17) Thoma, R. S., G. Holleschak, R. E. Hileman and J. D. Astwood (2001). Primary structural protein characterization of corn event MON 863 Cry3Bb1.11098 protein using N-terminal sequencing and MALDI time of flight mass spectrometric techniques. MSL-17154, an unpublished study conducted by Monsanto Company.

Monsanto Company
Product Safety Center
FINAL REPORT

REPORT NO.: **MSL - 17154**

DATE: **April 13, 2001**

TITLE: **Primary Structural Protein Characterization of Corn Event MON 863 Cry3Bb1.11098 Protein Using N-terminal Sequencing and MALDI Time of Flight Mass Spectrometric Techniques**

AUTHORS: **Richard S. Thoma, Gyula Holleschak, Ronald E. Hileman and James D. Astwood**

ABSTRACT: Insect protected corn (IPC) was engineered to express the *Bacillus thuringiensis* (*B.t.*) *cry3Bb1.11098* gene resulting in the accumulation of corn event MON 863 Cry3Bb1.11098 protein. Initial attempts to obtain direct primary structural data of this protein using Edman degradation chemistry to verify the identity of the full length 74 kilodalton (kDa), Cry3Bb1.11098 protein, resulted in no N-terminal sequence information. The lack of sequence data suggested that the protein was N-terminally blocked. However, N-terminal protein sequence was obtained from a truncated 66 kDa fragment. The 66 kDa fragment produced a ragged N-terminal sequence starting at amino acid positions 47, 50 and 61 of the corn event MON 863 Cry3Bb1.11098 protein.

To confirm the identity of the corn event MON 863 Cry3Bb1.11098 protein, immunoaffinity purified 74 and 66 kDa polypeptides were proteolytically digested with trypsin and characterized by Matrix Assisted Laser Desorption Ionization (MALDI) Time of Flight (TOF) mass spectrometry. This technique had the advantage that trypsin digested proteins fragment predominantly after the amino acids arginine and lysine. The resulting peptide mixture was then analyzed by MALDI-TOF mass spectrometric techniques. The peptide fragments produced a mass map, which were then compared to a computer generated listing of all possible trypsin digested peptides from the predicted protein sequence. For the corn event MON 863 Cry3Bb1.11098 protein, twenty four masses were identified which matched the computer generated listing. The peptides from the twenty four masses reflected 34.5% of the corn event MON 863 Cry3Bb1.11098 full length protein sequence. This large number of

(*Abstract continued on following page*)

masses indicated that the immunoaffinity purified sample of corn event MON 863 Cry3Bb1.11098 protein has been accurately identified.

Forty three amino acids corresponding to three peptide fragments matched the N-terminal region of the predicted corn event MON 863 Cry3Bb1.11098 full length protein. This included an N-terminal peptide with a mass of 727.4 (MH+) daltons and having the N-terminal methionine removed and the alanine at position 2 acetylated. A trypsin digest of the truncated 66 kDa band further corroborated that these peptide masses were derived from the N-terminal region of the corn event MON 863 Cry3Bb1.11098 protein. The mass map profile for the 66 kDa band was identical to that produced by the 74 kDa band with the exception of three unmatched masses corresponding to the N-terminal region of the protein.

The complimentary use of mass spectrometry and N-terminal analysis has yielded unambiguous data that confirmed the identity of the corn event MON 863 Cry3Bb1.11098 protein, including the N-terminus of the full length 74 kDa protein purified from grain.

Statement of No Data Confidentiality Claim

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(l)(A), (B), or (C).

"We submitted this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material by EPA according to FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA."

Company: Monsanto Company

Company Agent: _____

Title: _____

Signature: _____ Date: _____

Statement of Compliance

This study does not meet the requirements under GLP as specified in 40 CFR Part 160, however data was recorded following Monsanto Company Guidelines for Keeping Research Records.

[signature] Date: April 13, 2001
Principal Researcher

Ravinder S Sidhu Date: April 13, 2001
Sponsor

Date:
Submitter

Summary of Quality Control Review

This report was reviewed to ensure that it accurately reflects the raw data of the study. The raw data was audited for compliance to the Monsanto Company Guidelines for Keeping Research Records (GRR 10/1/99), and where applicable, to Monsanto SOP's.

Valerie A. Zink

Date: April 13, 2001

Quality Assurance Specialist
Monsanto Regulatory
Monsanto Company

MSL Number: 17154

Title: Primary Structural Protein Characterization of Corn Event MON 863 Cry3Bb1.11098 Protein Using N-terminal Sequencing and MALDI Time of Flight Mass Spectrometric Techniques

Facility: Monsanto Company
Product Safety Center
Biotechnology Regulatory Sciences
700 Chesterfield Parkway North
Chesterfield, Missouri 63198

Sponsor: Ravinder S. Sidhu

Authors: Richard S. Thoma
Gyula Holleschak
Ronald E. Hileman
James D. Astwood

Experimental Start Date: December 1, 1999

Study Completion Date: April 13, 2001

Records Retention: All study specific raw data and final report will be retained at Monsanto-St. Louis.

Signatures of Final Report Approval:

Richard S Thoma — Date: April 13, 2001
Principal Researcher

[signature] — Date: April 13, 2001
Director, Product Safety Center

Ravinder S Sidhu — Date: April 13, 2001
Sponsor

Table of Contents

Tables

Figures

Abbreviations and Definitions

α-cyano	alpha-cyano-4-hydroxy cinnamic acid
B.t.	*Bacillus thuringiensis*
IPC	Insect Protected Corn
IUPAC-IUB	International Union of Pure and Applied Chemistry - International Union of Biochemistry
kDa	kilodalton
MALDI	Matrix Assisted Laser Desorption and Ionization
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene difluoride
TFA	Trifluoroacetic acid
TOF	Time of Flight

1.0 Summary

Insect protected corn (IPC) was engineered to express the *Bacillus thuringiensis* (*B.t.*) *cry3Bb1.11098* gene resulting in the accumulation of corn event MON 863 Cry3Bb1.11098 protein. Initial attempts to obtain direct primary structural data of this protein using Edman degradation chemistry to verify the identity of the full length 74 kilodalton (kDa), Cry3Bb1.11098 protein, resulted in no N-terminal sequence information. The lack of sequence data suggested that the protein was N-terminally blocked. However, N-terminal protein sequence was obtained from a truncated 66 kDa fragment. The 66 kDa fragment produced a ragged N-terminal sequence starting at amino acid positions 47, 50 and 61 of the corn event MON 863 Cry3Bb1.11098 protein.

To confirm the identity of the corn event MON 863 Cry3Bb1.11098 protein, immunoaffinity purified 74 and 66 kDa polypeptides were proteolytically digested with trypsin and characterized by Matrix Assisted Laser Desorption Ionization (MALDI) Time of Flight (TOF) mass spectrometry. This technique had the advantage that trypsin digested proteins fragment predominantly after the amino acids arginine and lysine. The resulting peptide mixture was then analyzed by MALDI-TOF mass spectrometric techniques. The peptide fragments produced a mass map, which were then compared to a computer generated listing of all possible trypsin digested peptides from the predicted protein sequence. For the corn event MON 863 Cry3Bb1.11098 protein, twenty four masses were identified which matched the computer generated listing. The peptides from the twenty four masses reflected 34.5% of the corn event MON 863 Cry3Bb1.11098 full length protein sequence. This large number of masses indicated that the immunoaffinity purified sample of corn event MON 863 Cry3Bb1.11098 protein has been accurately identified.

Forty three amino acids corresponding to three peptide fragments matched the N-terminal region of the predicted corn event MON 863 Cry3Bb1.11098 full length protein. This included an N-terminal peptide with a mass of 727.4 (MH+) daltons and having the N-terminal methionine removed and the alanine at position 2 acetylated. A trypsin digest of the truncated 66 kDa band further corroborated that these peptide masses were derived from the N-terminal region of the corn event MON 863 Cry3Bb1.11098 protein. The mass map profile for the 66 kDa band was identical to that produced by the 74 kDa band with the exception of three unmatched masses corresponding to the N-terminal region of the protein.

The complimentary use of mass spectrometry and N-terminal analysis has yielded unambiguous data that confirmed the identity of the corn event MON 863 Cry3Bb1.11098 protein, including the N-terminus of the full length 74 kDa protein purified from grain.

2.0 Introduction

Insect protected corn (IPC) was engineered to express the *Bacillus thuringiensis* (*B.t.*) *cry3Bb1.11098* gene resulting in the accumulation of corn event MON 863 Cry3Bb1.11098 protein. Initial attempts to obtain direct primary structural data of this protein using Edman degradation chemistry to verify the identity of the full length 74 kilodalton (kDa) Cry3Bb1.11098 protein, resulted in no sequence information.

The Cry3Bb1 protein was previously referred to as CryIIIB2 (or Cry3B2) as well as Cry3Bb or CryIIIC. This protein should be referred to as the Cry3Bb1 protein according to the most recent and accepted nomenclature (Crickmore et al., 1998). A variant of the wild type *cry3Bb1* coding sequence (GenBank Accession No. M89794) was designed to encode a protein with enhanced insecticidal activity against corn rootworm. This *cry3Bb1* coding sequence variant was used to create recombinant *B.t.* strain EG11098. Expression of this gene in *Bacillus* results in the production of a protein, Cry3Bb1.11098, which contains a total of five amino acid differences from the wild type Cry3Bb1 protein sequence. This coding sequence was further manipulated to enhance expression in plants and placed into a vector used for the transformation of corn (*Zea mays*). Transformation event MON 863 produces a variant of the Cry3Bb1.11098 protein that differs from the wild type protein sequence by seven amino acids and from the bacillus-produced Cry3Bb1.11098 protein sequence by two amino acids. The protein produced in corn event MON 863 is also referred to simply as a Cry3Bb1 variant protein (Hileman and Astwood, 2001).

The exact amino acid differences of several specific Cry3Bb1 protein variants (alleles), including corn event MON 863 Cry3Bb1.11098 protein, are shown below.

Cry3Bb1 Allele		Amino acid substitutions relative to wild type Cry3Bb1 protein[a]					
Bacillus thuringiensis strain EG11098 Cry3Bb1.11098 protein		D165G	H231R	S311L	N313T	E317K	
Bacillus thuringiensis strain EG11231 Cry3Bb1.11231 protein			H231R	S311L	N313T	E317K	
Corn event MON 863 Cry3Bb1.11098 protein	A2	D166G	H232R	S312L	N314T	E318K	Q349R
Corn event MON 853 Cry3Bb1.11231 protein	A2		H232R	S312L	N314T	E318K	

[a] wild type Cry3Bb1 protein corresponds to Accession No. M89794.

2.1 Purpose

The purpose of this study was to confirm the identity of corn event MON 863 Cry3Bb1.11098 protein purified directly from corn tissues via immunoaffinity chromatography, including confirmation of the N-terminal amino acid sequence of the full length 74kDa protein. To obtain the necessary data, both N-terminal protein sequencing and MALDI-TOF mass spectrometry techniques were employed.

3.0 Test Material

The test material was corn event MON 863 Cry3Bb1.11098 protein purified from grain by immunoaffinity chromatography (lot 6321076).

4.0 Assay Control

A control trypsin digest was performed on an unstained, non-proteinaceous portion of the same gel used in this analysis. This control digest was carried through the mass spectrometric analysis. Masses observed from MALDI-TOF mass spectrometry of the control digest were either autocatalytic trypsin fragments or extraneous gel associated non-proteinaceous molecules. Non Cry3Bb1.11098 masses were eliminated from further analysis by comparing the protein's mass digest fingerprint to the control trypsin digest profile.

5.0 Methods

5.1 *In-gel digestion of the Cry3Bb1.11098 protein.* The immunoaffinity purified corn event MON863 Cry3Bb1.11098 protein was prepared for proteolytic digestion by running 4 lanes (~40 µg protein per lane) of the purified protein on a Novex, 4→20% gradient polyacrylamide mini-gel (San Diego, CA). Proteins were stained with Coomassie Brilliant Blue R (Sigma Chemical Co., No. B-8647, St. Louis, MO). Each of the Cry3Bb1.11098 bands were excised from the gel, reduced, alkylated and subjected to an in-gel trypsin (Promega, No. V5111, Madison, WI) digest. Briefly, each gel band was placed in a 500 µL Eppendorf tube and destained with 100 µL of destaining buffer [40% (v/v) methanol (Burdick and Jackson, No. 230-4, Muskegon, MI), 10% (v/v) glacial acetic acid (J.T. Baker, No. 9507-00, Phillipsburg, NJ) and 50% water (Burdick and Jackson, No. 365-4)] for 30 minutes. After each step, and all subsequent steps, solution was removed before the addition of the next reagent. This first step was repeated two additional times to remove remaining stain from the gel. Gel pieces were incubated in 100 µL 100 mM ammonium bicarbonate (J.T. Baker, No. 3003-01) solution for 30 minutes. The protein was reduced in 100 µL 100 mM ammonium

bicarbonate, 10 mM dithiothreitol (Calbiochem®, No. 233155, LaJolla, CA) for 2 hours at 37 °C. Alkylation of the protein was performed by the addition of 100 µL of 200 mM iodoacetic acid (Sigma Chemical Co., No. I-8136). Samples were incubated in the dark at room temperature for 20 minutes. Alkylating reagent was decanted and the gel pieces were incubated in 100 µL 100 mM ammonium bicarbonate solution for 30 minutes. The gel fragments were washed with 100 µL of 50% (v/v) acetonitrile (Perkin Elmer, No. 400315, Norwalk, CT) for 30 min at room temperature. The previous two washes and gel drying steps were repeated twice. Finally, the gel pieces were dried in a Speed Vac concentrator (Savant, Hollebrook, NY). Dried gel bands were rehydrated with 40 µL 25 mM ammonium bicarbonate solution containing 33 µg/mL trypsin. Trypsin digestion went for 16 hours at 37 °C. Peptides were extracted from the gel by the addition of 50 µL 70% (v/v) acetonitrile containing 0.1% (v/v) trifluoroacetic acid (TFA) (Perkin Elmer, No. 400003) for 1 hour at room temperature. This extraction was repeated two more times. Supernatants from each sample were combined into a single tube and dried in a Speed Vac concentrator. The sample was reconstituted in 20 µL 0.1% (v/v) TFA (Williams et al., 1997).

5.2 *N-terminal sequence analysis.* N-terminal sequence analysis was performed with a Procise™ 494 Protein Sequencing System (Perkin Elmer, Foster City, CA) capable of performing automated Edman degradation chemistry. Instrument operation and sample handling procedures are outlined in Searle Bioprocess Analytical Procedure 400.324 (Thoma, et al.,1998). Samples were excised from polyvinylidene difluoride (PVDF) membranes (NOVEX, No. LC2002, San Diego, CA) (Hunkapiller, et al, 1983) prior to analysis. Data was collected using Procise™ software. A twenty amino acid PTH standard (Perkin Elmer, No. 400879) was used to calibrate the chromatography prior to each sequence analysis.

5.3 *MALDI-TOF instrumentation and mass analysis.* Mass spectral analyses were performed using a Perseptive Voyager DE™ -RP (A division of Perkin Elmer, Foster City, CA) MALDI-TOF instrument. Mass calibration of the instrument was performed using a Porcine Somatotropin (Monsanto 70003PX, 1987) trypsin digested laboratory frozen stock mixture. For each sample, 0.3 µL was co-crystallized with 0.75 µL alpha-cyano-4-hydroxy cinnamic acid (α cyano) (Ciphergen Biosystems, No. 4003-1002, Palo Alto, CA). Samples were analyzed in reflector mode at a laser power attenuation step setting value of 1620 (MALDI-TOF instrument specific value), analyzing from 400 to 3000 mass units. Protonated (MH+) peptide masses were observed monoisotypically in reflector mode (Aebersold, 1993; Billeci and Stults, 1993). The 74 kDa sample was also analyzed in linear mode at a laser power attenuation step setting values of 1620 and 1800, analyzing from 2000 to ~5000 mass units. Linear data obtained was

mass (average). MacBioSpec™ software (PE Sciex Instruments, version 1.0.1 1992, Thornhill, Ontario, Canada) was used to compare the mass data obtained to the protein sequence deduced from the *cry3Bb1.11098* gene coding sequence.

6.0 Results and Discussion

Purified corn event MON 863 Cry3Bb1.11098 protein was initially submitted for N-terminal sequence analysis as four SDS-PAGE lanes of ~ 74 kDa and 66 kDa Coomassie Blue stained bands from a PVDF membrane blot. No N-terminal sequence was discerned from the 74 kDa, full length sample (Figure 1). During the attempted sequencing, background amino acid noise became more pronounced as the run progressed. These two factors suggested the N-terminus of the full length Cry3Bb1.11098 protein was blocked. N-terminal sequencing is not designed to elucidate the nature of the blockage nor can sequence be obtained from an intact protein if it is blocked. This result was in contrast to previous result obtained for the *Bacillus thuringiensis* (*B.t.*) purified Cry3Bb1 protein (Hileman, et al., 1999). For the bacterial produced Cry3Bb1 protein, the N-terminal sequence was obtained from the full length purified protein. The presence of a blocking group on the N-terminus of the full length corn protein is not entirely unexpected. In eukaryotic cells, post-translational modification of proteins is a common occurrence (Tsunasawa and Sakiyama, 1984). *B.t.*, a prokaryote, lacks the post-translational cellular machinery to add a blocking agent to the N-terminus of a protein.

N-terminal sequence was obtained from the 66 kDa band. This band was sequenced as an upper and lower half because it visually appeared as two overlapping bands. Sequence data produced multiple amino acids at each cycle for both halves and could only be interpreted after comparison to the known sequence for Cry3Bb1.11098. The data from this band indicated the presence of a ragged N-terminus starting at positions, 47, 50 and 61 (Figure 1). Based on N-terminal data, the 66 kDa band contained a mixture of truncated corn event MON 863 Cry3Bb1.11098 protein. The 66 kDa upper half contained sequence starting predominantly at positions 47 and 50. The lower half contained sequence starting predominantly at position 61 but also contained sequence starting at positions 47 and 50. The loss of 47, 50 or 61 amino acids from the N-terminal region of the protein may be sufficient to explain the ~8 kDa decrease from the full length protein observed by gel electrophoresis.

To verify the identity of the full length corn event MON 863 Cry3Bb1.11098 protein, MALDI-TOF mass spectrometry was employed. The protein was first proteolytically cleaved into peptide fragments following the in-gel trypsin digestion procedure as described above. Fragments generated therein were used to confirm the identity of the corn event MON 863 Cry3Bb1.11098 protein. In addition, the 66 kDa band was also subjected to the same proteolytic digest and mass spectrometric analysis.

The unfractionated tryptic digest for both bands were analyzed by MALDI-TOF mass spectrometry. The mass spectrum from the full length 74 kDa mixture is shown in Figure 2. Thirty four masses were analyzed using reflector mode mass spectrometry, having molecular weights ranging from 400 and 3000 mass units. An additional 3 masses greater than 2500 daltons were observed in linear mode. Each observed mass was compared to the theoretical trypsin digest map of Cry3Bb1.11098 protein generated using a computer (Table 1). Less than 1.0 mass unit difference between experimental and predicted digestion products was observed for 24 of the 37 peptides. Alone, this data strongly suggests the identity of the purified 74 kDa band to be Cry3Bb1.11098. Possibilities for the unmatched masses include, cleavage of the Cry3Bb1.11098 protein at non-trypsin specific sites (cuts other than post lysine or arginine), possible post-translational modifications to the corn event MON 863 Cry3Bb1.11098 protein, trypsin autocatalytic fragments or minor co-purified protein present in the original sample. Three identified mass fragments were known trypsin autocatalytic peptides.

The mass spectral data from the 74 kDa digest resulted in identification of 34.5% (225 amino acids out of 653) of the corn event MON 863 Cry3Bb1.11098 protein (Figure 4). This includes three fragments (43 amino acids), from the N-terminal region of the protein. A mass was identified, mass #12 (Table 1) at 727.4 (MH+) daltons corresponding to the N-terminal sequence of the Cry3Bb1 protein having the methionine cleaved and the alanine at position 2 acetylated. No other combination of amino acid sequence from the Cry3Bb1.11098 protein and/or blocking group was found to match this experimental mass. The remaining peptide fragments matched Cry3Bb1.11098 sequence scattered throughout the protein suggesting that the protein was significantly digested by trypsin.

Not all fragment masses, including the C-terminal peptide fragment, from the corn event MON 863 Cry3Bb1.11098 protein were identified using MALDI-TOF mass fingerprint. Thirteen masses were observed in this study not matching any theoretical peptide fragments. Non-specific trypsin cleavage of the Cry3Bb1 protein would result in unmatched masses from a mass fingerprint. Large peptide fragments may be underrepresented in the analysis simply because they eluted poorly from a gel. Small peptide fragments (< 6 amino acids) were not detected due to α-cyano matrix interference. Additionally some poorly ionized peptides were not detected during mass spectometric analysis.

The mass spectral data from the 66 kDa digest was nearly identical to that from the 74 kDa digest. These data represent supporting evidence that the 66 kDa polypeptide is derived from the full length 74 kDa Cry3Bb1.11098 protein. The only observed differences were three missing masses, 727.4 daltons, 829.4 daltons and 3445.7 daltons. All three corresponded to the N-terminal region of the corn event MON 863 Cry3Bb1.11098 protein: at positions 2-7, 8-14 and 15-44 respectively. As demonstrated from the N-terminal sequencing data, the 66 kDa polypeptide starts either at amino acid

47, 50 or 61. Therefore the three fragments observed from the 74 kDa band were not expected in the truncated 66 kDa band. The absence of these three masses from the 66 kDa mass spectral was interpreted as evidence for a truncation of corn event MON 863 Cry3Bb1.11098 protein.

7.0 Conclusion

The complimentary use of mass spectrometry and N-terminal analysis has yielded unambiguous data which confirmed the identity of the corn event MON 863 Cry3Bb1.11098 protein, including the N-terminus of the full length 74 kDa protein purified from grain.

8.0 References

Aebersold, R. 1993. Mass spectrometry of proteins and peptides in biotechnology. *Curr Opin Biotechnol* **4**, 412-9.

Billeci, T. M. and Stults, J. T. 1993. Tryptic mapping of recombinant proteins by matrix-assisted laser desorption/ionization mass spectrometry. *Anal Chem* **65**, 1709-16.

Crickmore, N., Zeigler, D.R., Feitelson, J., Schnepf, E., Van Rie, J. Lereclus, D., Baum, J. and Dean, D.H. (1998). Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiol Mol Biol Rev* **62**, 807-813.

Hileman, R.E., Pyla, P.D., Lee, T.C. and Astwood, J.D. (1999). Characterization of *B.t.* protein 11098 and *B.t.* protein 11231 produced by fermentation. Study number 98-01-39-05, MSL-15531, an internal publication of Monsanto Company.

Hileman, R.E. and Astwood, J.D. (2001). Additional characterization of the Cry3Bb1 protein produced in corn event MON 863. Technical Report MSL-17137, an internal publication of Monsanto Company.

Hunkapiller, M. W., Hewick, R. M., Dreyer, W. J. and Hood, L. E. 1983. High-sensitivity sequencing with a gas-phase sequenator. *Methods Enzymol* **91**, 399-413.

Thoma, R.S., Jennings, M.G. 1998. Operation of Applied Biosystems Model 494 Procise™ Protein Sequencing System. SOP 400.324, an internal publication of Monsanto Company.

Tsunasawa, S. and Sakiyama, F. 1984. Amino-terminal acetylation of proteins: an overview. *Methods Enzymol* **106**, 165-70.

Williams, K., LoPresti, M. and Stone, K. 1997. Internal protein sequencing of SDS-PAGE-separated proteins: Optimization of an in-gel digest protocol. *Tech in Protein Chem* **8**, 79-98.

Table 1. Peak list obtained from the direct analysis by MALDI-TOF mass spectrometry of Cry3Bb1.11098 protein after in-gel digestion with trypsin. Masses corresponding to each observed mass peak (Figure 1 and 2) are shown in the second and third column respectively. A predicted mass is shown if there is a corresponding match to the experimental mass. Masses 1 through 34 were observed under Reflector mode and are compared to MH+ mass. Masses 35 through 37 were observed under linear mode and are compared to Mass Average. The corresponding amino acid positions and sequences are also shown. Amino acids are shown using the IUPAC-IUB single letter code.

#	Mass (74 kDa)	Mass (66 kDa)	Mass (MH+)	Position (74 kDa)	Sequence
1	436.23	436.21	436.23	267-269	FNR
2	445.00				
3	447.20	N.D.	447.23	164-167	SQGR
4	478.28	478.30	478.29	270-272	FRR
5	515.35	515.35			
6	564.35	565.35	564.31	45-48	EFLR
7	603.37	603.41	603.33	163-167	RSQGR
8	619.41	619.43	619.34	299-303	TELTR
9	650.14	650.15			
10	656.16	656.19			
11	686.53	686.56	686.42	155-160	TPLSLR
12	727.48		727.35	2-7	Ac-ANPNNR
13	758.60	758.61			
14	814.67	814.69	814.52	154-160	KTPLSLR
15	829.58		829.41	8-14	SEHDTIK
16	842.68	842.70			trypsin autocatalytic fragment
17	847.60	847.63			
18	861.26	861.28			
19	925.68	925.70	925.47	573-580	YASTTNLR
20	937.74	937.77	937.53	348-355	LRPGYFGK
21	1019.76	1019.78			
22	1186.00	1186.02	1185.70	556-566	VTLNSAALLQR
23	1316.07				trypsin autocatalytic fragment
24	1350.98	1351.01	1350.64	170-180	ELFSQAESHFR
25	1363.02	1363.07	1363.73	110-121	AFMAQVEVLIDK
26	1407.98	1409.01			
27	1496.98	1497.03	1496.78	319-331	YGPTFLSIENSIR
28	1518.97	1519.00	1518.80	489-500	RGTIPFFTWTHR
29	1738.18	1738.23	1737.75	356-369	DSFNYWSGNYVETR
30	2001.52	2001.56	2001.04	332-347	KPHLFDYLQGIEFHTR
31	2099.46	2099.48			
32	2394.63	2394.69	2394.10	356-376	DSFNYWSGNYVETRPSIGSSK
33	2484.64	2484.73	2484.06	212-232	DAQVFGEEWGYSSEDVAEFYR
34	2552.73	2552.84	2552.20	237-257	LTQQYTDHCVNWYNVGLNGLR*

#	Mass (74kDa)	Mass (66kDa)	Mass (Ave.)	Position	Sequence
35	2640.56	N.D.	2641.8	212-233	DAQVFGEEWGYSSEDVAEFYRR
36	2809.40	N.D.			trypsin autocatalytic fragment
37	3445.79	N.D.	3445.7	15-44	VTPNSELQTNHNQYPLADNPNSTLEELNYK

* Carboxymethylated Cysteine in peptide N.D. (not determined)

SDS-PAGE gel	Position	Sequence
74 kDa band	-	no observed sequence
66 kDa band	47	LRMTEDXXTE
	50	TEDSSTEVLDNSTVK
	61	STVKDAVGTGISVVG

Figure 1. N-terminal sequence analysis of Cry3Bb1.11098 protein purified from IPC Event MON 863. The IUPAC-IUB single letter amino acid code is shown. Missing amino acid calls are denoted by an (X). Sequence data for the 66 kDa band was observed as a mixture of amino acids at each cycle.

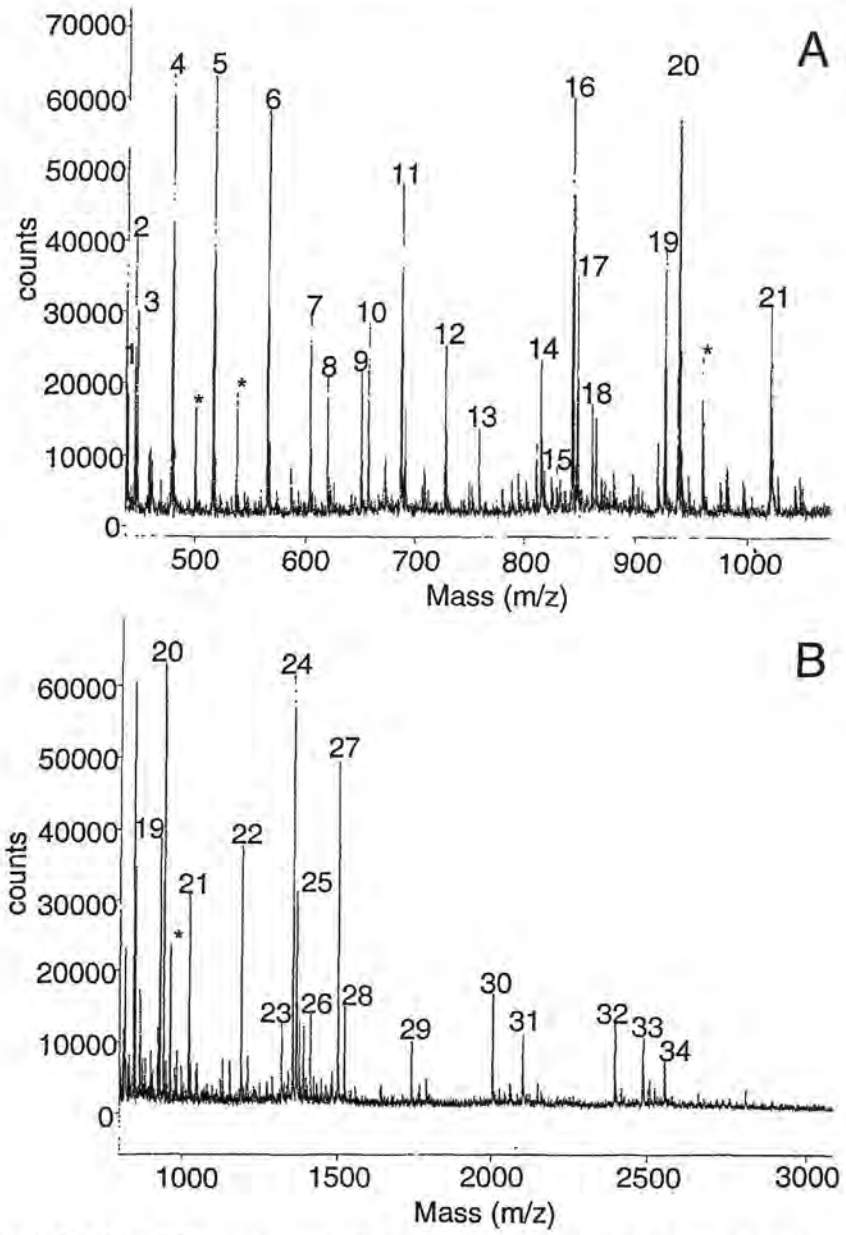

Figure 2. MALDI-TOF mass spectral fragment analysis of the tryptin digested 74 kDa band of Cry3Bb1.11098 protein purified from IPC Event MON 863. Numbered peaks correspond to the peak numbers shown in Table 1. Masses from 400 to 1000 daltons are observed in Panel A. Panel B's range is from 1000 to 3000 daltons. An (*) indicates a peptide containing a sodium salt (+22 daltons) mass.

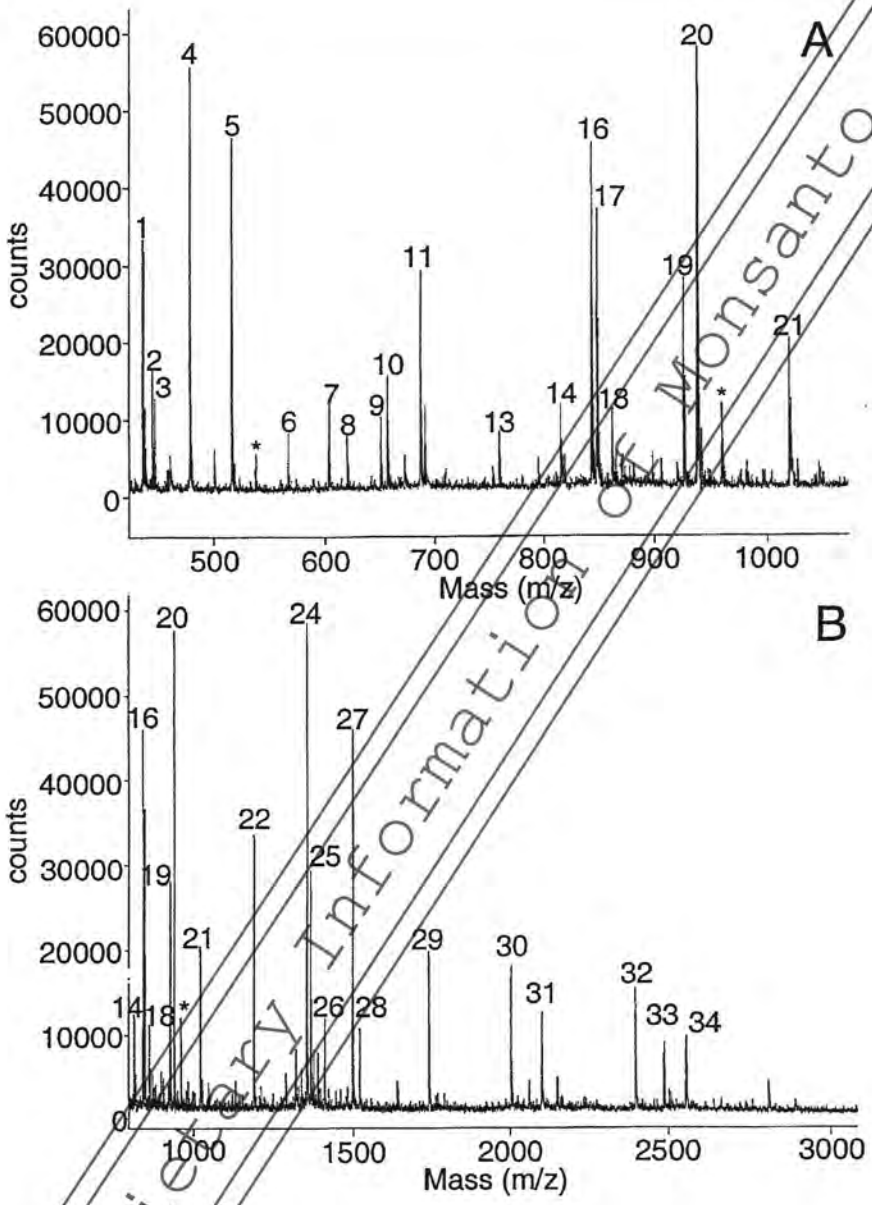

Figure 3. MALDI-TOF mass spectral fragment analysis of the tryptin digested 66 kDa band of Cry3Bb1.11098 protein purified from IPC Event MON 863.
Numbered peaks correspond to the peak numbers shown in Table 1. Masses from 400 to 1000 daltons are observed in Panel A. Panel B's range is from 1000 to 3000 daltons. An (*) indicates a peptide containing a sodium salt (+22 daltons) mass.

```
  1  MANPNNRSEH DTIKVTPNSE LQTNHNQYPL ADNPNSTLEE LNYKEFLRMT
 51  EDSSTEVLDN STVKDAVGTG ISVVGQILGV VGVPFAGALT SFYQSFLNTI
101  WPSDADPWKA FMAQVEVLID KKIEEYAKSK ALAELQGLQN NFEDYVNALN
151  SWKKTPLSLR SKRSQGRIRE LFSQAESHFR NSMPSFAVSK FEVLFLPTYA
201  QAANTHLLLL KDAQVFGEEW GYSSEDVAEF YRRQLKLTQQ YTDHCVNWYN
251  VGLNGLRGST YDAWVKFNRF RREMTLTVLD LIVLFPFYDI RLYSKGVKTE
301  LTRDIFTDPI FLLTTLQKYG PTFLSIENSI RKPHLFDYLQ GIEFHTRLRP
351  GYFGKDSFNY WSGNYVETRP SIGSSKTITS PFYGDKSTEP VQKLSFDGQK
401  VYRTIANTDV AAWPNGKVYL GVTKVDFSQY DDQKNETSTQ TYDSKRNNGH
451  VSAQDSIDQL PPETTDEPLE KAYSHQLNYA ECFLMQDRRG TIPFFTWTHR
501  SVDFFNTIDA EKITQLPVVK AYALSSGASI IEGPGFTGGN LLFLKESSNS
551  IAKFKVTLNS AALLQRYRVR IRYASTTNLR LFVQNSNNDF LVIYINKTMN
601  KDDDLTYQTF DLATTNSNMG FSGDKNELII GAESFVSNEK IYIDKIEFIP
651  VQL
```

Figure 4. Cry3Bb1.11098 sequence deduced from DNA. Fragments identified by mass using MALDI-TOF are highlighted and bolded. The single letter IUPAC-IUB amino acid code is shown.