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Abstract

Compositions and methods for conferring pesticidal activity to bacteria, plants, plant cells, tissues and seeds are provided. Compositions comprising a coding sequence for
5 pesticidal polypeptides are provided. The coding sequences can be used in DNA constructs or expression cassettes for transformation and expression in plants and bacteria. Compositions also comprise transformed bacteria, plants, plant cells, tissues, and seeds. In particular, isolated pesticidal nucleic acid molecules are provided. Additionally, amino acid sequences corresponding to the polynucleotides are encompassed. In particular, the present invention
10 provides for isolated nucleic acid molecules comprising nucleotide sequences encoding the amino acid sequence shown in SEQ ID NO:5, 2, or 10, the nucleotide sequence set forth in SEQ ID NO: 4, 1, 3, 4, 6, 9, or 11, or the nucleotide sequence deposited in a bacterial host as Accession No. B-50045, as well as variants and fragments thereof.

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AXMI-066 AND AXMI-076: DELTA-ENDOTOXIN PROTEINS AND METHODS
FOR THEIR USE

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FIELD OF THE INVENTION

This invention relates to the field of molecular biology. Provided are novel genes
5 that encode pesticidal proteins. These proteins and the nucleic acid sequences that encode
them are useful in preparing pesticidal formulations and in the production of transgenic
pest-resistant plants.

BACKGROUND OF THE INVENTION

10 *Bacillus thuringiensis* is a Gram-positive spore forming soil bacterium
characterized by its ability to produce crystalline inclusions that are specifically toxic to
certain orders and species of insects, but are harmless to plants and other non-targeted
organisms. For this reason, compositions including *Bacillus thuringiensis* strains or their
insecticidal proteins can be used as environmentally-acceptable insecticides to control
15 agricultural insect pests or insect vectors for a variety of human or animal diseases.

Crystal (Cry) proteins (delta-endotoxins) from *Bacillus thuringiensis* have potent
insecticidal activity against predominantly Lepidopteran, Dipteran, and Coleopteran
larvae. These proteins also have shown activity against *Hymenoptera*, *Homoptera*,
Phthiraptera, *Mallophaga*, and *Acari* pest orders, as well as other invertebrate orders
20 such as *Nemathelminthes*, *Platyhelminthes*, and *Sarcomastigophora* (Feitelson (1993)
The *Bacillus Thuringiensis* family tree. In *Advanced Engineered Pesticides*, Marcel
Dekker, Inc., New York, N.Y.) These proteins were originally classified as CryI to CryV
based primarily on their insecticidal activity. The major classes were *Lepidoptera*-
specific (I), *Lepidoptera*- and *Diptera*-specific (II), *Coleoptera*-specific (III), *Diptera*-
25 specific (IV), and nematode-specific (V) and (VI). The proteins were further classified
into subfamilies; more highly related proteins within each family were assigned divisional
letters such as *CryIA*, *CryIB*, *CryIC*, etc. Even more closely related proteins within each
division were given names such as *CryIC1*, *CryIC2*, etc.

A new nomenclature was recently described for the Cry genes based upon amino acid sequence homology rather than insect target specificity (Crickmore *et al.* (1998) *Microbiol. Mol. Biol. Rev.* 62:807-813). In the new classification, each toxin is assigned a unique name incorporating a primary rank (an Arabic number), a secondary rank (an
5 uppercase letter), a tertiary rank (a lowercase letter), and a quaternary rank (another Arabic number). In the new classification, Roman numerals have been exchanged for Arabic numerals in the primary rank. Proteins with less than 45% sequence identity have different primary ranks, and the criteria for secondary and tertiary ranks are 78% and 95%, respectively.

10 The crystal protein does not exhibit insecticidal activity until it has been ingested and solubilized in the insect midgut. The ingested protoxin is hydrolyzed by proteases in the insect digestive tract to an active toxic molecule. (Höfte and Whiteley (1989) *Microbiol. Rev.* 53:242-255). This toxin binds to apical brush border receptors in the midgut of the target larvae and inserts into the apical membrane creating ion channels or
15 pores, resulting in larval death.

Delta-endotoxins generally have five conserved sequence domains, and three conserved structural domains (see, for example, de Maagd *et al.* (2001) *Trends Genetics* 17:193-199). The first conserved structural domain consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II consists of three beta-
20 sheets arranged in a Greek key configuration, and domain III consists of two antiparallel beta-sheets in "jelly-roll" formation (de Maagd *et al.*, 2001, *supra*). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

Because of the devastation that insects can confer, and the improvement in yield
25 by controlling insect pests, there is a continual need to discover new forms of pesticidal toxins.

SUMMARY OF INVENTION

Compositions and methods for conferring pesticidal activity to bacteria, plants,
30 plant cells, tissues and seeds are provided. Compositions include nucleic acid molecules encoding sequences for pesticidal and insectidal polypeptides, vectors comprising those nucleic acid molecules, and host cells comprising the vectors. Compositions also include the pesticidal polypeptide sequences and antibodies to those polypeptides. The nucleotide sequences can be used in DNA constructs or expression cassettes for transformation and

expression in organisms, including microorganisms and plants. The nucleotide or amino acid sequences may be synthetic sequences that have been designed for expression in an organism including, but not limited to, a microorganism or a plant. Compositions also comprise transformed bacteria, plants, plant cells, tissues, and seeds.

5 In particular, isolated nucleic acid molecules are provided that encode a pesticidal protein. Additionally, amino acid sequences corresponding to the pesticidal protein are encompassed. In particular, the present invention provides for an isolated nucleic acid molecule comprising a nucleotide sequence encoding the amino acid sequence shown in SEQ ID NO:2 or 5, a nucleotide sequence set forth in SEQ ID NO:1, 3, 4, 6, 9, or 11, or
10 the delta-endotoxin nucleotide sequence of the DNA insert of the plasmid deposited in a bacterial host as Accession No. NRRL B-50045, as well as variants and fragments thereof. Nucleotide sequences that are complementary to a nucleotide sequence of the invention, or that hybridize to a sequence of the invention are also encompassed.

Methods are provided for producing the polypeptides of the invention, and for
15 using those polypeptides for controlling or killing a lepidopteran, coleopteran, nematode, or dipteran pest. Methods and kits for detecting the nucleic acids and polypeptides of the invention in a sample are also included.

The compositions and methods of the invention are useful for the production of organisms with enhanced pest resistance or tolerance. These organisms and compositions
20 comprising the organisms are desirable for agricultural purposes. The compositions of the invention are also useful for generating altered or improved proteins that have pesticidal activity, or for detecting the presence of pesticidal proteins or nucleic acids in products or organisms.

25 DESCRIPTION OF FIGURES

Figure 1 shows the DNA sequence of the *axmi-066* gene and its surrounding DNA region (SEQ ID NO:8). The first ATG (corresponding to the start site of SEQ ID NO:1; translation of which encodes AXMI-066 (SEQ ID NO:2)) is at nucleotide position 52 of the sequence shown in this figure. The second internal methionine (whose translation
30 encodes residues 14 through 637 of SEQ ID NO:2) is at position 91 of this figure. The TAA stop codon begins at position 1963 of the sequence in this figure. The ATG start codons and the TAA stop codon are shown in bold type. Two putative ribosome binding sites are shown in italics and underlined.

Figures 2A-2D show an alignment of AXMI-066_long (SEQ ID NO:2), AXMI-066 (SEQ ID NO:10), Cry2Aa1 (SEQ ID NO:14), Cry2Ab1 (SEQ ID NO:15), Cry2Ac1 (SEQ ID NO:16), Cry2Ad1 (SEQ ID NO:17), Cry2Ae1 (SEQ ID NO:18), Cry1Ac (SEQ ID NO:19), and Cry3Aa1 (SEQ ID NO:20). The alignment shows the most highly conserved amino acid residues highlighted in black, and highly conserved amino acid residues highlighted in gray.

DETAILED DESCRIPTION

The present invention is drawn to compositions and methods for regulating pest resistance or tolerance in organisms, particularly plants or plant cells. By “resistance” is intended that the pest (e.g., insect) is killed upon ingestion or other contact with the polypeptides of the invention. By “tolerance” is intended an impairment or reduction in the movement, feeding, reproduction, or other functions of the pest. The methods involve transforming organisms with a nucleotide sequence encoding a pesticidal protein of the invention. In particular, the nucleotide sequences of the invention are useful for preparing plants and microorganisms that possess pesticidal activity. Thus, transformed bacteria, plants, plant cells, plant tissues and seeds are provided. Compositions are pesticidal nucleic acids and proteins of *Bacillus* or other species. The sequences find use in the construction of expression vectors for subsequent transformation into organisms of interest, as probes for the isolation of other homologous (or partially homologous) genes, and for the generation of altered pesticidal proteins by methods known in the art, such as domain swapping or DNA shuffling. The proteins find use in controlling or killing lepidopteran, coleopteran, dipteran, and nematode pest populations and for producing compositions with pesticidal activity.

A plasmid containing the *axmi-066* nucleotide sequence of the invention was deposited in the permanent collection of the Agricultural Research Service Culture Collection, Northern Regional Research Laboratory (NRRL), 1815 North University Street, Peoria, Illinois 61604, United States of America, on May 29, 2007 under Accession No. NRRL B-50045. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Access to these deposits will be available during the pendency of the application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. Upon allowance of any claims in the application, the Applicants will make available to the public, pursuant to

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37 C.F.R. § 1.808, sample(s) of the deposit with the NRRL. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

By "pesticidal toxin" or "pesticidal protein" is intended a toxin that has toxic activity against one or more pests, including, but not limited to, members of the *Lepidoptera*, *Diptera*, and *Coleoptera* orders, or the *Nematoda* phylum, or a protein that has homology to such a protein. Pesticidal proteins have been isolated from organisms including, for example, *Bacillus sp.*, *Clostridium bifermentans* and *Paenibacillus popilliae*. Pesticidal proteins include amino acid sequences deduced from the full-length nucleotide sequences disclosed herein, and amino acid sequences that are shorter than the full-length sequences, either due to the use of an alternate downstream start site, or due to processing that produces a shorter protein having pesticidal activity. Processing may occur in the organism the protein is expressed in, or in the pest after ingestion of the protein.

Pesticidal proteins encompass delta-endotoxins. Delta-endotoxins include proteins identified as *cryI* through *cry43*, *cyl1* and *cyl2*, and Cyt-like toxin. There are currently over 250 known species of delta-endotoxins with a wide range of specificities and toxicities. For an expansive list see Crickmore *et al.* (1998), *Microbiol. Mol. Biol. Rev.* 62:807-813.

Thus, provided herein are novel isolated nucleotide sequences that confer pesticidal activity. These isolated nucleotide sequences encode polypeptides with homology to known delta-endotoxins or binary toxins. Also provided are the amino acid sequences of the pesticidal proteins. The protein resulting from translation of this gene allows cells to control or kill pests that ingest it.

Isolated Nucleic Acid Molecules, and Variants and Fragments Thereof

One aspect of the invention pertains to isolated or recombinant nucleic acid molecules comprising nucleotide sequences encoding pesticidal proteins and polypeptides or biologically active portions thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify nucleic acid molecules encoding proteins with regions of sequence homology. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., recombinant DNA, cDNA or genomic DNA) and RNA

molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An “isolated” or “purified” nucleic acid molecule or protein, or biologically active
5 portion thereof, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Preferably, an “isolated” nucleic acid is free of sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of
10 the organism from which the nucleic acid is derived. For purposes of the invention, “isolated” when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the isolated nucleic acid molecule encoding a pesticidal protein can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of
15 the cell from which the nucleic acid is derived. A pesticidal protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-pesticidal protein (also referred to herein as a “contaminating protein”).

Nucleotide sequences encoding the proteins of the present invention include the
20 sequence set forth in SEQ ID NO:1, 3, 4, 6, 9, or 11, or the nucleotide sequence deposited in a bacterial host as Accession No. B-50045, and variants, fragments, and complements thereof. By “complement” is intended a nucleotide sequence that is sufficiently complementary to a given nucleotide sequence such that it can hybridize to the given nucleotide sequence to thereby form a stable duplex. The corresponding amino acid
25 sequence for the pesticidal protein encoded by this nucleotide sequence are set forth in SEQ ID NO:2 or 5.

Nucleic acid molecules that are fragments of these nucleotide sequences encoding
pesticidal proteins are also encompassed by the present invention. By “fragment” is intended a portion of the nucleotide sequence encoding a pesticidal protein. A fragment
30 of a nucleotide sequence may encode a biologically active portion of a pesticidal protein, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. Nucleic acid molecules that are fragments of a nucleotide sequence encoding a pesticidal protein comprise at least about 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1350, 1400 contiguous nucleotides, or

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up to the number of nucleotides present in a full-length nucleotide sequence encoding a pesticidal protein disclosed herein, depending upon the intended use. By "contiguous" nucleotides is intended nucleotide residues that are immediately adjacent to one another. Fragments of the nucleotide sequences of the present invention will encode protein

5 fragments that retain the biological activity of the pesticidal protein and, hence, retain pesticidal activity. By "retains activity" is intended that the fragment will have at least about 30%, at least about 50%, at least about 70%, 80%, 90%, 95% or higher of the pesticidal activity of the pesticidal protein. In one embodiment, the pesticidal activity is coleopterocidal activity. In another embodiment, the pesticidal activity is lepidopterocidal

10 activity. In another embodiment, the pesticidal activity is nematocidal activity. In another embodiment, the pesticidal activity is dipterocidal activity. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Andrews *et al.* (1988) *Biochem. J.* 252:199-206; Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293; and U.S.

15 Patent No. 5,743,477.

A fragment of a nucleotide sequence encoding a pesticidal protein that encodes a biologically active portion of a protein of the invention will encode at least about 15, 25, 30, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450 contiguous amino acids, or up to the total number of amino acids present in a full-length pesticidal protein of the

20 invention.

Preferred pesticidal proteins of the present invention are encoded by a nucleotide sequence sufficiently identical to the nucleotide sequence of SEQ ID NO:1, 3, 4, 6, 9, or 11. By "sufficiently identical" is intended an amino acid or nucleotide sequence that has at least about 60% or 65% sequence identity, about 70% or 75% sequence identity, about

25 80% or 85% sequence identity, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity compared to a reference sequence using one of the alignment programs described herein using standard parameters. One of skill in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon

30 degeneracy, amino acid similarity, reading frame positioning, and the like.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent identity = number of identical positions/total number of

positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length. In another embodiment, the percent identity is calculated across the entirety of the reference sequence (i.e., the sequence disclosed herein as any of SEQ ID NO:1-13). The percent identity between two sequences can be determined using
5 techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A nonlimiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and
10 Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the BLASTN and BLASTX programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to pesticidal-like nucleic
15 acid molecules of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to pesticidal protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast
20 can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., BLASTX and BLASTN) can be used. Alignment may also be performed manually by inspection.

25 Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the ClustalW algorithm (Higgins *et al.* (1994) *Nucleic Acids Res.* 22:4673-4680). ClustalW compares sequences and aligns the entirety of the amino acid or DNA sequence, and thus can provide data about the sequence conservation of the entire amino acid sequence. The ClustalW algorithm is used in several commercially
30 available DNA/amino acid analysis software packages, such as the ALIGNX module of the Vector NTI Program Suite (Invitrogen Corporation, Carlsbad, CA). After alignment of amino acid sequences with ClustalW, the percent amino acid identity can be assessed. A non-limiting example of a software program useful for analysis of ClustalW alignments is GENEDOC™. GENEDOC™ (Karl Nicholas) allows assessment of amino acid (or

DNA) similarity and identity between multiple proteins. Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG Wisconsin Genetics Software
5 Package, Version 10 (available from Accelrys, Inc., 9685 Scranton Rd., San Diego, CA, USA). When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Unless otherwise stated, GAP Version 10, which uses the algorithm of Needleman
10 and Wunsch (1970) *J. Mol. Biol.* 48(3):443-453, will be used to determine sequence identity or similarity using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity or % similarity for an amino acid sequence using GAP weight of 8 and length weight of 2, and the BLOSUM62 scoring program.
15 Equivalent programs may also be used. By “equivalent program” is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

The invention also encompasses variant nucleic acid molecules. “Variants” of the
20 pesticidal protein encoding nucleotide sequences include those sequences that encode the pesticidal proteins disclosed herein but that differ conservatively because of the degeneracy of the genetic code as well as those that are sufficiently identical as discussed above. Naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization
25 techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the pesticidal proteins disclosed in the present invention as discussed below. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired biological activity of the
30 native protein, that is, pesticidal activity. By “retains activity” is intended that the variant will have at least about 30%, at least about 50%, at least about 70%, or at least about 80% of the pesticidal activity of the native protein. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *J. Econ. Entomol.* 83: 2480-2485; Andrews *et al.* (1988) *Biochem. J.* 252:199-206; Marrone *et al.* (1985) *J.*

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of *Economic Entomology* 78:290-293; and U.S. Patent No. 5,743,477.

In one embodiment, the variants encompass insertion of one or more amino acids into SEQ ID NO:2, 5, or 10. In another embodiment, the variants encompass insertion of one or more amino acids in apical loop 2 of SEQ ID NO:10. In another embodiment, the variants encompass insertion of one or more amino acids between residues 379 and 380 of SEQ ID NO:10. In another embodiment, the variants encompass insertion of at least a glycine residue between residues 379 and 380 of SEQ ID NO:10. In another embodiment, the variants encompass insertion of a glycine residue and one additional residue between residues 379 and 380 of SEQ ID NO:10. In another embodiment, the variants encompass insertion of two glycine residues, of a glycine and a threonine, a glycine and a serine, a glycine and a leucine, an arginine and a glycine, a glycine and an asparagine, a glycine and a lysine, a histidine and a glycine, a phenylalanine and a glycine, a leucine and a glycine, or an asparagine and a glycine residue between residues 379 and 380 of SEQ ID NO:10.

In yet another embodiment, the variant is selected from the group consisting of P83T, L250I, G319K, G319F, I322S, I322V, I322Q, I322A, L323F, Y376N, Y376I, Y376R, Y376S, Y376V, Y376A, R377E, R377Q, R377L, G378S, G378A, G378W, D379V, D379E, L380M, L380P, L380Y, Q381L, L401I, M406H, M406V, M406K, M406E, M406T, M406S, M406A, M406V, M406N, F407W, and F407R relative to SEQ ID NO:10.

The skilled artisan will further appreciate that changes can be introduced by mutation of the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded pesticidal proteins, without altering the biological activity of the proteins. Thus, variant isolated nucleic acid molecules can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, conservative amino acid substitutions may be made at one or more, predicted, nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a pesticidal protein without

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altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

Delta-endotoxins generally have five conserved sequence domains, and three conserved structural domains (see, for example, de Maagd *et al.* (2001) *Trends Genetics* 17:193-199). The first conserved structural domain consists of seven alpha helices and is involved in membrane insertion and pore formation. Domain II consists of three beta-sheets arranged in a Greek key configuration, and domain III consists of two antiparallel beta-sheets in "jelly-roll" formation (de Maagd *et al.*, 2001, *supra*). Domains II and III are involved in receptor recognition and binding, and are therefore considered determinants of toxin specificity.

AXMI-066 shows homology Cry2A family of proteins. The 3D structure of Cry2Aa has been determined (see, Morse *et al.* (2001) *Structure* 9:409-417), and domain swapping experiments between Cry2A and Cry2B have lead to the identification of specificity regions (see, for example, Liang and Dean (1994) *Molecular Microbiology*, 13 (4):569-575; Widner and Whiteley (1989) *J.Bacteriology*, 171(2):965-974; and Widner and Whiteley (1990) *J.Bacteriology*, 172(6):2826-2832).

Apical loops in Cry toxins have been implicated in receptor recognition, and Cry2Aa contains 2 apical loops. Loop 1 is found from about position 316 to about position 335 of SEQ ID NO:14. Loop 2 is found from about position 370 to about position 394 of SEQ ID NO:14. The corresponding residues in AXMI-066 can be found in the alignment of Figure 2.

Amino acid substitutions may be made in nonconserved regions that retain function. In general, such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif, where such residues are essential for protein activity. Examples of residues that are conserved and that may be essential for protein activity include, for example, residues that are identical

between all proteins contained in an alignment of similar or related toxins to the sequences of the invention (e.g., residues that are identical between all proteins contained in the alignment in Figure 7). Examples of residues that are conserved but that may allow conservative amino acid substitutions and still retain activity include, for example,

5 residues that have only conservative substitutions between all proteins contained in an alignment of similar or related toxins to the sequences of the invention (e.g., residues that have only conservative substitutions between all proteins contained in the alignment in Figure 7). However, one of skill in the art would understand that functional variants may have minor conserved or nonconserved alterations in the conserved residues.

10 Alternatively, variant nucleotide sequences can be made by introducing mutations randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for ability to confer pesticidal activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard
15 assay techniques.

Using methods such as PCR, hybridization, and the like corresponding pesticidal sequences can be identified, such sequences having substantial identity to the sequences of the invention. See, for example, Sambrook and Russell (2001) *Molecular Cloning: A Laboratory Manual*. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY) and Innis, *et al.* (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, NY).

In a hybridization method, all or part of the pesticidal nucleotide sequence can be used to screen cDNA or genomic libraries. Methods for construction of such cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook and
25 Russell, 2001, *supra*. The so-called hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P , or any other detectable marker, such as other radioisotopes, a fluorescent compound, an enzyme, or an enzyme co-factor. Probes for hybridization can be made by labeling synthetic oligonucleotides based on the known
30 pesticidal protein-encoding nucleotide sequence disclosed herein. Degenerate primers designed on the basis of conserved nucleotides or amino acid residues in the nucleotide sequence or encoded amino acid sequence can additionally be used. The probe typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, at least about 25, at least about 50, 75, 100, 125, 150, 175, or 200

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consecutive nucleotides of nucleotide sequence encoding a pesticidal protein of the invention or a fragment or variant thereof. Methods for the preparation of probes for hybridization are generally known in the art and are disclosed in Sambrook and Russell, 2001, *supra*.

5 For example, an entire pesticidal protein sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding pesticidal protein-like sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are preferably at least about 10 nucleotides in length, or at least about 20
10 nucleotides in length. Such probes may be used to amplify corresponding pesticidal sequences from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see,
15 for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree
20 than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that
25 lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10
30 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium

citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

- 5 Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl
 10 (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61$
 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a
 15 complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point
 20 (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12,
 25 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC
 30 concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York).

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See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York).

Isolated Proteins and Variants and Fragments Thereof

5 Pesticidal proteins are also encompassed within the present invention. By “pesticidal protein” is intended a protein having the amino acid sequence set forth in SEQ ID NO:2 or 5. Fragments, biologically active portions, and variants thereof are also provided, and may be used to practice the methods of the present invention.

10 “Fragments” or “biologically active portions” include polypeptide fragments comprising amino acid sequences sufficiently identical to the amino acid sequence set forth in SEQ ID NO:2 or 5, and that exhibit pesticidal activity. A biologically active portion of a pesticidal protein can be a polypeptide that is, for example, 10, 25, 50, 100, 150, 200, 250 or more amino acids in length. Such biologically active portions can be prepared by recombinant techniques and evaluated for pesticidal activity. Methods for
15 measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Andrews *et al.* (1988) *Biochem. J.* 252:199-206; Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293; and U.S. Patent No. 5,743,477.

As used here, a fragment comprises at least 8 contiguous amino acids of SEQ ID NO:2 or
20 5. The invention encompasses other fragments, however, such as any fragment in the protein greater than about 10, 20, 30, 50, 100, 150, 200, 250, or 300 amino acids.

By “variants” is intended proteins or polypeptides having an amino acid sequence that is at least about 60%, 65%, about 70%, 75%, about 80%, 85%, about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of
25 SEQ ID NO:2 or 5. Variants also include polypeptides encoded by a nucleic acid molecule that hybridizes to the nucleic acid molecule of SEQ ID NO:1, 3, 4, 6, 9, or 11, or a complement thereof, under stringent conditions. Variants include polypeptides that differ in amino acid sequence due to mutagenesis. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess the desired
30 biological activity of the native protein, that is, retaining pesticidal activity. Methods for measuring pesticidal activity are well known in the art. See, for example, Czapla and Lang (1990) *J. Econ. Entomol.* 83:2480-2485; Andrews *et al.* (1988) *Biochem. J.* 252:199-206; Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293; and U.S. Patent No. 5,743,477.

Bacterial genes, such as the *axmi* genes of this invention, quite often possess multiple methionine initiation codons in proximity to the start of the open reading frame. Often, translation initiation at one or more of these start codons will lead to generation of a functional protein. These start codons can include ATG codons. However, bacteria such as *Bacillus sp.* also recognize the codon GTG as a start codon, and proteins that initiate translation at GTG codons contain a methionine at the first amino acid. Furthermore, it is not often determined *a priori* which of these codons are used naturally in the bacterium. Thus, it is understood that use of one of the alternate methionine codons may also lead to generation of pesticidal proteins. These pesticidal proteins are encompassed in the present invention and may be used in the methods of the present invention.

Antibodies to the polypeptides of the present invention, or to variants or fragments thereof, are also encompassed. Methods for producing antibodies are well known in the art (see, for example, Harlow and Lane (1988) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; U.S. Patent No. 4,196,265).

15

Altered or Improved Variants

It is recognized that DNA sequences of a pesticidal protein may be altered by various methods, and that these alterations may result in DNA sequences encoding proteins with amino acid sequences different than that encoded by a pesticidal protein of the present invention. This protein may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions of one or more amino acids of SEQ ID NO:2 or 5, including up to about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 100, about 105, about 110, about 115, about 120, about 125, about 130, about 135, about 140, about 145, about 150, about 155, or more amino acid substitutions, deletions or insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of a pesticidal protein can be prepared by mutations in the DNA. This may also be accomplished by one of several forms of mutagenesis and/or in directed evolution. In some aspects, the changes encoded in the amino acid sequence will not substantially affect the function of the protein. Such variants will possess the desired pesticidal activity. However, it is understood that the ability of a pesticidal protein to confer pesticidal activity may be improved by the use of such techniques upon the compositions of this invention. For example, one may express a

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pesticidal protein in host cells that exhibit high rates of base misincorporation during DNA replication, such as XL-1 Red (Stratagene, La Jolla, CA). After propagation in such strains, one can isolate the DNA (for example by preparing plasmid DNA, or by amplifying by PCR and cloning the resulting PCR fragment into a vector), culture the
5 pesticidal protein mutations in a non-mutagenic strain, and identify mutated genes with pesticidal activity, for example by performing an assay to test for pesticidal activity. Generally, the protein is mixed and used in feeding assays. See, for example Marrone *et al.* (1985) *J. of Economic Entomology* 78:290-293. Such assays can include contacting plants with one or more pests and determining the plant's ability to survive and/or cause
10 the death of the pests. Examples of mutations that result in increased toxicity are found in Schnepf *et al.* (1998) *Microbiol. Mol. Biol. Rev.* 62:775-806.

Alternatively, alterations may be made to the protein sequence of many proteins at the amino or carboxy terminus without substantially affecting activity. This can include insertions, deletions, or alterations introduced by modern molecular methods, such as
15 PCR, including PCR amplifications that alter or extend the protein coding sequence by virtue of inclusion of amino acid encoding sequences in the oligonucleotides utilized in the PCR amplification. Alternatively, the protein sequences added can include entire protein-coding sequences, such as those used commonly in the art to generate protein fusions. Such fusion proteins are often used to (1) increase expression of a protein of
20 interest (2) introduce a binding domain, enzymatic activity, or epitope to facilitate either protein purification, protein detection, or other experimental uses known in the art (3) target secretion or translation of a protein to a subcellular organelle, such as the periplasmic space of Gram-negative bacteria, or the endoplasmic reticulum of eukaryotic cells, the latter of which often results in glycosylation of the protein.

25 Variant nucleotide and amino acid sequences of the present invention also encompass sequences derived from mutagenic and recombinogenic procedures such as DNA shuffling. With such a procedure, one or more different pesticidal protein coding regions can be used to create a new pesticidal protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of
30 related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between a pesticidal gene of the invention and other known pesticidal genes to obtain a new gene coding for a protein with an improved property of interest, such as an increased

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insecticidal activity. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA* 91:10747-10751; Stemmer (1994) *Nature* 370:389-391; Cramer *et al.* (1997) *Nature Biotech.* 15:436-438; Moore *et al.* (1997) *J. Mol. Biol.* 272:336-347; Zhang *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94:4504-4509; Cramer *et al.* (1998) *Nature* 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

Domain swapping or shuffling is another mechanism for generating altered pesticidal proteins. Domains may be swapped between pesticidal proteins, resulting in hybrid or chimeric toxins with improved pesticidal activity or target spectrum. Methods for generating recombinant proteins and testing them for pesticidal activity are well known in the art (see, for example, Naimov *et al.* (2001) *Appl. Environ. Microbiol.* 67:5328-5330; de Maagd *et al.* (1996) *Appl. Environ. Microbiol.* 62:1537-1543; Ge *et al.* (1991) *J. Biol. Chem.* 266:17954-17958; Schnepf *et al.* (1990) *J. Biol. Chem.* 265:20923-20930; Rang *et al.* (1999) *Appl. Environ. Microbiol.* 65:2918-2925).

Vectors

A pesticidal sequence of the invention may be provided in an expression cassette for expression in a plant of interest. By “plant expression cassette” is intended a DNA construct that is capable of resulting in the expression of a protein from an open reading frame in a plant cell. Typically these contain a promoter and a coding sequence. Often, such constructs will also contain a 3' untranslated region. Such constructs may contain a “signal sequence” or “leader sequence” to facilitate co-translational or post-translational transport of the peptide to certain intracellular structures such as the chloroplast (or other plastid), endoplasmic reticulum, or Golgi apparatus.

By “signal sequence” is intended a sequence that is known or suspected to result in cotranslational or post-translational peptide transport across the cell membrane. In eukaryotes, this typically involves secretion into the Golgi apparatus, with some resulting glycosylation. Insecticidal toxins of bacteria are often synthesized as protoxins, which are protolytically activated in the gut of the target pest (Chang (1987) *Methods Enzymol.* 153:507-516). In some embodiments of the present invention, the signal sequence is located in the native sequence, or may be derived from a sequence of the invention. By “leader sequence” is intended any sequence that when translated, results in an amino acid sequence sufficient to trigger co-translational transport of the peptide chain to a subcellular organelle. Thus, this includes leader sequences targeting transport and/or

glycosylation by passage into the endoplasmic reticulum, passage to vacuoles, plastids including chloroplasts, mitochondria, and the like.

By “plant transformation vector” is intended a DNA molecule that is necessary for efficient transformation of a plant cell. Such a molecule may consist of one or more plant expression cassettes, and may be organized into more than one “vector” DNA molecule. For example, binary vectors are plant transformation vectors that utilize two non-contiguous DNA vectors to encode all requisite cis- and trans-acting functions for transformation of plant cells (Hellens and Mullineaux (2000) *Trends in Plant Science* 5:446-451). “Vector” refers to a nucleic acid construct designed for transfer between different host cells. “Expression vector” refers to a vector that has the ability to incorporate, integrate and express heterologous DNA sequences or fragments in a foreign cell. The cassette will include 5' and 3' regulatory sequences operably linked to a sequence of the invention. By “operably linked” is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

“Promoter” refers to a nucleic acid sequence that functions to direct transcription of a downstream coding sequence. The promoter together with other transcriptional and translational regulatory nucleic acid sequences (also termed “control sequences”) are necessary for the expression of a DNA sequence of interest.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the pesticidal sequence to be under the transcriptional regulation of the regulatory regions.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a DNA sequence of the invention, and a translational and transcriptional termination region (i.e., termination region) functional in plants. The promoter may be native or analogous, or foreign or heterologous, to the plant host and/or to the DNA sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. Where the promoter is “native” or “homologous” to the plant host, it is

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intended that the promoter is found in the native plant into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the DNA sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked DNA sequence of the invention.

5 The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the DNA sequence of interest, the plant host, or any combination thereof). Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such
10 as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987) *Nucleic Acid Res.* 15:9627-9639.

15 Where appropriate, the gene(s) may be optimized for increased expression in the transformed host cell. That is, the genes can be synthesized using host cell-preferred codons for improved expression, or may be synthesized using codons at a host-preferred codon usage frequency. Generally, the GC content of the gene will be increased. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-
20 preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498.

In one embodiment, the pesticidal protein is targeted to the chloroplast for expression. In this manner, where the pesticidal protein is not directly inserted into the
25 chloroplast, the expression cassette will additionally contain a nucleic acid encoding a transit peptide to direct the pesticidal protein to the chloroplasts. Such transit peptides are known in the art. See, for example, Von Heijne *et al.* (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark *et al.* (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res. Commun.*
30 196:1414-1421; and Shah *et al.* (1986) *Science* 233:478-481.

The pesticidal gene to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be

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synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831.

Plant Transformation

5 Methods of the invention involve introducing a nucleotide construct into a plant. By "introducing" is intended to present to the plant the nucleotide construct in such a manner that the construct gains access to the interior of a cell of the plant. The methods of the invention do not require that a particular method for introducing a nucleotide construct to a plant is used, only that the nucleotide construct gains access to the interior
10 of at least one cell of the plant. Methods for introducing nucleotide constructs into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

 By "plant" is intended whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, propagules, embryos and progeny of the same. Plant cells can be
15 differentiated or undifferentiated (e.g. callus, suspension culture cells, protoplasts, leaf cells, root cells, phloem cells, pollen).

 "Transgenic plants" or "transformed plants" or "stably transformed" plants or cells or tissues refers to plants that have incorporated or integrated exogenous nucleic acid sequences or DNA fragments into the plant cell. These nucleic acid sequences
20 include those that are exogenous, or not present in the untransformed plant cell, as well as those that may be endogenous, or present in the untransformed plant cell. "Heterologous" generally refers to the nucleic acid sequences that are not endogenous to the cell or part of the native genome in which they are present, and have been added to the cell by infection, transfection, microinjection, electroporation, microprojection, or the like.

25 Transformation of plant cells can be accomplished by one of several techniques known in the art. The pesticidal gene of the invention may be modified to obtain or enhance expression in plant cells. Typically a construct that expresses such a protein would contain a promoter to drive transcription of the gene, as well as a 3' untranslated region to allow transcription termination and polyadenylation. The organization of such
30 constructs is well known in the art. In some instances, it may be useful to engineer the gene such that the resulting peptide is secreted, or otherwise targeted within the plant cell. For example, the gene can be engineered to contain a signal peptide to facilitate transfer of the peptide to the endoplasmic reticulum. It may also be preferable to engineer the

plant expression cassette to contain an intron, such that mRNA processing of the intron is required for expression.

Typically this “plant expression cassette” will be inserted into a “plant transformation vector”. This plant transformation vector may be comprised of one or more DNA vectors needed for achieving plant transformation. For example, it is a common practice in the art to utilize plant transformation vectors that are comprised of more than one contiguous DNA segment. These vectors are often referred to in the art as “binary vectors”. Binary vectors as well as vectors with helper plasmids are most often used for *Agrobacterium*-mediated transformation, where the size and complexity of DNA segments needed to achieve efficient transformation is quite large, and it is advantageous to separate functions onto separate DNA molecules. Binary vectors typically contain a plasmid vector that contains the cis-acting sequences required for T-DNA transfer (such as left border and right border), a selectable marker that is engineered to be capable of expression in a plant cell, and a “gene of interest” (a gene engineered to be capable of expression in a plant cell for which generation of transgenic plants is desired). Also present on this plasmid vector are sequences required for bacterial replication. The cis-acting sequences are arranged in a fashion to allow efficient transfer into plant cells and expression therein. For example, the selectable marker gene and the pesticidal gene are located between the left and right borders. Often a second plasmid vector contains the trans-acting factors that mediate T-DNA transfer from *Agrobacterium* to plant cells. This plasmid often contains the virulence functions (Vir genes) that allow infection of plant cells by *Agrobacterium*, and transfer of DNA by cleavage at border sequences and vir-mediated DNA transfer, as is understood in the art (Hellens and Mullineaux (2000) *Trends in Plant Science* 5:446-451). Several types of *Agrobacterium* strains (e.g. LBA4404, GV3101, EHA101, EHA105, etc.) can be used for plant transformation. The second plasmid vector is not necessary for transforming the plants by other methods such as microprojection, microinjection, electroporation, polyethylene glycol, etc.

In general, plant transformation methods involve transferring heterologous DNA into target plant cells (e.g. immature or mature embryos, suspension cultures, undifferentiated callus, protoplasts, etc.), followed by applying a maximum threshold level of appropriate selection (depending on the selectable marker gene) to recover the transformed plant cells from a group of untransformed cell mass. Explants are typically transferred to a fresh supply of the same medium and cultured routinely. Subsequently, the transformed cells are differentiated into shoots after placing on regeneration medium

supplemented with a maximum threshold level of selecting agent. The shoots are then transferred to a selective rooting medium for recovering rooted shoot or plantlet. The transgenic plantlet then grows into a mature plant and produces fertile seeds (e.g. Hiei *et al.* (1994) *The Plant Journal* 6:271-282; Ishida *et al.* (1996) *Nature Biotechnology* 14:745-750). Explants are typically transferred to a fresh supply of the same medium and cultured routinely. A general description of the techniques and methods for generating transgenic plants are found in Ayres and Park (1994) *Critical Reviews in Plant Science* 13:219-239 and Bommineni and Jauhar (1997) *Maydica* 42:107-120. Since the transformed material contains many cells; both transformed and non-transformed cells are present in any piece of subjected target callus or tissue or group of cells. The ability to kill non-transformed cells and allow transformed cells to proliferate results in transformed plant cultures. Often, the ability to remove non-transformed cells is a limitation to rapid recovery of transformed plant cells and successful generation of transgenic plants.

Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Generation of transgenic plants may be performed by one of several methods, including, but not limited to, microinjection, electroporation, direct gene transfer, introduction of heterologous DNA by *Agrobacterium* into plant cells (*Agrobacterium*-mediated transformation), bombardment of plant cells with heterologous foreign DNA adhered to particles, ballistic particle acceleration, aerosol beam transformation (U.S. Published Application No. 20010026941; U.S. Patent No. 4,945,050; International Publication No. WO 91/00915; U.S. Published Application No. 2002015066), *Lec1* transformation, and various other non-particle direct-mediated methods to transfer DNA.

Methods for transformation of chloroplasts are known in the art. See, for example, Svab *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:8526-8530; Svab and Maliga (1993) *Proc. Natl. Acad. Sci. USA* 90:913-917; Svab and Maliga (1993) *EMBO J.* 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:7301-7305.

Following integration of heterologous foreign DNA into plant cells, one then applies a maximum threshold level of appropriate selection in the medium to kill the untransformed cells and separate and proliferate the putatively transformed cells that survive from this selection treatment by transferring regularly to a fresh medium. By
5 continuous passage and challenge with appropriate selection, one identifies and proliferates the cells that are transformed with the plasmid vector. Molecular and biochemical methods can then be used to confirm the presence of the integrated heterologous gene of interest into the genome of the transgenic plant.

The cells that have been transformed may be grown into plants in accordance with
10 conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and
15 inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the present invention provides transformed seed (also referred to as "transgenic seed") having a nucleotide construct of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

20

Evaluation of Plant Transformation

Following introduction of heterologous foreign DNA into plant cells, the transformation or integration of heterologous gene in the plant genome is confirmed by various methods such as analysis of nucleic acids, proteins and metabolites associated
25 with the integrated gene.

PCR analysis is a rapid method to screen transformed cells, tissue or shoots for the presence of incorporated gene at the earlier stage before transplanting into the soil (Sambrook and Russell (2001) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). PCR is carried out using
30 oligonucleotide primers specific to the gene of interest or *Agrobacterium* vector background, etc.

Plant transformation may be confirmed by Southern blot analysis of genomic DNA (Sambrook and Russell, 2001, *supra*). In general, total DNA is extracted from the transformant, digested with appropriate restriction enzymes, fractionated in an agarose gel

and transferred to a nitrocellulose or nylon membrane. The membrane or “blot” is then probed with, for example, radiolabeled ^{32}P target DNA fragment to confirm the integration of introduced gene into the plant genome according to standard techniques (Sambrook and Russell, 2001, *supra*).

5 In Northern blot analysis, RNA is isolated from specific tissues of transformant, fractionated in a formaldehyde agarose gel, and blotted onto a nylon filter according to standard procedures that are routinely used in the art (Sambrook and Russell, 2001, *supra*). Expression of RNA encoded by the pesticidal gene is then tested by hybridizing the filter to a radioactive probe derived from a pesticidal gene, by methods known in the
10 art (Sambrook and Russell, 2001, *supra*).

Western blot, biochemical assays and the like may be carried out on the transgenic plants to confirm the presence of protein encoded by the pesticidal gene by standard procedures (Sambrook and Russell, 2001, *supra*) using antibodies that bind to one or more epitopes present on the pesticidal protein.

15

Pesticidal Activity in Plants

In another aspect of the invention, one may generate transgenic plants expressing a pesticidal protein that has pesticidal activity. Methods described above by way of example may be utilized to generate transgenic plants, but the manner in which the
20 transgenic plant cells are generated is not critical to this invention. Methods known or described in the art such as *Agrobacterium*-mediated transformation, biolistic transformation, and non-particle-mediated methods may be used at the discretion of the experimenter. Plants expressing a pesticidal protein may be isolated by common methods described in the art, for example by transformation of callus, selection of transformed
25 callus, and regeneration of fertile plants from such transgenic callus. In such process, one may use any gene as a selectable marker so long as its expression in plant cells confers ability to identify or select for transformed cells.

A number of markers have been developed for use with plant cells, such as resistance to chloramphenicol, the aminoglycoside G418, hygromycin, or the like. Other
30 genes that encode a product involved in chloroplast metabolism may also be used as selectable markers. For example, genes that provide resistance to plant herbicides such as glyphosate, bromoxynil, or imidazolinone may find particular use. Such genes have been reported (Stalker *et al.* (1985) *J. Biol. Chem.* 263:6310-6314 (bromoxynil resistance nitrilase gene); and Sathasivan *et al.* (1990) *Nucl. Acids Res.* 18:2188 (AHAS

imidazolinone resistance gene). Additionally, the genes disclosed herein are useful as markers to assess transformation of bacterial or plant cells. Methods for detecting the presence of a transgene in a plant, plant organ (e.g., leaves, stems, roots, etc.), seed, plant cell, propagule, embryo or progeny of the same are well known in the art. In one
 5 embodiment, the presence of the transgene is detected by testing for pesticidal activity.

Fertile plants expressing a pesticidal protein may be tested for pesticidal activity, and the plants showing optimal activity selected for further breeding. Methods are available in the art to assay for pest activity. Generally, the protein is mixed and used in feeding assays. See, for example Marrone *et al.* (1985) *J. of Economic Entomology*
 10 78:290-293.

The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (maize), sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape,
 15 *Brassica* sp., alfalfa, rye, millet, safflower, peanuts, sweet potato, cassava, coffee, coconut, pineapple, citrus trees, cocoa, tea, banana, avocado, fig, guava, mango, olive, papaya, cashew, macadamia, almond, oats, vegetables, ornamentals, and conifers.

Vegetables include, but are not limited to, tomatoes, lettuce, green beans, lima beans, peas, and members of the genus *Curcumis* such as cucumber, cantaloupe, and musk melon.
 20 Ornamentals include, but are not limited to, azalea, hydrangea, hibiscus, roses, tulips, daffodils, petunias, carnation, poinsettia, and chrysanthemum. Preferably, plants of the present invention are crop plants (for example, maize, sorghum, wheat, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, oilseed rape., etc.).

25

Use in Pesticidal Control

General methods for employing strains comprising a nucleotide sequence of the present invention, or a variant thereof, in pesticide control or in engineering other organisms as pesticidal agents are known in the art. See, for example U.S. Patent No.
 30 5,039,523 and EP 0480762A2.

The *Bacillus* strains containing a nucleotide sequence of the present invention, or a variant thereof, or the microorganisms that have been genetically altered to contain a pesticidal gene and protein may be used for protecting agricultural crops and products from pests. In one aspect of the invention, whole, i.e., unlysed, cells of a toxin

(pesticide)-producing organism are treated with reagents that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s).

Alternatively, the pesticide is produced by introducing a pesticidal gene into a cellular host. Expression of the pesticidal gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. In one aspect of this invention, these cells are then treated under conditions that prolong the activity of the toxin produced in the cell when the cell is applied to the environment of target pest(s). The resulting product retains the toxicity of the toxin. These naturally encapsulated pesticides may then be formulated in accordance with conventional techniques for application to the environment hosting a target pest, e.g., soil, water, and foliage of plants. See, for example EPA 0192319, and the references cited therein. Alternatively, one may formulate the cells expressing a gene of this invention such as to allow application of the resulting material as a pesticide.

The active ingredients of the present invention are normally applied in the form of compositions and can be applied to the crop area or plant to be treated, simultaneously or in succession, with other compounds. These compounds can be fertilizers, weed killers, cryoprotectants, surfactants, detergents, pesticidal soaps, dormant oils, polymers, and/or time-release or biodegradable carrier formulations that permit long-term dosing of a target area following a single application of the formulation. They can also be selective herbicides, chemical insecticides, virucides, microbicides, amoebicides, pesticides, fungicides, bacteriocides, nematocides, molluscicides or mixtures of several of these preparations, if desired, together with further agriculturally acceptable carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation. Suitable carriers and adjuvants can be solid or liquid and correspond to the substances ordinarily employed in formulation technology, e.g. natural or regenerated mineral substances, solvents, dispersants, wetting agents, tackifiers, binders or fertilizers. Likewise the formulations may be prepared into edible "baits" or fashioned into pest "traps" to permit feeding or ingestion by a target pest of the pesticidal formulation.

Methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention that contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention include leaf application, seed coating and soil application. The number of applications and the rate of application depend on the intensity of infestation by the corresponding pest.

The composition may be formulated as a powder, dust, pellet, granule, spray, emulsion, colloid, solution, or such like, and may be prepared by such conventional means as desiccation, lyophilization, homogenation, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of cells comprising the polypeptide. In all
5 such compositions that contain at least one such pesticidal polypeptide, the polypeptide may be present in a concentration of from about 1% to about 99% by weight.

Lepidopteran, dipteran, or coleopteran pests may be killed or reduced in numbers in a given area by the methods of the invention, or may be prophylactically applied to an environmental area to prevent infestation by a susceptible pest. Preferably the pest
10 ingests, or is contacted with, a pesticidally-effective amount of the polypeptide. By “pesticidally-effective amount” is intended an amount of the pesticide that is able to bring about death to at least one pest, or to noticeably reduce pest growth, feeding, or normal physiological development. This amount will vary depending on such factors as, for example, the specific target pests to be controlled, the specific environment, location,
15 plant, crop, or agricultural site to be treated, the environmental conditions, and the method, rate, concentration, stability, and quantity of application of the pesticidally-effective polypeptide composition. The formulations may also vary with respect to climatic conditions, environmental considerations, and/or frequency of application and/or severity of pest infestation.

20 The pesticide compositions described may be made by formulating either the bacterial cell, crystal and/or spore suspension, or isolated protein component with the desired agriculturally-acceptable carrier. The compositions may be formulated prior to administration in an appropriate means such as lyophilized, freeze-dried, desiccated, or in an aqueous carrier, medium or suitable diluent, such as saline or other buffer. The
25 formulated compositions may be in the form of a dust or granular material, or a suspension in oil (vegetable or mineral), or water or oil/water emulsions, or as a wettable powder, or in combination with any other carrier material suitable for agricultural application. Suitable agricultural carriers can be solid or liquid and are well known in the art. The term “agriculturally-acceptable carrier” covers all adjuvants, inert components,
30 dispersants, surfactants, tackifiers, binders, etc. that are ordinarily used in pesticide formulation technology; these are well known to those skilled in pesticide formulation. The formulations may be mixed with one or more solid or liquid adjuvants and prepared by various means, e.g., by homogeneously mixing, blending and/or grinding the pesticidal composition with suitable adjuvants using conventional formulation techniques. Suitable

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formulations and application methods are described in U.S. Patent No. 6,468,523.

"Pest" includes but is not limited to, insects, fungi, bacteria, nematodes, mites, ticks, and the like. Insect pests include insects selected from the orders *Coleoptera*,
 5 *Diptera*, *Hymenoptera*, *Lepidoptera*, *Mallophaga*, *Homoptera*, *Heniptera*, *Orthoptera*,
Thysanoptera, *Dermaptera*, *Isoptera*, *Anoplura*, *Siphonaptera*, *Trichoptera*, etc.,
 particularly *Coleoptera*, *Lepidoptera*, and *Diptera*.

The order *Coleoptera* includes the suborders *Adephaga* and *Polyphaga*. Suborder
Adephaga includes the superfamilies *Caraboidea* and *Gyrinoidea*, while suborder
 10 *Polyphaga* includes the superfamilies *Hydrophiloidea*, *Staphylinoidea*, *Cantharoidea*,
Cleroidea, *Elateroidea*, *Dascilloidea*, *Dryopoidea*, *Byrrhoidea*, *Cucujoidea*, *Meloidea*,
Mordelloidea, *Tenebrionoidea*, *Bostrichoidea*, *Scarabaeoidea*, *Cerambycoidea*,
Chrysomeloidea, and *Curculionoidea*. Superfamily *Caraboidea* includes the families
Cicindelidae, *Carabidae*, and *Dytiscidae*. Superfamily *Gyrinoidea* includes the family
 15 *Gyrinidae*. Superfamily *Hydrophiloidea* includes the family *Hydrophilidae*. Superfamily
Staphylinoidea includes the families *Silphidae* and *Staphylinidae*. Superfamily
Cantharoidea includes the families *Cantharidae* and *Lampyridae*. Superfamily *Cleroidea*
includes the families *Cleridae* and *Dermestidae*. Superfamily *Elateroidea* includes the
families *Elateridae* and *Buprestidae*. Superfamily *Cucujoidea* includes the family
 20 *Coccinellidae*. Superfamily *Meloidea* includes the family *Meloidae*. Superfamily
Tenebrionoidea includes the family *Tenebrionidae*. Superfamily *Scarabaeoidea* includes
the families *Passalidae* and *Scarabaeidae*. Superfamily *Cerambycoidea* includes the
family *Cerambycidae*. Superfamily *Chrysomeloidea* includes the family *Chrysomelidae*.
Superfamily *Curculionoidea* includes the families *Curculionidae* and *Scolytidae*.

25 The order *Diptera* includes the Suborders *Nematocera*, *Brachycera*, and
Cyclorrhapha. Suborder *Nematocera* includes the families *Tipulidae*, *Psychodidae*,
Culicidae, *Ceratopogonidae*, *Chironomidae*, *Simuliidae*, *Bibionidae*, and *Cecidomyiidae*.
Suborder *Brachycera* includes the families *Stratiomyidae*, *Tabanidae*, *Therevidae*,
Asilidae, *Mydidae*, *Bombyliidae*, and *Dolichopodidae*. Suborder *Cyclorrhapha* includes
 30 the Divisions *Aschiza* and *Aschiza*. Division *Aschiza* includes the families *Phoridae*,
Syrphidae, and *Conopidae*. Division *Aschiza* includes the Sections *Acalyptratae* and
Calypttratae. Section *Acalyptratae* includes the families *Otitidae*, *Tephritidae*,
Agromyzidae, and *Drosophilidae*. Section *Calypttratae* includes the families

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Hippoboscidae, *Oestridae*, *Tachinidae*, *Anthomyiidae*, *Muscidae*, *Calliphoridae*, and *Sarcophagidae*.

The order *Lepidoptera* includes the families *Papilionidae*, *Pieridae*, *Lycaenidae*, *Nymphalidae*, *Danaidae*, *Satyridae*, *Hesperiidae*, *Sphingidae*, *Saturniidae*, *Geometridae*,
5 *Arctiidae*, *Noctuidae*, *Lymantriidae*, *Sesiidae*, and *Tineidae*.

Insect pests of the invention for the major crops include: Maize: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Helicoverpa zea*, corn earworm; *Spodoptera frugiperda*, fall armyworm; *Diatraea grandiosella*, southwestern corn borer; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Diatraea saccharalis*,
10 sugarcane borer; *Diabrotica virgifera*, western corn rootworm; *Diabrotica longicornis barberi*, northern corn rootworm; *Diabrotica undecimpunctata howardi*, southern corn rootworm; *Melanotus* spp., wireworms; *Cyclocephala borealis*, northern masked chafer (white grub); *Cyclocephala immaculata*, southern masked chafer (white grub); *Popillia japonica*, Japanese beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus*
15 *maidis*, maize billbug; *Rhopalosiphum maidis*, corn leaf aphid; *Anuraphis maidiradicis*, corn root aphid; *Blissus leucopterus leucopterus*, chinch bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus sanguinipes*, migratory grasshopper; *Hylemya platura*, seedcorn maggot; *Agromyza parvicornis*, corn blot leafminer; *Anaphothrips obscurus*, grass thrips; *Solenopsis milesta*, thief ant; *Tetranychus urticae*, twospotted
20 spider mite; Sorghum: *Chilo partellus*, sorghum borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Feltia subterranea*, granulate cutworm; *Phyllophaga crinita*, white grub; *Eleodes*, *Conoderus*, and *Aeolus* spp., wireworms; *Oulema melanopus*, cereal leaf beetle; *Chaetocnema pulicaria*, corn flea beetle; *Sphenophorus maidis*, maize billbug;
25 *Rhopalosiphum maidis*; corn leaf aphid; *Sipha flava*, yellow sugarcane aphid; *Blissus leucopterus leucopterus*, chinch bug; *Contarinia sorghicola*, sorghum midge; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Wheat: *Pseudaletia unipunctata*, army worm; *Spodoptera frugiperda*, fall armyworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Agrotis orthogonia*, western
30 cutworm; *Elasmopalpus lignosellus*, lesser cornstalk borer; *Oulema melanopus*, cereal leaf beetle; *Hypera punctata*, clover leaf weevil; *Diabrotica undecimpunctata howardi*, southern corn rootworm; Russian wheat aphid; *Schizaphis graminum*, greenbug; *Macrosiphum avenae*, English grain aphid; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Melanoplus sanguinipes*,

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migratory grasshopper; *Mayetiola destructor*, Hessian fly; *Sitodiplosis mosellana*, wheat midge; *Meromyza americana*, wheat stem maggot; *Hylemya coarctata*, wheat bulb fly; *Frankliniella fusca*, tobacco thrips; *Cephus cinctus*, wheat stem sawfly; *Aceria tulipae*, wheat curl mite; Sunflower: *Suleima helianthana*, sunflower bud moth; *Homoeosoma electellum*, sunflower moth; *zygogramma exclamationis*, sunflower beetle; *Bothyrus gibbosus*, carrot beetle; *Neolasioptera murtfeldtiana*, sunflower seed midge; Cotton: *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Spodoptera exigua*, beet armyworm; *Pectinophora gossypiella*, pink bollworm; *Anthonomus grandis*, boll weevil; *Aphis gossypii*, cotton aphid; *Pseudatomoscelis seriatus*, cotton fleahopper; *Trialeurodes abutilonea*, bandedwinged whitefly; *Lygus lineolaris*, tarnished plant bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Thrips tabaci*, onion thrips; *Frankliniella fusca*, tobacco thrips; *Tetranychus cinnabarinus*, carmine spider mite; *Tetranychus urticae*, twospotted spider mite; Rice: *Diatraea saccharalis*, sugarcane borer; *Spodoptera frugiperda*, fall armyworm; *Helicoverpa zea*, corn earworm; *Colaspis brunnea*, grape colaspis; *Lissorhoptrus oryzophilus*, rice water weevil; *Sitophilus oryzae*, rice weevil; *Nephotettix nigropictus*, rice leafhopper; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; Soybean: *Pseudoplusia includens*, soybean looper; *Anticarsia gemmatilis*, velvetbean caterpillar; *Plathypena scabra*, green cloverworm; *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Spodoptera exigua*, beet armyworm; *Heliothis virescens*, cotton budworm; *Helicoverpa zea*, cotton bollworm; *Epilachna varivestis*, Mexican bean beetle; *Myzus persicae*, green peach aphid; *Empoasca fabae*, potato leafhopper; *Acrosternum hilare*, green stink bug; *Melanoplus femurrubrum*, redlegged grasshopper; *Melanoplus differentialis*, differential grasshopper; *Hylemya platura*, seedcorn maggot; *Sericothrips variabilis*, soybean thrips; *Thrips tabaci*, onion thrips; *Tetranychus turkestanii*, strawberry spider mite; *Tetranychus urticae*, twospotted spider mite; Barley: *Ostrinia nubilalis*, European corn borer; *Agrotis ipsilon*, black cutworm; *Schizaphis graminum*, greenbug; *Blissus leucopterus leucopterus*, chinch bug; *Acrosternum hilare*, green stink bug; *Euschistus servus*, brown stink bug; *Delia platura*, seedcorn maggot; *Mayetiola destructor*, Hessian fly; *Petrobia latens*, brown wheat mite; Oil Seed Rape: *Brevicoryne brassicae*, cabbage aphid; *Phyllotreta cruciferae*, Flea beetle; *Mamestra configurata*, Bertha armyworm; *Plutella xylostella*, Diamond-back moth; *Delia* ssp., Root maggots.

Nematodes include parasitic nematodes such as root-knot, cyst, and lesion nematodes, including *Heterodera* spp., *Meloidogyne* spp., and *Globodera* spp.; particularly members of the cyst nematodes, including, but not limited to, *Heterodera glycines* (soybean cyst nematode); *Heterodera schachtii* (beet cyst nematode);
 5 *Heterodera avenae* (cereal cyst nematode); and *Globodera rostochiensis* and *Globodera pailida* (potato cyst nematodes). Lesion nematodes include *Pratylenchus* spp.

Methods for increasing plant yield

Methods for increasing plant yield are provided. The methods comprise
 10 introducing into a plant or plant cell a polynucleotide comprising a pesticidal sequence disclosed herein. As defined herein, the “yield” of the plant refers to the quality and/or quantity of biomass produced by the plant. By “biomass” is intended any measured plant product. An increase in biomass production is any improvement in the yield of the measured plant product. Increasing plant yield has several commercial applications. For
 15 example, increasing plant leaf biomass may increase the yield of leafy vegetables for human or animal consumption. Additionally, increasing leaf biomass can be used to increase production of plant-derived pharmaceutical or industrial products. An increase in yield can comprise any statistically significant increase including, but not limited to, at least a 1% increase, at least a 3% increase, at least a 5% increase, at least a 10% increase,
 20 at least a 20% increase, at least a 30%, at least a 50%, at least a 70%, at least a 100% or a greater increase in yield compared to a plant not expressing the pesticidal sequence.

The following examples are offered by way of illustration and not by way of limitation.

25 EXPERIMENTAL

Example 1. Extraction of Plasmid DNA

A pure culture of strain ATX13046 was grown in large quantities of rich media. The culture was spun to harvest the cell pellet. The cell pellet was then prepared by
 30 treatment with SDS by methods known in the art, resulting in breakage of the cell wall and release of DNA. Proteins and large genomic DNA were then precipitated by a high salt concentration. The plasmid DNA was then precipitated by standard ethanol precipitation. The plasmid DNA was separated from any remaining chromosomal DNA by high-speed centrifugation through a cesium chloride gradient. The DNA was

visualized in the gradient by UV light and the band of lower density (i.e. the lower band) was extracted using a syringe. This band contained the plasmid DNA from Strain ATX13046. The quality of the DNA was checked by visualization on an agarose gel.

5 Example 2. Cloning of Genes

The purified plasmid DNA was sheared into 5-10 kb sized fragments and the 5' and 3' single stranded overhangs repaired using T4 DNA polymerase and Klenow fragment in the presence of all four dNTPs. Phosphates were then attached to the 5' ends by treatment with T4 polynucleotide kinase. The repaired DNA fragments were then
10 ligated overnight into a standard high copy vector (i.e. pBluescript SK+), suitably prepared to accept the inserts as known in the art (for example by digestion with a restriction enzyme producing blunt ends).

The quality of the library was analyzed by digesting a subset of clones with a restriction enzyme known to have a cleavage site flanking the cloning site. A high
15 percentage of clones were determined to contain inserts, with an average insert size of 5-6 kb.

Example 3. High Throughput Sequencing of Library Plates

Once the clone library quality was checked and confirmed, colonies were grown
20 in a rich broth in 2 ml 96-well blocks overnight at 37°C at a shaking speed of 350 rpm. The blocks were spun to harvest the cells to the bottom of the block. The blocks were then prepared by standard alkaline lysis prep in a high throughput format.

The end sequences of clones from this library were then determined for a large number of clones from each block in the following way: The DNA sequence of each
25 clone chosen for analysis was determined using the fluorescent dye terminator sequencing technique (Applied Biosystems) and standard primers flanking each side of the cloning site. Once the reactions had been carried out in the thermocycler, the DNA was precipitated using standard ethanol precipitation. The DNA was resuspended in water and loaded onto a capillary sequencing machine. Each library plate of DNA was
30 sequenced from either end of the cloning site, yielding two reads per plate over each insert.

Example 4. Assembly and Screening of Sequencing Data

DNA sequences obtained were compiled into an assembly project and aligned together to form contigs. This can be done efficiently using a computer program, such as Vector NTI, or alternatively by using the Phred/Phrap suite of DNA alignment and analysis programs. These contigs, along with any individual read that may not have been added to a contig, were compared to a compiled database of all classes of known pesticidal genes. Contigs or individual reads identified as having identity to a known endotoxin or pesticidal gene were analyzed further. Among the sequences obtained, DNA clones were identified as having homology to known endotoxin genes. Therefore, these clones were selected for further sequencing.

Example 5. Cloning of *axmi-076*

A fragment of DNA with homology to endotoxin genes was identified from Strain ATX14775. The full open reading frame was identified by Tail (Thermal Asymmetric Interlaced) PCR based methods as known in the art. Finally, using the DNA sequence of the full length open reading frame from the Tail (Thermal Asymmetric Interlaced) PCR product, the open reading frame was amplified directly by PCR from strain ATX14775 and cloned into a vector.

Example 6. Sequencing of clones

Primers were designed to anneal to the clones of interest in a manner such that DNA sequences generated from such primers will overlap existing DNA sequence of the clone(s). This process, known as “oligo walking,” is well known in the art. This process was utilized to determine the entire DNA sequence of the region exhibiting homology to a known endotoxin gene. In the case of the genes of the invention, this process was used to determine the DNA sequence of the entire open reading frame, resulting in a single nucleotide sequence for each. The completed DNA sequence was then placed back into the original large assembly for further validation. This allowed incorporation of more DNA sequence reads into the contig, resulting in multiple reads of coverage over the entire region.

Analysis of the DNA sequence of each clone by methods known in the art identified an open reading frame on each insert with homology to known delta endotoxin genes. The open reading frames were designated as *axmi-066* and *axmi-076*, respectively. The DNA sequence of *axmi-066* is provided in SEQ ID NO:1, and the amino acid

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sequence of the predicted protein is provided in SEQ ID NO:2. The DNA sequence of axmi-076 is provided in SEQ ID NO:4 and its predicted protein sequence is provided in SEQ ID NO:5.

The predicted open reading frame of *axmi-066* is 637 amino acids long. However, alignment with its closest endotoxin homologs suggests that the start of translation of *axmi-066* in *Bacillus* is likely to be at the internal ATG start codon thirty nine nucleotides downstream of the first ATG start codon (corresponding to nucleotide position 39 of SEQ ID NO:1). This coding sequence is set forth in SEQ ID NO:9. Translational initiation at this internal start codon will result in a 624 amino acid protein with a molecular weight of 71kD (SEQ ID NO:10). A possible but strong Shine-Dalgarno sequence is present 6 nucleotides upstream of this internal start codon, which supports the results of protein alignments.

Example 7. Synthetic nucleotide sequences encoding AXMI-066 and AXMI-076

The *optaxmi-066* gene (SEQ ID NO:3) represents a synthetic nucleotide sequence that upon translation, will encode the AXMI-066 protein.

The *optaxmi-076* gene (SEQ ID NO:6) and the *optaxmi-076v04* (SEQ ID NO:11) gene represent synthetic nucleotide sequences that upon translation, will encode the AXMI-076 protein.

Example 8 . Homology of AXMI-066 and AXMI-076 to Known Endotoxin Genes

A search of DNA and protein databases with the DNA sequences and amino acid sequences of AXMI-066 and AXMI-076 revealed that they are homologous to known delta-endotoxin proteins.

Figure 2 shows an alignment of AXMI-066 with several endotoxins. Blast searches identified members of the *cry2* endotoxin family as having the strongest homology to AXMI-066. Alignment of the AXMI-066 protein (SEQ ID NO:2) to a large set of endotoxin proteins confirmed that AXMI-066 has 74.8% identity to the Cry2Aa1 toxin.

Alignment of the AXMI-076 protein (SEQ ID NO:5) to a large set of endotoxin proteins confirmed that AXMI-076 has 93.1% identity to the Cry2Ae1 toxin and 91.0% identity to the Cry2Aa1 toxin.

Example 9. Expression of synthetic sequences in *Bacillus*

The *axmi-066*, *optaxmi-066*, *axmi-076*, *optaxmi-076v*, or *optaxmi-076v04* sequences (SEQ ID NO:1, 3, 4, 6, and 11, respectively) are amplified by PCR and cloned into the *Bacillus* expression vector such as pAX916 by methods well known in the art.

- 5 The resulting clone is assayed for expression of the AXMI protein after transformation into cells of a *cry(-)* *Bacillus thuringiensis* strain. A *Bacillus* strain containing the *axmi* clone and expressing the AXMI insecticidal protein is grown in, for example, CYS media (10 g/l Bacto-casitone; 3 g/l yeast extract; 6 g/l KH₂PO₄; 14 g/l K₂HPO₄; 0.5 mM MgSO₄; 0.05 mM MnCl₂; 0.05 mM FeSO₄), until sporulation is evident by microscopic
10 examination. Samples are prepared, and analyzed by polyacrylamide gel electrophoresis (PAGE).

- In the case of AXMI-066, the *axmi066* open reading frame starting from the internal ATG (corresponding to nucleotide positions 39-41 of SEQ ID NO:1) was amplified by PCR. The product was cloned into a *Bacillus* vector based on pAX916 as
15 well as *E. coli* expression vector based on pRSF1b (Invitrogen). The resulting clones were confirmed by restriction analysis and finally by complete sequencing of the cloned gene. The resulting constructs are called pAX2755 and pAX2757 respectively.

Example 10. Insecticidal Activity of AXMI-066 and AXMI-076

- 20 AXMI-066 and AXMI-076 were tested for activity against important lepidopteran pests by bioassay. Cultures of a *Bacillus* strain containing pAX2755 were grown to sporulation, pelleted, and tested on insect pests with appropriate controls. In these tests AXMI-066 and AXMI-076 demonstrated activity on several Lepidopteran pests, as summarized Table 1.

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Table 1. Insect activity of AXMI-066 and AXMI-076

Insect	AXMI-066	AXMI-076
European corn borer (<i>Ostrinia nubilalis</i>)	+/-	++++
Corn earworm (<i>Helicoverpa zea</i>)	++	+++
Tobacco budworm (<i>Heliothis virescens</i>)	++	++++
Fall armyworm (<i>Spodoptera frugiperda</i>)	++	++
Velvetbean caterpillar (<i>Anticarsia gemmatilis</i>)	+++	++++
Black cutworm (<i>Agrotis ipsilon</i>)	-	-

Example 11. AXMI-066 variants

- 5 Alignment of the AXMI-066 (SEQ ID NO:10) and Cry2Aa protein sequences indicates that the apical loops 1 and 2 of axmi-066 are both 2 amino acids shorter than loops 1 and 2 of Cry2Aa. AXMI-066 contains an additional loop 3 that is missing in Cry2Aa. Variant libraries of AXMI-066 have been generated containing insertions of 2 amino acids in loops 1 and 2, respectively. A deletion of loop 3 in AXMI-066 has also
- 10 been generated. AXMI-066 variants have been expressed and assayed for insecticidal activity on several Lepidopteran insects. Eleven AXMI-066 variants carrying insertions into loop 2 containing glycines have been identified that are active on several Lepidopteran insects.

- Variant libraries of axmi-66 were generated using the Quickchange LightningTM kit (Stratagene). Construct pAX5435 (His6-axmi-66 in pRSF1b) was mutagenized.
- 15 The 2 libraries consist of 2 codon insertions between Val320 and Pro321 (loop 1) and Gly378 and Asp379 (loop 2), respectively. Each library contains permutations of all 64 codons for the two inserted positions. A deletion of loop 3 was also carried out. The mutagenic sense oligos are as follows:

20

Loop 1:

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1. CTCCTTCGGCGTGNWNNWNCCCATCCTCGGCGGC (SEQ ID NO:21)

2. CTCCTTCGGCGTGNSNNSNCCCATCCTCGGCGGC (SEQ ID NO:22)

3. CTCCTTCGGCGTGNWNNNSNCCCATCCTCGGCGGC (SEQ ID NO:23)

4. CTCCTTCGGCGTGNSNNWNCCCATCCTCGGCGGC (SEQ ID NO:24)

5

Loop 2:

1. CGGCGTCTACAGAGGANWNNWNGATCTTCAGCACAACCTGG (SEQ ID NO:25)

2. CGGCGTCTACAGAGGANSNNSNGATCTTCAGCACAACCTGG (SEQ ID NO:26)

10 3. CGGCGTCTACAGAGGANSNNWNGATCTTCAGCACAACCTGG (SEQ ID NO:27)

4. CGGCGTCTACAGAGGANWNNNSNGATCTTCAGCACAACCTGG (SEQ ID NO:28)

Loop 3:

CGCCTTCCTCCTCTCAGTGAAGAGCAACTACTTCC (SEQ ID NO:29)

15

Mutagenesis reactions contain a sense oligo as described above and the corresponding antisense oligo. The libraries were cloned, and a number of clones were sequenced. Clones selected for functional characterization contained various combinations of positively charged, negatively charged, aromatic, polar and apolar amino acids. The selected insertion variants were expressed in *E. coli* and soluble extracts were prepared by bead beating in 50 mM Na-Carbonate pH 10.5, 1 mM DTT. The extracts were assayed for activity against Corn Earworm “Hz” (*Helicoverpa zea*), European Corn Borer “ECB” (*Ostrinia nubilalis*), Tobacco budworm “Hv” (*Heliothis virescens*), Fall Armyworm “FAW” (*Spodoptera frugiperda*), Black Cutworm “BCW” (*Agrotis ipsilon*), and Velvetbean caterpillar “VBC” (*Anticarsia gemmatilis*). Loop 2 insertion variants containing glycines were active against lepidopteran insects. The loop 2 GT insertion showed the highest toxicity of the variants tested. No activity was detected in the loop 1 insertion variants and the loop 3 deletion variants.

Single point mutations of AXMI-066 (SEQ ID NO:10) were also created and tested against lepidopteran pests. Table 2 lists the position and mutations that resulted in polypeptides having pesticidal activity equal to or greater than the pesticidal activity SEQ ID NO:10.

30

Table 2

Position relative to SEQ ID NO:10	Residue in SEQ ID NO:10	Active mutants
83	P	T
250	L	I
319	G	K, F
322	I	S, V, Q, A
323	L	F
376	Y	N, I, R, S, V, A
377	R	E, Q, L
378	G	S, A, W
379	D	V, E
380	L	M, P, Y
381	Q	L
401	L	I
406	M	H, V, K, E, T, S, A, V, N
407	F	W, R

Example 12. Assays for Pesticidal Activity

The *axmi-066*, *optaxmi-066*, *axmi-076*, and *optaxmi-076* nucleotide sequences of
 5 the invention can be tested for their ability to produce pesticidal proteins. The ability of a
 pesticidal protein to act as a pesticide upon a pest is often assessed in a number of ways.
 One way well known in the art is to perform a feeding assay. In such a feeding assay, one
 exposes the pest to a sample containing either compounds to be tested or control samples.
 Often this is performed by placing the material to be tested, or a suitable dilution of such
 10 material, onto a material that the pest will ingest, such as an artificial diet. The material to
 be tested may be composed of a liquid, solid, or slurry. The material to be tested may be
 placed upon the surface and then allowed to dry. Alternatively, the material to be tested
 may be mixed with a molten artificial diet, then dispensed into the assay chamber. The
 assay chamber may be, for example, a cup, a dish, or a well of a microtiter plate.

15 Assays for sucking pests (for example aphids) may involve separating the test
 material from the insect by a partition, ideally a portion that can be pierced by the sucking
 mouth parts of the sucking insect, to allow ingestion of the test material. Often the test

material is mixed with a feeding stimulant, such as sucrose, to promote ingestion of the test compound.

Other types of assays can include microinjection of the test material into the mouth, or gut of the pest, as well as development of transgenic plants, followed by test of
 5 the ability of the pest to feed upon the transgenic plant. Plant testing may involve isolation of the plant parts normally consumed, for example, small cages attached to a leaf, or isolation of entire plants in cages containing insects.

Other methods and approaches to assay pests are known in the art, and can be found, for example in Robertson and Preisler, eds. (1992) *Pesticide bioassays with*
 10 *arthropods*, CRC, Boca Raton, FL. Alternatively, assays are commonly described in the journals *Arthropod Management Tests* and *Journal of Economic Entomology* or by discussion with members of the Entomological Society of America (ESA).

Example 13. Vectoring of Genes for Plant Expression

15 The coding regions of the invention are connected with appropriate promoter and terminator sequences for expression in plants. Such sequences are well known in the art and may include the rice actin promoter or maize ubiquitin promoter for expression in monocots, the *Arabidopsis* UBQ3 promoter or CaMV 35S promoter for expression in dicots, and the nos or PinII terminators. Techniques for producing and confirming
 20 promoter – gene – terminator constructs also are well known in the art.

In one aspect of the invention, synthetic DNA sequences are designed and generated. These synthetic sequences have altered nucleotide sequence relative to the parent sequence, but encode proteins that are essentially identical to the parent AXMI-066 or AXMI-076 protein (e.g., SEQ ID NO:3 or 6).

25 In another aspect of the invention, modified versions of the synthetic genes are designed such that the resulting peptide is targeted to a plant organelle, such as the endoplasmic reticulum or the apoplast. Peptide sequences known to result in targeting of fusion proteins to plant organelles are known in the art. For example, the N-terminal region of the acid phosphatase gene from the White Lupin *Lupinus albus* (GENBANK®
 30 ID GI:14276838, Miller *et al.* (2001) *Plant Physiology* 127: 594-606) is known in the art to result in endoplasmic reticulum targeting of heterologous proteins. If the resulting fusion protein also contains an endoplasmic reticulum retention sequence comprising the peptide N-terminus-lysine-aspartic acid-glutamic acid-leucine (i.e., the “KDEL” motif, SEQ ID NO:7) at the C-terminus, the fusion protein will be targeted to the endoplasmic

reticulum. If the fusion protein lacks an endoplasmic reticulum targeting sequence at the C-terminus, the protein will be targeted to the endoplasmic reticulum, but will ultimately be sequestered in the apoplast.

Thus, this gene encodes a fusion protein that contains the N-terminal thirty-one amino acids of the acid phosphatase gene from the White Lupin *Lupinus albus* (GENBANK® ID GI:14276838, Miller *et al.*, 2001, *supra*) fused to the N-terminus of the AXMI-066 or AXMI-076 sequence, as well as the KDEL sequence at the C-terminus. Thus, the resulting protein is predicted to be targeted the plant endoplasmic reticulum upon expression in a plant cell.

A construct comprising a nucleotide sequence encoding a chloroplast transit peptide derived from *Chlamydomonas reinhardtii* linked to the *optaxmi-076v04* sequence is set forth in SEQ ID NO:12 (nucleotide sequence) and SEQ ID NO:13 (amino acid sequence).

The plant expression cassettes described above are combined with an appropriate plant selectable marker to aid in the selection of transformed cells and tissues, and ligated into plant transformation vectors. These may include binary vectors from *Agrobacterium*-mediated transformation or simple plasmid vectors for aerosol or biolistic transformation.

Example 14. Vectoring of *axmi-066*, *optaxmi-066*, *axmi-076*, and *optaxmi-076* genes for Plant Expression

The coding region DNA of the *axmi-066*, *optaxmi-066*, *axmi-076*, and *optaxmi-076* genes of the invention are operably connected with appropriate promoter and terminator sequences for expression in plants. Such sequences are well known in the art and may include the rice actin promoter or maize ubiquitin promoter for expression in monocots, the *Arabidopsis* UBQ3 promoter or CaMV 35S promoter for expression in dicots, and the nos or PinII terminators. Techniques for producing and confirming promoter – gene – terminator constructs also are well known in the art.

The plant expression cassettes described above are combined with an appropriate plant selectable marker to aid in the selections of transformed cells and tissues, and ligated into plant transformation vectors. These may include binary vectors from *Agrobacterium*-mediated transformation or simple plasmid vectors for aerosol or biolistic transformation.

Example 15. Transformation of Maize Cells with the pesticidal protein genes described herein

Maize ears are best collected 8-12 days after pollination. Embryos are isolated from the ears, and those embryos 0.8-1.5 mm in size are preferred for use in transformation. Embryos are plated scutellum side-up on a suitable incubation media, such as DN62A5S media (3.98 g/L N6 Salts; 1 mL/L (of 1000x Stock) N6 Vitamins; 800 mg/L L-Asparagine; 100 mg/L Myo-inositol; 1.4 g/L L-Proline; 100 mg/L Casamino acids; 50 g/L sucrose; 1 mL/L (of 1 mg/mL Stock) 2,4-D). However, media and salts other than DN62A5S are suitable and are known in the art. Embryos are incubated overnight at 25°C in the dark. However, it is not necessary *per se* to incubate the embryos overnight.

The resulting explants are transferred to mesh squares (30-40 per plate), transferred onto osmotic media for about 30-45 minutes, then transferred to a beaming plate (see, for example, PCT Publication No. WO/0138514 and U.S. Patent No. 5,240,842).

DNA constructs designed to the genes of the invention in plant cells are accelerated into plant tissue using an aerosol beam accelerator, using conditions essentially as described in PCT Publication No. WO/0138514. After beaming, embryos are incubated for about 30 min on osmotic media, and placed onto incubation media overnight at 25°C in the dark. To avoid unduly damaging beamed explants, they are incubated for at least 24 hours prior to transfer to recovery media. Embryos are then spread onto recovery period media, for about 5 days, 25°C in the dark, then transferred to a selection media. Explants are incubated in selection media for up to eight weeks, depending on the nature and characteristics of the particular selection utilized. After the selection period, the resulting callus is transferred to embryo maturation media, until the formation of mature somatic embryos is observed. The resulting mature somatic embryos are then placed under low light, and the process of regeneration is initiated by methods known in the art. The resulting shoots are allowed to root on rooting media, and the resulting plants are transferred to nursery pots and propagated as transgenic plants.

56148-3D1

Materials

DN62A5S Media

Components	Per Liter	Source
Chu's N6 Basal Salt Mixture (Prod. No. C 416)	3.98 g/L	Phytotechnology Labs
Chu's N6 Vitamin Solution (Prod. No. C 149)	1 mL/L (of 1000x Stock)	Phytotechnology Labs
L-Asparagine	800 mg/L	Phytotechnology Labs
Myo-inositol	100 mg/L	Sigma
L-Proline	1.4 g/L	Phytotechnology Labs
Casamino acids	100 mg/L	Fisher Scientific
Sucrose	50 g/L	Phytotechnology Labs
2,4-D (Prod. No. D-7299)	1 mL/L (of 1 mg/mL Stock)	Sigma

5

The pH of the solution is adjusted to pH 5.8 with 1N KOH/1N KCl, Gelrite (Sigma) is added at a concentration up to 3g/L, and the media is autoclaved. After cooling to 50°C, 2 ml/L of a 5 mg/ml stock solution of silver nitrate (Phytotechnology Labs) is added.

10

Example 16. Transformation of the *axmi-066*, *optaxmi-066*, *axmi-076*, and *optaxmi-076* genes of the invention in Plant Cells by *Agrobacterium*-Mediated Transformation

Ears are best collected 8-12 days after pollination. Embryos are isolated from the ears, and those embryos 0.8-1.5 mm in size are preferred for use in transformation.

- 15 Embryos are plated scutellum side-up on a suitable incubation media, and incubated overnight at 25°C in the dark. However, it is not necessary *per se* to incubate the embryos overnight. Embryos are contacted with an *Agrobacterium* strain containing the appropriate vectors for Ti plasmid mediated transfer for about 5-10 min, and then plated onto co-cultivation media for about 3 days (25°C in the dark). After co-cultivation,
- 20 explants are transferred to recovery period media for about five days (at 25°C in the dark). Explants are incubated in selection media for up to eight weeks, depending on the nature and characteristics of the particular selection utilized. After the selection period, the resulting callus is transferred to embryo maturation media, until the formation of mature somatic embryos is observed. The resulting mature somatic embryos are then
- 25 placed under low light, and the process of regeneration is initiated as known in the art.

All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention pertains.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 56148-3D1 Seq 19-DEC-16 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

<110> Daniel J. Tomso
Kimberly S. Sampson
Nalini Desai
Shruti Agarwal
Sandra Volrath
Volker Heinrichs

<120> AXMI-066 AND AXMI-076: DELTA-ENDOTOXIN
PROTEINS AND METHODS FOR THEIR USE

<130> 56148-3D1

<140> Divisional of CA 2,702,998

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ctc aat gct gat gaa tgg ggc atc ccg gcg gcg acg gtg agg acc tac	624
Leu Asn Ala Asp Glu Trp Gly Ile Pro Ala Ala Thr Val Arg Thr Tyr	
195 200 205	
agg gag cac ctc aag aga tac aca agg gac tac tca aac tac tgc atc	672
Arg Glu His Leu Lys Arg Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile	
210 215 220	
aac acc tac cag acg gcc ttc cgc ggc ctc aac aca agg cta cat gac	720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp	
225 230 235 240	
atg ctg gag ttc aga acc ttc atg ttc ctc aat gtg ctg gac tat gtg	768
Met Leu Glu Phe Arg Thr Phe Met Phe Leu Asn Val Leu Asp Tyr Val	
245 250 255	
agc atc tgg agc ctc ttc aag tac cag agc ttg atg gtg aca agc tcc	816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Thr Ser Ser	
260 265 270	
gcc aac ctc tat gct tct gga agc ggc agc aac cag ccc ttc acc gct	864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Ser Asn Gln Pro Phe Thr Ala	
275 280 285	
caa gat tgg ccc ttc ctc tac agc ctc ttc caa gtg aac tca aac tac	912
Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr	
290 295 300	
atc atg agc aac ttc ggc ggc aac cgt gaa act gct tcc ttc ggc gtg	960
Ile Met Ser Asn Phe Gly Gly Asn Arg Glu Thr Ala Ser Phe Gly Val	
305 310 315 320	

ccc atc ctc ggc ggc ttc atc atc aac ttc ctg ctg agc ttc cgc gtc	1008
Pro Ile Leu Gly Gly Phe Ile Ile Asn Phe Leu Leu Ser Phe Arg Val	
325 330 335	
aac tac act gga gga gtt tct tct ggg ctg ctg gga gtt gaa ggc atc	1056
Asn Tyr Thr Gly Gly Val Ser Ser Gly Leu Leu Gly Val Glu Gly Ile	
340 345 350	
agc aac aac ttc aac tgc aac tca agc ctc agc acg ccg gtg gtg agg	1104
Ser Asn Asn Phe Asn Cys Asn Ser Ser Leu Ser Thr Pro Val Val Arg	
355 360 365	
agc tgg ctg gac agc ggc gtc tac aga gga gat ctt cag cac aac tgg	1152
Ser Trp Leu Asp Ser Gly Val Tyr Arg Gly Asp Leu Gln His Asn Trp	
370 375 380	
agg acg gac atc ttc atg agg acc aac atc gtg cca tgc ggc gcc ttc	1200
Arg Thr Asp Ile Phe Met Arg Thr Asn Ile Val Pro Cys Gly Ala Phe	
385 390 395 400	
ctc ctc tca ttg gcc atg ttc cct gat gtg aag agc aac tac ttc ccg	1248
Leu Leu Ser Leu Ala Met Phe Pro Asp Val Lys Ser Asn Tyr Phe Pro	
405 410 415	
gac tac ttc atc agg aac atc agc ggc atc atc agg aac atc gac aac	1296
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Ile Ile Arg Asn Ile Asp Asn	
420 425 430	
atg aac ctc tca agg ccg ctg cac ttc aac gag gtg aga gat ctt cga	1344
Met Asn Leu Ser Arg Pro Leu His Phe Asn Glu Val Arg Asp Leu Arg	
435 440 445	
gac acc gag gtg gcc acc ttg gtg agc gtc cac aac agg aag aac aac	1392
Asp Thr Glu Val Ala Thr Leu Val Ser Val His Asn Arg Lys Asn Asn	
450 455 460	
atc tat gct gct cat gaa aat ggc acc atg atc cac ttc gcg cca gaa	1440
Ile Tyr Ala Ala His Glu Asn Gly Thr Met Ile His Phe Ala Pro Glu	
465 470 475 480	
ggc tac acc ggc ttc acc atc tca cca att tat gca act caa gtc aac	1488
Gly Tyr Thr Gly Phe Thr Ile Ser Pro Ile Tyr Ala Thr Gln Val Asn	
485 490 495	
aac caa aca agg acc ttc atc tcc gag aag ttt gga aat caa gga gat	1536
Asn Gln Thr Arg Thr Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp	
500 505 510	
tct ttg aga ttt gag cag acc aac acc acg gcg cgc tac acc ttc aga	1584
Ser Leu Arg Phe Glu Gln Thr Asn Thr Thr Ala Arg Tyr Thr Phe Arg	
515 520 525	
gga aat ggc aac aac tac aac ctc tac ctc cgc gtc agc agc caa ggc	1632
Gly Asn Gly Asn Asn Tyr Asn Leu Tyr Leu Arg Val Ser Ser Gln Gly	
530 535 540	

aac agc acc ttc cgc gtg acc atc aat ggc cgc gtc tac acc gtc tca	1680
Asn Ser Thr Phe Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Val Ser	
545 550 555 560	
aat gtc aac acc acc acc aac aat gat ggc gtc atc gac aat gga gca	1728
Asn Val Asn Thr Thr Thr Asn Asn Asp Gly Val Ile Asp Asn Gly Ala	
565 570 575	
aga ttc tcc gac atc cac atc ggc aac atc gtc gcc agc aac aac acc	1776
Arg Phe Ser Asp Ile His Ile Gly Asn Ile Val Ala Ser Asn Asn Thr	
580 585 590	
aac gtg ccg ctg gac atc aat gtc atc ctc aac agc ggc acc caa ttt	1824
Asn Val Pro Leu Asp Ile Asn Val Ile Leu Asn Ser Gly Thr Gln Phe	
595 600 605	
gag ctg atg aac atc atc ttc gtg cca acc aac atc ccg ccg ctc tac	1872
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aat gta gtg gcc cat gat cca ttt agt ttt gag cat aaa tca tta gat	96
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp	
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acc atc cga aaa gaa tgg atg gag tgg aaa aga aca gat cat agt tta	144
Thr Ile Arg Lys Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu	
35 40 45	
tat gta gct cct gta gtc gga act gtt tct agc ttt ctg cta aag aag	192
Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys	
50 55 60	
gtg ggg agt ctt att gga aaa agg ata ttg agt gaa tta tgg ggg tta	240
Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu	
65 70 75 80	
ata ttt cct agt ggt agc aca aat cta atg caa gat att tta agg gag	288
Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu	
85 90 95	

aca gaa caa ttc cta aat caa aga ctt aat aca gac act ctt gct cgt	336
Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg	
100 105 110	
gta aat gcg gaa ttg aca ggg ctg caa gcg aat ata agg gag ttt aat	384
Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn	
115 120 125	
caa caa gta gat aat ttt tta aat cct act caa aac cct gtt cct tta	432
Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu	
130 135 140	
tca ata act tct tca gtt aat aca atg cag caa tta ttt cta aat aga	480
Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg	
145 150 155 160	
tta ccc cag ttc cag ata caa gga tac caa ctg tta tta tta cct tta	528
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu	
165 170 175	
ttt gca cag gca gcc aat atg cat ctt tct ttt att aga gat gtt att	576
Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile	
180 185 190	
ctt aat gca gat gaa tgg gga att tca gca gca aca cta cgt acg tat	624
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr	
195 200 205	
cga gac tac ctg aga aat tat aca aga gat tat tct aat tat tgt ata	672
Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile	
210 215 220	
aat acg tat caa act gcg ttt aga ggg tta aac acc cgt tta cac gat	720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp	
225 230 235 240	
atg tta gaa ttt aga aca tat atg ttt tta aat gta ttt gaa tat gta	768
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val	
245 250 255	
tcc att tgg tca ttg ttt aaa tat cag agt ctt atg gta tct tct ggc	816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly	
260 265 270	
gct aat tta tat gct agt ggt agt gga cca cag cag acc caa tca ttt	864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe	
275 280 285	
att tca caa gac tgg cca ttt tta tat tct ctt ttc caa gtt aat tca	912
Ile Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser	
290 295 300	
aat tat gtg tta aat ggc ttt agt ggc gct agg aat act att aga ttc	960
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Asn Thr Ile Arg Phe	
305 310 315 320	

cca gct ggt ggt ggt tta tta cca cct ggt gtt act aca act cac gca	1008
Pro Ala Gly Gly Gly Leu Leu Pro Pro Gly Val Thr Thr Thr His Ala	
325 330 335	
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Leu Leu Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Tyr	
340 345 350	
ata ggc gct gtg ttt aat caa aat ttt aat tgt agc aca ctt ctc cca	1104
Ile Gly Ala Val Phe Asn Gln Asn Phe Asn Cys Ser Thr Leu Leu Pro	
355 360 365	
cct ttg tta aca cca ttt gtt agg agt tgg cta gat tca ggt aca gat	1152
Pro Leu Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp	
370 375 380	
cgg gag ggc gtt acc acc gtt aca aat tgg caa aca gaa tcc ttt aag	1200
Arg Glu Gly Val Thr Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Lys	
385 390 395 400	
aca act tta ggt tta agg tgt ggt gct ttt aca ccc cgt ggt aat tca	1248
Thr Thr Leu Gly Leu Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser	
405 410 415	
aac tat ttc cca gat tat ttt atc cgt aat att tct ggc gtt cct tta	1296
Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu	
420 425 430	
gtt gtt aga aac gaa gat tta aga aga ccg tta cac tat aat gaa ata	1344
Val Val Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile	
435 440 445	
aga aat ata gaa agt ccc tca gga aca cct ggt gga tta cga gct tat	1392
Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr	
450 455 460	
atg gta tct gtg cat aac aga aaa aat aat atc tat gcc gct cat gaa	1440
Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His Glu	
465 470 475 480	
aat ggt act atg att cat ttg gca ccg gaa gat tat aca gga ttt act	1488
Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr	
485 490 495	
atg ttg ccg ata cat gca act caa gtg aat aat caa acg cga aca ttt	1536
Met Leu Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe	
500 505 510	
att tct gaa aaa ttt gga aat caa ggt gat tcc tta aga ttt gaa caa	1584
Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln	
515 520 525	
agc gac acg aca gct cgt tat aca ctt aga ggg aat gga aat agt tac	1632
Ser Asp Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr	
530 535 540	

aat ctt tat tta aga gta tct tct cta gga aat tcc act att cga gtt 1680
 Asn Leu Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val
 545 550 555 560

act ata aac gga aga gtt tat act gtt cca aat gtt aat aca aat ata 1728
 Thr Ile Asn Gly Arg Val Tyr Thr Val Pro Asn Val Asn Thr Asn Ile
 565 570 575

aat aac gat gga gtc att gat aat gga gct cgt ttt tca gat att aat 1776
 Asn Asn Asp Gly Val Ile Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn
 580 585 590

atc ggt aat gta gta gca agt gat aat act aat gta ccg tta gat ata 1824
 Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile
 595 600 605

aac gtg aca tta agt tct gga act caa ttt gag ctt atg aat att atg 1872
 Asn Val Thr Leu Ser Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met
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<211> 634

<212> PRT

<213> *Bacillus thuringiensis*

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 35 40 45
 Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
 50 55 60
 Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu
 65 70 75 80
 Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
 85 90 95
 Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
 100 105 110
 Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
 115 120 125
 Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
 130 135 140
 Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
 145 150 155 160
 Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
 165 170 175
 Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile
 180 185 190
 Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr
 195 200 205

Arg	Asp	Tyr	Leu	Arg	Asn	Tyr	Thr	Arg	Asp	Tyr	Ser	Asn	Tyr	Cys	Ile
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Asn	Thr	Tyr	Gln	Thr	Ala	Phe	Arg	Gly	Leu	Asn	Thr	Arg	Leu	His	Asp
225					230					235					240
Met	Leu	Glu	Phe	Arg	Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val
				245					250					255	
Ser	Ile	Trp	Ser	Leu	Phe	Lys	Tyr	Gln	Ser	Leu	Met	Val	Ser	Ser	Gly
			260					265					270		
Ala	Asn	Leu	Tyr	Ala	Ser	Gly	Ser	Gly	Pro	Gln	Gln	Thr	Gln	Ser	Phe
		275				280						285			
Ile	Ser	Gln	Asp	Trp	Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser
	290					295					300				
Asn	Tyr	Val	Leu	Asn	Gly	Phe	Ser	Gly	Ala	Arg	Asn	Thr	Ile	Arg	Phe
305					310					315					320
Pro	Ala	Gly	Gly	Gly	Leu	Leu	Pro	Pro	Gly	Val	Thr	Thr	Thr	His	Ala
				325					330					335	
Leu	Leu	Ala	Ala	Arg	Val	Asn	Tyr	Ser	Gly	Gly	Val	Ser	Ser	Gly	Tyr
			340					345					350		
Ile	Gly	Ala	Val	Phe	Asn	Gln	Asn	Phe	Asn	Cys	Ser	Thr	Leu	Leu	Pro
	355					360						365			
Pro	Leu	Leu	Thr	Pro	Phe	Val	Arg	Ser	Trp	Leu	Asp	Ser	Gly	Thr	Asp
	370					375					380				
Arg	Glu	Gly	Val	Thr	Thr	Val	Thr	Asn	Trp	Gln	Thr	Glu	Ser	Phe	Lys
385					390					395					400
Thr	Thr	Leu	Gly	Leu	Arg	Cys	Gly	Ala	Phe	Thr	Pro	Arg	Gly	Asn	Ser
				405					410					415	
Asn	Tyr	Phe	Pro	Asp	Tyr	Phe	Ile	Arg	Asn	Ile	Ser	Gly	Val	Pro	Leu
			420					425					430		
Val	Val	Arg	Asn	Glu	Asp	Leu	Arg	Arg	Pro	Leu	His	Tyr	Asn	Glu	Ile
		435					440					445			
Arg	Asn	Ile	Glu	Ser	Pro	Ser	Gly	Thr	Pro	Gly	Gly	Leu	Arg	Ala	Tyr
	450					455					460				
Met	Val	Ser	Val	His	Asn	Arg	Lys	Asn	Asn	Ile	Tyr	Ala	Ala	His	Glu
465					470					475					480
Asn	Gly	Thr	Met	Ile	His	Leu	Ala	Pro	Glu	Asp	Tyr	Thr	Gly	Phe	Thr
				485					490					495	
Met	Leu	Pro	Ile	His	Ala	Thr	Gln	Val	Asn	Asn	Gln	Thr	Arg	Thr	Phe
			500					505					510		
Ile	Ser	Glu	Lys	Phe	Gly	Asn	Gln	Gly	Asp	Ser	Leu	Arg	Phe	Glu	Gln
	515					520						525			
Ser	Asp	Thr	Thr	Ala	Arg	Tyr	Thr	Leu	Arg	Gly	Asn	Gly	Asn	Ser	Tyr
	530					535					540				
Asn	Leu	Tyr	Leu	Arg	Val	Ser	Ser	Leu	Gly	Asn	Ser	Thr	Ile	Arg	Val
545					550					555					560
Thr	Ile	Asn	Gly	Arg	Val	Tyr	Thr	Val	Pro	Asn	Val	Asn	Thr	Asn	Ile
				565					570					575	
Asn	Asn	Asp	Gly	Val	Ile	Asp	Asn	Gly	Ala	Arg	Phe	Ser	Asp	Ile	Asn
			580					585					590		
Ile	Gly	Asn	Val	Val	Ala	Ser	Asp	Asn	Thr	Asn	Val	Pro	Leu	Asp	Ile
	595						600					605			
Asn	Val	Thr	Leu	Ser	Ser	Gly	Thr	Gln	Phe	Glu	Leu	Met	Asn	Ile	Met
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 <213> Artificial Sequence

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 aat gtg gtg gct cat gat ccc ttc agc ttc gag cac aag agc ttg gac 96
 Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
 20 25 30
 acc atc agg aag gag tgg atg gaa tgg aag agg aca gat cat tct ctt 144
 Thr Ile Arg Lys Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
 35 40 45
 tat gtg gcg ccg gtg gtg ggc acc gtc tca tcc ttc ctg ctg aag aag 192
 Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys
 50 55 60
 gtg ggc agc ctc atc ggc aag agg att ctt tct gag ctc tgg ggc ctc 240
 Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu
 65 70 75 80
 atc ttc cca agt gga tca aca aat ttg atg caa gac atc ctc cga gaa 288
 Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
 85 90 95
 aca gag cag ttc ctc aat caa agg ctc aac acc gac acc ttg gca agg 336
 Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
 100 105 110
 gtg aat gct gag ctg aca ggg cta caa gcc aac atc agg gag ttc aac 384
 Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn
 115 120 125
 cag cag gtg gac aac ttc ctc aac cca act caa aat cca gtg ccg ctg 432
 Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu
 130 135 140
 agc atc acc tcc tct gtt aac acc atg cag cag ctc ttc ctc aac agg 480
 Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
 145 150 155 160
 ctg ccg cag ttc cag atc caa ggc tac cag ctg ctg ctg ctg ccg cta 528
 Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
 165 170 175

ttt gct caa gct gcc aac atg cac ctc agc ttc atc aga gat gtc atc	576
Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile	
180 185 190	
ctc aat gct gat gaa tgg ggc atc tcg gcg gcg acg ctg agg acc tac	624
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr	
195 200 205	
aga gat tat ttg agg aac tac aca agg gac tac tca aac tac tgc atc	672
Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile	
210 215 220	
aac acc tac cag acg gcc ttc cgc ggc ctc aac aca agg ctg cat gac	720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp	
225 230 235 240	
atg ctg gag ttc aga acc tac atg ttc ctc aat gtt ttt gaa tat gtt	768
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val	
245 250 255	
tca att tgg agc ctc ttc aag tac cag agc ttg atg gtg agc agc ggc	816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly	
260 265 270	
gcc aac ctc tat gct tct gga agt ggg ccg cag caa act cag agc ttc	864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe	
275 280 285	
atc agc caa gat tgg ccc ttc ctc tac agc ctc ttc caa gtt aac agc	912
Ile Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser	
290 295 300	
aac tac gtg ctg aat ggc ttc tca gga gca agg aac acc atc aga ttt	960
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Asn Thr Ile Arg Phe	
305 310 315 320	
cct gct gga gga ggg ctg ctg ccg ccg ggc gtg aca aca act cat gct	1008
Pro Ala Gly Gly Gly Leu Leu Pro Pro Gly Val Thr Thr Thr His Ala	
325 330 335	
ctg ctg gcg gca aga gtt aac tac tct gga gga gtt tca agc ggc tac	1056
Leu Leu Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Tyr	
340 345 350	
atc ggc gcc gtc ttc aac cag aac ttc aac tgc tcg acg ctg ctg ccg	1104
Ile Gly Ala Val Phe Asn Gln Asn Phe Asn Cys Ser Thr Leu Leu Pro	
355 360 365	
ccg ctg ctg acg cca ttt gtg agg agc tgg ctg gac agc ggc acc gac	1152
Pro Leu Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp	
370 375 380	
aga gaa gga gtc acc acc gtc acc aac tgg caa act gag agc ttc aag	1200
Arg Glu Gly Val Thr Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Lys	
385 390 395 400	

acg acg ctg ggc ctc cgc tgc ggc gcc ttc acg ccg cgc ggc aac tca	1248
Thr Thr Leu Gly Leu Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser	
405 410 415	
aac tac ttc ccg gac tac ttc atc agg aac atc agc ggc gtg ccg ctg	1296
Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu	
420 425 430	
gtg gtg agg aat gaa gat ttg agg agg ccg ctg cac tac aat gag atc	1344
Val Val Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile	
435 440 445	
agg aac atc gag agc cca tca gga act cct gga ggc ctc cgc gcc tac	1392
Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr	
450 455 460	
atg gtt tct gtt cac aac agg aag aac aac atc tat gct gct cat gaa	1440
Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His Glu	
465 470 475 480	
aat ggc acc atg atc cac ctg gcg ccg gag gac tac acc ggc ttc acc	1488
Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr	
485 490 495	
atg cta ccc atc cat gca act caa gtc aac aac caa aca agg acc ttc	1536
Met Leu Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe	
500 505 510	
atc tcc gag aag ttt gga aat caa gga gat tct ttg aga ttt gag cag	1584
Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln	
515 520 525	
agc gac acc acg gcg cgc tac acc ttg aga gga aat ggc aac agc tac	1632
Ser Asp Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr	
530 535 540	
aac ctc tac ctc cgc gtc agc agc ctc ggc aac tca acc atc agg gtg	1680
Asn Leu Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val	
545 550 555 560	
acc atc aat ggc cgc gtc tac acc gtg cca aat gtc aac acc aac atc	1728
Thr Ile Asn Gly Arg Val Tyr Thr Val Pro Asn Val Asn Thr Asn Ile	
565 570 575	
aac aat gat ggc gtc atc gac aat gga gca aga ttc tcc gac atc aac	1776
Asn Asn Asp Gly Val Ile Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn	
580 585 590	
atc ggc aat gtg gtg gcc tcc gac aac acc aac gtg ccg ctg gac atc	1824
Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile	
595 600 605	
aat gtc acc ttg agc tca gga act caa ttt gag ctg atg aac atc atg	1872
Asn Val Thr Leu Ser Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met	
610 615 620	

ttc gtg cca aca aat ctt cct ccc atc tac
 Phe Val Pro Thr Asn Leu Pro Pro Ile Tyr
 625 630

1902

<210> 7
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Endoplasmic reticulum targeting sequence

<400> 7
 Lys Asp Glu Leu
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<210> 8
 <211> 1990
 <212> DNA
 <213> *Bacillus thuringiensis*

<400> 8
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 aataagtgtg atgcgtataa cgtagtgagg catgatccat ttagttttga gcataaatca 180
 ttagatacca tacagcaaga atggatggag tggaaaagaa ccgatcatag tttatatgta 240
 tctcctattg tgggaactat agctagtttt ctgctaaaga aaatagcggg gcttatagga 300
 aaaagaatat taagtgaagt aaagaattta atttttccta gtggtagtagt agaatacatg 360
 caagatatatt taagagggggc agaacaattc ctaaatacaa gacttgatgc agacaccttt 420
 gctcgggtag aggcagaatt gatagggctt caagcaaagt tagaggaatt taatcaacaa 480
 gtggacaatt ttttaaacc aaatacaaac cctgttcctt tagcaataat tgattcgggtt 540
 aatacaatgc aacaattatt cctaagtaga ttaccccgat tccagataca acgctatcag 600
 ctattattat tacctttatt tgcacaagca gccaatctac accttacctt tattagagat 660
 gttattctta atgcagatga atggggaata ccagcagcaa cagtgcgcac atatagagag 720
 cacctaaaaa gatatacacg cgattattcc aattattgta taaacacgta ccaaactgct 780
 ttccgagggt taaacactcg tttacatgat atgttagagt ttagaacatt tatgttttta 840
 aatgtattag actatgtatc tatctggctg ttgtttaaat atcaaagtct gatggttact 900
 tcaagtgcta atttatatgc ttcggggaagt ggtagtaatc aaccttttac tgcacaagac 960
 tggccatttt tatattctct tttccaagtg aattcaaatt atataatgtc taattttggt 1020
 ggtaaccgag agactgctag ttttggtggt cctattctgg ggggattcat aataaatttt 1080
 ttacttagtt ttaggggttaa ttatactgga ggagtttcat ctggtctcct aggtgttgaa 1140
 ggaatttcaa acaactttta ttgcaactcc tctttatcaa caccagttgt aagaagttgg 1200
 ctagattcag gtgtatatcg aggtgacctg caacacaatt ggcgaacaga catctttatg 1260
 aggactaata ttgtaccttg tgggtgcttt ctattatctc ttgctatggt tccagatggt 1320
 aaaagtaatt attttcctga ttatttcatt cgtaacattt ccggaattat tcgaaatatt 1380
 gataacatga atttgagtag accattacac tttaatgaag taagagattt aagagacact 1440
 gaagttgcta ctttagtatc tgtgcataat agaaaaaata atatctatgc tgctcatgaa 1500
 aatggtacta tgattcattt tgcgcgggaa gggtatacag gtttcacaat atcaccaata 1560
 tatgcaactc aagtaaataa tcaaacacga acgtttattt ctgaaaaatt cggaaatcaa 1620
 ggtgattcct tgagatttga acaaactaac acaacggctc gttatacgtt tagaggggaat 1680
 ggtaataatt ataactttta ttttaagagta tcctcacaag gaaattctac ttttcgagtt 1740
 actataaacg gtaggggtta tactgtttca aatgttaata ccactacaaa taatgatggg 1800
 gttattgata atggggctcg tttttcagat attcacatcg ggaatatagt ggcaagtaac 1860
 aataactaatg taccattaga tataaatgtg atacttaact ccggtactca atttgagctt 1920

atgaatatta tttttgttcc aactaacatt ccaccacttt attaagggttt gagtttctga 1980
 ggtaaataata 1990

<210> 9
 <211> 1872
 <212> DNA
 <213> *Bacillus thuringiensis*

<220>
 <221> CDS
 <222> (1)...(1872)

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 1 5 10 15
 aac gta gtg gcc cat gat cca ttt agt ttt gag cat aaa tca tta gat 96
 Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
 20 25 30
 acc ata cag caa gaa tgg atg gag tgg aaa aga acc gat cat agt tta 144
 Thr Ile Gln Gln Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
 35 40 45
 tat gta tct cct att gtg gga act ata gct agt ttt ctg cta aag aaa 192
 Tyr Val Ser Pro Ile Val Gly Thr Ile Ala Ser Phe Leu Leu Lys Lys
 50 55 60
 ata gcg ggg ctt ata gga aaa aga ata tta agt gag tta aag aat tta 240
 Ile Ala Gly Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Lys Asn Leu
 65 70 75 80
 att ttt cct agt ggt agt ata gaa tca atg caa gat att tta aga ggg 288
 Ile Phe Pro Ser Gly Ser Ile Glu Ser Met Gln Asp Ile Leu Arg Gly
 85 90 95
 gca gaa caa ttc cta aat caa aga ctt gat gca gac acc ttt gct cgg 336
 Ala Glu Gln Phe Leu Asn Gln Arg Leu Asp Ala Asp Thr Phe Ala Arg
 100 105 110
 gta gag gca gaa ttg ata ggg ctt caa gca aat gta gag gaa ttt aat 384
 Val Glu Ala Glu Leu Ile Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
 115 120 125
 caa caa gtg gac aat ttt tta aac cca aat caa aac cct gtt cct tta 432
 Gln Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
 130 135 140
 gca ata att gat tcg gtt aat aca atg caa caa tta ttc cta agt aga 480
 Ala Ile Ile Asp Ser Val Asn Thr Met Gln Gln Leu Phe Leu Ser Arg
 145 150 155 160
 tta ccc cag ttc cag ata caa cgc tat cag cta tta tta tta cct tta 528
 Leu Pro Gln Phe Gln Ile Gln Arg Tyr Gln Leu Leu Leu Leu Pro Leu
 165 170 175

ttt gca caa gca gcc aat tta cac ctt acc ttt att aga gat gtt att	576
Phe Ala Gln Ala Ala Asn Leu His Leu Thr Phe Ile Arg Asp Val Ile	
180 185 190	
ctt aat gca gat gaa tgg gga ata cca gca gca aca gtg cgc aca tat	624
Leu Asn Ala Asp Glu Trp Gly Ile Pro Ala Ala Thr Val Arg Thr Tyr	
195 200 205	
aga gag cac cta aaa aga tat aca cgc gat tat tcc aat tat tgt ata	672
Arg Glu His Leu Lys Arg Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile	
210 215 220	
aac acg tac caa act gct ttc cga ggt tta aac act cgt tta cat gat	720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp	
225 230 235 240	
atg tta gag ttt aga aca ttt atg ttt tta aat gta tta gac tat gta	768
Met Leu Glu Phe Arg Thr Phe Met Phe Leu Asn Val Leu Asp Tyr Val	
245 250 255	
tct atc tgg tcg ttg ttt aaa tat caa agt ctg atg gtt act tca agt	816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Thr Ser Ser	
260 265 270	
gct aat tta tat gct tcg gga agt ggt agt aat caa cct ttt act gca	864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Ser Asn Gln Pro Phe Thr Ala	
275 280 285	
caa gac tgg cca ttt tta tat tct ctt ttc caa gtg aat tca aat tat	912
Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr	
290 295 300	
ata atg tct aat ttt ggt ggt aac cga gag act gct agt ttt ggt gtt	960
Ile Met Ser Asn Phe Gly Gly Asn Arg Glu Thr Ala Ser Phe Gly Val	
305 310 315 320	
cct att ctg ggg gga ttc ata ata aat ttt tta ctt agt ttt agg gtt	1008
Pro Ile Leu Gly Gly Phe Ile Ile Asn Phe Leu Leu Ser Phe Arg Val	
325 330 335	
aat tat act gga gga gtt tca tct ggt ctc cta ggt gtt gaa gga att	1056
Asn Tyr Thr Gly Gly Val Ser Ser Gly Leu Leu Gly Val Glu Gly Ile	
340 345 350	
tca aac aac ttt aat tgc aac tcc tct tta tca aca cca gtt gta aga	1104
Ser Asn Asn Phe Asn Cys Asn Ser Ser Leu Ser Thr Pro Val Val Arg	
355 360 365	
agt tgg cta gat tca ggt gta tat cga ggt gac ctg caa cac aat tgg	1152
Ser Trp Leu Asp Ser Gly Val Tyr Arg Gly Asp Leu Gln His Asn Trp	
370 375 380	
cga aca gac atc ttt atg agg act aat att gta cct tgt ggt gct ttt	1200
Arg Thr Asp Ile Phe Met Arg Thr Asn Ile Val Pro Cys Gly Ala Phe	
385 390 395 400	

cta tta tct ctt gct atg ttt cca gat gtt aaa agt aat tat ttt cct	1248
Leu Leu Ser Leu Ala Met Phe Pro Asp Val Lys Ser Asn Tyr Phe Pro	
405 410 415	
gat tat ttc att cgt aac att tcc gga att att cga aat att gat aac	1296
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Ile Ile Arg Asn Ile Asp Asn	
420 425 430	
atg aat ttg agt aga cca tta cac ttt aat gaa gta aga gat tta aga	1344
Met Asn Leu Ser Arg Pro Leu His Phe Asn Glu Val Arg Asp Leu Arg	
435 440 445	
gac act gaa gtt gct act tta gta tct gtg cat aat aga aaa aat aat	1392
Asp Thr Glu Val Ala Thr Leu Val Ser Val His Asn Arg Lys Asn Asn	
450 455 460	
atc tat gct gct cat gaa aat ggt act atg att cat ttt gcg ccg gaa	1440
Ile Tyr Ala Ala His Glu Asn Gly Thr Met Ile His Phe Ala Pro Glu	
465 470 475 480	
ggt tat aca ggt ttc aca ata tca cca ata tat gca act caa gta aat	1488
Gly Tyr Thr Gly Phe Thr Ile Ser Pro Ile Tyr Ala Thr Gln Val Asn	
485 490 495	
aat caa aca cga acg ttt att tct gaa aaa ttc gga aat caa ggt gat	1536
Asn Gln Thr Arg Thr Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp	
500 505 510	
tcc ttg aga ttt gaa caa act aac aca acg gct cgt tat acg ttt aga	1584
Ser Leu Arg Phe Glu Gln Thr Asn Thr Thr Ala Arg Tyr Thr Phe Arg	
515 520 525	
ggg aat ggt aat aat tat aat ctt tat tta aga gta tcc tca caa gga	1632
Gly Asn Gly Asn Asn Tyr Asn Leu Tyr Leu Arg Val Ser Ser Gln Gly	
530 535 540	
aat tct act ttt cga gtt act ata aac ggt agg gtt tat act gtt tca	1680
Asn Ser Thr Phe Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Val Ser	
545 550 555 560	
aat gtt aat acc act aca aat aat gat ggg gtt att gat aat ggg gct	1728
Asn Val Asn Thr Thr Thr Asn Asn Asp Gly Val Ile Asp Asn Gly Ala	
565 570 575	
cgt ttt tca gat att cac atc ggg aat ata gtg gca agt aac aat act	1776
Arg Phe Ser Asp Ile His Ile Gly Asn Ile Val Ala Ser Asn Asn Thr	
580 585 590	
aat gta cca tta gat ata aat gtg ata ctt aac tcc ggt act caa ttt	1824
Asn Val Pro Leu Asp Ile Asn Val Ile Leu Asn Ser Gly Thr Gln Phe	
595 600 605	
gag ctt atg aat att att ttt gtt cca act aac att cca cca ctt tat	1872
Glu Leu Met Asn Ile Ile Phe Val Pro Thr Asn Ile Pro Pro Leu Tyr	
610 615 620	

<210> 10
 <211> 624
 <212> PRT
 <213> Bacillus thuringiensis

<400> 10
 Met Asn Asn Val Leu Asn Ser Glu Arg Thr Asn Lys Cys Asp Ala Tyr
 1 5 10 15
 Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
 20 25 30
 Thr Ile Gln Gln Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
 35 40 45
 Tyr Val Ser Pro Ile Val Gly Thr Ile Ala Ser Phe Leu Leu Lys Lys
 50 55 60
 Ile Ala Gly Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Lys Asn Leu
 65 70 75 80
 Ile Phe Pro Ser Gly Ser Ile Glu Ser Met Gln Asp Ile Leu Arg Gly
 85 90 95
 Ala Glu Gln Phe Leu Asn Gln Arg Leu Asp Ala Asp Thr Phe Ala Arg
 100 105 110
 Val Glu Ala Glu Leu Ile Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
 115 120 125
 Gln Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
 130 135 140
 Ala Ile Ile Asp Ser Val Asn Thr Met Gln Gln Leu Phe Leu Ser Arg
 145 150 155 160
 Leu Pro Gln Phe Gln Ile Gln Arg Tyr Gln Leu Leu Leu Leu Pro Leu
 165 170 175
 Phe Ala Gln Ala Ala Asn Leu His Leu Thr Phe Ile Arg Asp Val Ile
 180 185 190
 Leu Asn Ala Asp Glu Trp Gly Ile Pro Ala Ala Thr Val Arg Thr Tyr
 195 200 205
 Arg Glu His Leu Lys Arg Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile
 210 215 220
 Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp
 225 230 235 240
 Met Leu Glu Phe Arg Thr Phe Met Phe Leu Asn Val Leu Asp Tyr Val
 245 250 255
 Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Thr Ser Ser
 260 265 270
 Ala Asn Leu Tyr Ala Ser Gly Ser Gly Ser Asn Gln Pro Phe Thr Ala
 275 280 285
 Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr
 290 295 300
 Ile Met Ser Asn Phe Gly Gly Asn Arg Glu Thr Ala Ser Phe Gly Val
 305 310 315 320
 Pro Ile Leu Gly Gly Phe Ile Ile Asn Phe Leu Leu Ser Phe Arg Val
 325 330 335
 Asn Tyr Thr Gly Gly Val Ser Ser Gly Leu Leu Gly Val Glu Gly Ile
 340 345 350
 Ser Asn Asn Phe Asn Cys Asn Ser Ser Leu Ser Thr Pro Val Val Arg
 355 360 365
 Ser Trp Leu Asp Ser Gly Val Tyr Arg Gly Asp Leu Gln His Asn Trp
 370 375 380
 Arg Thr Asp Ile Phe Met Arg Thr Asn Ile Val Pro Cys Gly Ala Phe
 385 390 395 400

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Leu Leu Ser Leu Ala Met Phe Pro Asp Val Lys Ser Asn Tyr Phe Pro
      405                      410                      415
Asp Tyr Phe Ile Arg Asn Ile Ser Gly Ile Ile Arg Asn Ile Asp Asn
      420                      425                      430
Met Asn Leu Ser Arg Pro Leu His Phe Asn Glu Val Arg Asp Leu Arg
      435                      440                      445
Asp Thr Glu Val Ala Thr Leu Val Ser Val His Asn Arg Lys Asn Asn
      450                      455                      460
Ile Tyr Ala Ala His Glu Asn Gly Thr Met Ile His Phe Ala Pro Glu
465                      470                      475                      480
Gly Tyr Thr Gly Phe Thr Ile Ser Pro Ile Tyr Ala Thr Gln Val Asn
      485                      490                      495
Asn Gln Thr Arg Thr Phe Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp
      500                      505                      510
Ser Leu Arg Phe Glu Gln Thr Asn Thr Thr Ala Arg Tyr Thr Phe Arg
      515                      520                      525
Gly Asn Gly Asn Asn Tyr Asn Leu Tyr Leu Arg Val Ser Ser Gln Gly
      530                      535                      540
Asn Ser Thr Phe Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Val Ser
545                      550                      555                      560
Asn Val Asn Thr Thr Thr Asn Asn Asp Gly Val Ile Asp Asn Gly Ala
      565                      570                      575
Arg Phe Ser Asp Ile His Ile Gly Asn Ile Val Ala Ser Asn Asn Thr
      580                      585                      590
Asn Val Pro Leu Asp Ile Asn Val Ile Leu Asn Ser Gly Thr Gln Phe
      595                      600                      605
Glu Leu Met Asn Ile Ile Phe Val Pro Thr Asn Ile Pro Pro Leu Tyr
      610                      615                      620

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<210> 11
 <211> 1902
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic sequence encoding AXMI-076
 (optaxmi-076v04)

<220>
 <221> CDS
 <222> (1)...(1902)

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<400> 11
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Met Asn Asn Val Leu Asn Asn Gly Arg Thr Thr Asn Cys Asp Ala Tyr
  1                      5                      10                      15

aat gtt gtt gct cat gat cct ttc tca ttt gaa cac aag agc ttg gac      96
Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp
      20                      25                      30

acc atc aga aaa gaa tgg atg gaa tgg aaa aga aca gat cat tct ctc      144
Thr Ile Arg Lys Glu Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu
      35                      40                      45

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tat gtt gct cct gtt gtt gga act gtt agc agc ttc ttg ctg aag aag	192
Tyr Val Ala Pro Val Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys	
50 55 60	
gtt ggc agc ttg att gga aaa agg att ctt tca gag ctc tgg ggc ttg	240
Val Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu	
65 70 75 80	
atc ttt cct tct gga agc acc aac ttg atg caa gac atc ttg aga gaa	288
Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu	
85 90 95	
aca gag cag ttc ttg aac caa agg ctc aac aca gac acc ttg gca agg	336
Thr Glu Gln Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg	
100 105 110	
gtg aat gct gag ctc act ggc ctt caa gca aac atc aga gag ttc aac	384
Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn	
115 120 125	
caa caa gtt gac aac ttc ttg aat cca aca caa aat cct gtt cct ctc	432
Gln Gln Val Asp Asn Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu	
130 135 140	
tcc atc act tct tca gtg aac acc atg cag cag ctc ttc ttg aac agg	480
Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg	
145 150 155 160	
ctt cct cag ttc cag att caa gga tat cag ctg ctg ctg ctt cct ctc	528
Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu	
165 170 175	
ttt gct caa gct gca aac atg cat ctc tcc ttc atc aga gat gtc atc	576
Phe Ala Gln Ala Ala Asn Met His Leu Ser Phe Ile Arg Asp Val Ile	
180 185 190	
ttg aat gct gat gaa tgg ggc atc tct gct gcc acc ttg aga aca tac	624
Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr	
195 200 205	
aga gat tat ttg aga aac tac aca aga gat tat tca aac tac tgc atc	672
Arg Asp Tyr Leu Arg Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile	
210 215 220	
aac aca tat caa aca gct ttc aga ggc ctc aac aca agg ctt cat gac	720
Asn Thr Tyr Gln Thr Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp	
225 230 235 240	
atg ctg gag ttc aga aca tac atg ttc ttg aat gtt ttt gaa tat gtt	768
Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val	
245 250 255	
tcc atc tgg agc ttg ttc aag tac cag agc ttg atg gtt tct tct gga	816
Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly	
260 265 270	

gca aat tta tat gca tct gga tct gga cct caa caa aca cag agc ttc	864
Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe	
275 280 285	
atc tct caa gat tgg cca ttt ctc tac agc ttg ttc caa gtg aac agc	912
Ile Ser Gln Asp Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser	
290 295 300	
aac tat gtt ctt aat ggc ttc tct gga gca aga aac acc atc aga ttt	960
Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Asn Thr Ile Arg Phe	
305 310 315 320	
cct gct gga gga ggc ttg ctg cct cct ggt gtc acc acc acc cat gct	1008
Pro Ala Gly Gly Gly Leu Leu Pro Pro Gly Val Thr Thr Thr His Ala	
325 330 335	
ctt ctg gcg gcg cgc gtc aac tac tca gga gga gtt tct tct gga tac	1056
Leu Leu Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Tyr	
340 345 350	
att gga gct gtg ttc aac caa aac ttc aac tgc tcc acc ttg ctg ccg	1104
Ile Gly Ala Val Phe Asn Gln Asn Phe Asn Cys Ser Thr Leu Leu Pro	
355 360 365	
ccg ctg ctg aca cca ttt gtc aga agc tgg ctg gac tca gga act gac	1152
Pro Leu Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp	
370 375 380	
aga gaa gga gtc acc acc gtc acc aac tgg caa aca gag agc ttc aaa	1200
Arg Glu Gly Val Thr Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Lys	
385 390 395 400	
aca acc ttg ggc ctc cgc tgc ggc gcc ttc acg ccg cgc ggc aac agc	1248
Thr Thr Leu Gly Leu Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser	
405 410 415	
aac tac ttt cca gat tac ttc atc aga aac att tct gga gtt cct ttg	1296
Asn Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu	
420 425 430	
gtg gtg aga aat gaa gat ctg agg agg cct ctt cac tac aat gaa atc	1344
Val Val Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile	
435 440 445	
aga aac att gaa tct cca tca gga act cct gga ggc ctc cgc gcc tac	1392
Arg Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr	
450 455 460	
atg gtt tct gtt cac aac agg aag aac aac atc tat gct gct cat gaa	1440
Met Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala His Glu	
465 470 475 480	
aat gga aca atg att cat ctt gct cca gaa gat tac act ggc ttc acc	1488
Asn Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr	
485 490 495	

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atg ctg ccc atc cat gca aca caa gtc aac aac caa aca agg acc ttc 1536
Met Leu Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe
      500                      505                      510

att tca gaa aaa ttt gga aat caa gga gat tct ttg aga ttt gaa caa 1584
Ile Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln
      515                      520                      525

agc gac acc aca gca aga tac acc ttg aga gga aat gga aac agc tac 1632
Ser Asp Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr
      530                      535                      540

aac ttg tac ctc cgc gtc agc agc ttg gga aac agc acc atc agg gtg 1680
Asn Leu Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val
545                      550                      555                      560

acc atc aat gga aga gtc tac act gtt cca aat gtc aac acc aac atc 1728
Thr Ile Asn Gly Arg Val Tyr Thr Val Pro Asn Val Asn Thr Asn Ile
      565                      570                      575

aac aat gat ggt gtc att gac aat gga gca aga ttt tca gac atc aac 1776
Asn Asn Asp Gly Val Ile Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn
      580                      585                      590

att gga aat gtg gtg gct tct gac aac aca aat gtt cct ctg gac atc 1824
Ile Gly Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile
      595                      600                      605

aat gtc acc ctt tct tct gga aca caa ttt gag ctg atg aac atc atg 1872
Asn Val Thr Leu Ser Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met
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Phe Val Pro Thr Asn Leu Pro Pro Ile Tyr
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 chloroplast targeting peptide (optaxmi-076v04)

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  1                      5                      10                      15

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gca agc aaa aat caa caa gtg gcg ccg ctg gca tca agg cca gct tct	96
Ala Ser Lys Asn Gln Gln Val Ala Pro Leu Ala Ser Arg Pro Ala Ser	
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tct ctt tct gtt tct gct tct tct gtg gcg ccg gcg ccg gca tgc tct	144
Ser Leu Ser Val Ser Ala Ser Ser Val Ala Pro Ala Pro Ala Cys Ser	
35 40 45	
gct cct gct gga gct ggt aga aga gct gtg gtg gtg aga gct tct gca	192
Ala Pro Ala Gly Ala Gly Arg Arg Ala Val Val Val Arg Ala Ser Ala	
50 55 60	
aca aag gag aag gtg gag gag ctc acc atc cag atg aac aat gtt ctc	240
Thr Lys Glu Lys Val Glu Glu Leu Thr Ile Gln Met Asn Asn Val Leu	
65 70 75 80	
aac aat gga aga aca aca aac tgt gat gcc tac aat gtt gtt gct cat	288
Asn Asn Gly Arg Thr Thr Asn Cys Asp Ala Tyr Asn Val Val Ala His	
85 90 95	
gat cct ttc tca ttt gaa cac aag agc ttg gac acc atc aga aaa gaa	336
Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp Thr Ile Arg Lys Glu	
100 105 110	
tgg atg gaa tgg aaa aga aca gat cat tct ctc tat gtt gct cct gtt	384
Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu Tyr Val Ala Pro Val	
115 120 125	
gtt gga act gtt agc agc ttc ttg ctg aag aag gtt ggc agc ttg att	432
Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys Val Gly Ser Leu Ile	
130 135 140	
gga aaa agg att ctt tca gag ctc tgg ggc ttg atc ttt cct tct gga	480
Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu Ile Phe Pro Ser Gly	
145 150 155 160	
agc acc aac ttg atg caa gac atc ttg aga gaa aca gag cag ttc ttg	528
Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu Thr Glu Gln Phe Leu	
165 170 175	
aac caa agg ctc aac aca gac acc ttg gca agg gtg aat gct gag ctc	576
Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg Val Asn Ala Glu Leu	
180 185 190	
act ggc ctt caa gca aac atc aga gag ttc aac caa caa gtt gac aac	624
Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn Gln Gln Val Asp Asn	
195 200 205	
ttc ttg aat cca aca caa aat cct gtt cct ctc tcc atc act tct tca	672
Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu Ser Ile Thr Ser Ser	
210 215 220	
gtg aac acc atg cag cag ctc ttc ttg aac agg ctt cct cag ttc cag	720
Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg Leu Pro Gln Phe Gln	
225 230 235 240	

att caa gga tat cag ctg ctg ctg ctt cct ctc ttt gct caa gct gca	768
Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu Phe Ala Gln Ala Ala	
245 250 255	
aac atg cat ctc tcc ttc atc aga gat gtc atc ttg aat gct gat gaa	816
Asn Met His Leu Ser Phe Ile Arg Asp Val Ile Leu Asn Ala Asp Glu	
260 265 270	
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Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr Arg Asp Tyr Leu Arg	
275 280 285	
aac tac aca aga gat tat tca aac tac tgc atc aac aca tat caa aca	912
Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile Asn Thr Tyr Gln Thr	
290 295 300	
gct ttc aga ggc ctc aac aca agg ctt cat gac atg ctg gag ttc aga	960
Ala Phe Arg Gly Leu Asn Thr Arg Leu His Asp Met Leu Glu Phe Arg	
305 310 315 320	
aca tac atg ttc ttg aat gtt ttt gaa tat gtt tcc atc tgg agc ttg	1008
Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val Ser Ile Trp Ser Leu	
325 330 335	
ttc aag tac cag agc ttg atg gtt tct tct gga gca aat tta tat gca	1056
Phe Lys Tyr Gln Ser Leu Met Val Ser Ser Gly Ala Asn Leu Tyr Ala	
340 345 350	
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Ser Gly Ser Gly Pro Gln Gln Thr Gln Ser Phe Ile Ser Gln Asp Trp	
355 360 365	
cca ttt ctc tac agc ttg ttc caa gtg aac agc aac tat gtt ctt aat	1152
Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr Val Leu Asn	
370 375 380	
ggc ttc tct gga gca aga aac acc atc aga ttt cct gct gga gga ggc	1200
Gly Phe Ser Gly Ala Arg Asn Thr Ile Arg Phe Pro Ala Gly Gly Gly	
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Leu Leu Pro Pro Gly Val Thr Thr Thr His Ala Leu Leu Ala Ala Arg	
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Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Tyr Ile Gly Ala Val Phe	
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aac caa aac ttc aac tgc tcc acc ttg ctg ccg ccg ctg ctg aca cca	1344
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Phe Val Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg Glu Gly Val Thr	
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Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Lys Thr Thr Leu Gly Leu	
465 470 475 480	
cgc tgc ggc gcc ttc acg ccg cgc ggc aac agc aac tac ttt cca gat	1488
Arg Cys Gly Ala Phe Thr Pro Arg Gly Asn Ser Asn Tyr Phe Pro Asp	
485 490 495	
tac ttc atc aga aac att tct gga gtt cct ttg gtg gtg aga aat gaa	1536
Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val Val Arg Asn Glu	
500 505 510	
gat ctg agg agg cct ctt cac tac aat gaa atc aga aac att gaa tct	1584
Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile Arg Asn Ile Glu Ser	
515 520 525	
cca tca gga act cct gga ggc ctc cgc gcc tac atg gtt tct gtt cac	1632
Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr Met Val Ser Val His	
530 535 540	
aac agg aag aac aac atc tat gct gct cat gaa aat gga aca atg att	1680
Asn Arg Lys Asn Asn Ile Tyr Ala Ala His Glu Asn Gly Thr Met Ile	
545 550 555 560	
cat ctt gct cca gaa gat tac act ggc ttc acc atg ctg ccc atc cat	1728
His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Met Leu Pro Ile His	
565 570 575	
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Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile Ser Glu Lys Phe	
580 585 590	
gga aat caa gga gat tct ttg aga ttt gaa caa agc gac acc aca gca	1824
Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser Asp Thr Thr Ala	
595 600 605	
aga tac acc ttg aga gga aat gga aac agc tac aac ttg tac ctc cgc	1872
Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn Leu Tyr Leu Arg	
610 615 620	
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Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val Thr Ile Asn Gly Arg	
625 630 635 640	
gtc tac act gtt cca aat gtc aac acc aac atc aac aat gat ggt gtc	1968
Val Tyr Thr Val Pro Asn Val Asn Thr Asn Ile Asn Asn Asp Gly Val	
645 650 655	
att gac aat gga gca aga ttt tca gac atc aac att gga aat gtg gtg	2016
Ile Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile Gly Asn Val Val	
660 665 670	
gct tct gac aac aca aat gtt cct ctg gac atc aat gtc acc ctt tct	2064
Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile Asn Val Thr Leu Ser	
675 680 685	

tct gga aca caa ttt gag ctg atg aac atc atg ttt gtt cca aca aat 2112
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 690 695 700

ctt cct cca atc tac taa 2130
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 <213> Artificial Sequence

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 35 40 45
 Ala Pro Ala Gly Ala Gly Arg Arg Ala Val Val Val Arg Ala Ser Ala
 50 55 60
 Thr Lys Glu Lys Val Glu Glu Leu Thr Ile Gln Met Asn Asn Val Leu
 65 70 75 80
 Asn Asn Gly Arg Thr Thr Asn Cys Asp Ala Tyr Asn Val Val Ala His
 85 90 95
 Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asp Thr Ile Arg Lys Glu
 100 105 110
 Trp Met Glu Trp Lys Arg Thr Asp His Ser Leu Tyr Val Ala Pro Val
 115 120 125
 Val Gly Thr Val Ser Ser Phe Leu Leu Lys Lys Val Gly Ser Leu Ile
 130 135 140
 Gly Lys Arg Ile Leu Ser Glu Leu Trp Gly Leu Ile Phe Pro Ser Gly
 145 150 155 160
 Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu Thr Glu Gln Phe Leu
 165 170 175
 Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg Val Asn Ala Glu Leu
 180 185 190
 Thr Gly Leu Gln Ala Asn Ile Arg Glu Phe Asn Gln Gln Val Asp Asn
 195 200 205
 Phe Leu Asn Pro Thr Gln Asn Pro Val Pro Leu Ser Ile Thr Ser Ser
 210 215 220
 Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg Leu Pro Gln Phe Gln
 225 230 235 240
 Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu Phe Ala Gln Ala Ala
 245 250 255
 Asn Met His Leu Ser Phe Ile Arg Asp Val Ile Leu Asn Ala Asp Glu
 260 265 270
 Trp Gly Ile Ser Ala Ala Thr Leu Arg Thr Tyr Arg Asp Tyr Leu Arg
 275 280 285
 Asn Tyr Thr Arg Asp Tyr Ser Asn Tyr Cys Ile Asn Thr Tyr Gln Thr
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Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val	Ser	Ile	Trp	Ser	Leu
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Phe	Lys	Tyr	Gln	Ser	Leu	Met	Val	Ser	Ser	Gly	Ala	Asn	Leu	Tyr	Ala
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Ser	Gly	Ser	Gly	Pro	Gln	Gln	Thr	Gln	Ser	Phe	Ile	Ser	Gln	Asp	Trp
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Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser	Asn	Tyr	Val	Leu	Asn
	370					375					380				
Gly	Phe	Ser	Gly	Ala	Arg	Asn	Thr	Ile	Arg	Phe	Pro	Ala	Gly	Gly	Gly
385					390					395					400
Leu	Leu	Pro	Pro	Gly	Val	Thr	Thr	Thr	His	Ala	Leu	Leu	Ala	Ala	Arg
				405					410					415	
Val	Asn	Tyr	Ser	Gly	Gly	Val	Ser	Ser	Gly	Tyr	Ile	Gly	Ala	Val	Phe
			420					425					430		
Asn	Gln	Asn	Phe	Asn	Cys	Ser	Thr	Leu	Leu	Pro	Pro	Leu	Leu	Thr	Pro
		435					440					445			
Phe	Val	Arg	Ser	Trp	Leu	Asp	Ser	Gly	Thr	Asp	Arg	Glu	Gly	Val	Thr
	450					455				460					
Thr	Val	Thr	Asn	Trp	Gln	Thr	Glu	Ser	Phe	Lys	Thr	Thr	Leu	Gly	Leu
465					470					475					480
Arg	Cys	Gly	Ala	Phe	Thr	Pro	Arg	Gly	Asn	Ser	Asn	Tyr	Phe	Pro	Asp
				485					490					495	
Tyr	Phe	Ile	Arg	Asn	Ile	Ser	Gly	Val	Pro	Leu	Val	Val	Arg	Asn	Glu
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Asp	Leu	Arg	Arg	Pro	Leu	His	Tyr	Asn	Glu	Ile	Arg	Asn	Ile	Glu	Ser
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Pro	Ser	Gly	Thr	Pro	Gly	Gly	Leu	Arg	Ala	Tyr	Met	Val	Ser	Val	His
	530					535					540				
Asn	Arg	Lys	Asn	Asn	Ile	Tyr	Ala	Ala	His	Glu	Asn	Gly	Thr	Met	Ile
545					550					555					560
His	Leu	Ala	Pro	Glu	Asp	Tyr	Thr	Gly	Phe	Thr	Met	Leu	Pro	Ile	His
				565					570					575	
Ala	Thr	Gln	Val	Asn	Asn	Gln	Thr	Arg	Thr	Phe	Ile	Ser	Glu	Lys	Phe
			580					585					590		
Gly	Asn	Gln	Gly	Asp	Ser	Leu	Arg	Phe	Glu	Gln	Ser	Asp	Thr	Thr	Ala
		595					600					605			
Arg	Tyr	Thr	Leu	Arg	Gly	Asn	Gly	Asn	Ser	Tyr	Asn	Leu	Tyr	Leu	Arg
	610					615					620				
Val	Ser	Ser	Leu	Gly	Asn	Ser	Thr	Ile	Arg	Val	Thr	Ile	Asn	Gly	Arg
625					630					635					640
Val	Tyr	Thr	Val	Pro	Asn	Val	Asn	Thr	Asn	Ile	Asn	Asn	Asp	Gly	Val
				645					650					655	
Ile	Asp	Asn	Gly	Ala	Arg	Phe	Ser	Asp	Ile	Asn	Ile	Gly	Asn	Val	Val
			660					665					670		
Ala	Ser	Asp	Asn	Thr	Asn	Val	Pro	Leu	Asp	Ile	Asn	Val	Thr	Leu	Ser
		675					680					685			
Ser	Gly	Thr	Gln	Phe	Glu	Leu	Met	Asn	Ile	Met	Phe	Val	Pro	Thr	Asn
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Leu	Pro	Pro	Ile	Tyr											
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<210> 14

<211> 633

<212> PRT

<213> *Bacillus thuringiensis*

<400> 14

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Thr	Ile	Gln	Lys	Glu	Trp	Met	Glu	Trp	Lys	Arg	Thr	Asp	His	Ser	Leu
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Tyr	Val	Ala	Pro	Val	Val	Gly	Thr	Val	Ser	Ser	Phe	Leu	Leu	Lys	Lys
	50					55					60				
Val	Gly	Ser	Leu	Ile	Gly	Lys	Arg	Ile	Leu	Ser	Glu	Leu	Trp	Gly	Ile
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Ile	Phe	Pro	Ser	Gly	Ser	Thr	Asn	Leu	Met	Gln	Asp	Ile	Leu	Arg	Glu
				85					90					95	
Thr	Glu	Gln	Phe	Leu	Asn	Gln	Arg	Leu	Asn	Thr	Asp	Thr	Leu	Ala	Arg
			100					105					110		
Val	Asn	Ala	Glu	Leu	Ile	Gly	Leu	Gln	Ala	Asn	Ile	Arg	Glu	Phe	Asn
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Gln	Gln	Val	Asp	Asn	Phe	Leu	Asn	Pro	Thr	Gln	Asn	Pro	Val	Pro	Leu
	130					135					140				
Ser	Ile	Thr	Ser	Ser	Val	Asn	Thr	Met	Gln	Gln	Leu	Phe	Leu	Asn	Arg
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Leu	Pro	Gln	Phe	Gln	Ile	Gln	Gly	Tyr	Gln	Leu	Leu	Leu	Leu	Pro	Leu
				165				170						175	
Phe	Ala	Gln	Ala	Ala	Asn	Met	His	Leu	Ser	Phe	Ile	Arg	Asp	Val	Ile
			180					185					190		
Leu	Asn	Ala	Asp	Glu	Trp	Gly	Ile	Ser	Ala	Ala	Thr	Leu	Arg	Thr	Tyr
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Arg	Asp	Tyr	Leu	Arg	Asn	Tyr	Thr	Arg	Asp	Tyr	Ser	Asn	Tyr	Cys	Ile
	210					215					220				
Asn	Thr	Tyr	Gln	Thr	Ala	Phe	Arg	Gly	Leu	Asn	Thr	Arg	Leu	His	Asp
225					230					235					240
Met	Leu	Glu	Phe	Arg	Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val
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Ser	Ile	Trp	Ser	Leu	Phe	Lys	Tyr	Gln	Ser	Leu	Met	Val	Ser	Ser	Gly
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Thr	Ala	Gln	Asn	Trp	Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser
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Asn	Tyr	Ile	Leu	Ser	Gly	Ile	Ser	Gly	Thr	Arg	Leu	Ser	Ile	Thr	Phe
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Pro	Asn	Ile	Gly	Gly	Leu	Pro	Gly	Ser	Thr	Thr	Thr	His	Ser	Leu	Asn
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Ser	Ala	Arg	Val	Asn	Tyr	Ser	Gly	Gly	Val	Ser	Ser	Gly	Leu	Ile	Gly
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Ala	Thr	Asn	Leu	Asn	His	Asn	Phe	Asn	Cys	Ser	Thr	Val	Leu	Pro	Pro
		355					360					365			
Leu	Ser	Thr	Pro	Phe	Val	Arg	Ser	Trp	Leu	Asp	Ser	Gly	Thr	Asp	Arg
	370					375					380				
Glu	Gly	Val	Ala	Thr	Ser	Thr	Asn	Trp	Gln	Thr	Glu	Ser	Phe	Gln	Thr
385					390					395					400
Thr	Leu	Ser	Leu	Arg	Cys	Gly	Ala	Phe	Ser	Ala	Arg	Gly	Asn	Ser	Asn
				405					410					415	

Tyr Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val
 420 425 430
 Ile Arg Asn Glu Asp Leu Thr Arg Pro Leu His Tyr Asn Gln Ile Arg
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 Asn Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Ala Arg Ala Tyr Leu
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 Val Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Ala Asn Glu Asn
 465 470 475 480
 Gly Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile
 485 490 495
 Ser Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile
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 Ser Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser
 515 520 525
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 Leu Tyr Leu Arg Val Ser Ser Ile Gly Asn Ser Thr Ile Arg Val Thr
 545 550 555 560
 Ile Asn Gly Arg Val Tyr Thr Val Ser Asn Val Asn Thr Thr Thr Asn
 565 570 575
 Asn Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Ser Asp Ile Asn Ile
 580 585 590
 Gly Asn Ile Val Ala Ser Asp Asn Thr Asn Val Thr Leu Asp Ile Asn
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<210> 15

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<212> PRT

<213> *Bacillus thuringiensis*

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 35 40 45
 Tyr Leu Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
 50 55 60
 Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
 65 70 75 80
 Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Gln Asp Ile Leu Arg Glu
 85 90 95
 Thr Glu Lys Phe Leu Asn Gln Arg Leu Asn Thr Asp Thr Leu Ala Arg
 100 105 110
 Val Asn Ala Glu Leu Thr Gly Leu Gln Ala Asn Val Glu Glu Phe Asn
 115 120 125
 Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Arg Asn Ala Val Pro Leu
 130 135 140
 Ser Ile Thr Ser Ser Val Asn Thr Met Gln Gln Leu Phe Leu Asn Arg
 145 150 155 160

Leu	Pro	Gln	Phe	Gln	Met	Gln	Gly	Tyr	Gln	Leu	Leu	Leu	Leu	Pro	Leu	
				165					170					175		
Phe	Ala	Gln	Ala	Ala	Asn	Leu	His	Leu	Ser	Phe	Ile	Arg	Asp	Val	Ile	
			180					185					190			
Leu	Asn	Ala	Asp	Glu	Trp	Gly	Ile	Ser	Ala	Ala	Thr	Leu	Arg	Thr	Tyr	
		195				200						205				
Arg	Asp	Tyr	Leu	Lys	Asn	Tyr	Thr	Arg	Asp	Tyr	Ser	Asn	Tyr	Cys	Ile	
	210					215					220					
Asn	Thr	Tyr	Gln	Ser	Ala	Phe	Lys	Gly	Leu	Asn	Thr	Arg	Leu	His	Asp	
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Met	Leu	Glu	Phe	Arg	Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val	
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Ser	Ile	Trp	Ser	Leu	Phe	Lys	Tyr	Gln	Ser	Leu	Leu	Val	Ser	Ser	Gly	
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Ala	Asn	Leu	Tyr	Ala	Ser	Gly	Ser	Gly	Pro	Gln	Gln	Thr	Gln	Ser	Phe	
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Thr	Ser	Gln	Asp	Trp	Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser	
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Asn	Tyr	Val	Leu	Asn	Gly	Phe	Ser	Gly	Ala	Arg	Leu	Ser	Asn	Thr	Phe	
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Pro	Asn	Ile	Val	Gly	Leu	Pro	Gly	Ser	Thr	Thr	Thr	His	Ala	Leu	Leu	
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Ala	Ser	Pro	Phe	Asn	Gln	Asn	Phe	Asn	Cys	Ser	Thr	Phe	Leu	Pro	Pro	
		355				360						365				
Leu	Leu	Thr	Pro	Phe	Val	Arg	Ser	Trp	Leu	Asp	Ser	Gly	Ser	Asp	Arg	
	370					375				380						
Glu	Gly	Val	Ala	Thr	Val	Thr	Asn	Trp	Gln	Thr	Glu	Ser	Phe	Glu	Thr	
385					390					395					400	
Thr	Leu	Gly	Leu	Arg	Ser	Gly	Ala	Phe	Thr	Ala	Arg	Gly	Asn	Ser	Asn	
			405					410					415			
Tyr	Phe	Pro	Asp	Tyr	Phe	Ile	Arg	Asn	Ile	Ser	Gly	Val	Pro	Leu	Val	
			420					425					430			
Val	Arg	Asn	Glu	Asp	Leu	Arg	Arg	Pro	Leu	His	Tyr	Asn	Glu	Ile	Arg	
		435				440						445				
Asn	Ile	Ala	Ser	Pro	Ser	Gly	Thr	Pro	Gly	Gly	Ala	Arg	Ala	Tyr	Met	
	450					455					460					
Val	Ser	Val	His	Asn	Arg	Lys	Asn	Asn	Ile	His	Ala	Val	His	Glu	Asn	
465					470					475					480	
Gly	Ser	Met	Ile	His	Leu	Ala	Pro	Asn	Asp	Tyr	Thr	Gly	Phe	Thr	Ile	
			485						490					495		
Ser	Pro	Ile	His	Ala	Thr	Gln	Val	Asn	Asn	Gln	Thr	Arg	Thr	Phe	Ile	
			500					505					510			
Ser	Glu	Lys	Phe	Gly	Asn	Gln	Gly	Asp	Ser	Leu	Arg	Phe	Glu	Gln	Asn	
		515					520					525				
Asn	Thr	Thr	Ala	Arg	Tyr	Thr	Leu	Arg	Gly	Asn	Gly	Asn	Ser	Tyr	Asn	
	530					535					540					
Leu	Tyr	Leu	Arg	Val	Ser	Ser	Ile	Gly	Asn	Ser	Thr	Ile	Arg	Val	Thr	
545					550					555					560	
Ile	Asn	Gly	Arg	Val	Tyr	Thr	Ala	Thr	Asn	Val	Asn	Thr	Thr	Thr	Asn	
			565					570						575		
Asn	Asp	Gly	Val	Asn	Asp	Asn	Gly	Ala	Arg	Phe	Ser	Asp	Ile	Asn	Ile	
			580					585					590			
Gly	Asn	Val	Val	Ala	Ser	Ser	Asn	Ser	Asp	Val	Pro	Leu	Asp	Ile	Asn	
		595					600					605				

Val Thr Leu Asn Ser Gly Thr Gln Phe Asp Leu Met Asn Ile Met Leu
 610 615 620
 Val Pro Thr Asn Ile Ser Pro Leu Tyr
 625 630

<210> 16
 <211> 622
 <212> PRT
 <213> *Bacillus thuringiensis*

<400> 16
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 1 5 10 15
 Asn Val Val Ala His Asp Pro Phe Ser Phe Glu His Lys Ser Leu Asn
 20 25 30
 Thr Ile Glu Lys Glu Trp Lys Glu Trp Lys Arg Thr Asp His Ser Leu
 35 40 45
 Tyr Val Ala Pro Ile Val Gly Thr Val Gly Ser Phe Leu Leu Lys Lys
 50 55 60
 Val Gly Ser Leu Val Gly Lys Arg Ile Leu Ser Glu Leu Gln Asn Leu
 65 70 75 80
 Ile Phe Pro Ser Gly Ser Ile Asp Leu Met Gln Glu Ile Leu Arg Ala
 85 90 95
 Thr Glu Gln Phe Ile Asn Gln Arg Leu Asn Ala Asp Thr Leu Gly Arg
 100 105 110
 Val Asn Ala Glu Leu Ala Gly Leu Gln Ala Asn Val Ala Glu Phe Asn
 115 120 125
 Arg Gln Val Asp Asn Phe Leu Asn Pro Asn Gln Asn Pro Val Pro Leu
 130 135 140
 Ala Ile Ile Asp Ser Val Asn Thr Leu Gln Gln Leu Phe Leu Ser Arg
 145 150 155 160
 Leu Pro Gln Phe Gln Ile Gln Gly Tyr Gln Leu Leu Leu Leu Pro Leu
 165 170 175
 Phe Ala Gln Ala Ala Asn Phe Asn Leu Ser Phe Ile Arg Gly Val Ile
 180 185 190
 Leu Asn Ala Asp Glu Trp Gly Ile Ser Ala Ala Thr Val Arg Thr Tyr
 195 200 205
 Arg Asp His Leu Arg Lys Phe His Arg Asp Tyr Ser Asn Tyr Cys Ile
 210 215 220
 Asn Pro Tyr Gln Thr Ala Phe Arg Gly Leu Asn His Arg Leu Pro Asp
 225 230 235 240
 Met Leu Glu Phe Arg Thr Tyr Met Phe Leu Asn Val Phe Glu Tyr Val
 245 250 255
 Ser Ile Trp Ser Leu Phe Lys Tyr Gln Ser Leu Leu Val Ser Ser Gly
 260 265 270
 Ala Asn Leu Tyr Ala Ser Gly Ser Gly Pro Thr Gln Ser Phe Thr Ala
 275 280 285
 Gln Asn Trp Pro Phe Leu Tyr Ser Leu Phe Gln Val Asn Ser Asn Tyr
 290 295 300
 Val Leu Asn Gly Leu Ser Gly Ala Arg Thr Thr Ile Thr Phe Pro Asn
 305 310 315 320
 Ile Gly Gly Leu Pro Val Tyr His Asn Ser Thr Leu His Phe Ala Arg
 325 330 335
 Ile Asn Tyr Arg Gly Gly Val Ser Ser Ser Arg Ile Gly Gln Ala Asn
 340 345 350

Leu Asn Gln Asn Phe Asn Ile Ser Thr Leu Phe Asn Pro Leu Gln Thr
 355 360 365
 Pro Phe Ile Arg Ser Trp Leu Asp Ser Gly Thr Asp Arg Glu Gly Val
 370 375 380
 Ala Thr Ser Thr Asn Trp Gln Ser Gly Ala Phe Glu Thr Thr Leu Leu
 385 390 395 400
 Arg Phe Ser Ile Phe Ser Ala Arg Gly Asn Ser Asn Phe Phe Pro Asp
 405 410 415
 Tyr Phe Ile Arg Asn Ile Ser Gly Val Val Gly Thr Ile Ser Asn Ala
 420 425 430
 Asp Leu Ala Arg Pro Leu His Phe Asn Glu Ile Arg Asp Ile Gly Thr
 435 440 445
 Thr Ala Val Ala Ser Leu Val Thr Val His Asn Arg Lys Asn Asn Ile
 450 455 460
 Tyr Asp Thr His Glu Asn Gly Thr Met Ile His Leu Ala Pro Asn Asp
 465 470 475 480
 Tyr Thr Gly Phe Thr Val Ser Pro Ile His Ala Thr Gln Val Asn Asn
 485 490 495
 Gln Ile Arg Thr Phe Ile Ser Glu Lys Tyr Gly Asn Gln Gly Asp Ser
 500 505 510
 Leu Arg Phe Glu Leu Ser Asn Pro Thr Ala Arg Tyr Thr Leu Arg Gly
 515 520 525
 Asn Gly Asn Ser Tyr Asn Leu Tyr Leu Arg Val Ser Ser Ile Gly Ser
 530 535 540
 Ser Thr Ile Arg Val Thr Ile Asn Gly Arg Val Tyr Thr Ala Asn Val
 545 550 555 560
 Asn Thr Thr Thr Asn Asn Asp Gly Val Leu Asp Asn Gly Ala Arg Phe
 565 570 575
 Ser Asp Ile Asn Ile Gly Asn Val Val Ala Ser Ala Asn Thr Asn Val
 580 585 590
 Pro Leu Asp Ile Gln Val Thr Phe Asn Gly Asn Pro Gln Phe Glu Leu
 595 600 605
 Met Asn Ile Met Phe Val Pro Thr Asn Leu Pro Pro Leu Tyr
 610 615 620

<210> 17

<211> 633

<212> PRT

<213> *Bacillus thuringiensis*

<400> 17

Met Asn Ser Val Leu Asn Ser Gly Arg Asn Thr Ile Cys Asp Ala Tyr
 1 5 10 15
 Asn Val Val Val His Asp Pro Phe Ser Phe Gln His Lys Ser Leu Asp
 20 25 30
 Thr Ile Gln Lys Glu Trp Met Glu Trp Lys Lys Asp Asn His Ser Leu
 35 40 45
 Tyr Val Asp Pro Ile Val Gly Thr Val Ala Ser Phe Leu Leu Lys Lys
 50 55 60
 Leu Gly Ser Leu Ile Gly Lys Arg Ile Leu Ser Glu Leu Arg Asn Leu
 65 70 75 80
 Ile Phe Pro Ser Gly Ser Thr Asn Leu Met Glu Asp Ile Leu Arg Glu
 85 90 95
 Thr Glu Lys Phe Leu Asn Gln Lys Leu Asn Thr Asp Thr Leu Ser Arg
 100 105 110

Val	Asn	Ala	Glu	Leu	Thr	Gly	Leu	Gln	Ala	Asn	Val	Glu	Glu	Phe	Asn
		115					120					125			
Arg	Gln	Val	Asp	Asn	Phe	Leu	Asn	Pro	Asn	Arg	Asn	Ala	Val	Pro	Leu
	130					135					140				
Ser	Ile	Thr	Ser	Ser	Val	Asn	Thr	Met	Gln	Gln	Leu	Phe	Leu	Asn	Arg
145					150					155					160
Leu	Ser	Gln	Phe	Gln	Met	Gln	Gly	Tyr	Gln	Leu	Leu	Leu	Leu	Pro	Leu
				165					170					175	
Phe	Ala	Gln	Ala	Ala	Asn	Leu	His	Leu	Ser	Phe	Ile	Arg	Asp	Val	Ile
			180					185					190		
Leu	Asn	Ala	Glu	Glu	Trp	Gly	Ile	Ser	Ala	Ala	Thr	Leu	Arg	Thr	Tyr
	195					200						205			
Gln	Asn	His	Leu	Arg	Asn	Tyr	Thr	Arg	Asp	Tyr	Ser	Asn	Tyr	Cys	Ile
	210				215						220				
Asp	Thr	Tyr	Gln	Thr	Ala	Phe	Arg	Gly	Leu	Asn	Thr	Arg	Leu	His	Asp
225					230					235					240
Met	Leu	Glu	Phe	Arg	Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val
				245					250					255	
Ser	Ile	Trp	Ser	Leu	Phe	Lys	Tyr	Gln	Ser	Leu	Leu	Val	Ser	Ser	Gly
		260					265						270		
Ala	Asn	Leu	Tyr	Ala	Ser	Gly	Ser	Gly	Pro	Gln	Gln	Thr	Gln	Leu	Phe
	275					280						285			
Thr	Ser	Gln	Asp	Trp	Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser
	290					295					300				
Asn	Tyr	Val	Leu	Ser	Gly	Phe	Ser	Gly	Ala	Ser	Leu	Phe	Thr	Thr	Phe
305					310					315					320
Pro	Asn	Ile	Gly	Gly	Leu	Pro	Gly	Ser	Thr	Thr	Thr	Gln	Ala	Leu	Leu
			325					330						335	
Ala	Ala	Arg	Val	Asn	Tyr	Ser	Gly	Gly	Ile	Thr	Ser	Gly	Ser	Ile	Gly
			340					345					350		
Gly	Ser	Asn	Phe	Asn	Gln	Asn	Phe	Asn	Cys	Asn	Thr	Ile	Ser	Pro	Pro
	355					360					365				
Leu	Ser	Thr	Ser	Phe	Val	Arg	Ser	Trp	Leu	Asp	Ser	Gly	Ser	Asp	Arg
	370					375					380				
Gln	Gly	Val	Asn	Thr	Val	Thr	Asn	Trp	Gln	Thr	Glu	Ser	Phe	Glu	Thr
385					390					395					400
Thr	Ser	Gly	Leu	Arg	Cys	Gly	Ala	Phe	Thr	Pro	Arg	Gly	Asn	Ser	Asn
				405					410					415	
Tyr	Tyr	Pro	Gly	Tyr	Phe	Ile	Arg	Asn	Ile	Ser	Gly	Val	Ser	Leu	Val
		420					425						430		
Leu	Arg	Asn	Glu	Asp	Leu	Lys	Arg	Pro	Leu	Tyr	Tyr	Asn	Glu	Lys	Arg
	435					440						445			
Asn	Ile	Glu	Ser	Pro	Ser	Gly	Thr	Pro	Gly	Gly	Ala	Arg	Ala	Tyr	Met
	450					455					460				
Val	Ser	Val	His	Asn	Lys	Lys	Asn	Asn	Ile	Tyr	Ala	Val	His	Glu	Asn
465					470					475					480
Gly	Thr	Met	Ile	His	Leu	Ala	Pro	Glu	Asp	Asn	Thr	Gly	Phe	Thr	Ile
				485					490					495	
Ser	Pro	Ile	His	Ala	Thr	Gln	Val	Asn	Asn	Gln	Thr	Arg	Thr	Phe	Ile
			500					505					510		
Ser	Glu	Lys	Phe	Gly	Asn	Gln	Gly	Asp	Ser	Leu	Arg	Phe	Glu	Gln	Ser
	515					520						525			
Asn	Thr	Thr	Ala	Arg	Tyr	Thr	Leu	Arg	Gly	Asn	Gly	Asn	Ser	Tyr	Asn
	530					535					540				
Leu	Tyr	Leu	Arg	Val	Ser	Ser	Ile	Gly	Asn	Ser	Thr	Ile	Arg	Val	Thr
545					550				555						560

[illegible]

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<210> 18
<211> 632
<212> PRT
<213> Bacillus thuringiensis
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<400> 18																
Met	Asn	Asn	Val	Leu	Asn	Asn	Gly	Arg	Thr	Thr	Ile	Cys	Asp	Ala	Tyr	
1				5					10					15		
Asn	Val	Val	Ala	His	Asp	Pro	Phe	Ser	Phe	Glu	His	Lys	Ser	Leu	Asp	
			20					25					30			
Thr	Ile	Arg	Lys	Glu	Trp	Met	Glu	Trp	Lys	Arg	Thr	Asp	His	Ser	Leu	
		35					40					45				
Tyr	Val	Ala	Pro	Ile	Val	Gly	Thr	Val	Ser	Ser	Phe	Leu	Leu	Lys	Lys	
	50					55					60					
Val	Gly	Ser	Leu	Ile	Gly	Lys	Arg	Ile	Leu	Ser	Glu	Leu	Trp	Gly	Leu	
65					70					75					80	
Ile	Phe	Pro	Ser	Gly	Ser	Thr	Asn	Leu	Met	Gln	Asp	Ile	Leu	Arg	Glu	
				85					90					95		
Thr	Glu	Gln	Phe	Leu	Asn	Gln	Arg	Leu	Asn	Thr	Asp	Thr	Leu	Ala	Arg	
			100					105					110			
Val	Asn	Ala	Glu	Leu	Glu	Gly	Leu	Gln	Ala	Asn	Ile	Arg	Glu	Phe	Asn	
		115					120					125				
Gln	Gln	Val	Asp	Asn	Phe	Leu	Asn	Pro	Thr	Gln	Asn	Pro	Val	Pro	Leu	
	130					135					140					
Ser	Ile	Thr	Ser	Ser	Val	Asn	Thr	Met	Gln	Gln	Leu	Phe	Leu	Asn	Arg	
145				150					155						160	
Leu	Pro	Gln	Phe	Arg	Val	Gln	Gly	Tyr	Gln	Leu	Leu	Leu	Leu	Pro	Leu	
				165					170					175		
Phe	Ala	Gln	Ala	Ala	Asn	Met	His	Leu	Ser	Phe	Ile	Arg	Asp	Val	Val	
			180				185						190			
Leu	Asn	Ala	Asp	Glu	Trp	Gly	Ile	Ser	Ala	Ala	Thr	Leu	Arg	Thr	Tyr	
		195				200						205				
Gln	Asn	Tyr	Leu	Lys	Asn	Tyr	Thr	Thr	Glu	Tyr	Ser	Asn	Tyr	Cys	Ile	
	210					215					220					
Asn	Thr	Tyr	Gln	Thr	Ala	Phe	Arg	Gly	Leu	Asn	Thr	Arg	Leu	His	Asp	
225					230					235					240	
Met	Leu	Glu	Phe	Arg	Thr	Tyr	Met	Phe	Leu	Asn	Val	Phe	Glu	Tyr	Val	
				245					250					255		
Ser	Ile	Trp	Ser	Leu	Phe	Lys	Tyr	Gln	Ser	Leu	Leu	Val	Ser	Ser	Gly	
			260					265					270			
Ala	Asn	Leu	Tyr	Ala	Ser	Gly	Ser	Gly	Pro	Gln	Gln	Thr	Gln	Ser	Phe	
		275					280					285				
Thr	Ser	Gln	Asp	Trp	Pro	Phe	Leu	Tyr	Ser	Leu	Phe	Gln	Val	Asn	Ser	
	290					295						300				

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Asn Tyr Val Leu Asn Gly Phe Ser Gly Ala Arg Leu Thr Gln Thr Phe
305                      310                      315                      320
Pro Asn Ile Gly Gly Leu Pro Gly Thr Thr Thr Thr His Ala Leu Leu
                      325                      330                      335
Ala Ala Arg Val Asn Tyr Ser Gly Gly Val Ser Ser Gly Asp Ile Gly
                      340                      345                      350
Ala Val Phe Asn Gln Asn Phe Ser Cys Ser Thr Phe Leu Pro Pro Leu
                      355                      360                      365
Leu Thr Pro Phe Val Arg Ser Trp Leu Asp Ser Gly Ser Asp Arg Gly
                      370                      375                      380
Gly Val Asn Thr Val Thr Asn Trp Gln Thr Glu Ser Phe Glu Ser Thr
385                      390                      395                      400
Leu Gly Leu Arg Cys Gly Ala Phe Thr Ala Arg Gly Asn Ser Asn Tyr
                      405                      410                      415
Phe Pro Asp Tyr Phe Ile Arg Asn Ile Ser Gly Val Pro Leu Val Val
                      420                      425                      430
Arg Asn Glu Asp Leu Arg Arg Pro Leu His Tyr Asn Glu Ile Arg Asn
                      435                      440                      445
Ile Glu Ser Pro Ser Gly Thr Pro Gly Gly Leu Arg Ala Tyr Met Val
                      450                      455                      460
Ser Val His Asn Arg Lys Asn Asn Ile Tyr Ala Val His Glu Asn Gly
465                      470                      475                      480
Thr Met Ile His Leu Ala Pro Glu Asp Tyr Thr Gly Phe Thr Ile Ser
                      485                      490                      495
Pro Ile His Ala Thr Gln Val Asn Asn Gln Thr Arg Thr Phe Ile Ser
                      500                      505                      510
Glu Lys Phe Gly Asn Gln Gly Asp Ser Leu Arg Phe Glu Gln Ser Asn
                      515                      520                      525
Thr Thr Ala Arg Tyr Thr Leu Arg Gly Asn Gly Asn Ser Tyr Asn Leu
                      530                      535                      540
Tyr Leu Arg Val Ser Ser Leu Gly Asn Ser Thr Ile Arg Val Thr Ile
545                      550                      555                      560
Asn Gly Arg Val Tyr Thr Ala Ser Asn Val Asn Thr Thr Thr Asn Asn
                      565                      570                      575
Asp Gly Val Asn Asp Asn Gly Ala Arg Phe Leu Asp Ile Asn Met Gly
                      580                      585                      590
Asn Val Val Ala Ser Asp Asn Thr Asn Val Pro Leu Asp Ile Asn Val
                      595                      600                      605
Thr Phe Asn Ser Gly Thr Gln Phe Glu Leu Met Asn Ile Met Phe Val
                      610                      615                      620
Pro Thr Asn Leu Pro Pro Ile Tyr
625                      630

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<210> 19

<211> 1178

<212> PRT

<213> *Bacillus thuringiensis*

<400> 19

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Met Asp Asn Asn Pro Asn Ile Asn Glu Cys Ile Pro Tyr Asn Cys Leu
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Ser Asn Pro Glu Val Glu Val Leu Gly Gly Glu Arg Ile Glu Thr Gly
                      20                      25                      30
Tyr Thr Pro Ile Asp Ile Ser Leu Ser Leu Thr Gln Phe Leu Leu Ser
                      35                      40                      45

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Glu	Phe	Val	Pro	Gly	Ala	Gly	Phe	Val	Leu	Gly	Leu	Val	Asp	Ile	Ile	50	55	60
Trp	Gly	Ile	Phe	Gly	Pro	Ser	Gln	Trp	Asp	Ala	Phe	Leu	Val	Gln	Ile	65	70	75
Glu	Gln	Leu	Ile	Asn	Gln	Arg	Ile	Glu	Glu	Phe	Ala	Arg	Asn	Gln	Ala	85	90	95
Ile	Ser	Arg	Leu	Glu	Gly	Leu	Ser	Asn	Leu	Tyr	Gln	Ile	Tyr	Ala	Glu	100	105	110
Ser	Phe	Arg	Glu	Trp	Glu	Ala	Asp	Pro	Thr	Asn	Pro	Ala	Leu	Arg	Glu	115	120	125
Glu	Met	Arg	Ile	Gln	Phe	Asn	Asp	Met	Asn	Ser	Ala	Leu	Thr	Thr	Ala	130	135	140
Ile	Pro	Leu	Phe	Ala	Val	Gln	Asn	Tyr	Gln	Val	Pro	Leu	Leu	Ser	Val	145	150	155
Tyr	Val	Gln	Ala	Ala	Asn	Leu	His	Leu	Ser	Val	Leu	Arg	Asp	Val	Ser	165	170	175
Val	Phe	Gly	Gln	Arg	Trp	Gly	Phe	Asp	Ala	Ala	Thr	Ile	Asn	Ser	Arg	180	185	190
Tyr	Asn	Asp	Leu	Thr	Arg	Leu	Ile	Gly	Asn	Tyr	Thr	Asp	Tyr	Ala	Val	195	200	205
Arg	Trp	Tyr	Asn	Thr	Gly	Leu	Glu	Arg	Val	Trp	Gly	Pro	Asp	Ser	Arg	210	215	220
Asp	Trp	Val	Arg	Tyr	Asn	Gln	Phe	Arg	Arg	Glu	Leu	Thr	Leu	Thr	Val	225	230	235
Leu	Asp	Ile	Val	Ala	Leu	Phe	Pro	Asn	Tyr	Asp	Ser	Arg	Arg	Tyr	Pro	245	250	255
Ile	Arg	Thr	Val	Ser	Gln	Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asn	Pro	Val	260	265	270
Leu	Glu	Asn	Phe	Asp	Gly	Ser	Phe	Arg	Gly	Ser	Ala	Gln	Gly	Ile	Glu	275	280	285
Arg	Ser	Ile	Arg	Ser	Pro	His	Leu	Met	Asp	Ile	Leu	Asn	Ser	Ile	Thr	290	295	300
Ile	Tyr	Thr	Asp	Ala	His	Arg	Gly	Tyr	Tyr	Tyr	Trp	Ser	Gly	His	Gln	305	310	315
Ile	Met	Ala	Ser	Pro	Val	Gly	Phe	Ser	Gly	Pro	Glu	Phe	Thr	Phe	Pro	325	330	335
Leu	Tyr	Gly	Thr	Met	Gly	Asn	Ala	Ala	Pro	Gln	Gln	Arg	Ile	Val	Ala	340	345	350
Gln	Leu	Gly	Gln	Gly	Val	Tyr	Arg	Thr	Leu	Ser	Ser	Thr	Leu	Tyr	Arg	355	360	365
Arg	Pro	Phe	Asn	Ile	Gly	Ile	Asn	Asn	Gln	Gln	Leu	Ser	Val	Leu	Asp	370	375	380
Gly	Thr	Glu	Phe	Ala	Tyr	Gly	Thr	Ser	Ser	Asn	Leu	Pro	Ser	Ala	Val	385	390	395
Tyr	Arg	Lys	Ser	Gly	Thr	Val	Asp	Ser	Leu	Asp	Glu	Ile	Pro	Pro	Gln	405	410	415
Asn	Asn	Asn	Val	Pro	Pro	Arg	Gln	Gly	Phe	Ser	His	Arg	Leu	Ser	His	420	425	430
Val	Ser	Met	Phe	Arg	Ser	Gly	Phe	Ser	Asn	Ser	Ser	Val	Ser	Ile	Ile	435	440	445
Arg	Ala	Pro	Met	Phe	Ser	Trp	Ile	His	Arg	Ser	Ala	Glu	Phe	Asn	Asn	450	455	460
Ile	Ile	Ala	Ser	Asp	Ser	Ile	Thr	Gln	Ile	Pro	Ala	Val	Lys	Gly	Asn	465	470	475
Phe	Leu	Phe	Asn	Gly	Ser	Val	Ile	Ser	Gly	Pro	Gly	Phe	Thr	Gly	Gly	485	490	495

Asp	Leu	Val	Arg	Leu	Asn	Ser	Ser	Gly	Asn	Asn	Ile	Gln	Asn	Arg	Gly	
			500					505					510			
Tyr	Ile	Glu	Val	Pro	Ile	His	Phe	Pro	Ser	Thr	Ser	Thr	Arg	Tyr	Arg	
		515					520						525			
Val	Arg	Val	Arg	Tyr	Ala	Ser	Val	Thr	Pro	Ile	His	Leu	Asn	Val	Asn	
		530				535					540					
Trp	Gly	Asn	Ser	Ser	Ile	Phe	Ser	Asn	Thr	Val	Pro	Ala	Thr	Ala	Thr	
545					550					555					560	
Ser	Leu	Asp	Asn	Leu	Gln	Ser	Ser	Asp	Phe	Gly	Tyr	Phe	Glu	Ser	Ala	
				565					570					575		
Asn	Ala	Phe	Thr	Ser	Ser	Leu	Gly	Asn	Ile	Val	Gly	Val	Arg	Asn	Phe	
			580					585					590			
Ser	Gly	Thr	Ala	Gly	Val	Ile	Ile	Asp	Arg	Phe	Glu	Phe	Ile	Pro	Val	
		595					600					605				
Thr	Ala	Thr	Leu	Glu	Ala	Glu	Tyr	Asn	Leu	Glu	Arg	Ala	Gln	Lys	Ala	
		610				615						620				
Val	Asn	Ala	Leu	Phe	Thr	Ser	Thr	Asn	Gln	Leu	Gly	Leu	Lys	Thr	Asn	
625					630					635					640	
Val	Thr	Asp	Tyr	His	Ile	Asp	Gln	Val	Ser	Asn	Leu	Val	Thr	Tyr	Leu	
				645					650					655		
Ser	Asp	Glu	Phe	Cys	Leu	Asp	Glu	Lys	Arg	Glu	Leu	Ser	Glu	Lys	Val	
			660					665					670			
Lys	His	Ala	Lys	Arg	Leu	Ser	Asp	Glu	Arg	Asn	Leu	Leu	Gln	Asp	Ser	
		675					680						685			
Asn	Phe	Lys	Asp	Ile	Asn	Arg	Gln	Pro	Glu	Arg	Gly	Trp	Gly	Gly	Ser	
		690				695					700					
Thr	Gly	Ile	Thr	Ile	Gln	Gly	Gly	Asp	Asp	Val	Phe	Lys	Glu	Asn	Tyr	
705					710					715					720	
Val	Thr	Leu	Ser	Gly	Thr	Phe	Asp	Glu	Cys	Tyr	Pro	Thr	Tyr	Leu	Tyr	
				725					730					735		
Gln	Lys	Ile	Asp	Glu	Ser	Lys	Leu	Lys	Ala	Phe	Thr	Arg	Tyr	Gln	Leu	
			740					745					750			
Arg	Gly	Tyr	Ile	Glu	Asp	Ser	Gln	Asp	Leu	Glu	Ile	Tyr	Leu	Ile	Arg	
		755					760					765				
Tyr	Asn	Ala	Lys	His	Glu	Thr	Val	Asn	Val	Pro	Gly	Thr	Gly	Ser	Leu	
		770				775					780					
Trp	Pro	Leu	Ser	Ala	Gln	Ser	Pro	Ile	Gly	Lys	Cys	Gly	Glu	Pro	Asn	
785					790					795					800	
Arg	Cys	Ala	Pro	His	Leu	Glu	Trp	Asn	Pro	Asp	Leu	Asp	Cys	Ser	Cys	
				805					810					815		
Arg	Asp	Gly	Glu	Lys	Cys	Ala	His	His	Ser	His	His	Phe	Ser	Leu	Asp	
			820					825					830			
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		835					840					845				
Ile	Phe	Lys	Ile	Lys	Thr	Gln	Asp	Gly	His	Ala	Arg	Leu	Gly	Asn	Leu	
		850				855					860					
Glu	Phe	Leu	Glu	Glu	Lys	Pro	Leu	Val	Gly	Glu	Ala	Leu	Ala	Arg	Val	
865					870					875					880	
Lys	Arg	Ala	Glu	Lys	Lys	Trp	Arg	Asp	Lys	Arg	Glu	Lys	Leu	Glu	Trp	
				885					890					895		
Glu	Thr	Asn	Ile	Val	Tyr	Lys	Glu	Ala	Lys	Glu	Ser	Val	Asp	Ala	Leu	
			900					905					910			
Phe	Val	Asn	Ser	Gln	Tyr	Asp	Gln	Leu	Gln	Ala	Asp	Thr	Asn	Ile	Ala	
		915					920					925				
Met	Ile	His	Ala	Ala	Asp	Lys	Arg	Val	His	Ser	Ile	Arg	Glu	Ala	Tyr	
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Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala Ile Phe Glu
 945 950 955 960
 Glu Leu Glu Gly Arg Ile Phe Thr Ala Phe Ser Leu Tyr Asp Ala Arg
 965 970 975
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 980 985 990
 Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn Gln Arg Ser Val
 995 1000 1005
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 1025 1030 1035 1040
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 20 25 30
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 35 40 45
 Asn Tyr Lys Glu Phe Leu Arg Met Thr Ala Asp Asn Asn Thr Glu Ala
 50 55 60
 Leu Asp Ser Ser Thr Thr Lys Asp Val Ile Gln Lys Gly Ile Ser Val
 65 70 75 80
 Val Gly Asp Leu Leu Gly Val Val Gly Phe Pro Phe Gly Gly Ala Leu
 85 90 95
 Val Ser Phe Tyr Thr Asn Phe Leu Asn Thr Ile Trp Pro Ser Glu Asp
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 Pro Trp Lys Ala Phe Met Glu Gln Val Glu Ala Leu Met Asp Gln Lys
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 Ile Ala Asp Tyr Ala Lys Asn Lys Ala Leu Ala Glu Leu Gln Gly Leu
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Gln	Asn	Asn	Val	Glu	Asp	Tyr	Val	Ser	Ala	Leu	Ser	Ser	Trp	Gln	Lys
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Leu	Phe	Ser	Gln	Ala	Glu	Ser	His	Phe	Arg	Asn	Ser	Met	Pro	Ser	Phe
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Ala	Ile	Ser	Gly	Tyr	Glu	Val	Leu	Phe	Leu	Thr	Thr	Tyr	Ala	Gln	Ala
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Ala	Asn	Thr	His	Leu	Phe	Leu	Leu	Lys	Asp	Ala	Gln	Ile	Tyr	Gly	Glu
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Glu	Trp	Gly	Tyr	Glu	Lys	Glu	Asp	Ile	Ala	Glu	Phe	Tyr	Lys	Arg	Gln
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Val	Gly	Leu	Asp	Lys	Leu	Arg	Gly	Ser	Ser	Tyr	Glu	Ser	Trp	Val	Asn
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Phe	Asn	Arg	Tyr	Arg	Arg	Glu	Met	Thr	Leu	Thr	Val	Leu	Asp	Leu	Ile
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Thr	Glu	Leu	Thr	Arg	Asp	Val	Leu	Thr	Asp	Pro	Ile	Val	Gly	Val	Asn
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Asn	Leu	Arg	Gly	Tyr	Gly	Thr	Thr	Phe	Ser	Asn	Ile	Glu	Asn	Tyr	Ile
			325					330						335	
Arg	Lys	Pro	His	Leu	Phe	Asp	Tyr	Leu	His	Arg	Ile	Gln	Phe	His	Thr
			340					345					350		
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Gly	Asn	Tyr	Val	Ser	Thr	Arg	Pro	Ser	Ile	Gly	Ser	Asn	Asp	Ile	Ile
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Thr	Ser	Pro	Phe	Tyr	Gly	Asn	Lys	Ser	Ser	Glu	Pro	Val	Gln	Asn	Leu
385					390					395					400
Glu	Phe	Asn	Gly	Glu	Lys	Val	Tyr	Arg	Ala	Val	Ala	Asn	Thr	Asn	Leu
			405					410						415	
Ala	Val	Trp	Pro	Ser	Ala	Val	Tyr	Ser	Gly	Val	Thr	Lys	Val	Glu	Phe
			420					425					430		
Ser	Gln	Tyr	Asn	Asp	Gln	Thr	Asp	Glu	Ala	Ser	Thr	Gln	Thr	Tyr	Asp
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Ser	Lys	Arg	Asn	Val	Gly	Ala	Val	Ser	Trp	Asp	Ser	Ile	Asp	Gln	Leu
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Pro	Pro	Glu	Thr	Thr	Asp	Glu	Pro	Leu	Glu	Lys	Gly	Tyr	Ser	His	Gln
465					470					475					480
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			485					490						495	
Pro	Val	Leu	Thr	Trp	Thr	His	Lys	Ser	Val	Asp	Phe	Phe	Asn	Met	Ile
			500					505					510		
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Gln	Ser	Gly	Ala	Ser	Val	Val	Ala	Gly	Pro	Arg	Phe	Thr	Gly	Gly	Asp
	530					535					540				
Ile	Ile	Gln	Cys	Thr	Glu	Asn	Gly	Ser	Ala	Ala	Thr	Ile	Tyr	Val	Thr
545					550					555					560
Pro	Asp	Val	Ser	Tyr	Ser	Gln	Lys	Tyr	Arg	Ala	Arg	Ile	His	Tyr	Ala
			565					570						575	
Ser	Thr	Ser	Gln	Ile	Thr	Phe	Thr	Leu	Ser	Leu	Asp	Gly	Ala	Pro	Phe
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Asn Gln Tyr Tyr Phe Asp Lys Thr Ile Asn Lys Gly Asp Thr Leu Thr
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 Tyr Asn Ser Phe Asn Leu Ala Ser Phe Ser Thr Pro Phe Glu Leu Ser
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35

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THAT WHICH IS CLAIMED:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an amino acid sequence having pesticidal activity, wherein said nucleotide sequence is selected from the group consisting of:
 - a) the nucleotide sequence set forth in SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
 - b) a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence of SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
 - c) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:4;
 - d) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 2, or 10;
 - e) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:2 or 10;
 - f) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:5; and,
 - g) the nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. B-50045.

2. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:10, and wherein said amino acid sequence comprises an insertion of one or two amino acids between residues 379 and 380 of SEQ ID NO:10.

3. The nucleic acid molecule of claim 2, wherein said amino acid sequence comprises an insertion of two amino acids between residues 379 and 380 of SEQ ID NO:10, and wherein said insertion is selected from the group consisting of a glycine and a glycine, a glycine and a threonine, a glycine and a serine, a glycine and a leucine, an arginine and a glycine, a glycine and an asparagine, a glycine and a lysine, a histidine and a glycine, a phenylalanine and a glycine, a leucine and a glycine, and an asparagine and a glycine residue.

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4. The isolated nucleic acid molecule of claim 1, wherein said nucleotide sequence is a synthetic sequence that has been designed for expression in a plant.
5. A vector comprising the nucleic acid molecule of claim 1.
6. The vector of claim 5, further comprising a nucleic acid molecule encoding a heterologous polypeptide.
7. A host cell that contains the vector of claim 5.
8. The host cell of claim 7 that is a bacterial host cell.
9. The host cell of claim 7 that is a plant cell.
10. A transgenic plant comprising the host cell of claim 9.
11. The transgenic plant of claim 10, wherein said plant is selected from the group consisting of maize, sorghum, wheat, cabbage, sunflower, tomato, crucifers, peppers, potato, cotton, rice, soybean, sugarbeet, sugarcane, tobacco, barley, and oilseed rape.
12. A transgenic seed comprising the nucleic acid molecule of claim 1.
13. An isolated polypeptide with pesticidal activity, selected from the group consisting of:
 - a) a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 2, or 10;
 - b) a polypeptide comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:2 or 10;
 - c) a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:5;
 - d) a polypeptide that is encoded by SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;

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e) a polypeptide that is encoded by a nucleotide sequence that is at least 90% identical to the nucleotide sequence of SEQ ID NO:9, 1, 3, 6, or 11;

f) a polypeptide that is encoded by a nucleotide sequence that is at least 95% identical to the nucleotide sequence of SEQ ID NO:4; and,

5 f) a polypeptide encoded by the nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. B-50045.

14. The polypeptide of claim 13, wherein said polypeptide comprises a sequence having at least 90% sequence identity to the amino acid sequence set forth in
10 SEQ ID NO:10, and wherein said amino acid sequence comprises an insertion of one or two amino acids between residues 379 and 380 of SEQ ID NO:10.

15. The polypeptide of claim 14, wherein said amino acid sequence comprises an insertion of two amino acids between residues 379 and 380 of SEQ ID NO:10, and
15 wherein said insertion is selected from the group consisting of a glycine and a glycine, a glycine and a threonine, a glycine and a serine, a glycine and a leucine, an arginine and a glycine, a glycine and an asparagine, a glycine and a lysine, a histidine and a glycine, a phenylalanine and a glycine, a leucine and a glycine, and an asparagine and a glycine residue.

20

16. The polypeptide of claim 13 further comprising heterologous amino acid sequences.

17. A composition comprising the polypeptide of claim 13.

25

18. The composition of claim 17, wherein said composition is selected from the group consisting of a powder, dust, pellet, granule, spray, emulsion, colloid, and solution.

30 19. The composition of claim 17, wherein said composition is prepared by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of bacterial cells.

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20. The composition of claim 17, comprising from about 1% to about 99% by weight of said polypeptide.

21. A method for controlling a lepidopteran, coleopteran, nematode, or dipteran pest population comprising contacting said population with a pesticidally-effective amount of a polypeptide of claim 13.

22. A method for killing a lepidopteran, coleopteran, nematode, or dipteran pest, comprising contacting said pest with, or feeding to said pest, a pesticidally-effective amount of a polypeptide of claim 13.

23. A method for producing a polypeptide with pesticidal activity, comprising culturing the host cell of claim 6 under conditions in which the nucleic acid molecule encoding the polypeptide is expressed.

15

24. A plant having stably incorporated into its genome a DNA construct comprising a nucleotide sequence that encodes a protein having pesticidal activity, wherein said nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
- b) a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence of SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
- c) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:4;
- d) a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:5, 2, or 10;
- e) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:2 or 10;
- f) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:5; and,
- g) the nucleotide sequence of the DNA insert of the plasmid deposited as Accession No. B-50045;

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wherein said nucleotide sequence is operably linked to a promoter that drives expression of a coding sequence in a plant cell.

25. The plant of claim 24, wherein said nucleic acid molecule encodes a
5 sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:10, and wherein said amino acid sequence comprises an insertion of one or two amino acids between residues 379 and 380 of SEQ ID NO:10.

26. The plant of claim 25, wherein said amino acid sequence comprises an
10 insertion of two amino acids between residues 379 and 380 of SEQ ID NO:10, and wherein said insertion is selected from the group consisting of a glycine and a glycine, a glycine and a threonine, a glycine and a serine, a glycine and a leucine, an arginine and a glycine, a glycine and an asparagine, a glycine and a lysine, a histidine and a glycine, a phenylalanine and a glycine, a leucine and a glycine, and an asparagine and a glycine
15 residue.

27. The plant of claim 24, wherein said plant is a plant cell.

28. A method for protecting a plant from a pest, comprising introducing into
20 said plant or cell thereof at least one expression vector comprising a nucleotide sequence that encodes a pesticidal polypeptide, wherein said nucleotide sequence is selected from the group consisting of:

- a) the nucleotide sequence set forth in SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
- 25 b) a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence of SEQ ID NO:4, 1, 3, 4, 6, 9, or 11;
- c) a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence of SEQ ID NO:4;
- d) a nucleotide sequence that encodes a polypeptide comprising the
30 amino acid sequence of SEQ ID NO:5, 2, or 10;
- e) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:2 or 10;

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- f) a nucleotide sequence that encodes a polypeptide comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:5; and,
- g) the nucleotide sequence of the DNA insert of the plasmid deposited
5 as Accession No. B-50045.

29. The method of claim 28, wherein said plant produces a pesticidal polypeptide having pesticidal activity against a lepidopteran, coleopteran, nematode, or dipteran pest.

10

AGACAGGTTCTTAAACATACTTGTTATTATCAAGAGAGTATTGTCTCTTTA**ATG**TATTATTATATAACCC
AATATTTCAAGGAGGATTTTAT**ATGA**ATAATGTATTGAATAGCGAAAGAACTAATAAGTGTGATGCGT
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GTTTCTGAGGTAAATATA

FIG. 1

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Axmi066-lo	:	----	MLLYNPIFQGGFY	ADNVVAD	SEINK	DAVWVAHDP	SEFEHKS	LDTHQ	QEDM	QEDR	DHSLVS	PIVGT	:	69
Axmi-066	:	----	----	----	----	----	----	----	----	----	----	----	:	56
Cry2Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	56
Cry2Ab1	:	----	----	----	----	----	----	----	----	----	----	----	:	56
Cry2Ac1	:	----	----	----	----	----	----	----	----	----	----	----	:	56
Cry2Ad1	:	----	----	----	----	----	----	----	----	----	----	----	:	56
Cry2Ae1	:	----	----	----	----	----	----	----	----	----	----	----	:	56
cry1Ac	:	----	----	----	----	----	----	----	----	----	----	----	:	41
cry3Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	75
Axmi066-lo	:	80	100	120	140	*							:	139
Axmi-066	:	----	----	----	----	----	----	----	----	----	----	----	:	126
Cry2Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	126
Cry2Ab1	:	----	----	----	----	----	----	----	----	----	----	----	:	126
Cry2Ac1	:	----	----	----	----	----	----	----	----	----	----	----	:	126
Cry2Ad1	:	----	----	----	----	----	----	----	----	----	----	----	:	126
Cry2Ae1	:	----	----	----	----	----	----	----	----	----	----	----	:	126
cry1Ac	:	----	----	----	----	----	----	----	----	----	----	----	:	109
cry3Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	150
Axmi066-lo	:	160	180	200	220	*							:	210
Axmi-066	:	----	----	----	----	----	----	----	----	----	----	----	:	197
Cry2Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	197
Cry2Ab1	:	----	----	----	----	----	----	----	----	----	----	----	:	197
Cry2Ac1	:	----	----	----	----	----	----	----	----	----	----	----	:	197
Cry2Ad1	:	----	----	----	----	----	----	----	----	----	----	----	:	197
Cry2Ae1	:	----	----	----	----	----	----	----	----	----	----	----	:	197
cry1Ac	:	----	----	----	----	----	----	----	----	----	----	----	:	181
cry3Aa1	:	----	----	----	----	----	----	----	----	----	----	----	:	225

FIG. 2A
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Axmi066-10	MCIPAAIVKTYREHDKPVRNATSMNCIMVQTATFRGLM	TRHEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	281
Axmi-066	MCIPAAIVKTYREHDKPVRNATSMNCIMVQTATFRGLM	TRHEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
Cry2Aa1	MCISAAITLRTYRDYMARNTQDTSMNCIMVQTATFRGLM	TRLEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
Cry2Ab1	MCISAAITLRTYRDYMARNTQDTSMNCIMVQTATFRGLM	TRLEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
Cry2Ac1	MCISAAITLRTYRDYMARNTQDTSMNCIMVQTATFRGLM	TRLEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
Cry2Ad1	MCISAAITLRTYRDYMARNTQDTSMNCIMVQTATFRGLM	TRLEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
Cry2Ae1	MCISAAITLRTYRDYMARNTQDTSMNCIMVQTATFRGLM	TRLEDHNEFRFETARINWLDVSTIUSLFRKQSSAM	268
cry1Ac	MCFDAATINSTRYNDLIRLIGVITDAWRWANNIGLERWGPDSRDVVRNQHRELLTLDLINALFPNWDSTRYP		256
cry3Aa1	MEYERKEDLAEYKRQLKLIQEFNTHGKQWNVGLDKRGSSYE SUVQFNREHRENTLTDLDLHALFPNWDVRLYP		300
Axmi066-10	WPS SAAILKASEGES	WPS SAAILKASEGES	345
Axmi-066	WPS SAAILKASEGES	WPS SAAILKASEGES	332
Cry2Aa1	WSS SAAILKASEGES	WSS SAAILKASEGES	336
Cry2Ab1	WSS SAAILKASEGES	WSS SAAILKASEGES	336
Cry2Ac1	WSS SAAILKASEGES	WSS SAAILKASEGES	333
Cry2Ad1	WSS SAAILKASEGES	WSS SAAILKASEGES	336
Cry2Ae1	WSS SAAILKASEGES	WSS SAAILKASEGES	336
cry1Ac	WPSVVSQITREIYINPV-LENQDGSFRGSAQGIERSIRSPHEMDILN--SITLITDAHRCYY-----YUSGHOIM		322
cry3Aa1	KEVKTELITDVLTDPIVGANNLRGYGTTFSNIENYIRKPHLFDYLRH--IQPHIRFQPGYGNDSFNYUSGNYGS		373
Axmi066-10	SPRNNVTGKVS	SPRNNVTGKVS	415
Axmi-066	SPRNNVTGKVS	SPRNNVTGKVS	402
Cry2Aa1	SARNNVSGEVS	SARNNVSGEVS	411
Cry2Ab1	SARNNVSGEVS	SARNNVSGEVS	411
Cry2Ac1	SARNNVSGEVS	SARNNVSGEVS	407
Cry2Ad1	SARNNVSGEVS	SARNNVSGEVS	411
Cry2Ae1	SARNNVSGEVS	SARNNVSGEVS	410
cry1Ac	ASPNGESEPFIFFPYGIMGNAAPQQR-IVAQIGQGVYKILSSILYRSPFNIGINNOQLSVLDGDEFAYGTSSNL		396
cry3Aa1	TRPSIGSNDIIISPFYGNKSSPEVQNLDFNCKEKVYRAVANTINLAVWPSAVYSGVTKVDFSQNDQIDEASTQTYD		448

FIG. 2B

Axm1066-10	: SLAMF-PDVKSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ	: 460	*	480	*	500	*	520	: 483
Axm1-066	: SLAMF-PDVKSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 470
Cry2Aa1	: -----RGHSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 479
Cry2Ab1	: -----RGHSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 479
Cry2Ac1	: -----RGHSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 469
Cry2Ad1	: -----RGHSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 479
Cry2Ae1	: -----RGHSNFFDVFIRNISGIRNMDHESRPLFHEVDDPRDT-----EVAITLVSVHNRKCHIVAARQ								: 478
Cry1Ac	: PSAYRKSGTVSLDEIPQANVPPROGFSHRSHVSHRSRSGFSNSVSIIRAPHFSWDRSAEFMIIASDSI								: 471
Cry3Aa1	: SKR-NVGAVSLSIDQLPETIDEPLEKGYSHQANVYCHLHQGSRGILP-----VLTTHKSYQFPNIDSKKI								: 517
Axm1066-10	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ	: 540	*	560	*	580	*	600	: 556
Axm1-066	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 543
Cry2Aa1	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 552
Cry2Ab1	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 552
Cry2Ac1	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 542
Cry2Ad1	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 552
Cry2Ae1	: NGTHHFAPEGTIGFTLSP-IVATQVHMQKIFISEKFGHC-GDSIRFEQDNTFAETIPFENGCHMNNINARVSSQ								: 551
Cry1Ac	: TQIPAVKGNFLQESVLSG-PGFHGGVLRLNSSCHNIQRGYIEPIHFPSTETVRFSVRYASVTPPIHLNHHV								: 545
Cry3Aa1	: TQLP[AVKQYKLOSEAEVACPRFNGGIIQQENGSAATY-----VTPDVSY[Q[TRASIHVAS[TSQITFTLSL								: 587

FIG. 2C

Axmi066-10	: GNSIFRVTINGCRVYIVS	620	*	640	*	660	*	617
Axmi-066	: GNSIFRVTINGCRVYIVS							604
Cry2Aa1	: GNSIFRVTINGCRVYIVS							613
Cry2Ab1	: GNSIFRVTINGCRVYIVS							613
Cry2Ac1	: GNSIFRVTINGCRVYIVS							602
Cry2Ad1	: GNSIFRVTINGCRVYIVS							613
Cry2Ae1	: GNSIFRVTINGCRVYIVS							612
Cry1Ac	: GNSIFRVTINGCRVYIVS							618
Cry3Aa1	: DG--APFNQYFDKILNKGMILYYSFNLASFSSTPFEISGNMILQIGVTGLS							645
Axmi066-10	: GTOPELMMRLIGVPTNIPRLY	680	*	700	*	720	*	637
Axmi-066	: GTOPELMMRLIGVPTNIPRLY							624
Cry2Aa1	: GTPEDLMMRLIGVPTNIPRLY							633
Cry2Ab1	: GTPEDLMMRLIGVPTNIPRLY							633
Cry2Ac1	: NPQPELMMRLIGVPTNIPRLY							622
Cry2Ad1	: GTPEDLMMRLIGVPTNIPRLY							633
Cry2Ae1	: GTOPELMMRLIGVPTNIPRLY							632
Cry1Ac	: ERAQKAVTAALETSTHQLGKTNVTDYHIDQVSNLYTSLDEFCLDEKRELSEKVKHAKRLSDERNILQDSNFKDI							693
Cry3Aa1	: IEFIPEN							652

FIG. 2D

AGACAGGTTCTTAAACATACTTGTTATTATCAAGAGAGTATTGTCTCTTTAATGTTATTATATAACCC
AATATTTCAAGGAGGATTTTATATGAATAATGTATTGAATAGCGAAAGAACTAATAAGTGTGATGCGT
ATAACGTAGTGGCCCATGATCCATTTAGTTTTGAGCATAAATCATTAGATACCATACAGCAAGAATGG
ATGGAGTGGAAAAGAACCGATCATAGTTTATATGTATCTCCTATTGTGGGAACTATAGCTAGTTTTCT
GCTAAAGAAAATAGCGGGGCTTATAGGAAAAAGAATATTAAGTGAGTTAAAGAATTTAATTTTTCTTA
GTGGTAGTATAGAATCAATGCAAGATATTTTAAGAGGGGGCAGAACAAATTCCTAAATCAAAGACTTGAT
GCAGACACCTTTGCTCGGGTAGAGGCAGAATTGATAGGGCTTCAAGCAAATGTAGAGGAATTTAATCA
ACAAGTGGACAATTTTTTAAACCCCAAATCAAAACCCTGTTTCCTTTAGCAATAATTGATTCGGTTAATA
CAATGCAACAATTATTCCTAAGTAGATTACCCAGTTCCAGATACAACGCTATCAGCTATTATTATTA
CCTTTATTTGCACAAGCAGCCAATTTACACCTTACCTTTATTAGAGATGTTATTCTTAATGCAGATGA
ATGGGGGAATACCAGCAGCAACAGTGCGCACATATAGAGAGCACCTAAAAAGATATACACGCGATTATT
CCAATTATTGTATAAACACGTACCAAACCTGCTTTCCGAGGTTTAAACACTCGTTTACATGATATGTTA
GAGTTTAGAACATTTATGTTTTTAAATGTATTAGACTATGTATCTATCTGGTCGTTGTTTTAAATATCA
AAGTCTGATGGTTACTTCAAGTGCTAATTTATATGCTTCGGGAAGTGGTAGTAATCAACCTTTTACTG
CACAAGACTGGCCATTTTTTATATTCTCTTTTCCAAGTGAATTCAAATTATATAATGTCTAATTTTGGT
GGTAACCGAGAGACTGCTAGTTTTGGTGTTTCCTATTCTGGGGGGATTTCATAATAAATTTTTTACTTAG
TTTTAGGGTTAATTATACTGGAGGAGTTTCATCTGGTCTCCTAGGTGTTGAAGGAATTTCAAACAACCT
TTAATTGCAACTCCTCTTTATCAACACCAGTTGTAAGAAGTTGGCTAGATTCAGGTGTATATCGAGGT
GACCTGCAACACAATTGGCGAACAGACATCTTTATGAGGACTAATATTGTACCTTGTGGTGCTTTTCT
ATTATCTCTTGCTATGTTTCCAGATGTTAAAAGTAATTATTTTCCTGATTATTTTCATTCGTAACATTT
CCGGAATTATTCGAAATATTGATAACATGAATTTGAGTAGACCATTACACTTTAATGAAGTAAGAGAT
TTAAGAGACACTGAAGTTGCTACTTTAGTATCTGTGCATAATAGAAAAAATAATATCTATGCTGCTCA
TGAAAATGGTACTATGATTCATTTTGCGCCGGAAGGTTATACAGGTTTCACAATATCACCAATATATG
CAACTCAAGTAAATAATCAAACACGAACGTTTATTTCTGAAAAATTTCGGAAATCAAGGTGATTCCTTG
AGATTTGAACAAACTAACACAACGGCTCGTTATACGTTTAGAGGGAATGGTAATAATTATAATCTTTA
TTAAGAGTATCCTCACAAGGAAATTCTACTTTTCGAGTTACTATAAACGGTAGGGTTTATACTGTTT
CAAATGTTAATACCACTACAAATAATGATGGGGTTATTGATAATGGGGCTCGTTTTTTCAGATATTCAC
ATCGGGGAATATAGTGGCAAGTAACAATACTAATGTACCATTAGATATAAATGTGATACTTAACTCCGG
TACTCAATTTGAGCTTATGAATATTATTTTTGTTCCAACATAACATTCCACCACTTTATTAAGGTTTGA
GTTTCTGAGGTAAATATA