGASIFNRRGYFSDPALILMHელიHV LHGLYGIKVDDLPIV		
Consensus	(201)			
		251		300
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(251)	PNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNFGRGIV		
Consensus	(251)			
		301		350
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(301)	DRLNKVLVCISDPNININIIYKNKFKDKYKFVEDSEGKYSIDVESFDKLYK		
Consensus	(301)			
		351		400
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(351)	SLMFGFTETNIAENYKIKTRASYFSDSLPPVKIKNLLDNEIYTIEEGFNI		
Consensus	(351)			
		401		450
Cry1C 630aa	(1)	-----		
neurotoxin B1INP5	(401)	SDKDMEKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSVKAPGICIDVD		
Consensus	(401)			
		451		500
Cry1C 630aa	(1)	-----MEENNQOCIPYCLSNPEE-----VLLDGERIST		
neurotoxin B1INP5	(451)	NEDLFFIADKNSFSDLSKNERIEYNTQSNYIEINDFPINELLDLTLISK		
Consensus	(451)	DD N I YN SN E LILD D IS		
		501		550
Cry1C 630aa	(31)	GNS-----S-IDIISL-----		
neurotoxin B1INP5	(501)	IELPSENTESLTDNFVDVPEYKQPAIKKIIFTDENTIFQYLYSQTFPLDI		
Consensus	(501)	IDI L		
		551		600
Cry1C 630aa	(41)	--LVQFLVSNFVPG--G--GGFLVGLIDFVWGVGIVGPSQWDAFLVQIEQLINE		
neurotoxin B1INP5	(551)	RDLSLTSSFDALLFSNKVYSFFSMIDYIKTANKVVEAGLFAGWVKQIVND		
Consensus	(551)	I F IDFI F I QIIND		
		601		650
Cry1C 630aa	(86)	RIAEFARNAAIANLEGLGNNFNIIYVEAFKEWEEDPN-NP-ETRTRVIDRFR		
neurotoxin B1INP5	(601)	FVIEANKSNTMDKLIADISLIVPYIGLALNVGNETAKGNFENAFELIAGASI		
Consensus	(601)	I E K I I I A E N E I		
		651		700
Cry1C 630aa	(135)	ILDGLLERDIPSFRIISGFVPLLSVYAQAANHLAHLRDSVIFGERWGLT		
neurotoxin B1INP5	(651)	ILDFIPELLIPVVGAFLLSYLDNKNKI IKTIDNALTKRNEKWSDMYGLI		
Consensus	(651)	ILD I E IP E I I AI K F D WGL		
		701		750
Cry1C 630aa	(185)	TINVNENYNRLIRHIDFAYADHCANTYNRGLNNEPKSTYQDVIIT-----		
neurotoxin B1INP5	(701)	VAQWLSTVNTQFYTKKGMKALNYQAQALEEIKYRYNINISEKEKSNIN		
Consensus	(701)	N N I E N AL I K YN W		
		751		800

Cry1C 630aa	(228)	-----Y <span style="background-color: yellow;">N</span> R <span style="background-color: green;">L</span> R <span style="background-color: green;">R</span> D <span style="background-color: green;">L</span> T <span style="background-color: green;">L</span> T <span style="background-color: green;">V</span> L <span style="background-color: green;">D</span> I <span style="background-color: green;">A</span> A <span style="background-color: green;">F</span> F <span style="background-color: yellow;">P</span> N <span style="background-color: green;">Y</span> D <span style="background-color: green;">N</span> R <span style="background-color: green;">R</span> R <span style="background-color: green;">Y</span> P <span style="background-color: green;">I</span> Q <span style="background-color: green;">P</span> V <span style="background-color: green;">G</span> Q <span style="background-color: green;">L</span> T <span style="background-color: green;">R</span> E <span style="background-color: green;">V</span> Y <span style="background-color: green;">T</span> D <span style="background-color: green;">P</span> L <span style="background-color: green;">I</span>
neurotoxin B1INP5	(751)	ID <span style="background-color: green;">F</span> N <span style="background-color: green;">D</span> I <span style="background-color: green;">N</span> S <span style="background-color: green;">K</span> L <span style="background-color: green;">N</span> E <span style="background-color: green;">G</span> I <span style="background-color: green;">N</span> Q <span style="background-color: green;">A</span> I <span style="background-color: green;">D</span> N <span style="background-color: green;">I</span> N <span style="background-color: green;">N</span> F <span style="background-color: green;">I</span> N <span style="background-color: green;">G</span> C <span style="background-color: green;">S</span> V <span style="background-color: green;">S</span> L <span style="background-color: green;">M</span> K <span style="background-color: green;">K</span> M <span style="background-color: green;">I</span> P <span style="background-color: green;">L</span> A <span style="background-color: green;">V</span> E <span style="background-color: green;">K</span> L <span style="background-color: green;">L</span> D <span style="background-color: green;">F</span> D <span style="background-color: green;">N</span>
Consensus	(751)	N      D      ID      F  N      Y  I  M  L  E  D
Cry1C 630aa	(273)	N <span style="background-color: green;">F</span> N <span style="background-color: green;">P</span> Q <span style="background-color: green;">L</span> Q <span style="background-color: green;">S</span> V <span style="background-color: green;">A</span> Q <span style="background-color: green;">L</span> P <span style="background-color: green;">T</span> F <span style="background-color: green;">N</span> V <span style="background-color: green;">M</span> E <span style="background-color: green;">S</span> S <span style="background-color: green;">R</span> I <span style="background-color: green;">R</span> N-----P <span style="background-color: green;">H</span> L <span style="background-color: green;">F</span> D <span style="background-color: green;">I</span> L <span style="background-color: green;">N</span> N <span style="background-color: green;">L</span> T <span style="background-color: green;">I</span> I <span style="background-color: green;">F</span> T
neurotoxin B1INP5	(801)	TL <span style="background-color: green;">K</span> K <span style="background-color: green;">N</span> L <span style="background-color: green;">L</span> N <span style="background-color: green;">Y</span> I <span style="background-color: green;">D</span> E <span style="background-color: green;">N</span> K <span style="background-color: green;">L</span> Y <span style="background-color: green;">L</span> I <span style="background-color: green;">G</span> S <span style="background-color: green;">A</span> E <span style="background-color: green;">Y</span> E <span style="background-color: green;">K</span> S <span style="background-color: green;">K</span> V <span style="background-color: green;">N</span> K <span style="background-color: green;">Y</span> L <span style="background-color: green;">K</span> T <span style="background-color: green;">I</span> M <span style="background-color: green;">P</span> F <span style="background-color: green;">D</span> L <span style="background-color: green;">S</span> I <span style="background-color: green;">Y</span> T <span style="background-color: green;">N</span> D <span style="background-color: green;">T</span> I <span style="background-color: green;">L</span> I
Consensus	(801)	NL          LI  SA          P      I  N  TI
Cry1C 630aa	(312)	D <span style="background-color: green;">W</span> F <span style="background-color: green;">S</span> V <span style="background-color: green;">G</span> R <span style="background-color: green;">N</span> F <span style="background-color: green;">Y</span> W <span style="background-color: green;">G</span> G <span style="background-color: green;">H</span> R <span style="background-color: green;">V</span> I <span style="background-color: green;">S</span> S <span style="background-color: green;">L</span> I <span style="background-color: green;">G</span> G <span style="background-color: green;">N</span> I <span style="background-color: green;">T</span> S <span style="background-color: green;">P</span> I <span style="background-color: green;">Y</span> G <span style="background-color: green;">R</span> E <span style="background-color: green;">A</span> N <span style="background-color: green;">Q</span> E <span style="background-color: green;">P</span> P <span style="background-color: green;">R</span> S <span style="background-color: green;">F</span> T <span style="background-color: green;">F</span> N <span style="background-color: green;">G</span> P <span style="background-color: green;">V</span> F
neurotoxin B1INP5	(851)	E <span style="background-color: green;">M</span> F <span style="background-color: green;">N</span> K <span style="background-color: green;">Y</span> N <span style="background-color: green;">S</span> E <span style="background-color: green;">I</span> L <span style="background-color: green;">N</span> N <span style="background-color: green;">I</span> I <span style="background-color: green;">L</span> N <span style="background-color: green;">L</span> R <span style="background-color: green;">Y</span> K <span style="background-color: green;">D</span> N <span style="background-color: green;">N</span> L <span style="background-color: green;">I</span> D <span style="background-color: green;">L</span> S <span style="background-color: green;">G</span> Y <span style="background-color: green;">G</span> A <span style="background-color: green;">K</span> V <span style="background-color: green;">E</span> V <span style="background-color: green;">Y</span> D <span style="background-color: green;">G</span> V <span style="background-color: green;">E</span> L <span style="background-color: green;">N</span> D <span style="background-color: green;">K</span> N <span style="background-color: green;">Q</span> F <span style="background-color: green;">K</span>
Consensus	(851)	D  F                  L          I      Y  G
Cry1C 630aa	(362)	R <span style="background-color: green;">T</span> L <span style="background-color: green;">S</span> N <span style="background-color: green;">P</span> L <span style="background-color: green;">R</span> L <span style="background-color: green;">L</span> Q <span style="background-color: green;">Q</span> P-----W <span style="background-color: green;">P</span> A <span style="background-color: green;">P</span> P <span style="background-color: green;">F</span> N <span style="background-color: green;">L</span> R <span style="background-color: green;">S</span> V <span style="background-color: green;">E</span> G <span style="background-color: green;">V</span> E <span style="background-color: green;">F</span> S <span style="background-color: green;">T</span> P
neurotoxin B1INP5	(901)	L <span style="background-color: green;">T</span> S <span style="background-color: green;">S</span> A <span style="background-color: green;">N</span> S <span style="background-color: green;">K</span> I <span style="background-color: green;">R</span> V <span style="background-color: green;">T</span> O <span style="background-color: green;">N</span> Q <span style="background-color: green;">N</span> I <span style="background-color: green;">I</span> F <span style="background-color: green;">N</span> S <span style="background-color: green;">V</span> F <span style="background-color: green;">L</span> D <span style="background-color: green;">F</span> S <span style="background-color: green;">V</span> S <span style="background-color: green;">F</span> W <span style="background-color: green;">I</span> R <span style="background-color: green;">I</span> P <span style="background-color: green;">K</span> Y <span style="background-color: green;">K</span> N <span style="background-color: green;">D</span> S <span style="background-color: green;">I</span> Q <span style="background-color: green;">N</span> Y <span style="background-color: green;">I</span> H <span style="background-color: green;">N</span> E <span style="background-color: green;">Y</span>
Consensus	(901)	T  S  S      L  Q                          P  F      G  I
Cry1C 630aa	(395)	T <span style="background-color: green;">N</span> S <span style="background-color: green;">F</span> T <span style="background-color: green;">Y</span> R <span style="background-color: green;">G</span> R <span style="background-color: green;">T</span> V <span style="background-color: green;">D</span> S <span style="background-color: green;">L</span> T <span style="background-color: green;">E</span> L <span style="background-color: green;">P</span> P <span style="background-color: green;">E</span> D <span style="background-color: green;">N</span> S <span style="background-color: green;">V</span> P <span style="background-color: green;">P</span> R <span style="background-color: green;">E</span> G <span style="background-color: green;">Y</span> S <span style="background-color: green;">H</span> R <span style="background-color: green;">I</span> C <span style="background-color: green;">H</span> A <span style="background-color: green;">T</span> F <span style="background-color: green;">Q</span> R <span style="background-color: green;">S</span> G <span style="background-color: green;">I</span> P <span style="background-color: green;">F</span> L <span style="background-color: green;">T</span>
neurotoxin B1INP5	(951)	T <span style="background-color: green;">I</span> I <span style="background-color: green;">N</span> C <span style="background-color: green;">M</span> K <span style="background-color: green;">N</span> N <span style="background-color: green;">S</span> G <span style="background-color: green;">W</span> K <span style="background-color: green;">I</span> S <span style="background-color: green;">I</span> R <span style="background-color: green;">G</span> N <span style="background-color: green;">R</span> I <span style="background-color: green;">I</span> W <span style="background-color: green;">L</span> I <span style="background-color: green;">D</span> I <span style="background-color: green;">N</span> G <span style="background-color: green;">K</span> I <span style="background-color: green;">K</span> S <span style="background-color: green;">V</span> F <span style="background-color: green;">F</span> E <span style="background-color: green;">Y</span> N <span style="background-color: green;">I</span> R <span style="background-color: green;">E</span> D <span style="background-color: green;">I</span> S <span style="background-color: green;">E</span> Y <span style="background-color: green;">I</span> N
Consensus	(951)	T          K                  S  L      G  S  L      I      S  F  I
Cry1C 630aa	(445)	T <span style="background-color: green;">G</span> V <span style="background-color: green;">V</span> F <span style="background-color: green;">S</span> W <span style="background-color: green;">T</span> D <span style="background-color: green;">R</span> S <span style="background-color: green;">A</span> T <span style="background-color: green;">L</span> T <span style="background-color: green;">N</span> T <span style="background-color: green;">I</span> D <span style="background-color: green;">P</span> E <span style="background-color: green;">R</span> I <span style="background-color: green;">N</span> O <span style="background-color: green;">I</span> P <span style="background-color: green;">L</span> V <span style="background-color: green;">K</span> G <span style="background-color: green;">F</span> R <span style="background-color: green;">V</span> W <span style="background-color: green;">G</span> G <span style="background-color: green;">T</span> S <span style="background-color: green;">V</span> I <span style="background-color: green;">T</span> G <span style="background-color: green;">P</span> G <span style="background-color: green;">P</span> --F <span style="background-color: green;">T</span> G
neurotoxin B1INP5	(1001)	R <span style="background-color: green;">W</span> F <span style="background-color: green;">F</span> V <span style="background-color: green;">T</span> I <span style="background-color: green;">I</span> N <span style="background-color: green;">N</span> L <span style="background-color: green;">N</span> N <span style="background-color: green;">A</span> K <span style="background-color: green;">I</span> Y <span style="background-color: green;">I</span> N <span style="background-color: green;">G</span> K <span style="background-color: green;">L</span> E <span style="background-color: green;">S</span> N <span style="background-color: green;">T</span> D <span style="background-color: green;">I</span> K <span style="background-color: green;">D</span> I <span style="background-color: green;">R</span> E <span style="background-color: green;">V</span> I <span style="background-color: green;">A</span> N <span style="background-color: green;">G</span> E <span style="background-color: green;">I</span> I <span style="background-color: green;">F</span> K <span style="background-color: green;">L</span> D <span style="background-color: green;">G</span> D <span style="background-color: green;">I</span> D <span style="background-color: green;">R</span>
Consensus	(1001)	S  T          I      N  I      V  A      I  I
Cry1C 630aa	(493)	G <span style="background-color: green;">D</span> I <span style="background-color: green;">L</span> R <span style="background-color: green;">R</span> N <span style="background-color: green;">T</span> F <span style="background-color: green;">G</span> D <span style="background-color: green;">F</span> V <span style="background-color: green;">S</span> L <span style="background-color: green;">Q</span> V <span style="background-color: green;">N</span> I <span style="background-color: green;">N</span> S <span style="background-color: green;">P</span> I <span style="background-color: green;">T</span> O <span style="background-color: green;">R</span> Y <span style="background-color: green;">R</span> L <span style="background-color: green;">R</span> F <span style="background-color: green;">R</span> Y <span style="background-color: green;">A</span> S <span style="background-color: green;">S</span> R <span style="background-color: green;">D</span> A <span style="background-color: green;">R</span> V <span style="background-color: green;">I</span> V <span style="background-color: green;">L</span> T <span style="background-color: green;">G</span> A <span style="background-color: green;">A</span> S <span style="background-color: green;">T</span>
neurotoxin B1INP5	(1051)	T <span style="background-color: green;">Q</span> F <span style="background-color: green;">I</span> W <span style="background-color: green;">M</span> K <span style="background-color: green;">Y</span> E <span style="background-color: green;">S</span> I <span style="background-color: green;">F</span> N <span style="background-color: green;">T</span> E <span style="background-color: green;">L</span> S <span style="background-color: green;">Q</span> S <span style="background-color: green;">N</span> I <span style="background-color: green;">E</span> E <span style="background-color: green;">R</span> Y <span style="background-color: green;">K</span> I <span style="background-color: green;">Q</span> S <span style="background-color: green;">Y</span> S <span style="background-color: green;">E</span> L <span style="background-color: green;">K</span> D <span style="background-color: green;">F</span> W <span style="background-color: green;">G</span> N <span style="background-color: green;">P</span> L <span style="background-color: green;">M</span> Y <span style="background-color: green;">N</span> K <span style="background-color: green;">E</span> Y <span style="background-color: green;">Y</span> M
Consensus	(1051)	I      F      F  S      N  N      K      Y      A      I  M
Cry1C 630aa	(543)	G <span style="background-color: green;">V</span> G <span style="background-color: green;">C</span> O <span style="background-color: green;">V</span> S <span style="background-color: green;">V</span> M <span style="background-color: green;">P</span> L <span style="background-color: green;">Q</span> T <span style="background-color: green;">M</span> E <span style="background-color: green;">I</span> G <span style="background-color: green;">E</span> N <span style="background-color: green;">L</span> T <span style="background-color: green;">S</span> R <span style="background-color: green;">T</span> E <span style="background-color: green;">R</span> --Y <span style="background-color: green;">T</span> D <span style="background-color: green;">F</span> S <span style="background-color: green;">N</span> P <span style="background-color: green;">F</span> S <span style="background-color: green;">F</span> R <span style="background-color: green;">A</span> N <span style="background-color: green;">P</span> D <span style="background-color: green;">I</span> I <span style="background-color: green;">G</span> I <span style="background-color: green;">S</span>
neurotoxin B1INP5	(1101)	F <span style="background-color: green;">N</span> A <span style="background-color: green;">G</span> N <span style="background-color: green;">K</span> N <span style="background-color: green;">S</span> Y <span style="background-color: green;">L</span> K <span style="background-color: green;">L</span> K <span style="background-color: green;">D</span> S <span style="background-color: green;">P</span> V <span style="background-color: green;">G</span> E <span style="background-color: green;">I</span> L <span style="background-color: green;">T</span> R <span style="background-color: green;">S</span> K <span style="background-color: green;">Y</span> N <span style="background-color: green;">Q</span> N <span style="background-color: green;">S</span> K <span style="background-color: green;">Y</span> I <span style="background-color: green;">N</span> Y <span style="background-color: green;">R</span> D <span style="background-color: green;">L</span> I <span style="background-color: green;">G</span> E <span style="background-color: green;">K</span> F <span style="background-color: green;">I</span> I <span style="background-color: green;">R</span> R <span style="background-color: green;">K</span>
Consensus	(1101)	A <span style="background-color: green;">G</span> N      I  L  K      I <span style="background-color: green;">G</span> E  L <span style="background-color: green;">T</span> F          F  A      I  I
Cry1C 630aa	(591)	--E <span style="background-color: green;">Q</span> P <span style="background-color: green;">L</span> F <span style="background-color: green;">G</span> A <span style="background-color: green;">G</span> S <span style="background-color: green;">I</span> S <span style="background-color: green;">S</span> G <span style="background-color: green;">E</span> L <span style="background-color: green;">Y</span> I <span style="background-color: green;">D</span> K <span style="background-color: green;">I</span> E <span style="background-color: green;">I</span> L <span style="background-color: green;">A</span> D <span style="background-color: green;">A</span> T <span style="background-color: green;">F</span> E <span style="background-color: green;">A</span> E <span style="background-color: green;">S</span> D <span style="background-color: green;">L</span> E <span style="background-color: green;">R</span> A <span style="background-color: green;">Q</span> K
neurotoxin B1INP5	(1151)	S <span style="background-color: green;">N</span> S <span style="background-color: green;">Q</span> S <span style="background-color: green;">I</span> N <span style="background-color: green;">D</span> D <span style="background-color: green;">I</span> V <span style="background-color: green;">R</span> K <span style="background-color: green;">E</span> D <span style="background-color: green;">Y</span> L <span style="background-color: green;">Y</span> I <span style="background-color: green;">D</span> F <span style="background-color: green;">F</span> N <span style="background-color: green;">L</span> N <span style="background-color: green;">Q</span> E <span style="background-color: green;">W</span> R <span style="background-color: green;">V</span> Y <span style="background-color: green;">T</span> Y <span style="background-color: green;">K</span> Y <span style="background-color: green;">F</span> K <span style="background-color: green;">K</span> E <span style="background-color: green;">E</span> E <span style="background-color: green;">K</span>
Consensus	(1151)	Q  I          I <span style="background-color: green;">Y</span> I <span style="background-color: green;">D</span> I      F          K



Perfringolysin O (C. perfringens Type A) versus Cry1C identity 12.2%

```

1                               50
Cry1C 630aa (1) ---MEENNQNCIPYNCLSNPEEVLDDGERISTGNSSIDISLSLVOFLVS
perfringolysin O AAA23270 (1) MIRFKKTKLIASIAMALCLFSQPVISFSKDIIDKNQSIDSGISLSYNNRN
Consensus (1) I VI IS N SID IS L F

51                               100
Cry1C 630aa (48) NFPVGGGFLVGLIDFVWGVGQWDAFLVQIEQLINERIAEFARNAAIA
perfringolysin O AAA23270 (51) EVLASN-----GDKIESFVPEKGGK
Consensus (51) L DK I F A

101                               150
Cry1C 630aa (98) NLEGLGNNFNIIYVEAKKEWEEDNNPETRTRVIDRFRILLDGLLERDIPSF
perfringolysin O AAA23270 (71) ----AGNKFIVVERQKRLTTSVVDISIIDSND--RTYPGALQLADKAF
Consensus (101) GN F I K P V D R G L AF

151                               200
Cry1C 630aa (148) RISGFVPLLSVYAAQAAHLHLALRDSVIFGCEWGLITINVNENYNRLLIR
perfringolysin O AAA23270 (115) VEN---RPTLLMVKEAANINIDLP----GLKGENSIKVDPTYKQVSG
Consensus (151) P I M A N I I I G K S Y K L

201                               250
Cry1C 630aa (198) HIDEYADHCANTYNRGLNNLPKSTYQDWITYNLRRDLTLTLDIAAFFP
perfringolysin O AAA23270 (156) AIDELVSKWNEKYSSTHTLPARTQYSESIVVYSQISSALNN-----
Consensus (201) IDE Y KS Y D I Y K L V

251                               300
Cry1C 630aa (248) NYDNRRYPIQPVGQLTREYVTDPLINFPNQLQSVAAQLPTFNVMSSRIRN
perfringolysin O AAA23270 (199) -----AKVLENSLGVDFNAWANNKVMILAYKQIFYYTYSADLPKN
Consensus (251) V S V L N L LA F M A KN

301                               350
Cry1C 630aa (298) PHLFDLNNLTIPTDWFVGRNRFYWGGRVLSLIGGGNITSPIYGRGAN
perfringolysin O AAA23270 (240) P--SDFDSDSVTFNDLKKQG-----VSNAPPLMVSNAVYGRTIY
Consensus (301) P DI F D G IS IS YGR

351                               400
Cry1C 630aa (348) QEPPRSFTFNGPVFRTLSNPTLRLIQQPWAPPFVLRGVEGVFSTPTNS
perfringolysin O AAA23270 (278) VK-----LETTSSKDVQAAFKAIIKNTDIKNS
Consensus (351) L A N I S NS

401                               450
Cry1C 630aa (398) FTYRGRGTVDSLTELPEDNSVPPREGYSHRLCHATFVQRSGTPTLTGV
perfringolysin O AAA23270 (306) QQYKDIYENS SFTAVVLGGDAQEHNKVVTKDFDEIRKVIKDNATFSTKNP
Consensus (401) YK S T L A S V K F T

451                               500
Cry1C 630aa (448) VFSWIDRSATLINTIDPERINQIPLVKGFRVWGGTSVITGPGFTCGDILR
perfringolysin O AAA23270 (356) AVPISYTSVFLKDNSVAAVHNKTDYLETSTSEYSGKIN-LDHSQAYVAQ
Consensus (451) F S S L N I I SGA I

501                               550
Cry1C 630aa (498) RNTFGDFVSLQVNNISPTIQRYRLRFRYASSRDARVIVLTGAASTGVGGQ
perfringolysin O AAA23270 (405) FEVAWDEVSYDKEGNEVLTHKTDWGNVYQDKTAHYSTVIPLEANAR-----
Consensus (501) D VS N IT K S II A A

551                               600
Cry1C 630aa (548) VSVNMPLOKTMETIGENLTSRTFRYTDPSNPFFRANPDIIGISEQPLFGA
perfringolysin O AAA23270 (450) ---NIRIKARECTGLAWEWWRDVISEYDVPILNNINVSILWGTI---LYPG
Consensus (551) NI I G SDF P S N I G S LF A

601                               633
Cry1C 630aa (598) GSTSSGELYIDKIEIILADATFEAESDLERAQK
perfringolysin O AAA23270 (494) SSITYN-----
Consensus (601) SIS

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Pneumolysin (*S. pneumoniae*) versus Cry1C identity 12%

		1		50
Cry1C 630aa	(1)	MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV		
pneumolysin ABO21376	(1)	-----MANKAVNDFELAMNYDKKKLL		
Consensus	(1)	AN AI I MNF L		
		51		100
Cry1C 630aa	(51)	PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE		
pneumolysin ABO21376	(22)	THQ-----GESIENRFIKEGNQLPDEFVVIERKKRSLSD		
Consensus	(51)	G S FI QL E I K AIA		
		101		150
Cry1C 630aa	(101)	GLGNFNLIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLIERDISPSFRIS		
pneumolysin ABO21376	(55)	--TNTSDISVTATNDSRLYPG-----ALLVVDDELLENPP		
Consensus	(101)	N I V A D P ILD L P		
		151		200
Cry1C 630aa	(151)	GFEVPLLSVYAQAANLHLAIRDSVIFGERWGLTININENYNRLLRHID		
pneumolysin ABO21376	(88)	--TLAADRAPMTYSIDLPLASSDSFLQVEDPSNSSVGVAVNDLLAKWH		
Consensus	(151)	L L I L L S F S V N LI		
		201		250
Cry1C 630aa	(201)	EYADHCANTYNRGLNNLPKSTYQDWITYNRRRDLTLTLVLDIAAFFPNYD		
pneumolysin ABO21376	(136)	QDYG-----QNNVPARMQYKITAHSMEQLKVKFGSDFEKTGNSLD		
Consensus	(201)	LNNLP D IT L D D		
		251		300
Cry1C 630aa	(251)	NRRYPQPVGQLTREVYTDPLINFNPOLOSVAQLPTFNWESSRIRNPHL		
pneumolysin ABO21376	(178)	IDFNSVHSG-----EKIQIIVNFKQIYYTISVDAVKNP		
Consensus	(251)	I QIQ V F M IKNP		
		301		350
Cry1C 630aa	(301)	FDILNNTLIFTDFWFSVGRNFWGGHRVISSLIGGGNIITSPIYGRLEANQ--		
pneumolysin ABO21376	(211)	GDVFDQTVTVEDLKQRG-----ISAEERPLVYISSVAYGRQVYLKL		
Consensus	(301)	DI N D G ISA ISS YGR		
		351		400
Cry1C 630aa	(349)	EPPRSFTFNGPVFRTLSNPTLRLLLQPPWPAPPFNLRGVEGVFSTPINSF		
pneumolysin ABO21376	(251)	ETTSKSDEVEAAFEALIKGVKVAPQTEWKQILDNTEVKAVILGGDPS-SG		
Consensus	(351)	E F L Q W N I PS S		
		401		450
Cry1C 630aa	(399)	TYRGRGIVDSLTELPEEDNSVPPREGYSHRLCHATFVQRSGTFFLITGVV		
pneumolysin ABO21376	(300)	ARVVTGKVDMVELIQE-----GSRFTADHPGLPISYTTISFLRDNVV		
Consensus	(401)	G VD L DL E G H T FL VV		
		451		500
Cry1C 630aa	(449)	FSWTDRSATLINTDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRR		
pneumolysin ABO21376	(342)	ATFQNSIDYVETKVTAYRN-----GDILLD		
Consensus	(451)	SF S L I R GDIL		
		501		550
Cry1C 630aa	(499)	NIFGDFVSLQVNNINSPIQRYRLRFYASSRDARVIVLTGAASTGVGGQV		
pneumolysin ABO21376	(367)	HSGAYVAQYYITWDELSYDHQKVEVLPKAWDRNGQDLTAHFTTSLPLKG		
Consensus	(501)	S A I A D LTA ST I		
		551		600
Cry1C 630aa	(549)	SVNMPLQKTMEIGENITSRITFRYIDFSNPFSTRANPDIIGISEQPLFGAG		
pneumolysin ABO21376	(417)	NVRN-----LSVKIRECTGLAWEMWRTVYEKTDLPVLRKRTISLWGT		
Consensus	(551)	V I I T DF K II IFG		
		601		632
Cry1C 630aa	(599)	SISSGELYIDKIEIILADATFEAESDLERAQK		
pneumolysin ABO21376	(460)	LYPQVEDKVENDE-----		
Consensus	(601)	E ID		

Ricin (Ricinus communis) versus Cry1C identity 13%

		1		50
Cry1C 630aa	(1)	MEENNQNCIPYNCLSNPEEVLLDGERISTGNSSIDI	SLSLVQFLVSNFV	
ricinP02879	(1)	-----MKPGGN-----	TTVWVWYAVATWL	
Consensus	(1)		I G SI I F VA FL	
		51		100
Cry1C 630aa	(51)	PGGGFLVGLIDFVWGI	VGPSQDAFLVQIEQLINERIAE	FARNAALANLE
ricinP02879	(20)	CFGSTSGWSFTLEDNNL	FPKQYPIINFTTAGATVQSYTNE	IR--AVRGRL
Consensus	(51)	G	I P QW	F R AI
		101		150
Cry1C 630aa	(101)	GLGNNFNIIYVEAFKEWEED	PNNPETRTRVDRFRIL	LDGLERDIPSPRIS
ricinP02879	(68)	TTGADVRHEIPVLPNRVGL	PIINQRFILVELSNHAELS	SVTLALDVTNAYV
Consensus	(101)	G	I P N I L L DI I	
		151		200
Cry1C 630aa	(151)	GFVEVPLLSVYAQAAN	LHLAAILRDSVLF	GERWGLTINVNENY
ricinP02879	(118)	GYRAGNSAYEFHPDN	QEDAEAIT-HLFTD	VQNRYTFAFGGNYDR
Consensus	(151)	GF	A F N A I F D T	NY R ID
		201		250
Cry1C 630aa	(201)	EYADHCANTYNRGLN	LNPKSTYQDNI	TYNRLRRDLTLTVL
ricinP02879	(163)	QLAGNLRENIELGN	GLEEAISALYYSTGGT	QLPTLARSFI
Consensus	(201)	A	G L A W	TL I D
		251		300
Cry1C 630aa	(251)	NRRYPIQPVGQL	TRVYTDPLINFNPQLQSV	AQLPTFNVMES
ricinP02879	(213)	AAEFQYIEGEMR	TRIRYN-----RRSA	PDPSVITLNSWGR
Consensus	(251)	RF	TR Y	A PS LE S R
		301		350
Cry1C 630aa	(301)	FDILNNLTIFTDFW	SVGRNFYWGHRV	ISSLI
ricinP02879	(253)	IQESNGAFASPIQ	LQRNNGSKFSVYD	VSLIP---IIL
Consensus	(301)	NN	S RN IS LI	I A I R A P
		351		400
Cry1C 630aa	(351)	PRSFTFNGP	VFRVLSNPTLRL	LQQF
ricinP02879	(298)	PSSQF	SLLLRPVV	PNFNADVCMDEP
Consensus	(351)	P S F I L N L P P	R G V F	
		401		450
Cry1C 630aa	(396)	NSFTYRGRG	TVDLSLTELPPEDNS	VPPREGYSHRL
ricinP02879	(347)	AIQLWPCKSNT	DANQLWTLKRDNTIR	---SNGKCLT
Consensus	(401)	W K DA		S C TF G L
		451		500
Cry1C 630aa	(446)	GVVFSW	TDRS---ATLNT	IDPERINQI
ricinP02879	(393)	DCNTA	TDATRWQIWDNGT	IINPRSSLVLAATSGNS
Consensus	(451)	A T D S	TI R I	SV T
		501		550
Cry1C 630aa	(493)	GDILRRN	TFGDVFSLQV	NLNSPITQRYRLRF
ricinP02879	(443)	QGWLPT	NNTQPFVTTIV	GLYGLCLQAN---
Consensus	(501)	L N FVS V I Q		D A
		551		600
Cry1C 630aa	(543)	GVGQV	SVNMPLQKTME	EIGENLTSRTFR
ricinP02879	(490)	YADGS	TRPCQNRDNCL	TSDSNIRETVV
Consensus	(551)	G I N L NI K S		S
		601		638
Cry1C 630aa	(593)	PLFG	AGSIS	SSGELYIDKIEIIL
ricinP02879	(540)	NLYS	GLVLDVRASDPSL	KQIILYPLHGDPNQI
Consensus	(601)	LF A I		IIL D

Shigella (S. dysenteriae) toxin versus Cry1C identity 14.6% (89 aa)

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466                                     515
Cry1C 630aa (466) RINQIPLVKGFRVWGGSVITIGPGFTGDI LRRTNFGDFVSLQVWINSPI
shigella toxin (1) MKKTLIAASLSFFSASALATPDCVTCCKVEYTKYNDDDTFTVKVGDKELF
Consensus (466) I I F ASAL T TG K D SL V

516                                     554
Cry1C 630aa (516) TORVRIIRFRYASSRDARVILTGAASTGVGGQVSVNMPL
shigella toxin (51) TNRWNLQSLLSAQITGMTVTIKTNACHNGGGSSEVIFR
Consensus (516) TNRW L SA M V A GG S I

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Tetanus toxin (Clostridium tetani) versus Cry1C identity 12.6%

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1                                     50
Cry1C 630aa (1) -----MEENNQICIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQF
tetanus toxin AAA23282 (1) RSLEYQVDAIKKIIDYEYKIYSGEDKEQIADENLNKN-KLEEKANKAMI
Consensus (1) MD Y S PD I E N ID

51                                     100
Cry1C 630aa (45) LVSNFVPGGGFLVGLIDFVWGIVGPSOWDAFLVQIEOLINERIAEFARNA
tetanus toxin AAA23282 (50) NINIFMRES--SRSFVNQMINEAKKQLLEDTQSKNIMQYTKANSKFI
Consensus (51) I FM I A Q F Q NII I AK

101                                    150
Cry1C 630aa (95) AIANLEGNGNFMNIYVEAFKEEEDPNNPETRTRVTD RFRILDGLLEEDI
tetanus toxin AAA23282 (98) GITELKKLESKINQVSTPIPIESYSKN----LDCWVDNEEDIIDVILKKST
Consensus (101) AI L L N F N ID ID IL K

151                                    200
Cry1C 630aa (145) PSFRISGFVPLL SVYAQAANLHLAALRDSVIFGFRWGLTIINENYNR
tetanus toxin AAA23282 (144) -----LNLDINNDIISISGFNSVITYPDAQ
Consensus (151) L I I D G S I

201                                    250
Cry1C 630aa (195) LIRHIDEYADHCANTYNR--GNNLPKSTYODWITYNRLRRDITITVLDI
tetanus toxin AAA23282 (172) LVPGLNGKAHILVNNESSEVIVHKAMDIEYNDMFNNFTVSFWLRVPKVSA
Consensus (201) LI I A H N L YND L L L L

251                                    300
Cry1C 630aa (243) AAFPNYDNRYPITQPVGQLTREVYTDPIINFN-----PQLQVQIPLT
tetanus toxin AAA23282 (222) SHLEQYGTNEYSIISSMKKHSLSIGGWSVSLKGNLIWTLDKSAEVRQ
Consensus (251) A N I M S I S I S A L

301                                    350
Cry1C 630aa (287) FNVMESSRIRNPHLFDILNNTITFTDFWFSVGRNPFYWG GHRVISSLIGGN
tetanus toxin AAA23282 (272) ITFRDLPDKFNALANKVWFTITINDRLSSANLYING-----VLMGSAE
Consensus (301) D N HL ITI D S A F G LIG A

351                                    400
Cry1C 630aa (337) ITSPIYGRFANPEPPSFTFNGPVFRTLSNPTIRLQQPWPAPPFNLRGV
tetanus toxin AAA23282 (316) ITGLGAIREDNNTITLKLDRCN-----NQYVSDKFRIFCKALNPKEL
Consensus (351) IT RE NN K N N L I N K I

401                                    450
Cry1C 630aa (387) EGFSTPINSFTYRGRGTVDLSLTELPPEDNSVPPREGYSRRLCHATFVQ
tetanus toxin AAA23282 (360) EKLYTSYLSITFLR-----DFWGNPLRYDTEYLLIPVASS
Consensus (401) E L S S SF D P R H L

451                                    500
Cry1C 630aa (437) RSGTPFLTGVVFSWTDTRSATNTIDPERINQIPLVKGFRVWGTSVIT
tetanus toxin AAA23282 (396) K-----DVQLKNIIDYNYLTNAPSYTNGKLNLYRRLYGLKFFI
Consensus (451) K S L T P N I RLW G I

501                                    550
Cry1C 630aa (487) GPGFTGGDILRRNTFGDFVSLQVWINSPIITORYRIRFRYASSRDARVIVL
tetanus toxin AAA23282 (436) KRYPTNNEIDSFVKS GDFIKLYVSYNN---NEHIGYPKDGNAFNNDRI
Consensus (501) DI GDFI L V N N H L F L I

551                                    600
Cry1C 630aa (537) TGAASTGVGGQVSVNMPLQKIMEIGENLTSRTFRYTDFSNPFSTRANPDI
tetanus toxin AAA23282 (483) LRVSYNAPGIPYKMEAVKLRDLKT--YSVQLKLYDDKNASLGLVGTN
Consensus (551) A A G L M K DI S K D N

601                                    644
Cry1C 630aa (587) IGTSEQPLFGAGSISGELYIDKIEITLADATFEESDLERAQK
tetanus toxin AAA23282 (531) GQIGNDENRDILIASNWFNHLKDKILGCDWYEVPTDEGWTND-
Consensus (601) I P S K II D F D

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Theta toxin (*C. perfringens* Type A) versus Cry1C identity 12.5%

		1		50
Cry1C 630aa	(1)	---	MEENNQNQC	IPYNCLSNPEEVLLDGERISTGNSSSIDISLSLVQPLVLS
theta toxin P0C2E9	(1)	MIRFKKTKLIAS	IAMALCLFSQPVLSFSKD	IIDKNQSIDSGLSSLSYNRN
Consensus	(1)		I	VI IS N SID IS L F
		51		100
Cry1C 630aa	(48)	NFVPGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINER	IAEFARNAAIA	
theta toxin P0C2E9	(51)	EVLASN	-----	GDKIESEFVPKEK
Consensus	(51)	L		DKI F A
		101		150
Cry1C 630aa	(98)	NLEGLGNENIYVEAFKEWEEDNPNP	ETRTRVIDRFRILDGLLERDIP	SEF
theta toxin P0C2E9	(71)	---	TGNKFIIVVERQKSLTTSFVDISI	IDSND--RTYPGALQLADKAF
Consensus	(101)		GN F I K P	V D R G L AF
		151		200
Cry1C 630aa	(148)	RISGFVPLLSVYAQAANLHLAILRDSVIFGERW	GLTTIINVNEN-YNRI	
theta toxin P0C2E9	(115)	VENRPTILMVKRKPININIDLPGK	-----	GENSIKVDPTYGKVS
Consensus	(151)		I LL	NI L LK G SI V D Y KL
		201		250
Cry1C 630aa	(197)	RHIDEYADHCANTYNRGLNLPKSTYQDWITYNL	RRLDILTIVLDIAAF	
theta toxin P0C2E9	(156)	GAIDELVSKWNEKYSSTHTLPARTQYSESMVYSK	SQISSAINVN-----	
Consensus	(201)		IDE	Y KS Y D I Y K L V
		251		300
Cry1C 630aa	(247)	PNYDNRRYPIQPVGQVREVTDFLINFNPQIQSVA	QLPTINVMESRIR	
theta toxin P0C2E9	(200)	-----	AKVLENSLGVDFNAVANNKKMILLAYKQIF	YTVSADLPK
Consensus	(251)		V S V	L N L LA F M A K
		301		350
Cry1C 630aa	(297)	NPHLFDLILNNLTIFDWFVSVGRNFYWGHRVIS	SLIGGGNITSPIYGRE	EA
theta toxin P0C2E9	(240)	NP--	SDLFDDSVTFNDLQKKG-----	VSNAPPLMVSNAVYGR
Consensus	(301)		NP	DI F D G IS IS YGR
		351		400
Cry1C 630aa	(347)	NQEPPRSFTFNGPVPFRILSNPTLRLLQQPWP	PPFNLRGVEGVEFSTPTN	
theta toxin P0C2E9	(278)	YVK-----	LETTSSKDVQAAFKALKNTDIKN	
Consensus	(351)			L A N I S N
		401		450
Cry1C 630aa	(397)	SFTYRGRGTVDLSLELPPEDNSVPPREGYS	HRLCHATFVQSGTFFLTITG	
theta toxin P0C2E9	(306)	SQYKDIYENS	SFTAIVLGGDAQEHNKVVTKDFDEIRKVIKDNAT	FSTKN
Consensus	(401)		S YK	S T L A S V K F T
		451		500
Cry1C 630aa	(447)	VVFSWIDRSATLINTIDPERINQIPLVKGFRVW	GGTSVITGPGFTGGDIL	
theta toxin P0C2E9	(356)	PAYPISYTSVFLKDNSVAAVHNTDYETTSTEY	SKGKIN-LDHS	SGAYVA
Consensus	(451)		F S S L	N I I SG A I
		501		550
Cry1C 630aa	(497)	RRNTFGDFVS	LQVNI NSPLTQRYRLRFRYASSRDARVIVLTGA	AASTGVGG
theta toxin P0C2E9	(405)	QFEVAWDEVS	YDKEGNEVLTHTKTWDGN	YQDKTAHYSTVI
Consensus	(501)		D VS	N IT K S II A A
		551		600
Cry1C 630aa	(547)	QVSVNMP	LQKTMEIGENLTSRTFRYIDFSNPF	SFRANPD IIGISEQPLFG
theta toxin P0C2E9	(451)	---	NIRIKARECTGLAWEW	RRDVISEYDVPLININVS IWCITL---
Consensus	(551)		NI I	G SDF P S N I G S LF
		601		634
Cry1C 630aa	(597)	AGSISSGELYIDKIEIILADATFEAESDLERAQK		
theta toxin P0C2E9	(494)	SSITYN	-----	
Consensus	(601)		A	SIS

