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(19) (CA) **CANADIAN PATENT** (12)

(54) Hybrid Bacillus Thuringiensis Gene, Plasmid, and Transformed Pseudomonas Fluorescens

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Abstract of the Disclosure

A novel hybrid B.t. toxin gene toxic to lepidopteran insects has been cloned. The DNA encoding the B.t. toxin can be used to transform various prokaryotic and eukaryotic microbes to express the B.t. toxin. These recombinant microbes can be used to control lepidopteran insects in various environments.

DESCRIPTION

NOVEL HYBRID *BACILLUS THURINGIENSIS* GENE, PLASMID, AND TRANSFORMED *PSEUDOMONAS FLUORESCENS*

Background of the Invention

The most widely used microbial pesticides are derived from the bacterium Bacillus thuringiensis. This bacterial agent is used to control a wide range of leaf-eating caterpillars, Japanese beetles and mosquitos. Bacillus thuringiensis produces a proteinaceous paraspore or crystal which is toxic upon ingestion by a susceptible insect host. For example, B. thuringiensis var. kurstaki HD-1 produces a crystal called a delta toxin which is toxic to the larvae of a number of lepidopteran insects. The cloning and expression of this B.t. crystal protein gene in Escherichia coli has been described in the published literature (Schnepf, H.E. and Whitely, H.R. [1981] Proc. Natl. Acad. Sci. USA 78:2893-2897). U.S. Patent 4,448,885 and U.S. Patent 4,467,036 both disclose the expression of B.t. crystal protein in E. coli. In U.S. 4,467,036 B. thuringiensis var. kurstaki HD-1 is disclosed as being available from the well-known NRRL culture repository at Peoria, Illinois. Its accession number there is NRRL B-3792. B. thuringiensis var. kurstaki HD-73 is also available from NRRL. Its accession number is NRRL B-4488.

Brief Summary of the Invention

Disclosed and claimed is a novel hybrid toxin gene toxic to lepidopteran insects. This toxin gene has been transferred to a Pseudomonas fluorescens host via a plasmid vector.

Specifically, the invention comprises a novel hybrid delta endotoxin gene comprising part of the B.

thuringiensis var. kurstaki strain HD-73 toxin gene and part of the toxin gene from B. thuringiensis var. thuringiensis strain Berliner 1715 (DNA 5:305-314, 1986). This hybrid gene was inserted into a suitable transfer vector which was then used to transform a Pseudomonas fluorescens host. The P. fluorescens host can be used as an insecticide active against lepidopteran insects.

More specifically, the subject invention concerns a novel hybrid toxin gene (DNA) encoding a novel protein having activity against lepidopteran insects. Table 1 discloses the DNA encoding the novel toxin. Table 2 discloses the amino acid sequence of the novel hybrid toxin. Table 3 is a composite of Tables 1 and 2.

Detailed Disclosure of the Invention

The novel hybrid toxin gene of the subject invention comprises part of the B. thuringiensis var. kurstaki strain HD-73 toxin gene and part of a B. thuringiensis var. thuringiensis strain Berliner 1715 toxin gene. In general, the B.t.k. HD-73 gene portion was initially combined with DNA segments derived from (1) the known E. coli plasmid pBR322, and (2) a DNA segment representing the hybrid Tac promoter. Next, the 3' part of the Berliner toxin gene was added followed by a DNA segment conferring the ability to replicate in Pseudomonas from the known plasmid pRO1614,

The resulting hybrid gene was contained in a plasmid named pM3,130-7. This plasmid was used to transform a Pseudomonas fluorescens microbe to give the transformed strain named MR420.

The toxin gene of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of lepidopteran insects where they will proliferate and be ingested by the

insects. The result is a control of the unwanted insects. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of target pest(s). The resulting product retains the toxicity of the B.t. toxin.

Where the B.t. toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes; fungi, particularly yeast, e.g., genera Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula, and Aureobasidium. Of particular interest are such phytosphere bacterial species as Pseudomonas syringae.

Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacterium tumefaciens, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioti, Alcaligenes entrophus, and Azotobacter vinlandii; and phytosphere yeast species such as Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces roseus, S. odorus, Kluyveromyces veronae, and Aureobasidium pollulans. Of particular interest are the pigmented microorganisms.

A wide variety of ways are available for introducing the B.t. gene expressing the toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the toxin gene, the toxin gene under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, whereby integration or stable maintenance will occur.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In some instances, it may be desirable to provide for regulative expression of the toxin, where expression of the toxin will only occur after release into the environment. This can be achieved with operators or a region binding to an activator or enhancers, which are capable of induction upon a change in the physical or chemical environment of the microorganisms. For example, a temperature sensitive regulatory region may be employed, where the organisms may be grown up in the laboratory without expression of a toxin, but upon release into the environment, expression would begin. Other techniques may employ a specific nutrient medium in the laboratory, which inhibits the

expression of the toxin, where the nutrient medium in the environment would allow for expression of the toxin. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The initiation and translational termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal.

In the direction of transcription, namely in the 5' to 3' direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and the promoter, where the regulatory region may be either 5' or 3' of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence involving a marker, where the second DNA sequence may be joined to the toxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototropy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the

constructs, as well as for modifying the host. The organisms may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the toxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the toxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, and usually not more than about 1000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the toxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a toxin gene is lost, the resulting organism will be likely to also lose the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the trp gene, lac gene, gal gene, the lambda left and right promoters, the Tac promoter, the naturally-occurring promoters associated with the toxin gene, where functional in the

host. See for example, U.S. Patent Nos. 4,332,898, 4,342,832 and 4,356,270. The termination region may be the termination region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system which is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus which is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pRO1614, and the like. See for example, Olson et al., (1982) J. Bacteriol. 150:6069, and Bagdasarian et al., (1981) Gene 16:237, and U.S. Patent Nos. 4,356,270, 4,362,817, and 4,371,625.

The B.t. gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms,

when present. The transformants then can be tested for pesticidal activity.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi. Illustrative prokaryotes, both Gram-negative and -positive, include Enterobacteriaceae, such as Escherichia, Erwinia, Shigella, Salmonella, and Proteus; Bacillaceae; Rhizobiceae, such as Rhizobium; Spirillaceae, such as photobacterium, Zymomonas, Serratia, Aeromonas, Vibrio, Desulfovibrio, Spirillum; Lactobacillaceae; Pseudomonadaceae, such as Pseudomonas and Acetobacter; Azotobacteraceae and Nitrobacteraceae. Among eukaryotes are fungi, such as Phycomycetes and Ascomycetes, which includes yeast, such as Saccharomyces and Schizosaccharomyces; and Basidiomycetes yeast, such as Rhodotorula, Aureobasidium, Sporobolomyces, and the like.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the B.t. gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion

bodies; leaf affinity; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

Host organisms of particular interest include yeast, such as Rhodotorula sp., Aureobasidium sp., Saccharomyces sp., and Sporobolomyces sp.; phylloplane organisms such as Pseudomonas sp., Erwinia sp. and Flavobacterium sp.; or such other organisms as Escherichia, Lactobacillus sp., Bacillus sp., and the like. Specific organisms include Pseudomonas aeruginosa, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus thuringiensis, Escherichia coli, Bacillus subtilis, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the B.t. toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a

combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bio-availability or bioactivity of the toxin.

The cellular host containing the B.t. insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the B.t. gene. These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

The B.t. cells may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions,

emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

The pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the lepidopteran pest(s), e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 - Construction of Novel Hybrid Toxin Gene and Transformation into *Pseudomonas fluorescens*

A portion of the B. thuringiensis var. kurstaki HD-73 gene including all of the toxin-encoding DNA from the starting ATG (i.e., start methionine) to the HindIII site was inserted into the Tac-promoted plasmid pKK223-3 (Pharmacia). This was done by making a blunt fusion of this gene just downstream from the ribosome binding site in pKK223-3. This formed plasmid pKK2. Plasmid pKK2 was completely digested with HindIII which cleaves at the ultimate 3' end of the toxin gene. The HindIII overhang was made blunt by filling it in with deoxynucleotides using Klenow fragment. Next, PstI linker was added to the new

blunt site followed by digestion with PstI and closing up of the plasmid with DNA ligase. Then the 3' portion of the Berliner toxin sequence (DNA 5:305-314, 1986) was cloned as a SacI to PstI fragment into the modified pKK2/SacI + PstI thus making a recombinant toxin gene in a new plasmid named pKK73BB-9. This gene is of Berliner origin for all sequences beyond (3' to) the SacI site.

Next, pKK73BB-9 was completely digested with PstI, which cleaves at the ultimate 3' end of the new chimeric toxin gene, and treated with bacterial alkaline phosphatase. The PstI DNA fragment, from plasmid pRO1614, which confers the ability to replicate in Pseudomonas was then inserted into the pKK73BB-9/PstI vector to form plasmid pM2,107-1. Plasmid pM2,107-1 was completely digested with the restriction endonuclease Tth111I, the open site overhang was made blunt by treatment with Klenow fragment plus deoxynucleotides, linkered for BamH1, BamH1 digested, ligated, transformed, and screened to find a plasmid which had deleted the useless partial tetracycline resistance gene (derived from pKK223-3). This gave plasmid pM3,123-1. Plasmid pM3,123-1 was cleaved with the restriction endonuclease PvuI, digested very briefly with Bal31, made blunt by treatment with Klenow fragment and deoxynucleoside triphosphates, and used as a vector to clone the similarly blunted tetracycline resistance gene from pBR322 (map positions EcoRI and AvaI). Screening of tetracycline resistant colonies produced the desired plasmid called pM3,130-7. This plasmid was then transformed, using standard procedures, into a Pseudomonas fluorescens. The resulting transformed strain is called MR420.

The above cloning procedures were conducted using standard procedures unless otherwise noted.

The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. These procedures are all described in Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York. Thus, it is within the skill of those in the genetic engineering art to extract DNA from microbial cells, perform restriction enzyme digestions, electrophorese DNA fragments, tail and anneal plasmid and insert DNA, ligate DNA, transform cells, prepare plasmid DNA, electrophorese proteins, and sequence DNA.

The restriction enzymes disclosed herein can be purchased from Bethesda Research Laboratories, Gaithersburg, MD, or New England Biolabs, Beverly, MA. The enzymes are used according to the instructions provided by the supplier.

Plasmid pM3,130-7, containing the B.t. toxin gene, can be removed from the transformed host microbe by use of standard well-known procedures. For example, P. fluorescens (pM3,130-7) can be subjected to cleared lysate isopycnic density gradient procedures, and the like, to recover pM3,130-7.

A subculture of P. fluorescens (pM3,130-7) has been deposited in the permanent collection of the Agricultural Research Service Patent Culture Collection (NRRL), Regional Research Center, 1815 North University Street, Peoria, Illinois 61604 USA on February 22, 1988. The culture was assigned the accession number NRRL B-18332 by the repository. This deposit is available to the public upon the grant of a patent disclosing it. The deposit is also available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license

to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the culture. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing the deposit.

Example 2 - Insertion of Toxin Gene Into Plants

The novel gene coding for the novel insecticidal toxin, as disclosed herein, can be inserted into plant cells using the Ti plasmid from Agrobacter tumefaciens. Plant cells can then be caused to regenerate into plants (Zambryski, P., Joos, H., Gentello, C., Leemans, J., Van Montague, M. and Schell, J [1983] Cell 32:1033-1043). A particularly useful vector in this regard is pEND4K (Klee, H.J., Yanofsky, M.F. and Nester, E.W. [1985] Bio/Technology 3:637-642). This plasmid can replicate both in plant cells and in bacteria and has multiple cloning sites for passenger genes. The toxin gene, for example, can be inserted into the BamHI site of pEND4K, propagated in E. coli, and transformed into appropriate plant cells.

Example 3 - Cloning of Novel Hybrid *B. thuringiensis* Genes Into Baculoviruses

The novel hybrid gene of the invention can be cloned into baculoviruses such as Autographa californica nuclear polyhedrosis virus (AcNPV). Plasmids can be constructed that contain the AcNPV genome cloned into a commercial cloning vector such as pUC8. The AcNPV genome is modified so that the coding region of the polyhedrin gene is removed and a unique cloning site for a passenger gene is placed directly behind the polyhedrin promoter. Examples of such vectors are pGP-B6874, described by Pennock et al. (Pennock, G.D., Shoemaker, C. and Miller, L.K. [1984] Mol. Cell. Biol. 4:399-406), and pAC380, described by Smith et al. (Smith, G.E., Summers, M.D. and Fraser, M.J. [1983] Mol Cell. Biol. 3:2156-2165). The gene coding for the novel protein toxin of the invention can be modified with BamHI linkers at appropriate regions both upstream and downstream from the coding region and inserted into the passenger site of one of the AcNPV vectors.

As disclosed previously, the nucleotide sequence encoding the novel B.t. toxin gene is shown in Table 1. The deduced amino acid sequence is shown in Table 2.

It is well known in the art that the amino acid sequence of a protein is determined by the nucleotide sequence of the DNA. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins, different nucleotide sequences can code for a particular amino acid. Thus, the genetic code can be depicted as follows:

Phenylalanine (Phe)	TTK	Histidine (His)	CAK
Leucine (Leu)	XTY	Glutamine (Gln)	CAJ
Isoleucine (Ile)	ATM	Asparagine (Asn)	AAK
Methionine (Met)	ATG	Lysine (Lys)	AAJ
Valine (Val)	GTL	Aspartic acid (Asp)	GAK
Serine (Ser)	QRS	Glutamic acid (Glu)	GAJ
Proline (Pro)	CCL	Cysteine (Cys)	TGK

Threonine (Thr)	ACL	Tryptophan (Trp)	TGG
Alanine (Ala)	GCL	Arginine (Arg)	WGZ
Tyrosine (Tyr)	TAK	Glycine (Gly)	GGL
Termination signal	TAJ		

Key: Each 3-letter deoxynucleotide triplet corresponds to a trinucleotide of mRNA, having a 5'-end on the left and a 3'-end on the right. All DNA sequences given herein are those of the strand whose sequence correspond to the mRNA sequence, with thymine substituted for uracil. The letters stand for the purine or pyrimidine bases forming the deoxynucleotide sequence.

A = adenine

G = guanine

C = cytosine

T = thymine

X = T or C if Y is A or G

X = C if Y is C or T

Y = A, G, C or T if X is C

Y = A or G if X is T

W = C or A if Z is A or G

W - C if Z is C or T

Z = A, G, C or T if W is C

Z = A or G if W is A

QR = TC if S is A, G, C or T; alternatively

QR = AG if S is T or C

J = A or G

K = T or C

L = A, T, C or G

M = A, C or T

The above shows that the novel amino acid sequence of the B.t. toxin can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein. Accordingly, the subject invention includes such equivalent nucleotide sequences. In addition it has been

shown that proteins of identified structure and function may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E.T. and Kezdy, F.J. [1984] Science 223:249-255). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is retained to some degree.

Table 1

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                10          20          30          40          50          60
  1 ATGGATAACA ATCCGAACAT CAATGAATGC ATTCCTTATA ATTGTTTAAG TAACCCTGAA
 61 GTAGAAGTAT TAGGTGGAGA AAGAATAGAA ACTGGTTACA CCCCAATCGA TATTTCCTTG
121 TCGCTAACGC AATTTCTTTT GAGTGAATTT GTTCCCGGTG CTGGATTTGT GTTAGGACTA
181 GTTGATATAA TATGGGGAAT TTTTGGTCCC TCTCAATGGG ACGCATTTCT TGTACAAATT
241 GAACAGTTAA TTAACCAAAG AATAGAAGAA TTCGCTAGGA ACCAAGCCAT TTCTAGATTA

              310         320         330         340         350         360
301 GAAGGACTAA GCAATCTTTA TCAAATTTAC GCAGAATCTT TTAGAGAGTG GGAAGCAGAT
361 CCTACTAATC CAGCATTAAG AGAAGAGATG CGTATTCAAT TCAATGACAT GAACAGTGCC
421 CTTACAACCG CTATTCCTCT TTTTGCAGTT CAAAATTATC AAGTTCCTCT TTTATCAGTA
481 TATGTTCAAG CTGCAAATTT ACATTTATCA GTTTTGAGAG ATGTTTCAGT GTTTGGACAA
541 AGGTGGGGAT TTGATGCCGC GACTATCAAT AGTCGTTATA ATGATTTAAC TAGGCTTATT

              610         620         630         640         650         660
601 GGCAACTATA CAGATTATGC TGTACGCTGG TACAATACGG GATTAGAACG TGTATGGGGA
661 CCGGATTCTA GAGATTGGGT AAGGTATAAT CAATTTAGAA GAGAATTAAC ACTAACTGTA
721 TTAGATATCG TTGCTCTGTT CCCGAATTAT GATAGTAGAA GATATCCAAT TCGAACAGTT
781 TCCCAATTAA CAAGAGAAAT TTATACAAAC CCAGTATTAG AAAATTTTGA TGGTAGTTTT
841 CGAGGCTCGG CTCAGGGCAT AGAAAGAAGT ATTAGGAGTC CACATTTGAT GGATATACTT

              910         920         930         940         950         960
901 AACAGTATAA CCATCTATAC GGATGCTCAT AGGGGTTATT ATTATTGGTC AGGGCATCAA
 961 ATAATGGCTT CTCCTGTAGG GTTTTCGGGG CCAGAATTCA CTTTTCCGCT ATATGGAACT
1021 ATGGGAAATG CAGCTCCACA ACAACGTATT GTTGCTCAAC TAGGTCAGGG CGTGTATAGA
1081 ACATTATCGT CCACTTTATA TAGAAGACCT TTTAATATAG GGATAAATAA TCAACAACTA
1141 TCTGTTCTTG ACGGGACAGA ATTTGCTTAT GGAACCTCCT CAAATTTGCC ATCCGCTGTA

             1210        1220        1230        1240        1250        1260
1201 TACAGAAAAA GCGGAACGGT AGATTCGCTG GATGAAATAC CGCCACAGAA TAACAACGTG
1261 CCACCTAGGC AAGGATTTAG TCATCGATTA AGCCATGTTT CAATGTTTCG TTCAGGCTTT
1321 AGTAATAGTA GTGTAAGTAT AATAAGAGCT CCTATGTTCT CTTGGATACA TCGTAGTGCT
1381 GAATTTAATA ATATAATTCC TTCATCACAA ATTACACAAA TACCTTTAAC AAAATCTACT
1441 AATCTTGGCT CTGGAACTTC TGTCGTTAAA GGACCAGGAT TTACAGGAGG AGATATTCTT

             1510        1520        1530        1540        1550        1560
1501 CGAAGAACTT CACCTGGCCA GATTTCAACC TTAAGAGTAA ATATTACTGC ACCATTATCA
1561 CAAAGATATC GGGTAAGAAT TCGCTACGCT TCTACCACAA ATTTACAATT CCATACATCA
1621 ATTGACGGAA GACCTATTAA TCAGGGGAAT TTTTCAGCAA CTATGAGTAG TGGGAGTAAT
1681 TTACAGTCCG GAAGCTTTAG GACTGTAGGT TTTACTACTC CGTTTAACTT TTCAAATGGA
1741 TCAAGTGTAT TTACGTTAAG TGCTCATGTC TTCAATTCAG GCAATGAAGT TTATATAGAT

             1810        1820        1830        1840        1850        1860
1801 CGAATTGAAT TTGTTCCGGC AGAAGTAACC TTTGAGGCAG AATATGATTT AGAAAGAGCA
1861 CAAAAGGCGG TGAATGAGCT GTTTACTTCT TCCAATCAAA TCGGGTTAAA AACAGATGTG
1921 ACGGATTATC ATATTGATCA AGTATCCAAT TTAGTTGAGT GTTTATCTGA TGAATTTTGT
1981 CTGGATGAAA AAAAAGAATT GTCCGAGAAA GTCAAACATG CGAAGCGACT TAGTGATGAG
2041 CGGAATTTAC TTCAAGATCC AAACTTTAGA GGGATCAATA GACAACTAGA CCGTGGCTGG

             2110        2120        2130        2140        2150        2160
2101 AGAGGAAGTA CGGATATTAC CATCCAAGGA GGCGATGACG TATTCAAAGA GAATTACGTT
2161 ACGCTATTGG GTACCTTTGA TGAGTGCTAT CCAACGTATT TATATCAAAA AATAGATGAG
2221 TCGAAATTAA AAGCCTATAC CCGTTACCAA TTAAGAGGGT ATATCGAAGA TAGTCAAGAC
2281 TTAGAAATCT ATTTAATTCG CTACAATGCC AAACACGAAA CAGTAAATGT GCCAGGTACG
2341 GGTTCCTTAT GGCCGCTTTC AGCCCCAAGT CCAATCGGAA AATGTGCCCA TCATTCCCAT
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Table 1 (cont'd)

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                2410        2420        2430        2440        2450        2460
2401 CATTTCTCCT TGGACATTGA TGTTGGATGT ACAGACTTAA ATGAGGACTT AGGTGTATGG
2461 GTGATATTCA AGATTAAGAC GCAAGATGGC CATGCAAGAC TAGGAAATCT AGAATTTCTC
2521 GAAGAGAAAC CATTAGTAGG AGAAGCACTA GCTCGTGTGA AAAGAGCGGA GAAAAAATGG
2581 AGAGACAAAC GTGAAAAATT GGAATGGGAA ACAAATATTG TTTATAAAGA GGCAAAAGAA
2641 TCTGTAGATG CTTTATTTGT AAACTCTCAA TATGATAGAT TACAAGCGGA TACCAACATC

                2710        2720        2730        2740        2750        2760
2701 GCGATGATTC ATGCGGCAGA TAAACGCGTT CATAGCATTC GAGAAGCTTA TCTGCCTGAG
2761 CTGTCTGTGA TTCCGGGTGT CAATGCGGCT ATTTTTGAAG AATTAGAAGG GCGTATTTTC
2821 ACTGCATTCT CCCTATATGA TGCGAGAAAT GTCATTAAAA ATGGTGATTT TAATAATGGC
2881 TTATCCTGCT GGAACGTGAA AGGGCATGTA GATGTAGAAG AACAAAACAA CCACCGTTCG
2941 GTCCTTGTTG TTCCGGAATG GGAAGCAGAA GTGTCACAAG AAGTTCGTGT CTGTCCGGGT

                3010        3020        3030        3040        3050        3060
3001 CGTGGCTATA TCCTTCGTGT CACAGCGTAC AAGGAGGGAT ATGGAGAAGG TTGCGTAACC
3061 ATTCATGAGA TCGAGAACAA TACAGACGAA CTGAAGTTTA GCAACTGTGT AGAAGAGGAA
3121 GTATATCCAA ACAACACGGT AACGTGTAAT GATTATACTG CGACTCAAGA AGAATATGAG
3181 GGTACGTACA CTTCTCGTAA TCGAGGATAT GACGGAGCCT ATGAAAGCAA TTCTTCTGTA
3241 CCAGCTGATT ATGCATCAGC CTATGAAGAA AAAGCATATA CAGATGGACG AAGAGACAAT

                3310        3320        3330        3340        3350        3360
3301 CCTTGTGAAT CTAACAGAGG ATATGGGGAT TACACACCAC TACCAGCTGG CTATGTGACA
3361 AAAGAATTAG AGTACTTCCC AGAAACCGAT AAGGTATGGA TTGAGATCGG AGAAACGGAA
3421 GGAACATTCA TCGTGGACAG CGTGGAATTA CTTCTTATGG AGGAA*
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Table 2

	5							10							15
1	Met	Asp	Asn	Asn	Pro	Asn	Ile	Asn	Glu	Cys	Ile	Pro	Tyr	Asn	Cys
16	Leu	Ser	Asn	Pro	Glu	Val	Glu	Val	Leu	Gly	Gly	Glu	Arg	Ile	Glu
31	Thr	Gly	Tyr	Thr	Pro	Ile	Asp	Ile	Ser	Leu	Ser	Leu	Thr	Gln	Phe
46	Leu	Leu	Ser	Glu	Phe	Val	Pro	Gly	Ala	Gly	Phe	Val	Leu	Gly	Leu
61	Val	Asp	Ile	Ile	Trp	Gly	Ile	Phe	Gly	Pro	Ser	Gln	Trp	Asp	Ala
76	Phe	Leu	Val	Gln	Ile	Glu	Gln	Leu	Ile	Asn	Gln	Arg	Ile	Glu	Glu
91	Phe	Ala	Arg	Asn	Gln	Ala	Ile	Ser	Arg	Leu	Glu	Gly	Leu	Ser	Asn
106	Leu	Tyr	Gln	Ile	Tyr	Ala	Glu	Ser	Phe	Arg	Glu	Trp	Glu	Ala	Asp
121	Pro	Thr	Asn	Pro	Ala	Leu	Arg	Glu	Glu	Met	Arg	Ile	Gln	Phe	Asn
136	Asp	Met	Asn	Ser	Ala	Leu	Thr	Thr	Ala	Ile	Pro	Leu	Phe	Ala	Val
151	Gln	Asn	Tyr	Gln	Val	Pro	Leu	Leu	Ser	Val	Tyr	Val	Gln	Ala	Ala
166	Asn	Leu	His	Leu	Ser	Val	Leu	Arg	Asp	Val	Ser	Val	Phe	Gly	Gln
181	Arg	Trp	Gly	Phe	Asp	Ala	Ala	Thr	Ile	Asn	Ser	Arg	Tyr	Asn	Asp
196	Leu	Thr	Arg	Leu	Ile	Gly	Asn	Tyr	Thr	Asp	Tyr	Ala	Val	Arg	Trp
211	Tyr	Asn	Thr	Gly	Leu	Glu	Arg	Val	Trp	Gly	Pro	Asp	Ser	Arg	Asp
226	Trp	Val	Arg	Tyr	Asn	Gln	Phe	Arg	Arg	Glu	Leu	Thr	Leu	Thr	Val
241	Leu	Asp	Ile	Val	Ala	Leu	Phe	Pro	Asn	Tyr	Asp	Ser	Arg	Arg	Tyr
256	Pro	Ile	Arg	Thr	Val	Ser	Gln	Leu	Thr	Arg	Glu	Ile	Tyr	Thr	Asn
271	Pro	Val	Leu	Glu	Asn	Phe	Asp	Gly	Ser	Phe	Arg	Gly	Ser	Ala	Gln
286	Gly	Ile	Glu	Arg	Ser	Ile	Arg	Ser	Pro	His	Leu	Met	Asp	Ile	Leu
301	Asn	Ser	Ile	Thr	Ile	Tyr	Thr	Asp	Ala	His	Arg	Gly	Tyr	Tyr	Tyr
316	Trp	Ser	Gly	His	Gln	Ile	Met	Ala	Ser	Pro	Val	Gly	Phe	Ser	Gly
331	Pro	Glu	Phe	Thr	Phe	Pro	Leu	Tyr	Gly	Thr	Met	Gly	Asn	Ala	Ala
346	Pro	Gln	Gln	Arg	Ile	Val	Ala	Gln	Leu	Gly	Gln	Gly	Val	Tyr	Arg
361	Thr	Leu	Ser	Ser	Thr	Leu	Tyr	Arg	Arg	Pro	Phe	Asn	Ile	Gly	Ile
376	Asn	Asn	Gln	Gln	Leu	Ser	Val	Leu	Asp	Gly	Thr	Glu	Phe	Ala	Tyr
391	Gly	Thr	Ser	Ser	Asn	Leu	Pro	Ser	Ala	Val	Tyr	Arg	Lys	Ser	Gly
406	Thr	Val	Asp	Ser	Leu	Asp	Glu	Ile	Pro	Pro	Gln	Asn	Asn	Asn	Val
421	Pro	Pro	Arg	Gln	Gly	Phe	Ser	His	Arg	Leu	Ser	His	Val	Ser	Met
436	Phe	Arg	Ser	Gly	Phe	Ser	Asn	Ser	Ser	Val	Ser	Ile	Ile	Arg	Ala
451	Pro	Met	Phe	Ser	Trp	Ile	His	Arg	Ser	Ala	Glu	Phe	Asn	Asn	Ile
466	Ile	Pro	Ser	Ser	Gln	Ile	Thr	Gln	Ile	Pro	Leu	Thr	Lys	Ser	Thr
481	Asn	Leu	Gly	Ser	Gly	Thr	Ser	Val	Val	Lys	Gly	Pro	Gly	Phe	Thr
496	Gly	Gly	Asp	Ile	Leu	Arg	Arg	Thr	Ser	Pro	Gly	Gln	Ile	Ser	Thr
511	Leu	Arg	Val	Asn	Ile	Thr	Ala	Pro	Leu	Ser	Gln	Arg	Tyr	Arg	Val
526	Arg	Ile	Arg	Tyr	Ala	Ser	Thr	Thr	Asn	Leu	Gln	Phe	His	Thr	Ser
541	Ile	Asp	Gly	Arg	Pro	Ile	Asn	Gln	Gly	Asn	Phe	Ser	Ala	Thr	Met
556	Ser	Ser	Gly	Ser	Asn	Leu	Gln	Ser	Gly	Ser	Phe	Arg	Thr	Val	Gly
571	Phe	Thr	Thr	Pro	Phe	Asn	Phe	Ser	Asn	Gly	Ser	Ser	Val	Phe	Thr
586	Leu	Ser	Ala	His	Val	Phe	Asn	Ser	Gly	Asn	Glu	Val	Tyr	Ile	Asp
601	Arg	Ile	Glu	Phe	Val	Pro	Ala	Glu	Val	Thr	Phe	Glu	Ala	Glu	Tyr
616	Asp	Leu	Glu	Arg	Ala	Gln	Lys	Ala	Val	Asn	Glu	Leu	Phe	Thr	Ser
631	Ser	Asn	Gln	Ile	Gly	Leu	Lys	Thr	Asp	Val	Thr	Asp	Tyr	His	Ile
646	Asp	Gln	Val	Ser	Asn	Leu	Val	Glu	Cys	Leu	Ser	Asp	Glu	Phe	Cys
661	Leu	Asp	Glu	Lys	Lys	Glu	Leu	Ser	Glu	Lys	Val	Lys	His	Ala	Lys
676	Arg	Leu	Ser	Asp	Glu	Arg	Asn	Leu	Leu	Gln	Asp	Pro	Asn	Phe	Arg
691	Gly	Ile	Asn	Arg	Gln	Leu	Asp	Arg	Gly	Trp	Arg	Gly	Ser	Thr	Asp

Table 2 (cont'd)

706 Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val
721 Thr Leu Leu Gly Thr Phe Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr
736 Gln Lys Ile Asp Glu Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Gln
751 Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp Leu Glu Ile Tyr Leu
766 Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
781 Gly Ser Leu Trp Pro Leu Ser Ala Pro Ser Pro Ile Gly Lys Cys
796 Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys
811 Thr Asp Leu Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile
826 Lys Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu
841 Glu Glu Lys Pro Leu Val Gly Glu Ala Leu Ala Arg Val Lys Arg
856 Ala Glu Lys Lys Trp Arg Asp Lys Arg Glu Lys Leu Glu Trp Glu
871 Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu Ser Val Asp Ala Leu
886 Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile
901 Ala Met Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg Glu
916 Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly Val Asn Ala Ala
931 Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe Thr Ala Phe Ser Leu
946 Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly
961 Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln
976 Asn Asn His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu
991 Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu
1006 Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr
1021 Ile His Glu Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn
1036 Cys Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn
1051 Asp Tyr Thr Ala Thr Gln Glu Glu Tyr Glu Gly Thr Tyr Thr Ser
1066 Arg Asn Arg Gly Tyr Asp Gly Ala Tyr Glu Ser Asn Ser Ser Val
1081 Pro Ala Asp Tyr Ala Ser Ala Tyr Glu Glu Lys Ala Tyr Thr Asp
1096 Gly Arg Arg Asp Asn Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp
1111 Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr
1126 Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu
1141 Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu

Table 3

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                5                  10                  15                  20
Met Asp Asn Asn Pro Asn Ile Asn Glu Cys Ile Pro Tyr Asn Cys Leu Ser Asn Pro Glu
ATG GAT AAC AAT CCG AAC ATC AAT GAA TGC ATT CCT TAT AAT TGT TTA AGT AAC CCT GAA

                25                  30                  35                  40
Val Glu Val Leu Gly Gly Glu Arg Ile Glu Thr Gly Tyr Thr Pro Ile Asp Ile Ser Leu
GTA GAA GTA TTA GGT GGA GAA AGA ATA GAA ACT GGT TAC ACC CCA ATC GAT ATT TCC TTG

                45                  50                  55                  60
Ser Leu Thr Gln Phe Leu Leu Ser Glu Phe Val Pro Gly Ala Gly Phe Val Leu Gly Leu
TCG CTA ACG CAA TTT CTT TTG AGT GAA TTT GTT CCC GGT GCT GGA TTT GTG TTA GGA CTA

                65                  70                  75                  80
Val Asp Ile Ile Trp Gly Ile Phe Gly Pro Ser Gln Trp Asp Ala Phe Leu Val Gln Ile
GTT GAT ATA ATA TGG GGA ATT TTT GGT CCC TCT CAA TGG GAC GCA TTT CTT GTA CAA ATT

                85                  90                  95                  100
Glu Gln Leu Ile Asn Gln Arg Ile Glu Glu Phe Ala Arg Asn Gln Ala Ile Ser Arg Leu
GAA CAG TTA ATT AAC CAA AGA ATA GAA GAA TTC GCT AGG AAC CAA GCC ATT TCT AGA TTA

                105                 110                 115                 120
Glu Gly Leu Ser Asn Leu Tyr Gln Ile Tyr Ala Glu Ser Phe Arg Glu Trp Glu Ala Asp
GAA GGA CTA AGC AAT CTT TAT CAA ATT TAC GCA GAA TCT TTT AGA GAG TGG GAA GCA GAT

                125                 130                 135                 140
Pro Thr Asn Pro Ala Leu Arg Glu Glu Met Arg Ile Gln Phe Asn Asp Met Asn Ser Ala
CCT ACT AAT CCA GCA TTA AGA GAA GAG ATG CGT ATT CAA TTC AAT GAC ATG AAC AGT GCC

                145                 150                 155                 160
Leu Thr Thr Ala Ile Pro Leu Phe Ala Val Gln Asn Tyr Gln Val Pro Leu Leu Ser Val
CTT ACA ACC GCT ATT CCT CTT TTT GCA GTT CAA AAT TAT CAA GTT CCT CTT TTA TCA GTA

                165                 170                 175                 180
Tyr Val Gln Ala Ala Asn Leu His Leu Ser Val Leu Arg Asp Val Ser Val Phe Gly Gln
TAT GTT CAA GCT GCA AAT TTA CAT TTA TCA GTT TTG AGA GAT GTT TCA GTG TTT GGA CAA

                185                 190                 195                 200
Arg Trp Gly Phe Asp Ala Ala Thr Ile Asn Ser Arg Tyr Asn Asp Leu Thr Arg Leu Ile
AGG TGG GGA TTT GAT GCC GCG ACT ATC AAT AGT CGT TAT AAT GAT TTA ACT AGG CTT ATT

                205                 210                 215                 220
Gly Asn Tyr Thr Asp Tyr Ala Val Arg Trp Tyr Asn Thr Gly Leu Glu Arg Val Trp Gly
GGC AAC TAT ACA GAT TAT GCT GTA CGC TGG TAC AAT ACG GGA TTA GAA CGT GTA TGG GGA

                225                 230                 235                 240
Pro Asp Ser Arg Asp Trp Val Arg Tyr Asn Gln Phe Arg Arg Glu Leu Thr Leu Thr Val
CCG GAT TCT AGA GAT TGG GTA AGG TAT AAT CAA TTT AGA AGA GAA TTA ACA CTA ACT GTA

                245                 250                 255                 260
Leu Asp Ile Val Ala Leu Phe Pro Asn Tyr Asp Ser Arg Arg Tyr Pro Ile Arg Thr Val
TTA GAT ATC GTT GCT CTG TTC CCG AAT TAT GAT AGT AGA AGA TAT CCA ATT CGA ACA GTT

                265                 270                 275                 280
Ser Gln Leu Thr Arg Glu Ile Tyr Thr Asn Pro Val Leu Glu Asn Phe Asp Gly Ser Phe
TCC CAA TTA ACA AGA GAA ATT TAT ACA AAC CCA GTA TTA GAA AAT TTT GAT GGT AGT TTT
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Table 3 (cont'd)

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                285                 290                 295                 300
Arg Gly Ser Ala Gln Gly Ile Glu Arg Ser Ile Arg Ser Pro His Leu Met Asp Ile Leu
CGA GGC TCG GCT CAG GGC ATA GAA AGA AGT ATT AGG AGT CCA CAT TTG ATG GAT ATA CTT

                305                 310                 315                 320
Asn Ser Ile Thr Ile Tyr Thr Asp Ala His Arg Gly Tyr Tyr Tyr Trp Ser Gly His Gln
AAC AGT ATA ACC ATC TAT ACG GAT GCT CAT AGG GGT TAT TAT TAT TGG TCA GGG CAT CAA

                325                 330                 335                 340
Ile Met Ala Ser Pro Val Gly Phe Ser Gly Pro Glu Phe Thr Phe Pro Leu Tyr Gly Thr
ATA ATG GCT TCT CCT GTA GGG TTT TCG GGG CCA GAA TTC ACT TTT CCG CTA TAT GGA ACT

                345                 350                 355                 360
Met Gly Asn Ala Ala Pro Gln Gln Arg Ile Val Ala Gln Leu Gly Gln Gly Val Tyr Arg
ATG GGA AAT GCA GCT CCA CAA CAA CGT ATT GTT GCT CAA CTA GGT CAG GGC GTG TAT AGA

                365                 370                 375                 380
Thr Leu Ser Ser Thr Leu Tyr Arg Arg Pro Phe Asn Ile Gly Ile Asn Asn Gln Gln Leu
ACA TTA TCG TCC ACT TTA TAT AGA AGA CCT TTT AAT ATA GGG ATA AAT AAT CAA CAA CTA

                385                 390                 395                 400
Ser Val Leu Asp Gly Thr Glu Phe Ala Tyr Gly Thr Ser Ser Asn Leu Pro Ser Ala Val
TCT GTT CTT GAC GGG ACA GAA TTT GCT TAT GGA ACC TCC TCA AAT TTG CCA TCC GCT GTA

                405                 410                 415                 420
Tyr Arg Lys Ser Gly Thr Val Asp Ser Leu Asp Glu Ile Pro Pro Gln Asn Asn Asn Val
TAC AGA AAA AGC GGA ACG GTA GAT TCG CTG GAT GAA ATA CCG CCA CAG AAT AAC AAC GTG

                425                 430                 435                 440
Pro Pro Arg Gln Gly Phe Ser His Arg Leu Ser His Val Ser Met Phe Arg Ser Gly Phe
CCA CCT AGG CAA GGA TTT AGT CAT CGA TTA AGC CAT GTT TCA ATG TTT CGT TCA GGC TTT

                445                 450                 455                 460
Ser Asn Ser Ser Val Ser Ile Ile Arg Ala Pro Met Phe Ser Trp Ile His Arg Ser Ala
AGT AAT AGT AGT GTA AGT ATA ATA AGA GCT CCT ATG TTC TCT TGG.ATA CAT CGT AGT GCT

                465                 470                 475                 480
Glu Phe Asn Asn Ile Ile Pro Ser Ser Gln Ile Thr Gln Ile Pro Leu Thr Lys Ser Thr
GAA TTT AAT AAT ATA ATT CCT TCA TCA CAA ATT ACA CAA ATA CCT TTA ACA AAA TCT ACT

                485                 490                 495                 500
Asn Leu Gly Ser Gly Thr Ser Val Val Lys Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu
AAT CTT GGC TCT GGA ACT TCT GTC GTT AAA GGA CCA GGA TTT ACA GGA GGA GAT ATT CTT

                505                 510                 515                 520
Arg Arg Thr Ser Pro Gly Gln Ile Ser Thr Leu Arg Val Asn Ile Thr Ala Pro Leu Ser
CGA AGA ACT TCA CCT GGC CAG ATT TCA ACC TTA AGA GTA AAT ATT ACT GCA CCA TTA TCA

                525                 530                 535                 540
Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asn Leu Gln Phe His Thr Ser
CAA AGA TAT CGG GTA AGA ATT CGC TAC GCT TCT ACC ACA AAT TTA CAA TTC CAT ACA TCA

                545                 550                 555                 560
Ile Asp Gly Arg Pro Ile Asn Gln Gly Asn Phe Ser Ala Thr Met Ser Ser Gly Ser Asn
ATT GAC GGA AGA CCT ATT AAT CAG GGG AAT TTT TCA GCA ACT ATG AGT AGT GGG AGT AAT

                565                 570                 575                 580
Leu Gln Ser Gly Ser Phe Arg Thr Val Gly Phe Thr Thr Pro Phe Asn Phe Ser Asn Gly
TTA CAG TCC GGA AGC TTT AGG ACT GTA GGT TTT ACT ACT CCG TTT AAC TTT TCA AAT GGA
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Table 3 (cont'd)

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                    585                 590                 595                 600
Ser Ser Val Phe Thr Leu Ser Ala His Val Phe Asn Ser Gly Asn Glu Val Tyr Ile Asp
TCA AGT GTA TTT ACG TTA AGT GCT CAT GTC TTC AAT TCA GGC AAT GAA GTT TAT ATA GAT

                    605                 610                 615                 620
Arg Ile Glu Phe Val Pro Ala Glu Val Thr Phe Glu Ala Glu Tyr Asp Leu Glu Arg Ala
CGA ATT GAA TTT GTT CCG GCA GAA GTA ACC TTT GAG GCA GAA TAT GAT TTA GAA AGA GCA

                    625                 630                 635                 640
Gln Lys Ala Val Asn Glu Leu Phe Thr Ser Ser Asn Gln Ile Gly Leu Lys Thr Asp Val
CAA AAG GCG GTG AAT GAG CTG TTT ACT TCT TCC AAT CAA ATC GGG TTA AAA ACA GAT GTG

                    645                 650                 655                 660
Thr Asp Tyr His Ile Asp Gln Val Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe Cys
ACG GAT TAT CAT ATT GAT CAA GTA TCC AAT TTA GTT GAG TGT TTA TCT GAT GAA TTT TGT

                    665                 670                 675                 680
Leu Asp Glu Lys Lys Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu
CTG GAT GAA AAA AAA GAA TTG TCC GAG AAA GTC AAA CAT GCG AAG CGA CTT AGT GAT GAG

                    685                 690                 695                 700
Arg Asn Leu Leu Gln Asp Pro Asn Phe Arg Gly Ile Asn Arg Gln Leu Asp Arg Gly Trp
CGG AAT TTA CTT CAA GAT CCA AAC TTT AGA GGG ATC AAT AGA CAA CTA GAC CGT GGC TGG

                    705                 710                 715                 720
Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val
AGA GGA AGT ACG GAT ATT ACC ATC CAA GGA GGC GAT GAC GTA TTC AAA GAG AAT TAC GTT

                    725                 730                 735                 740
Thr Leu Leu Gly Thr Phe Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu
ACG CTA TTG GGT ACC TTT GAT GAG TGC TAT CCA ACG TAT TTA TAT CAA AAA ATA GAT GAG

                    745                 750                 755                 760
Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Gln Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
TCG AAA TTA AAA GCC TAT ACC CGT TAC CAA TTA AGA GGG TAT ATC GAA GAT AGT CAA GAC

                    765                 770                 775                 780
Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn Val Pro Gly Thr
TTA GAA ATC TAT TTA ATT CGC TAC AAT GCC AAA CAC GAA ACA GTA AAT GTG CCA GGT ACG

                    785                 790                 795                 800
Gly Ser Leu Trp Pro Leu Ser Ala Pro Ser Pro Ile Gly Lys Cys Ala His His Ser His
GGT TCC TTA TGG CCG CTT TCA GCC CCA AGT CCA ATC GGA AAA TGT GCC CAT CAT TCC CAT

                    805                 810                 815                 820
His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn Glu Asp Leu Gly Val Trp
CAT TTC TCC TTG GAC ATT GAT GTT GGA TGT ACA GAC TTA AAT GAG GAC TTA GGT GTA TGG

                    825                 830                 835                 840
Val Ile Phe Lys Ile Lys Thr Gln Asp Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu
GTG ATA TTC AAG ATT AAG ACG CAA GAT GGC CAT GCA AGA CTA GGA AAT CTA GAA TTT CTC

                    845                 850                 855                 860
Glu Glu Lys Pro Leu Val Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp
GAA GAG AAA CCA TTA GTA GGA GAA GCA CTA GCT CGT GTG AAA AGA GCG GAG AAA AAA TGG

                    865                 870                 875                 880
Arg Asp Lys Arg Glu Lys Leu Glu Trp Glu Thr Asn Ile Val Tyr Lys Glu Ala Lys Glu
AGA GAC AAA CGT GAA AAA TTG GAA TGG GAA ACA AAT ATT GTT TAT AAA GAG GCA AAA GAA
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Table 3 (cont'd)

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                    885                 890                 895                 900
Ser Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile
TCT GTA GAT GCT TTA TTT GTA AAC TCT CAA TAT GAT AGA TTA CAA GCG GAT ACC AAC ATC

                    905                 910                 915                 920
Ala Met Ile His Ala Ala Asp Lys Arg Val His Ser Ile Arg Glu Ala Tyr Leu Pro Glu
GCG ATG ATT CAT GCG GCA GAT AAA CGC GTT CAT AGC ATT CGA GAA GCT TAT CTG CCT GAG

                    925                 930                 935                 940
Leu Ser Val Ile Pro Gly Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Arg Ile Phe
CTG TCT GTG ATT CCG GGT GTC AAT GCG GCT ATT TTT GAA GAA TTA GAA GGG CGT ATT TTC

                    945                 950                 955                 960
Thr Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn Asn Gly
ACT GCA TTC TCC CTA TAT GAT GCG AGA AAT GTC ATT AAA AAT GGT GAT TTT AAT AAT GGC

                    965                 970                 975                 980
Leu Ser Cys Trp Asn Val Lys Gly His Val Asp Val Glu Glu Gln Asn Asn His Arg Ser
TTA TCC TGC TGG AAC GTG AAA GGG CAT GTA GAT GTA GAA GAA CAA AAC AAC CAC CGT TCG

                    985                 990                 995                1000
Val Leu Val Val Pro Glu Trp Glu Ala Glu Val Ser Gln Glu Val Arg Val Cys Pro Gly
GTC CTT GTT GTT CCG GAA TGG GAA GCA GAA GTG TCA CAA GAA GTT CGT GTC TGT CCG GGT

                   1005                1010                1015                1020
Arg Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr
CGT GGC TAT ATC CTT CGT GTC ACA GCG TAC AAG GAG GGA TAT GGA GAA GGT TGC GTA ACC

                   1025                1030                1035                1040
Ile His Glu Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys Val Glu Glu Glu
ATT CAT GAG ATC GAG AAC AAT ACA GAC GAA CTG AAG TTT AGC AAC TGT GTA GAA GAG GAA

                   1045                1050                1055                1060
Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp Tyr Thr Ala Thr Gln Glu Glu Tyr Glu
GTA TAT CCA AAC AAC ACG GTA ACG TGT AAT GAT TAT ACT GCG ACT CAA GAA GAA TAT GAG

                   1065                1070                1075                1080
Gly Thr Tyr Thr Ser Arg Asn Arg Gly Tyr Asp Gly Ala Tyr Glu Ser Asn Ser Ser Val
GGT ACG TAC ACT TCT CGT AAT CGA GGA TAT GAC GGA GCC TAT GAA AGC AAT TCT TCT GTA

                   1085                1090                1095                1100
Pro Ala Asp Tyr Ala Ser Ala Tyr Glu Glu Lys Ala Tyr Thr Asp Gly Arg Arg Asp Asn
CCA GCT GAT TAT GCA TCA GCC TAT GAA GAA AAA GCA TAT ACA GAT GGA CGA AGA GAC AAT

                   1105                1110                1115                1120
Pro Cys Glu Ser Asn Arg Gly Tyr Gly Asp Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr
CCT TGT GAA TCT AAC AGA GGA TAT GGG GAT TAC ACA CCA CTA CCA GCT GGC TAT GTG ACA

                   1125                1130                1135                1140
Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu
AAA GAA TTA GAG TAC TTC CCA GAA ACC GAT AAG GTA TGG ATT GAG ATC GGA GAA ACG GAA

                   1145                1150                1155
Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
GGA ACA TTC ATC GTG GAC AGC GTG GAA TTA CTT CTT ATG GAG GAA
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THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. DNA encoding a B.t. var kurstaki strain HD-73 and var thuringiensis strain Berliner 1715 toxin having the amino acid sequence shown in Table 2.

2. DNA, according to claim 1, having the nucleotide sequence shown in Table 1, wherein said sequence terminates at the stop codon.

3. Toxin active against lepidopteran insects having the amino acid sequence shown in Table 2.

4. A recombinant DNA transfer vector comprising DNA encoding a B.t. var kurstaki strain HD-73 and var thuringiensis strain Berliner 1715 having all or part of the nucleotide sequence which codes for the amino acid sequence shown in Table 2.

5. The DNA transfer vector, according to claim 4, transferred to and replicated in a prokaryotic or eukaryotic host.

6. A bacterial host transformed to express a B.t. var kurstaki strain HD-73 and var thuringiensis strain Berliner 1715 toxin having the amino acid sequence shown in Table 2.

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7. Pseudomonas fluorescens, according to claim 6, transformed with a plasmid vector containing the B.t. toxin gene encoding the B.t. toxin having the amino acid sequence shown in Table 2.

8. Pseudomonas fluorescens (pM3,130-7), having the identifying characteristic of NRRL B-18332, a Pseudomonas fluorescens according to claim 7.

9. A microorganism according to claim 6, which is a species of Pseudomonas, Azotobacter, Erwinia, Serratia, Klebsiella, Rhizobium, Rhodopseudomonas, Methylophilius, Agrobacterium, Acetobacter or Alcaligenes.

10. A microorganism according to claim 9, wherein said microorganism is pigmented and phylloplane adherent.

11. A method for controlling lepidopteran insects which comprises administering to said insects or to the environment of said insects a microorganism according to claim 9.

12. A method according to claim 11, wherein said administration is to the rhizosphere.

13. A method according to claim ~~12~~ 11, wherein said administration is to the phylloplane.

14. A method according to claim 11, wherein said administration is to a body of water.

15. An insectididal composition comprising insecticide containing substantially intact, chemically or physically

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treated cells having prolonged pesticidal activity when applied to the environment of a target pest, wherein said insecticide is a polypeptide toxic to lepidopteran insects, is intracellular, and is produced as a result of expression of a transformed microbe capable of expressing the B.t. var kurstaki strain HD-73 and var thuringiensis strain Berliner 1715 toxin having the amino acid sequence shown in Table 2.

16. The insecticidal composition, according to claim 15, wherein said cells are prokaryotes or lower eukaryotes.

17. The insecticidal composition, according to claim 16, wherein said prokaryote cells are selected from the group consisting of Enterobacteriaceae, Bacillaceae, Rhizobiaceae, Spirillaceae, Lactobacillaceae, Pseudomonadaceae, Azotobacteraceae, and Nitrobacteraceae.

18. The insecticidal composition, according to claim 16, wherein said lower eukaryote cells are selected from the group consisting of Phycomycetes, Ascomycetes, and Basidiomycetes.

19. The insecticidal composition, according to claim 15, wherein said cell is a pigmented bacterium, yeast, or fungus.

20. Treated, substantially intact unicellular microorganism cells containing an intracellular toxin,

which toxin is a result of expression of a B.t. var kurstaki strain HD-73 and var thuringiensis strain Berliner 1715 toxin gene toxic to lepidopteran insects which codes for a polypeptide toxin having the amino acid sequence shown in Table 2, wherein said cells are chemically or physically treated under conditions which prolong the insecticidal activity when said cell is applied to the environment of a target insect.

21. The cells according to claim 20, wherein said microorganism is Pseudomonas.

22. Pseudomonas cells according to claim 21, wherein said cells are treated with iodine.

23. The cells, according to claim 20, which are Pseudomonas fluorescens.

24. The cells, according to claim 23, which are Pseudomonas fluorescens (pM3,130-7).

25. A plasmid selected from the group consisting of pM2,107-1 pM3,123-1 and pM3,130-7.

26. Plasmid pM3,130-7, according to claim 25.

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