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

Submitted to

Review Committee on Genetic Modifications

Department of Biotechnology

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16 January 2009

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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[1]. 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[2]. 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[3]. Relevant extract of this report is attached as A**nnexure I**. In summary, in a number of studies to assess the acute toxicity and pathogenicity of commercial Bta formulations in birds (young mallards, *Anas platyrhynchus*; 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 Bta strains or *Pseudomonas fluorescens* engineered to express Cry1C protein (A**nnexure 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[4].

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.[5] 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.

Gene designation	Accession No.	Reference
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[6]. 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[7], 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[8], 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[9] and more recently the group

led Soberon[10] 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 in brought out in a recent article by Gomez[11] 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[12].

The reason for different insect specificities of the Cry1C protein as compared to other Cry proteins has been studies using binding assays with ^{125}I – or biotin labeled toxins and larval brush border membrane vesicles (BBMV)[13]. In the same study the pore-forming activity of the Cry proteins on BBMV were determined using voltage-sensitive fluorescent dye $DiSC_3$[14]. 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.[15] have tried to understand the role of the protein domain of Cry1C protein that confers it 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 cytocidal 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)[16]. 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[17]. 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	MEENNQNQCIP	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	TREVY	457	TDRSAT
278	FNPQL	458	L
284	QSVAQL	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	TELPPEDNSVPPREGY	619	F
434	SHRLCHATF	624	EAESD
441	VQRSGTP	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	TREVYTDP
89	INERIAE	273	LIN
90	F	278	FNPQL
98	ARNAAIAN	284	QSVAQL
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	TELPPEDNSVPPREGYSHRLCHATF	625	L
441	VQRSGTP	630	ERAQK
443	FL		
448	TTGVV		
449	F		
457	SWTDRSAT		
458	L		
471	TNTIDPERINQIP		
475	LVKG		
490	FRVWGGTSVITGPGF		
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	NAAIANLEGLGNNFNIYVEAFK
126	EWEEDPNNPETR
128	TR
132	VIDR
134	FR
142	ILDGLLER
148	DIPSFR
172	ISGFEVPLLSVYAQAANLHLAILR
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	VISSLIGGGNITSPIYGR
352	EANQEPPR
362	SFTFNGPVFR
370	TLSNPTLR
384	LLQQPWPAPPFNLR
401	GVEGVEFSTPTNSFTYR
403	GR
422	GTVDSLTELPPEDNSVPPR
428	EGYSHR
437	LCHATFVQR
454	SGTPFLTTGVVFSWTDR
466	SATLTNTIDPER
474	INQIPLVK
477	GFR
497	VWGGTSVITGPGFTGGDILR
498	R
518	NTFGDFVSLQVNINSPITQR
520	YR
522	LR
524	FR
529	YASSR
532	DAR
556	VIVLTGAASTGVGGQVSVNMPLQK
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

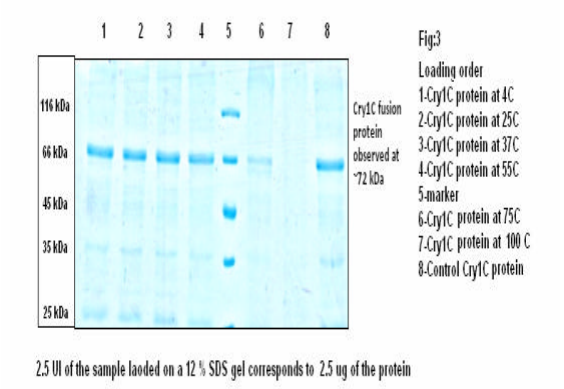
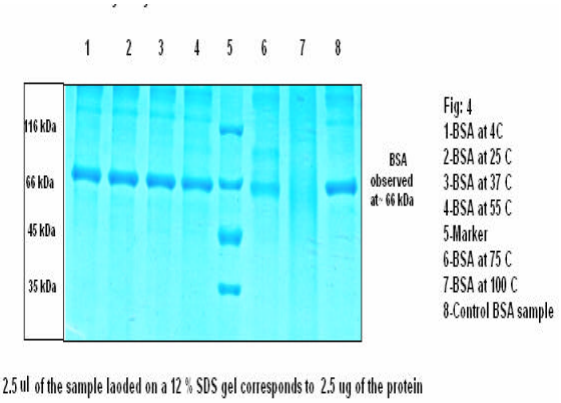


Figure 1 (b) – thermal stability profile of control protein (BSA)



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[18]. 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)[19] recommends performing the digestion at pH 1.2. However, the FAO/WHO (2001)[20] 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)[21] and by Thomas *et al.* (2004)[18] 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

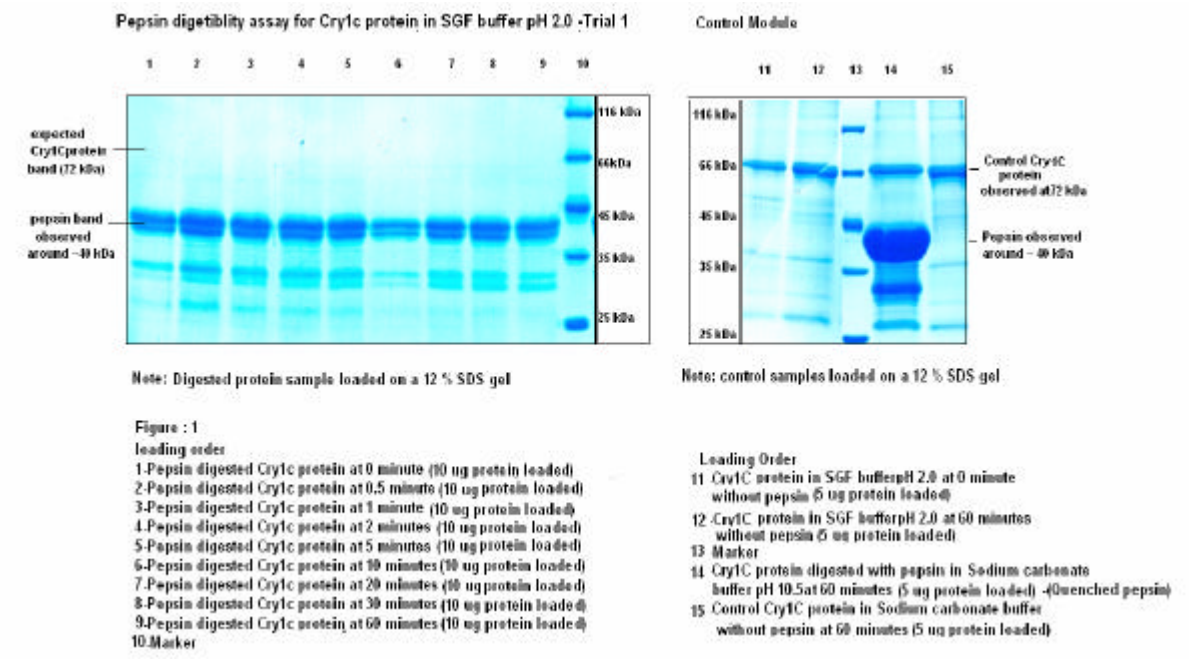
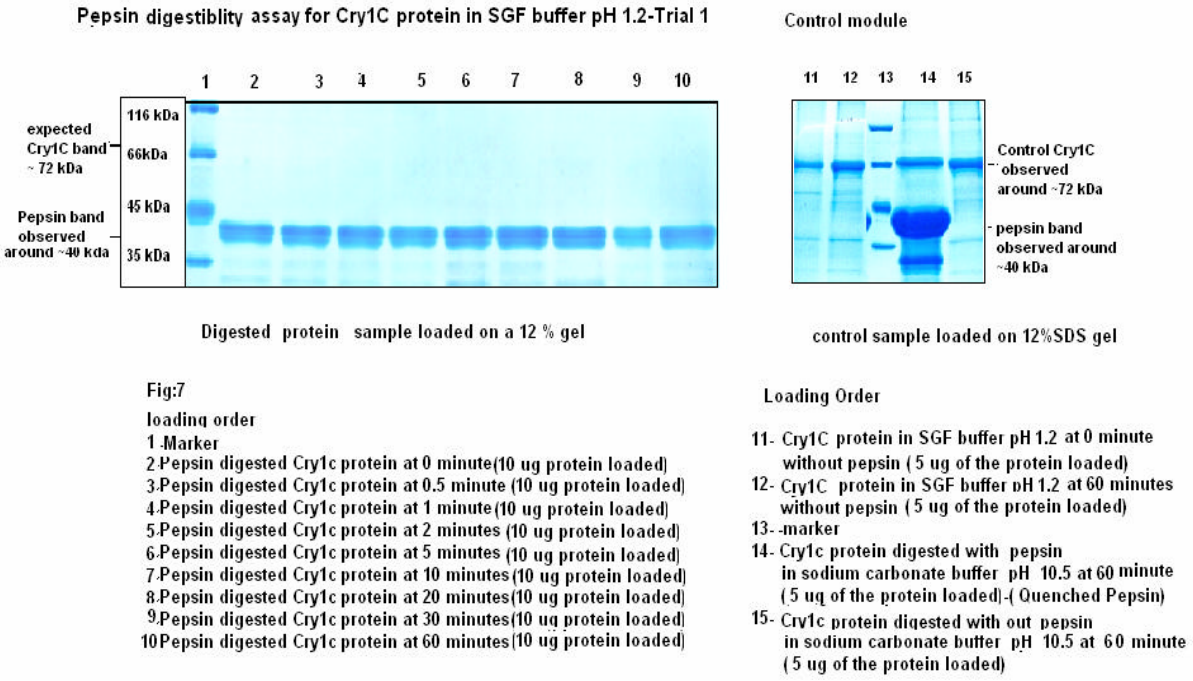


Figure 2 (b) – Pepsin digestibility of Cry1C recombinant protein at pH 1.2



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[22]. 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[23], and in case of cattle, at 587 and 418 kgs for Holstein and Jersey breeds[24]. 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	
Ration intake/day?		@ 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 dietaryproteins 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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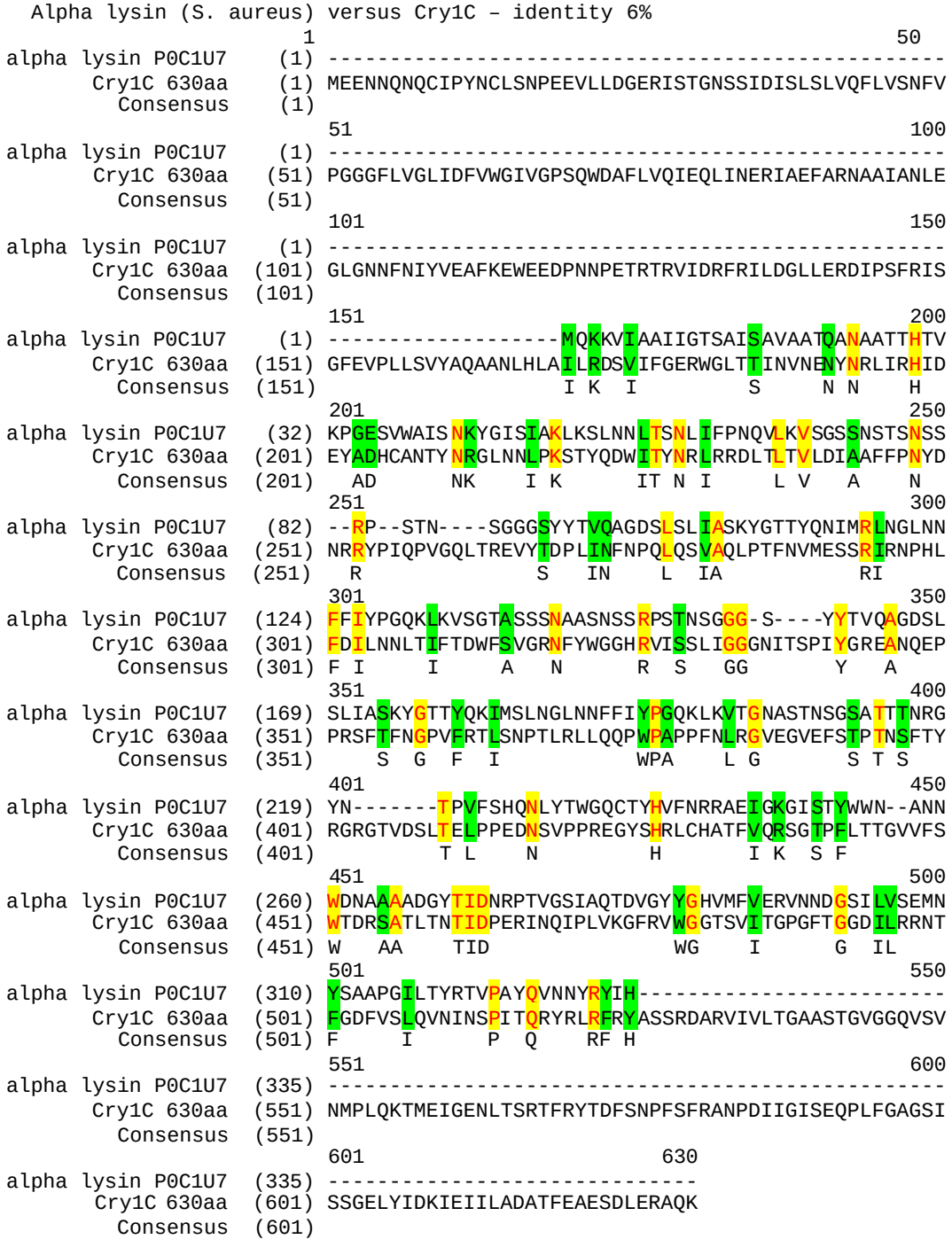
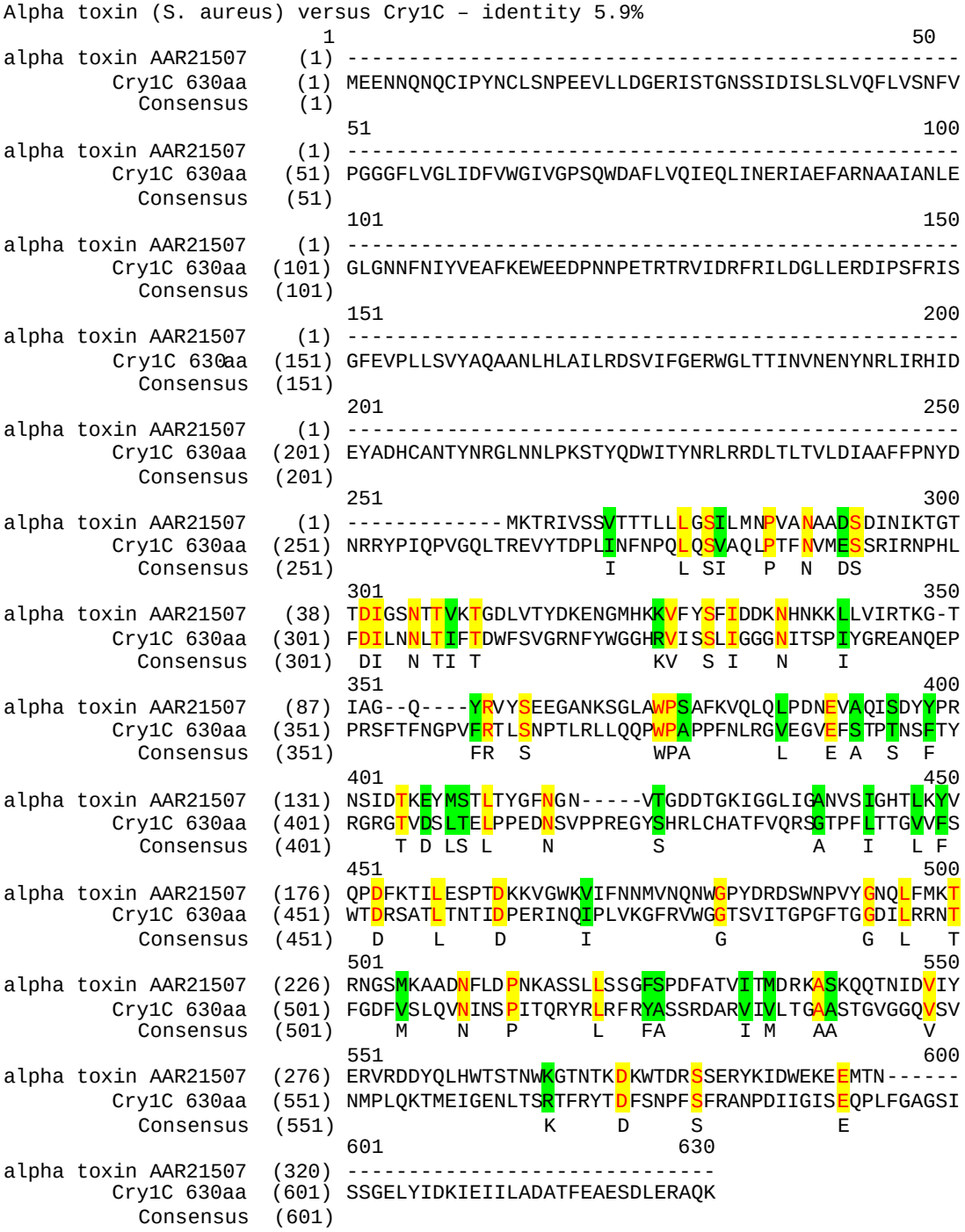
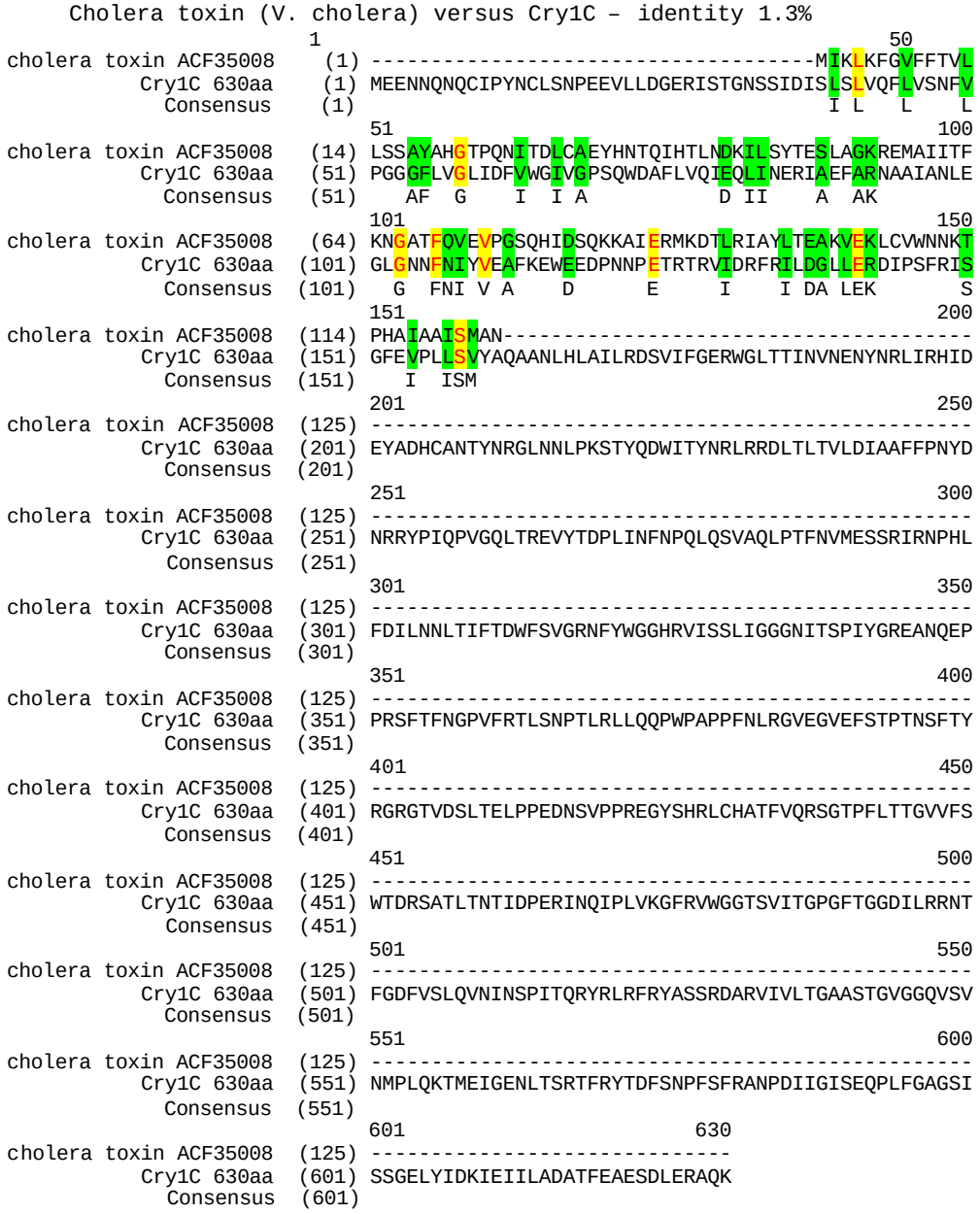
PAIRWISE ALIGNMENTS OF DIFFERENT TOXINS TO CRY1C

Aerolysin (Aeromonas hydrophila) versus Cry1C – identity 12.2%

```
                        1                                                  50
aerolysin ABA28308    (1) ----------------------MQKLKITGLSLIISGLLMAQAHAAEPVY
       Cry1C 630aa    (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
         Consensus    (1)                       L   KIS     I   L
                         51                                                 100
aerolysin ABA28308   (29) PDQLRLFSLGQEVCGDKYRPITREEAQSVKSNIVNMMGQWQISGLANGWV
       Cry1C 630aa   (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
         Consensus   (51) P    L  L   V G             I   I   IA F   A
                         101                                                150
aerolysin ABA28308   (79) IMGPGYNGEIKPGSASNTWCYPINP-----VTGEIPTLSALDIPDGDEVD
       Cry1C 630aa  (101) GLGNNFN--IYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFR
         Consensus  (101)  LG  FN  I   A       P NP     V      L AL   D
                        151                                                200
aerolysin ABA28308  (124) VQWRLVHDSANFIKPTSYLAHYLGYAWVGGNHSQYVGEDMDVTRDGDGWV
       Cry1C 630aa  (149) ISGFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRH
         Consensus  (151) I    V   A F          L  A I G         M
                        201                                                250
aerolysin ABA28308  (174) IRGNNDGGCEGYRCGEK----------------------TAIKVSNFAYN
       Cry1C 630aa  (199) IDEYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPN
         Consensus  (201) I    D     Y  G                        T I IA F  N
                        251                                                300
aerolysin ABA28308  (202) LDPDSFKHGDVTQSDRQLVKTVVG---------WAINDSDTPQSG-----
       Cry1C 630aa  (249) YDNRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRNP
         Consensus  (251)  D   F    V Q  R L    L            I       S
                        301                                                350
aerolysin ABA28308  (238) --YDVTLRYDTATNWSKTN----------------------TYGLSEKV
       Cry1C 630aa  (299) HLFDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQ
         Consensus  (301)   FDI       T W                           YG
                        351                                                400
aerolysin ABA28308  (263) TTKN--KFKWPLVGETELSIEIAANQSWASQ---------NGGSTTTSL
       Cry1C 630aa  (349) EPPRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTNSF
         Consensus  (351)        F  PL            NQ W A              S T S
                        401                                                450
aerolysin ABA28308  (301) SQSVRSTVPARSKIPVKIELYKADISYPYEFKADVSYDLT----------
       Cry1C 630aa  (399) TYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTGVV
         Consensus  (401) S   R TV A S IP           Y H          S
                        451                                                500
aerolysin ABA28308  (341) ----------------------LSGFLRWGGNAWYTHPDNRP-NWNHT
       Cry1C 630aa  (449) FSWTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRR
         Consensus  (451)                         L GF  WGG A  T P
                        501                                                550
aerolysin ABA28308  (366) FVIGPYKDKASSIRYQWDKRYIP--GEVKWWDWNWTIQQNGLSTMQNNLA
       Cry1C 630aa  (499) NTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAASTGVGGQV
         Consensus  (501)    G F      I      RY          D    I   A ST
                        551                                                600
aerolysin ABA28308  (414) RVLRPVRAGITGDFSAESQFAGNIEIGAPVPLAADSKVRRTRS--VDGAG
       Cry1C 630aa  (549) SVNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQPLFGAG
         Consensus  (551)  V  PL   I       S      D   P    A   I       L GAG
                        601                                632
aerolysin ABA28308  (462) QGLRLEIPLDAQELSGLGFSNVSLSVTPVANQ
       Cry1C 630aa  (599) SISSGELYIDKIEIILADATFEAESDLERAQK
         Consensus  (601)      EI ID  EI     S  A S    AN
```

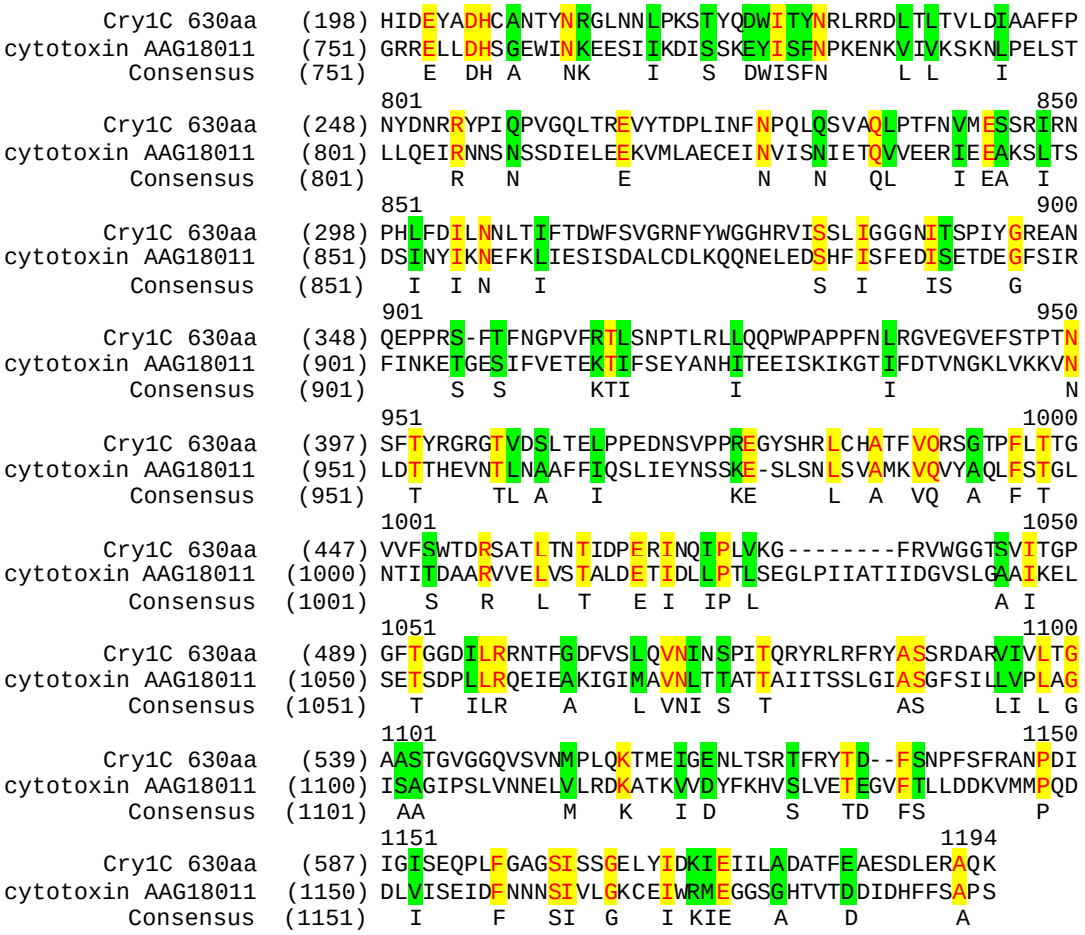

Alpha lysin (S. aureus) versus Cry1C – identity 6%
alpha lysin P0C1U7
Cry1C 630aa
Consensus


Alpha toxin (S. aureus) versus Cry1C – identity 5.9%
alpha toxin AAR21507
Cry1C 630aa
Consensus


Cholera toxin (V. cholera) versus Cry1C – identity 1.3%
cholera toxin ACF35008
Cry1C 630aa
Consensus

Cytotoxin (Clostridium difficile) versus Cry1C identity – 6.4%

```
                          1                                                  50
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011      (1) MSLVNRKQLEKMANVRFRVQEDEYVAILDALEEYHNMSENTVVEKYLKLK
         Consensus     (1)

                         51                                                 100
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011     (51) DINSLTDTYIDTYKKSGRNKALKKFKEYLVIEILELENSNLTPVEKNLHF
         Consensus     (51)

                        101                                                 150
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011    (101) IWIGGQINDTAINYINQWKDVNSDYNVNVFYDSNAFLINTLKKTIIESAS
         Consensus    (101)

                        151                                                 200
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011    (151) NDTLESFRENLNDPEFNHTAFFRKRMQIIYDKQQNFINYYKAQKEENPDL
         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) INKPDSVKTAVDWEEMQLEAIMKHKEYIPEYTSKHFDTLDEEVQSSFESV
         Consensus    (301)

                        351                                                 400
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011    (351) LASKSDKSEIFLPLGDIEVSPLEVKIAFAKGSIINQALISAKDSYCSDLL
         Consensus    (351)

                        401                                                 450
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011    (401) IKQIQNRYKILNDTLGPIISQGNDFNTTMNNFGESLGAIANEENISFIAK
         Consensus    (401)

                        451                                                 500
       Cry1C 630aa     (1) --------------------------------------------------
cytotoxin AAG18011    (451) IGSYLRVGFYPEANTTVTLSGPTIYAGAYKDLLTFKEMSIDTSILSSELR
         Consensus    (451)

                        501                                                 550
       Cry1C 630aa     (1) ------------------------------------------MEENNQNQ
cytotoxin AAG18011    (501) NFEFPKVNISQATEQEKNSLWQFNEERAKIQFEEYKKNYFEGALGEDDNL
         Consensus    (501)                                              N

                        551                                                 600
       Cry1C 630aa     (9) CIPYNCLSNPEEVLLDGERISTGN-----S--S-IDISLSLVQFLVSNFV
cytotoxin AAG18011    (551) DFSQNTVTDKEYLLEKISSSTKSSERGYVHYIVQLQGDKISYEAACNLFA
         Consensus    (551)     N LS  E LL      S             I            F

                        601                                                 650
       Cry1C 630aa    (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
cytotoxin AAG18011    (601) KNPYDSILFQKNIEDSEVAYYYNPTDSEIQEIDKYRIPDRISDRPKIKLT
         Consensus    (601)       I     I        W      I  I   RI D         L

                        651                                                 700
       Cry1C 630aa    (101) GLGN-NFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDG-LLERDIPSFR
cytotoxin AAG18011    (651) FIGHGKAEFNTDIFAGLDVDSLSSEIETAIGLAKEDISPKSIEINLLGCN
         Consensus    (651)  IG        D F   D D    E  T I      I    IE  I

                        701                                                 750
       Cry1C 630aa    (149) ISGFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIR-
cytotoxin AAG18011    (701) MFSYSVNVEETYPGKLLLRVKDKVSELMPSMSQDSIIVSANQYEVRINSE
         Consensus    (701) I  F V L   Y     L L      LI         I  N    RI

                        751                                                 800
```


Cry1C 630aa (198) HIDEYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFP
cytotoxin AAG18011 (751) GRRELLDHSGEWINKEESIIKDISSKEYISFNPKENKVIVKSKNLPELST
Consensus (751) E DH A NK I S DWISFN L L I
801 850
Cry1C 630aa (248) NYDNRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRN
cytotoxin AAG18011 (801) LLQEIRNNSNSSDIELEEKVMLAECEINVISNIETQVVEERIEEAKSLTS
Consensus (801) R N E N N QL I EA I
851 900
Cry1C 630aa (298) PHLFDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREAN
cytotoxin AAG18011 (851) DSINYIKNEFKLIESISDALCDLKQQNELEDSHFISFEDISETDEGFSIR
Consensus (851) I I N I S I IS G
901 950
Cry1C 630aa (348) QEPPRS-FTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTN
cytotoxin AAG18011 (901) FINKETGESIFVETEKTIFSEYANHITEEISKIKGTIFDTVNGKLVKKVN
Consensus (901) S S KTI I I N
951 1000
Cry1C 630aa (397) SFTYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTG
cytotoxin AAG18011 (951) LDTTHEVNTLNAAFFIQSLIEYNSSKE-SLSNLSVAMKVQVYAQLFSTGL
Consensus (951) T TL A I KE L A VQ A F T
1001 1050
Cry1C 630aa (447) VVFSWTDRSATLTNTIDPERINQIPLVKG--------FRVWGGTSVITGP
cytotoxin AAG18011 (1000) NTITDAARVVELVSTALDETIDLLPTLSEGLPIIATIIDGVSLGAAIKEL
Consensus (1001) S R L T E I IP L A I
1051 1100
Cry1C 630aa (489) GFTGGDILRRNTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTG
cytotoxin AAG18011 (1050) SETSDPLLRQEIEAKIGIMAVNLTTATTAIITSSLGIASGFSILLVPLAG
Consensus (1051) T ILR A L VNI S T AS LI L G
1101 1150
Cry1C 630aa (539) AASTGVGGQVSVNMPLQKTMEIGENLTSRTFRYTD--FSNPFSFRANPDI
cytotoxin AAG18011 (1100) ISAGIPSLVNNELVLRDKATKVVDYFKHVSLVETEGVFTLLDDKVMMPQD
Consensus (1101) AA M K I D S TD FS P
1151 1194
Cry1C 630aa (587) IGISEQPLFGAGSISSGELYIDKIEIILADATFEAESDLERAQK
cytotoxin AAG18011 (1150) DLVISEIDFNNNSIVLGKCEIWRMEGGSGHTVTDDIDHFFSAPS
Consensus (1151) I F SI G I KIE A D A

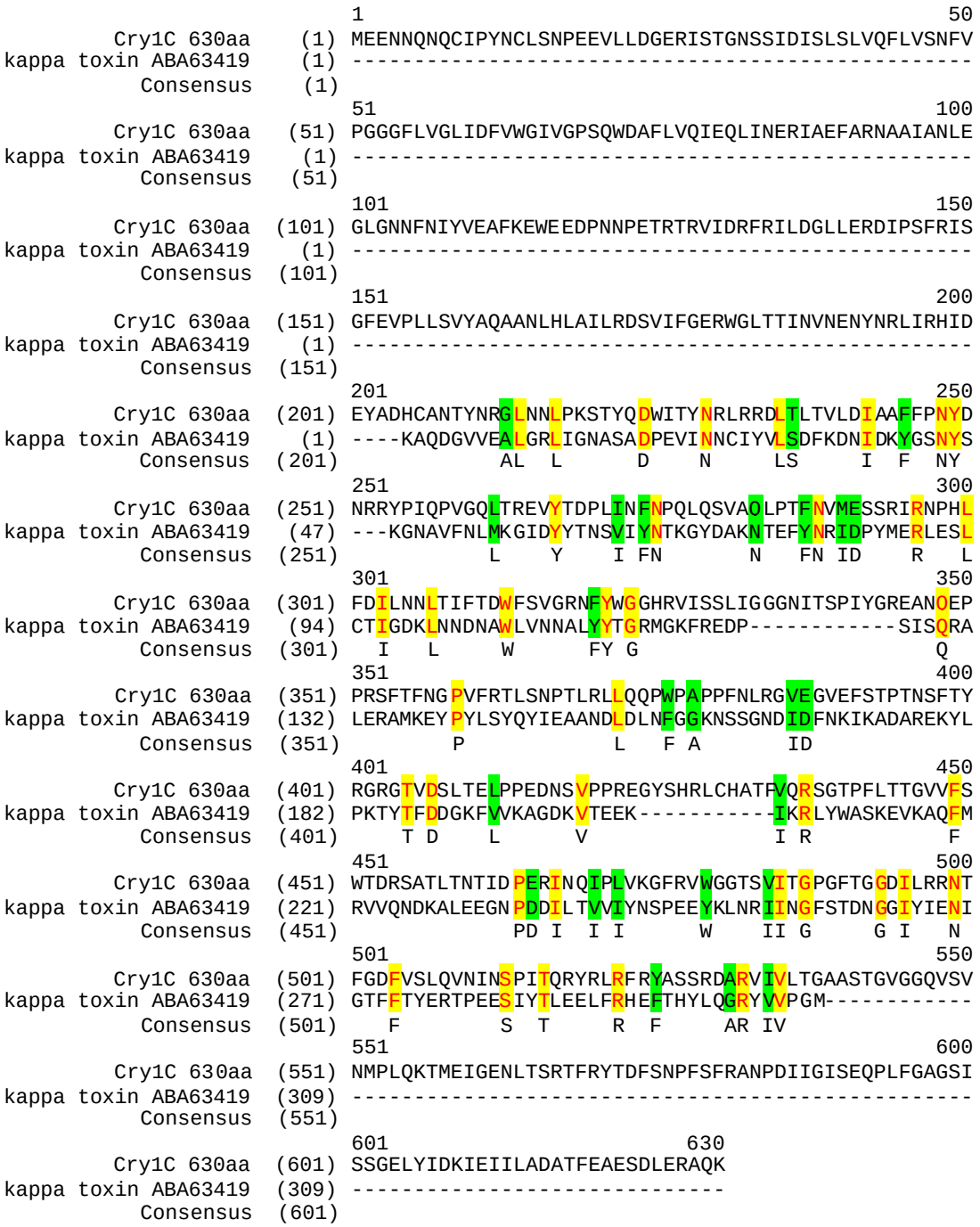
Delta lysin (26 aa long; S. aureus) versus Cry1C identity – 14.8%

```
                  230                       256
         Cry1C 630aa  (230) RLRRDLTLTVLDIAAFFPNYDNRRYPI
delta lysin ABQ49855    (1) -MAQDIISTIGDLVKWIIDTVNKFTKK
           Consensus  (230)  L  DI  TI DI  F     NK
```

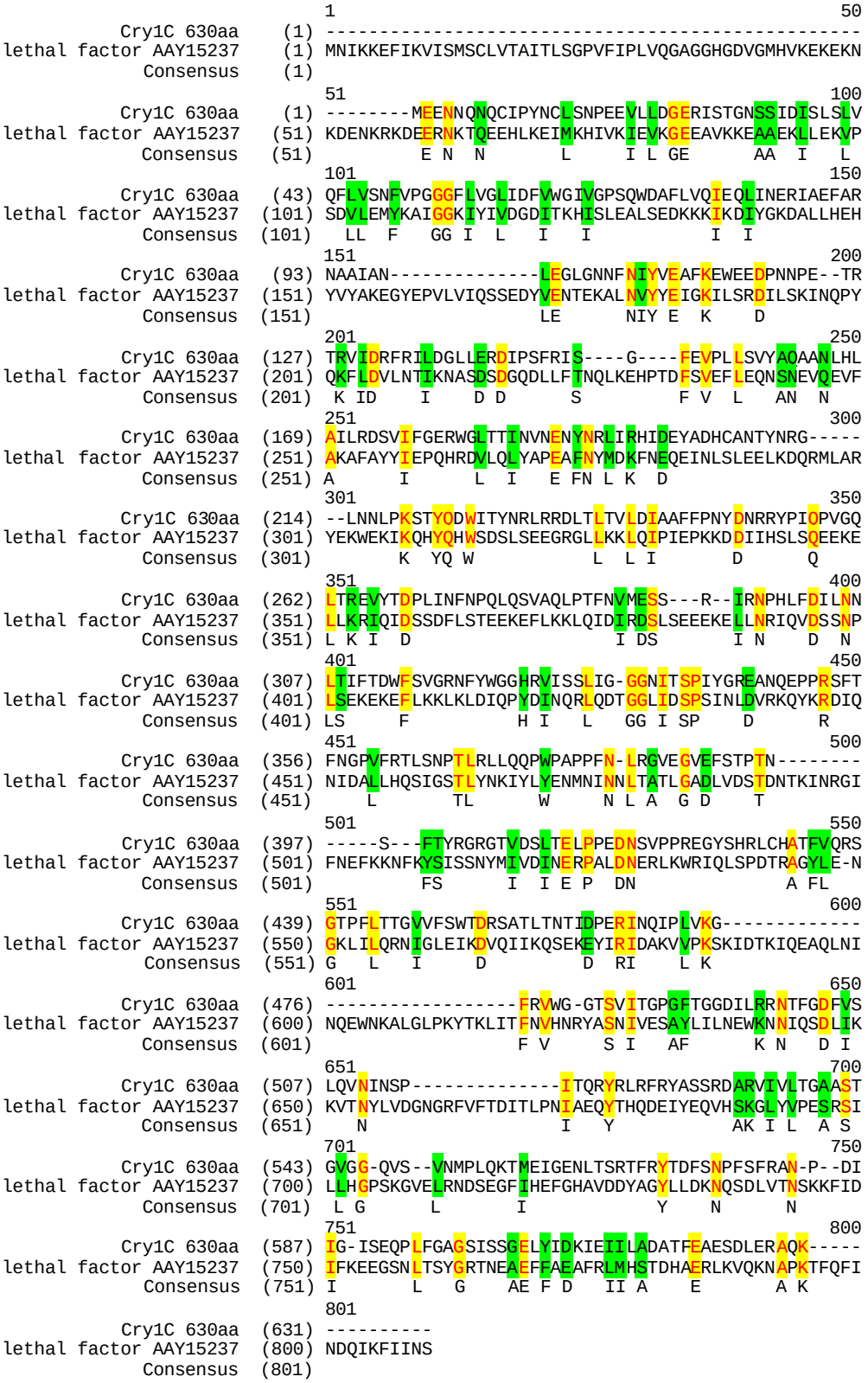
Enterotoxin A (Clostridium perfringens, Type A) versus Cry1C identity – 12.8%

```
                          1                                                  50
          Cry1C 630aa   (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
enterotoxin A AAG18010  (1) ------NEYYPEIIVLNPN----------TFHKKVNINLDSSSFEYKWST
             Consensus  (1)       N   P   L NP           T    I I L    F
                         51                                                 100
          Cry1C 630aa   (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
enterotoxin A AAG18010  (35) EGS------------------DFILVRYLEESNKKILQKIRIKGILSNT
             Consensus  (51)  G                   D  LV      N KI    R  AI
                         101                                                150
          Cry1C 630aa   (101) GLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRIS
enterotoxin A AAG18010  (66) KSFNKMSIDFKDIKKLSLG------------------------------
             Consensus  (101)    N   I     K
                         151                                                200
          Cry1C 630aa   (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHID
enterotoxin A AAG18010  (85) ------------------------------------YIMSNFKSFNSENE
             Consensus  (151)                                     I  NF       D
                         201                                                250
          Cry1C 630aa   (201) EYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
enterotoxin A AAG18010  (99) LDRDHLG---------------------------------------FKIID
             Consensus  (201)    DH A                                     F   D
                         251                                                300
          Cry1C 630aa   (251) NRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRN-PH
enterotoxin A AAG18010  (111) NKTYYYDEASKLVKG-----LINININNSLFYFDPIESNLVTGWQTINGKKY
             Consensus  (251) NK Y       L K      LIN N  L     I S  V     I    H
                         301                                                350
          Cry1C 630aa   (300) LFDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQE
enterotoxin A AAG18010  (156) YFDINTGAASTSYKIINGKHFYFNNNGVMQLGVFKG--------------
             Consensus  (301)  FDI       S     GK FYF    VI   I  G
                         351                                                400
          Cry1C 630aa   (350) PPRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTNSFT
enterotoxin A AAG18010  (192) -PDGFEYFAPANTQNNN------------------IEGQAIVYQSKFLT
             Consensus  (351)  P  F F AP      N                  IEG      S   T
                         401                                                450
          Cry1C 630aa   (400) YRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTGVVF
enterotoxin A AAG18010  (222) LNGK--------KYYFDNDSKAVTGWQTIDGKKYYFNLNTAEAATG---W
             Consensus  (401)   GK            DN      GW        F N       T    F
                         451                                                500
          Cry1C 630aa   (450) SWTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRRN
enterotoxin A AAG18010  (261) QTIDGKKYYFNTNTSIASTGYTIINGKHFYFNTDGIMQIGVFKG----PN
             Consensus  (451)    D      NT          II G   W  T  I   G   G     N
                         501                                                550
          Cry1C 630aa   (500) TFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAASTGVGGQVS
enterotoxin A AAG18010  (307) GFEYFAPANTDANNIEGQAIRYQNRFLYLHDN--IYYFGNNSKAVTGWQT
             Consensus  (501)  F  F   N   N    Q  R   RF    D   I   G  S AV G  S
                         551                                                600
          Cry1C 630aa   (550) VNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQPLFGAGS
enterotoxin A AAG18010  (355) INGNVYYFMPDTAMAAAGGLFEIDGVIYFFG-----VDGVKAPGIYG---
             Consensus  (551) IN  L   M       A      D    F       I GI    IFG
                         601                        631
          Cry1C 630aa   (600) ISSGELYIDKIEIILADATFEAESDLERAQK
enterotoxin A AAG18010  (397) -------------------------------
             Consensus  (601)
```

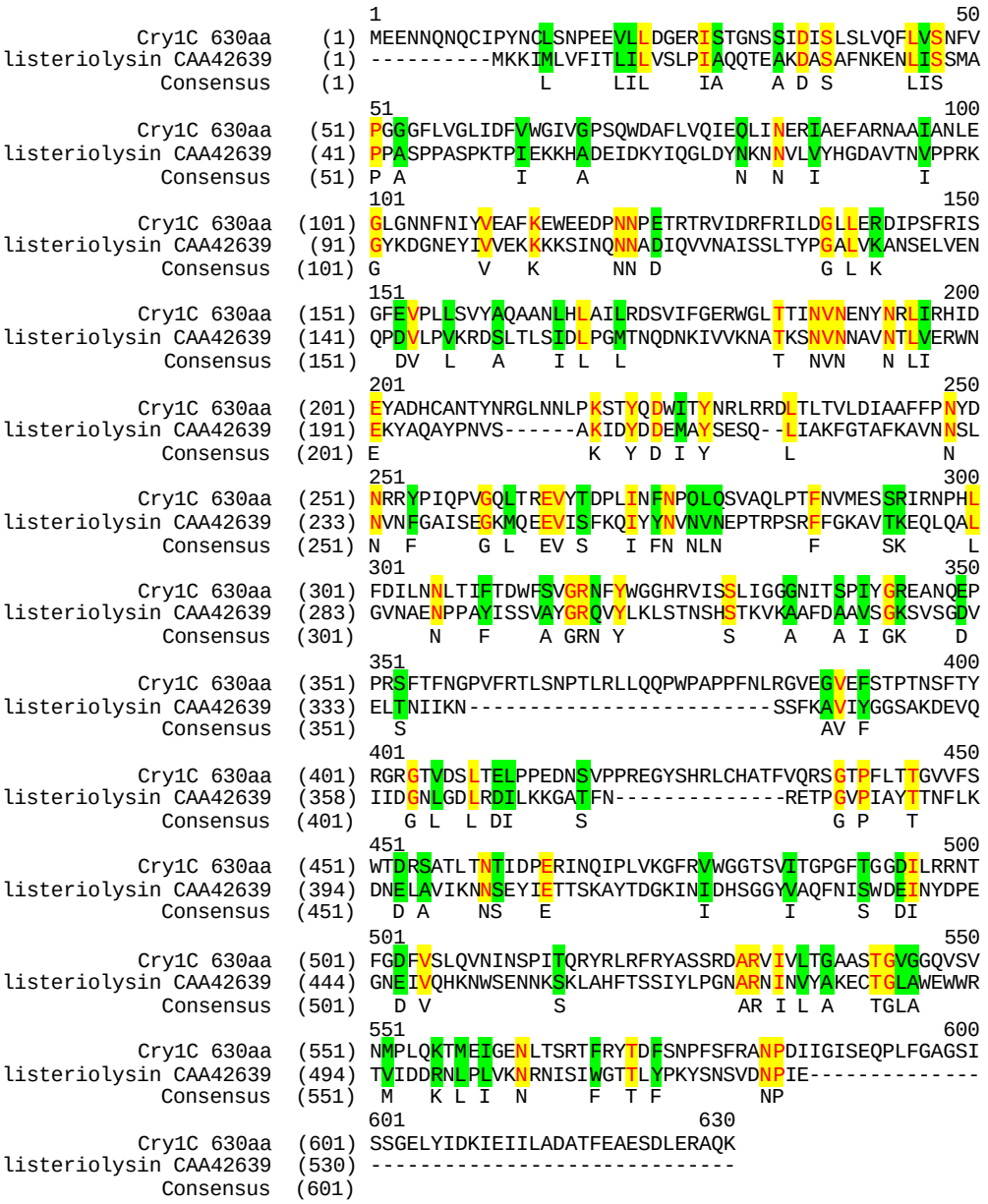
Kappa toxin (C. perfringens Type A) versus Cry1C identity 6.2%



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



Listeriolysin (Listeria monocytogenes) versus Cry1C identity 10%



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

```
                          2                                                  51
      Cry1C 630aa     (1) ----------------------------------MEENNQNQCIPYNCL
neurotoxin A5HZZ9     (2) PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTF
        Consensus     (2)                                    N
                         52                                                 101
      Cry1C 630aa    (16) SNPEE--------------VLLDGERISTGNSSIDISLSLVQFLVSNFVP
neurotoxin A5HZZ9    (52) TNPEEGDLNPPPEAKQVPVSYYDSTYLSTDNEKDNYLKGVTKLFERIYST
        Consensus    (52) SNPEE                D   IST N        L       F
                        102                                                 151
      Cry1C 630aa    (52) GGG--FLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANL
neurotoxin A5HZZ9   (102) DLGRMLLTSIVRGIPFWGGSTIDTELKVIDTNCINVIQPDGSYRSEELNL
        Consensus   (102)   G   L  II  I    G S      V   N IN    D A  A   NL
                        152                                                 201
      Cry1C 630aa   (100) EGLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRI
neurotoxin A5HZZ9   (152) VIIGPSADIIQFECKSFGHEVLN-LTRNGYGSTQYIRFSPDFTFGFEESL
        Consensus   (152)   IG    I     K F  D  N  TR        I            I
                        202                                                 251
      Cry1C 630aa   (150) SGFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHI
neurotoxin A5HZZ9   (201) EVDTNPLLGAGKFATDPAVTLAHELIHAGHRLYGIAINPNRVFKVNTNAY
        Consensus   (202)      PLL     A    L I  D I  G R     IN N  F
                        252                                                 301
      Cry1C 630aa   (200) DEYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNY
neurotoxin A5HZZ9   (251) YEMSGLEVSFEELRTFGGHDAKFIDSLQENEFRLYYYNKFKDIASTLNKA
        Consensus   (252)  E A                A F D I  N  R        DIAA
                        302                                                 351
      Cry1C 630aa   (250) DNRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRNPH
neurotoxin A5HZZ9   (301) KSIVGTTASLQYMKNVFKEKYLLSEDTSGKFSVDKLKFDKLYK------M
        Consensus   (302)          L  L         LI              F  L
                        352                                                 401
      Cry1C 630aa   (300) LFDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQE
neurotoxin A5HZZ9   (345) LTEIYTEDNFVKFFKVLNRKTYLNFDKAVFKINIVPKVNYTIYDGFNLRN
        Consensus   (352) L DI          F  L R  Y    K I  I     I   IY
                        402                                                 451
      Cry1C 630aa   (350) PPRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTNSFT
neurotoxin A5HZZ9   (395) TNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSKTKSLDK
        Consensus   (402)    A  FNG           KL N       F L  V GI  S
                        452                                                 501
      Cry1C 630aa   (400) YRGRGTVDSLTELPPEDNSVPP-REGYSHRLCHATFVQRSGTPFLTTGVV
neurotoxin A5HZZ9   (445) GYNKALNDLCIKVNNWDLFFSPSEDNFTNDLNKGEEITSDTNIEAAEENI
        Consensus   (452)    KA  D    L   D    P  D FS  L  A  I           I
                        502                                                 551
      Cry1C 630aa   (449) FSWTDRSATLTNTIDPER-----------INQIPLVKGFRVWGGTSVIT
neurotoxin A5HZZ9   (495) SLDLIQQYYLTFNFDNEPENISIENLSSDIIGQLELMPNIERFPNGKKYE
        Consensus   (502)          LT   D E             I QI LM     F
                        552                                                 601
      Cry1C 630aa   (487) GPGFTGGDILRRNTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVL
neurotoxin A5HZZ9   (545) LDKYTMFHYLRAQEFEHGKSRIALTNSVNEALLNPSRVYTFFSSDYVKKV
        Consensus   (552)    FT    LR N F    S     NS           Y       V  L
                        602                                                 651
      Cry1C 630aa   (537) TGAASTGVGGQVSVNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDI
neurotoxin A5HZZ9   (595) NKATEAAMFLGWVEQLVYDFTDETSEVSTTDKIADITIIIPYIGPALNIG
        Consensus   (602)   A   AM       NL    T E  E  TS          PF   A
                        652                                        695
      Cry1C 630aa   (587) IGISEQPLFGAGSISSGELYIDKIEIILADATFEAESDLERAQK
neurotoxin A5HZZ9   (645) NMLYKDDFVGALIFSGAVILLEFIPEIAIPVLGTFALVSYIANK
        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) MPVTINNFNYNDPIDNNNIIMMEPPFARGTGRYYKAFKITDRIWIIPERY
        Consensus     (1)
                         51                                                 100
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5    (51) TFGYKPEDFNKSSGIFNRDVCEYYDPDYLNTNDKKNIFLQTMIKLFNRIK
        Consensus    (51)
                        101                                                 150
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5   (101) SKPLGEKLLEMIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNPGEVER
        Consensus   (101)
                        151                                                 200
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5   (151) KKGIFANLIIFGPGPVLNENETIDIGIQNHFASREGFGGIMQMKFCPEYV
        Consensus   (151)
                        201                                                 250
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5   (201) SVFNNVQENKGASIFNRRGYFSDPALILMHELIHVLHGLYGIKVDDLPIV
        Consensus   (201)
                        251                                                 300
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5   (251) PNEKKFFMQSTDAIQAEELYTFGGQDPSIITPSTDKSIYDKVLQNFRGIV
        Consensus   (251)
                        301                                                 350
      Cry1C 630aa     (1) --------------------------------------------------
neurotoxin B1INP5   (301) DRLNKVLVCISDPNININIYKNKFKDKYKFVEDSEGKYSIDVESFDKLYK
        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) ------------MEENNQNQCIPYNCLSNPEE-------VLLDGERIST
neurotoxin B1INP5   (451) NEDLFFIADKNSFSDDLSKNERIEYNTQSNYIENDFPINELILDTDLISK
        Consensus   (451)              DD   N  I YN  SN  E       LILD D IS
                        501                                                 550
      Cry1C 630aa    (31) GNS----------S-IDISLS----------------------------
neurotoxin B1INP5   (501) IELPSENTESLTDFNVDVPVYEKQPAIKKIFTDENTIFQYLYSQTFPLDI
        Consensus   (501)               IDI L
                        551                                                 600
      Cry1C 630aa    (41) --LVQFLVSNFVP--G-GGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINE
neurotoxin B1INP5   (551) RDISLTSSFDDALLFSNKVYSFFSMDYIKTANKVVEAGLFAGWVKQIVND
        Consensus   (551)   I               F    IDFI           F   I QIIND
                        601                                                 650
      Cry1C 630aa    (86) RIAEFARNAAIANLEGLGNNFNIYVEAFKEWEEDPN-NPETRTRVIDRFR
neurotoxin B1INP5   (601) FVIEANKSNTMDKIADISLIVPYIGLALNVGNETAKGNFENAFEIAGASI
        Consensus   (601)  I E  K   I  I  I         A     E    N E    I
                        651                                                 700
      Cry1C 630aa   (135) ILDGLLERDIPSFRISGFEVPLLSVYAQAANLHLAILRDSVIFGERWGLT
neurotoxin B1INP5   (651) LLEFIPELLIPVVGAFLLESYIDNKNKIIKTIDNALTKRNEKWSDMYGLI
        Consensus   (651) ILD I E  IP       E  I         I  AI K    F D WGL
                        701                                                 750
      Cry1C 630aa   (185) TINVNENYNRLIRHIDEYADHCANTYNRGLNNLPKSTYQDWIT-------
neurotoxin B1INP5   (701) VAQWLSTVNTQFYTIKEGMYKALNYQAQALEEIIKYRYNIYSEKEKSNIN
        Consensus   (701)   N     N     I E      N    AL  I K  YN W
                        751                                                 800
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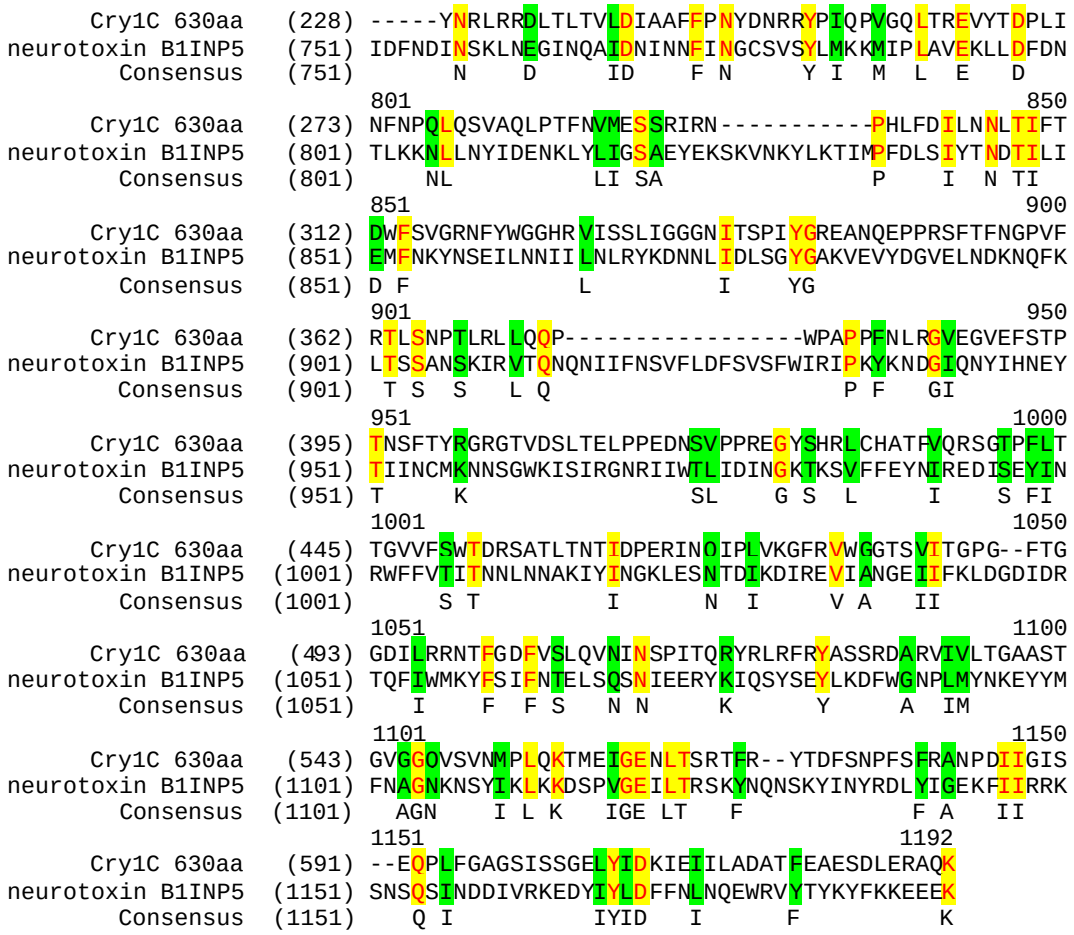
Cry1C 630aa (228) -----YNRLRRDLTLTVLDIAAFFPNYDNRRYPIQPVGQLTREVYTDPLI
neurotoxin B1INP5 (751) IDFNDINSKLNEGINQAIDNINNFINGCSVSYLMKKMIPLAVEKLLDFDN
Consensus (751) N D ID F N Y I M L E D
801 850
Cry1C 630aa (273) NFNPQLQSVAQLPTFNVMESSRIRN----------PHLFDILNNLTIFT
neurotoxin B1INP5 (801) TLKKNLLNYIDENKLYLIGSAEYEKSKVNKYLKTIMPFDLSIYTNDTILI
Consensus (801) NL LI SA P I N TI
851 900
Cry1C 630aa (312) DWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQEPPRSFTFNGPVF
neurotoxin B1INP5 (851) EMFNKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFK
Consensus (851) D F L I YG
901 950
Cry1C 630aa (362) RTLSNPTLRLLQQP----------------WPAPPFNLRGVEGVEFSTP
neurotoxin B1INP5 (901) LTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEY
Consensus (901) T S S L Q P F GI
951 1000
Cry1C 630aa (395) TNSFTYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLT
neurotoxin B1INP5 (951) TIINCMKNNSGWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYIN
Consensus (951) T K SL G S L I S FI
1001 1050
Cry1C 630aa (445) TGVVFSWTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPG--FTG
neurotoxin B1INP5 (1001) RWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDR
Consensus (1001) S T I N I V A II
1051 1100
Cry1C 630aa (493) GDILRRNTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAAST
neurotoxin B1INP5 (1051) TQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNPLMYNKEYYM
Consensus (1051) I F F S N N K Y A IM
1101 1150
Cry1C 630aa (543) GVGGQVSVNMPLQKTMEIGENLTSRTFR--YTDFSNPFSFRANPDIIGIS
neurotoxin B1INP5 (1101) FNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRK
Consensus (1101) AGN I L K IGE LT F F A II
1151 1192
Cry1C 630aa (591) --EQPLFGAGSISSGELYIDKIEIILADATFEAESDLERAQK
neurotoxin B1INP5 (1151) SNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEK
Consensus (1151) Q I IYID I F K

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

```
                                1                                                  50
                Cry1C 630aa    (1) ---MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVS
perfringolysin O AAA23270      (1) MIRFKKTKLIASIAMALCLFSQPVISFSKDITDKNQSIDSGISSLSYNRN
                  Consensus    (1)            I          VI     IS  N SID  IS L F
                                51                                                 100
                Cry1C 630aa   (48) NFVPGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIA
perfringolysin O AAA23270     (51) EVLASN-----------------------------GDKIESFVPKEGKK
                  Consensus   (51)   L                                  DKI  F    A
                               101                                                 150
                Cry1C 630aa   (98) NLEGLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSF
perfringolysin O AAA23270     (71) ----AGNKFIVVERQKRSLTTSPVDISIIDSVND--RTYPGALQLADKAF
                  Consensus  (101)      GN F I     K     P        V D  R   G L     AF
                               151                                                 200
                Cry1C 630aa  (148) RISGFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIR
perfringolysin O AAA23270    (115) VEN---RPTILMVKEAININIDLP------GLKGENSIKVDDPTYGKVSG
                  Consensus  (151)        P I M   A NI I I       G K   S       Y KL
                               201                                                 250
                Cry1C 630aa  (198) HIDEYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFP
perfringolysin O AAA23270    (156) AIDELVSKWNEKYSSTHTLPARTQYSESMVYSKSQISSALNVN-------
                  Consensus  (201)  IDE        Y        KS Y D I Y K      L V
                               251                                                 300
                Cry1C 630aa  (248) NYDNRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRN
perfringolysin O AAA23270    (199) ---------AKVLENSLGVDFNAVANNEKKVMILAYKQIFYTVSADLPKN
                  Consensus  (251)            V   S  V    L N    L  LA    F  M A   KN
                               301                                                 350
                Cry1C 630aa  (298) PHLFDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREAN
perfringolysin O AAA23270    (240) P--SDLFDDSVTFNDLKQKG----------VSNEAPPLMVSNVAYGRTIY
                  Consensus  (301) P   DI      F D    G          IS       IS   YGR
                               351                                                 400
                Cry1C 630aa  (348) QEPPRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTNS
perfringolysin O AAA23270    (278) VK----------------------LETTSSSKDVQAAFKALIKNTDIKNS
                  Consensus  (351)                          L     A   N      I  S   NS
                               401                                                 450
                Cry1C 630aa  (398) FTYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTGV
perfringolysin O AAA23270    (306) QQYKDIYENSSFTAVVLGGDAQEHNKVVTKDFDEIRKVIKDNATFSTKNP
                  Consensus  (401)   YK      S T L     A       S        V K    F T
                               451                                                 500
                Cry1C 630aa  (448) VFSWTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILR
perfringolysin O AAA23270    (356) AYPISYTSVFLKDNSVAAVHNKTDYIETTSTEYSKGKIN-LDHSGAYVAQ
                  Consensus  (451)  F  S  S  L         N    I           I     SGA I
                               501                                                 550
                Cry1C 630aa  (498) RNTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAASTGVGGQ
perfringolysin O AAA23270    (405) FEVAWDEVSYDKEGNEVLTHKTWDGNYQDKTAHYSTVIPLEANAR-----
                  Consensus  (501)      D VS     N  IT K         S     II   A A
                               551                                                 600
                Cry1C 630aa  (548) VSVNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQPLFGA
perfringolysin O AAA23270    (450) ---NIRIKARECTGLAWEWWRDVISEYDVPLTNNINVSIWGTT---LYPG
                  Consensus  (551)    NI I      G          SDF  P S   N  I G S   LF A
                               601                          633
                Cry1C 630aa  (598) GSISSGELYIDKIEIILADATFEAESDLERAQK
perfringolysin O AAA23270    (494) SSITYN--------------------------
                  Consensus  (601)  SIS
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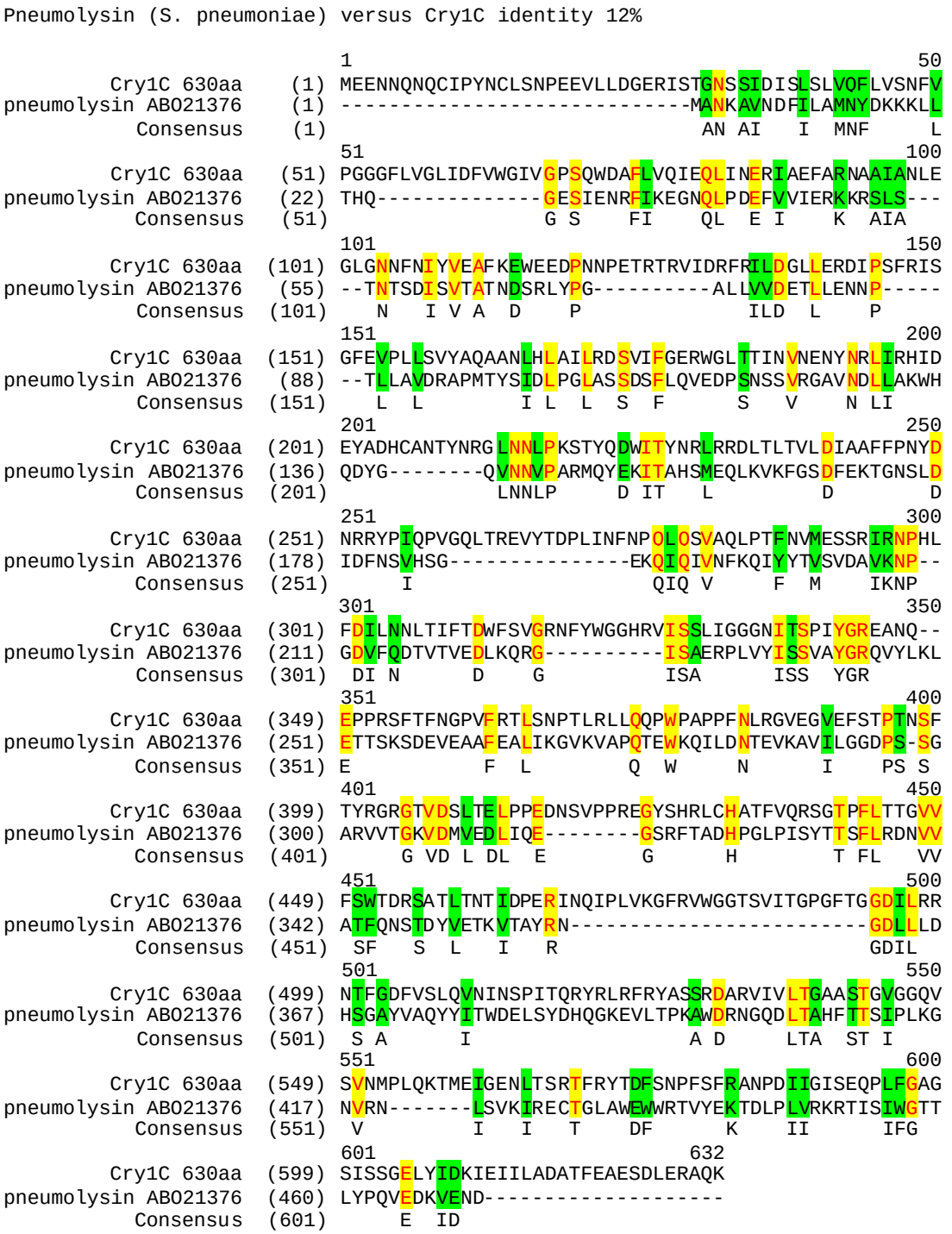
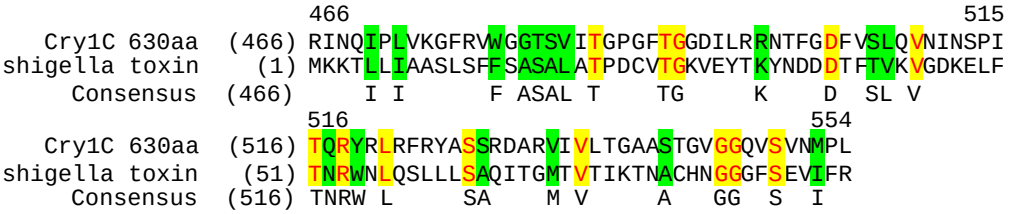

Pneumolysin (S. pneumoniae) versus Cry1C identity 12%
1 50
Cry1C 630aa (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
pneumolysin ABO21376 (1) ----------MANKAVNDFILAMNYDKKKLL
Consensus (1) AN AI I MNF L
51 100
Cry1C 630aa (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
pneumolysin ABO21376 (22) THQ----------GESIENRFIKEGNQLPDEFVVIERKKRSLS---
Consensus (51) G S FI QL E I K AIA
101 150
Cry1C 630aa (101) GLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRIS
pneumolysin ABO21376 (55) --TNTSDISVTATNDSRLYPG----------ALLVVDETLLENNP-----
Consensus (101) N I V A D P ILD L P
151 200
Cry1C 630aa (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHID
pneumolysin ABO21376 (88) --TLLAVDRAPMTYSIDLPGLASSDSFLQVEDPSNSSVRGAVNDLLAKWH
Consensus (151) L L I L L S F S V N LI
201 250
Cry1C 630aa (201) EYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
pneumolysin ABO21376 (136) QDYG--------QVNNVPARMQYEKITAHSMEQLKVKFGSDFEKTGNSLD
Consensus (201) LNNLP D IT L D D
251 300
Cry1C 630aa (251) NRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRNPHL
pneumolysin ABO21376 (178) IDFNSVHSG---------------EKQIQIVNFKQIYYTVSVDAVKNP--
Consensus (251) I QIQ V F M IKNP
301 350
Cry1C 630aa (301) FDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQ--
pneumolysin ABO21376 (211) GDVFQDTVTVEDLKQRG----------ISAERPLVYISSVAYGRQVYLKL
Consensus (301) DI N D G ISA ISS YGR
351 400
Cry1C 630aa (349) EPPRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLRGVEGVEFSTPTNSF
pneumolysin ABO21376 (251) ETTSKSDEVEAAFEALIKGVKVAPQTEWKQILDNTEVKAVILGGDPS-SG
Consensus (351) E F L Q W N I PS S
401 450
Cry1C 630aa (399) TYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTTGVV
pneumolysin ABO21376 (300) ARVVTGKVDMVEDLIQE--------GSRFTADHPGLPISYTTSFLRDNVV
Consensus (401) G VD L DL E G H T FL VV
451 500
Cry1C 630aa (449) FSWTDRSATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTGGDILRR
pneumolysin ABO21376 (342) ATFQNSTDYVETKVTAYRN------------------------GDLLLD
Consensus (451) SF S L I R GDIL
501 550
Cry1C 630aa (499) NTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAASTGVGGQV
pneumolysin ABO21376 (367) HSGAYVAQYYITWDELSYDHQGKEVLTPKAWDRNGQDLTAHFTTSIPLKG
Consensus (501) S A I A D LTA ST I
551 600
Cry1C 630aa (549) SVNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQPLFGAG
pneumolysin ABO21376 (417) NVRN-------LSVKIRECTGLAWEWWRTVYEKTDLPLVRKRTISIWGTT
Consensus (551) V I I T DF K II IFG
601 632
Cry1C 630aa (599) SISSGELYIDKIEIILADATFEAESDLERAQK
pneumolysin ABO21376 (460) LYPQVEDKVEND-------------------
Consensus (601) E ID

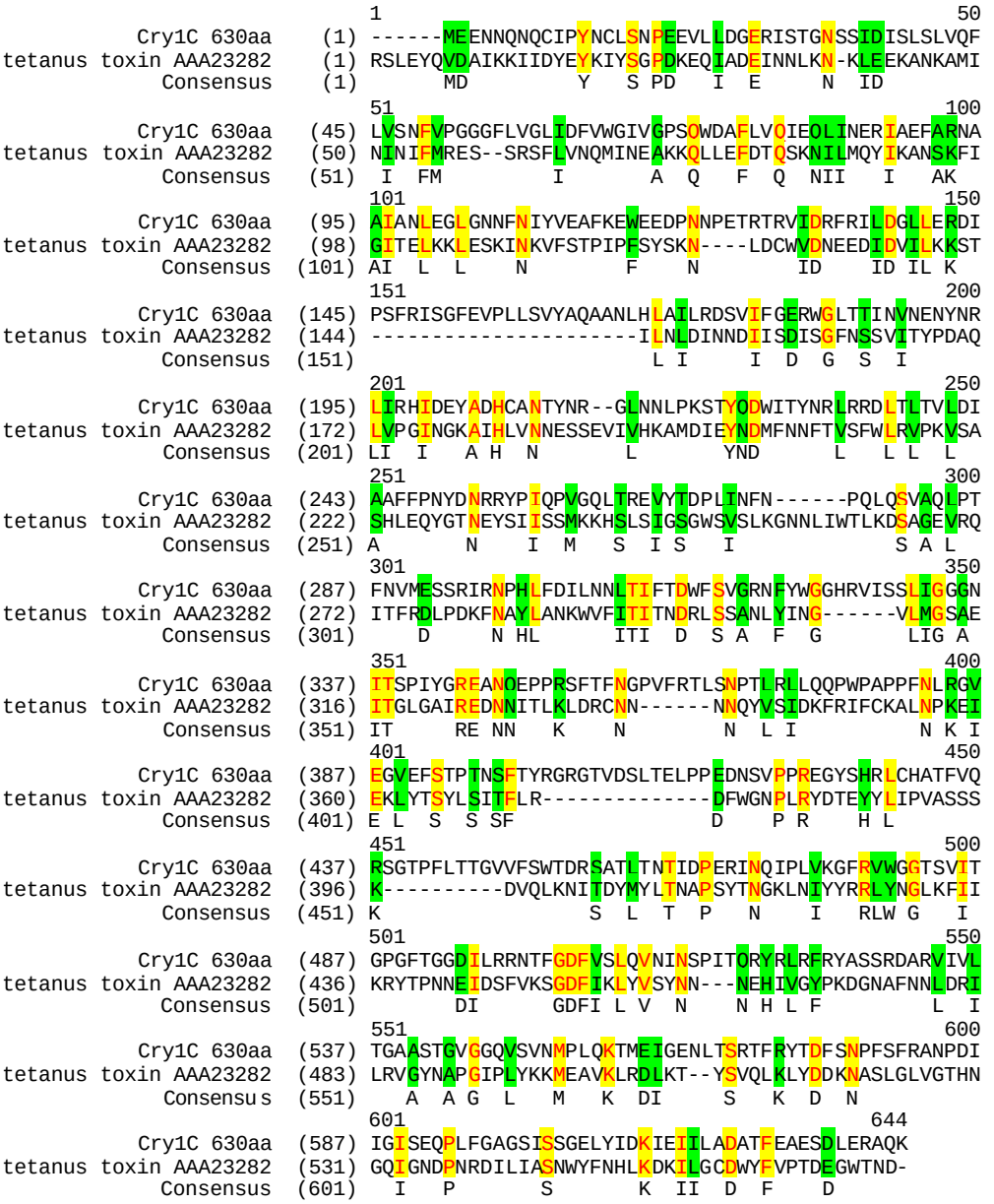
Ricin (Ricinus comminis) versus Cry1C identity 13%

```
                  1                                                  50
Cry1C 630aa     (1) MEENNQNQCIPYNCLSNPEEVLLDGERISTGNSSIDISLSLVQFLVSNFV
ricinP02879     (1) ---------------------------MKPGGN----TIVIWMYAVATWL
  Consensus     (1)                            I  G      SI I  F VA FL
                 51                                                 100
Cry1C 630aa    (51) PGGGFLVGLIDFVWGIVGPSQWDAFLVQIEQLINERIAEFARNAAIANLE
ricinP02879    (20) CFGSTSGWSFTLEDNNIFPKQYPIINFTTAGATVQSYTNFIR--AVRGRL
  Consensus    (51)   G             I P QW                F R  AI
                101                                                 150
Cry1C 630aa   (101) GLGNNFNIYVEAFKEWEEDPNNPETRTRVIDRFRILDGLLERDIPSFRIS
ricinP02879    (68) TTGADVRHEIPVLPNRVGLPINQRFILVELSNHAELSVTLALDVTNAYVV
  Consensus   (101)   G      I          P N       I     L   L  DI    I
                151                                                 200
Cry1C 630aa   (151) GFEVPLLSVYAQAANLHLAILRDSVIFGERWGLTTINVNENYNRLIRHID
ricinP02879   (118) GYRAGNSAYFFHPDNQEDAEAIT-HLFTDVQNRYTFAFGGNYDR----LE
  Consensus   (151) GF     A F    N   A      IF D     T     NY R    ID
                201                                                 250
Cry1C 630aa   (201) EYADHCANTYNRGLNNLPKSTYQDWITYNRLRRDLTLTVLDIAAFFPNYD
ricinP02879   (163) QLAGNLRENIELGNGPLEEAISALYYYSTGGTQLPTLARSFIICIQMISE
  Consensus   (201)   A         G   L  A    W          TL    I       D
                251                                                 300
Cry1C 630aa   (251) NRRYPIQPVGQLTREVYTDPLINFNPQLQSVAQLPTFNVMESSRIRNPHL
ricinP02879   (213) AARFQYIEGEMRTRIRYN----------RRSAPDPSVITLENSWGRLSTA
  Consensus   (251)   RF        TR  Y              A  PS   LE S  R
                301                                                 350
Cry1C 630aa   (301) FDILNNLTIFTDWFSVGRNFYWGGHRVISSLIGGGNITSPIYGREANQEP
ricinP02879   (253) IQESNQGAFASPIQLQRRNGSKFSVYDVSILIP---IIALMVYRCAP--P
  Consensus   (301)     NN    S      RN         IS LI    I A I  R A   P
                351                                                 400
Cry1C 630aa   (351) PRSFTFNGPVFRTLSNPTLRLLQQPWPAPPFNLR-G----VEGVEFSTPT
ricinP02879   (298) P-SSQFSLLIRPVVPNFNADVCMDPEPIVRIVGRNGLCVDVRDGRFHNGN
  Consensus   (351) P S  F   I   L N    L   P P      R G    V    F
                401                                                 450
Cry1C 630aa   (396) NSFTYRGRGTVDSLTELPPEDNSVPPREGYSHRLCHATFVQRSGTPFLTT
ricinP02879   (347) AIQLWPCKSNTDANQLWTLKRDNTIR----SNGKCLTTYGYSPGVYVMIY
  Consensus   (401)     W  K   DA                S   C  TF    G   L
                451                                                 500
Cry1C 630aa   (446) GVVFSWTDRS---ATLTNTIDPERINQIPLVKGFRVWGGTSVITGPGFTG
ricinP02879   (393) DCNTAATDATRWQIWDNGTIINPRSSLVLAATSGNSGTTLTVQTNIYAVS
  Consensus   (451)     A TD S        TI   R   I           SV T
                501                                                 550
Cry1C 630aa   (493) GDILRRNTFGDFVSLQVNINSPITQRYRLRFRYASSRDARVIVLTGAAST
ricinP02879   (443) QGWLPTNNTQPFVTTIVGLYGLCLQAN---SGQVWIEDCSSEKAEQQWAL
  Consensus   (501)    L  N    FVS  V I     Q           D          A
                551                                                 600
Cry1C 630aa   (543) GVGGQVSVNMPLQKTMEIGENLTSRTFRYTDFSNPFSFRANPDIIGISEQ
ricinP02879   (490) YADGSIRPQQNRDNCLTSDSNIRETVVKILSCGPASSGQRWMFKNDGTIL
  Consensus   (551)    G I  N      L    NI     K        S          S
                601                                 638
Cry1C 630aa   (593) PLFGAGSISSGELYIDKIEIILADATFEAESDLERAQK
ricinP02879   (540) NLYSGLVLDVRASDPSLKQIILYPLHGDPNQIWLPLF-
  Consensus   (601)  LF A  I           IIL     D
```

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



Tetanus toxin (Clostridium tetani) versus Cry1C identity 12.6%



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