

NOVEL PESTICIDAL PROTEINS AND STRAINS

5 The present invention is a continuation-in-part application of U.S. application serial number 08/037,057 filed March 25, 1993, the disclosures of which are herein incorporated by reference.

FIELD OF THE INVENTION

10 The present invention is drawn to methods and compositions for controlling plant and non-plant pests.

BACKGROUND OF THE INVENTION

15 Insect pests are a major factor in the loss of the world's commercially important agricultural crops. Broad spectrum chemical pesticides have been used extensively to control or eradicate pests of agricultural importance. There is, however, substantial interest in developing effective alternative pesticides.

20 Microbial pesticides have played an important role as alternatives to chemical pest control. The most extensively used microbial product is based on the bacterium Bacillus thuringiensis (Bt). Bt is a gram-positive spore forming Bacillus which produces an insecticidal crystal protein (ICP) during sporulation.

25 Numerous varieties of Bt are known that produce more than 25 different but related ICP's. The ICP's made by Bt are toxic to larvae of certain insects in the orders Lepidoptera,



Diptera and Coleoptera. In general, when the ICP is ingested by a susceptible insect the crystal is solubilized and transformed into a toxic moiety by the insect gut proteases. None of the ICP's active against coleopteran larvae have demonstrated significant effects on the genus Diabrotica particularly Diabrotica virgifera virgifera, the western corn rootworm (WCRW) or Diabrotica longicornis barberi, the northern corn rootworm.

Bt is closely related to Bacillus cereus (Bc). A major distinguishing characteristic is the lack of a parasporal crystal in Bc. Bc is a widely distributed bacterium that is commonly found in soil and has been isolated from a variety of foods and drugs. The organism has been implicated in the spoilage of food.

Although Bt has been very useful in controlling insect pests, there is a need to expand the number of potential biological control agents.

SUMMARY OF THE INVENTION

The present invention is drawn to compositions and methods for controlling plant and non-plant pests. Particularly, new pesticidal proteins are disclosed which are isolatable from the vegetative growth stage of Bacillus. Bacillus strains, proteins, and genes encoding the proteins are provided.

The methods and compositions of the invention may be used in a variety of systems for controlling plant and non-plant pests.

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DETAILED DESCRIPTION OF THE INVENTION

Compositions and methods for controlling plant pests are provided. In particular, novel pesticidal proteins are provided which are produced during vegetative growth of Bacillus strains.

5 The proteins are useful as pesticidal agents.

The present invention recognizes that pesticidal proteins are produced during vegetative growth of Bacillus strains. For the purpose of the present invention vegetative growth is defined as that period of time before the onset of sporulation. In the case of Bt, this vegetative growth occurs before production of ICPs. Genes encoding such proteins can be isolated, cloned and
10 transformed into various delivery vehicles for use in pest management programs.

For purposes of the present invention, pests include but are not limited to insects, fungi, bacteria, nematodes, mites, ticks, protozoan pathogens, animal-parasitic liver flukes, and the like. Insect pests include insects selected from the orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Mallophaga, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera,
15 Isoptera, Anoplura, Siphonaptera, Trichoptera, etc.

Tables 1 - 10 gives a list of pests associated with major crop plants and pests of human and veterinary importance. Such pests are included within the scope of the present invention.

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TABLE 1
Lepidoptera (Butterflies and Moths)

5	Maize		Sunflower
	<u>Ostrinia nubilalis</u> , European corn borer	35	<u>Suleima helianthana</u> , sunflower bud moth
	<u>Agrotis ipsilon</u> , black cutworm		<u>Homoeosoma electellum</u> , sunflower moth
	<u>Helicoverpa zea</u> , corn earworm		
10	<u>Spodoptera frugiperda</u> , fall armyworm		Cotton
	<u>Diatraea grandiosella</u> , southwestern corn borer	40	<u>Heliothis virescens</u> , cotton boll worm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer		<u>Helicoverpa zea</u> , cotton bollworm
15	<u>Diatraea saccharalis</u> , sugarcane borer		<u>Spodoptera exigua</u> , beet armyworm
			<u>Pectinophora gossypiella</u> , pink bollworm
	Sorghum	45	Rice
	<u>Chilo partellus</u> , sorghum borer		<u>Diatraea saccharalis</u> , sugarcane borer
	<u>Spodoptera frugiperda</u> , fall armyworm		<u>Spodoptera frugiperda</u> , fall armyworm
20	<u>Helicoverpa zea</u> , corn earworm		<u>Helicoverpa zea</u> , corn earworm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer	50	Soybean
	<u>Feltia subterranea</u> , granulate cutworm		<u>Pseudoplusia includens</u> , soybean looper
			<u>Anticarsia gemmatilis</u> , velvetbean caterpillar
25	Wheat		<u>Plathypena scabra</u> , green cloverworm
	<u>Pseudaletia unipunctata</u> , army worm		<u>Ostrinia nubilalis</u> , European corn borer
	<u>Spodoptera frugiperda</u> , fall armyworm	55	<u>Agrotis ipsilon</u> , black cutworm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer		<u>Spodoptera exigua</u> , beet armyworm
30	<u>Agrotis orthogonia</u> , pale western cutworm		<u>Heliothis virescens</u> , cotton boll worm
	<u>Elasmopalpus lignosellus</u> , lesser cornstalk borer	60	<u>Helicoverpa zea</u> , cotton bollworm
			Barley
			<u>Ostrinia nubilalis</u> , European corn borer
			<u>Agrotis ipsilon</u> , black cutworm

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TABLE 2

Coleoptera (Beetles)

Maize	
5	<u>Diabrotica virgifera virgifera</u> , western corn rootworm
	<u>Diabrotica longicornis barberi</u> , northern corn rootworm
	<u>Diabrotica undecimpunctata howardi</u> , southern corn rootworm
	<u>Melanotus</u> spp., wireworms
	<u>Cyclocephala borealis</u> , northern masked chafer (white grub)
10	<u>Cyclocephala immaculata</u> , southern masked chafer (white grub)
	<u>Popillia japonica</u> , Japanese beetle
	<u>Chaetocnema pulicaria</u> , corn flea beetle
	<u>Sphenophorus maidis</u> , maize billbug
Sorghum	
15	<u>Phyllophaga crinita</u> , white grub
	<u>Eleodes</u> , <u>Conoderus</u> , and <u>Aeolus</u> spp., wireworms
	<u>Oulema melanopus</u> , cereal leaf beetle
	<u>Chaetocnema pulicaria</u> , corn flea beetle
20	<u>Sphenophorus maidis</u> , maize billbug
Wheat	
	<u>Oulema melanopus</u> , cereal leaf beetle
	<u>Hypera punctata</u> , clover leaf weevil
25	<u>Diabrotica undecimpunctata howardi</u> , southern corn rootworm
Sunflower	
	<u>Zygogramma exclamationis</u> , sunflower beetle
	<u>Bothyrus gibbosus</u> , carrot beetle
30	
Cotton	
	<u>Anthonomus grandis</u> , boll weevil
Rice	
35	<u>Colaspis brunnea</u> , grape colaspis
	<u>Lissorhoptrus oryzophilus</u> , rice water weevil
	<u>Sitophilus oryzae</u> , rice weevil
Soybean	
40	<u>Epilachna varivestis</u> , Mexican bean beetle

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TABLE 3

Homoptera (Whiteflies, Aphids etc..)

5	Maize	<u>Rhopalosiphum maidis</u> , corn leaf aphid <u>Anuraphis maidiradicis</u> , corn root aphid
	Sorghum	
10		<u>Rhopalosiphum maidis</u> , corn leaf aphid <u>Sipha flava</u> , yellow sugarcane aphid
	Wheat	
15		Russian wheat aphid <u>Schizaphis graminum</u> , greenbug <u>Macrosiphum avenae</u> , English grain aphid
	Cotton	
20		<u>Aphis gossypii</u> , cotton aphid <u>Pseudatomoscelis seriatus</u> , cotton fleahopper <u>Trialeurodes abutilonea</u> , bandedwinged whitefly
	Rice	
25		<u>Nephotettix nigropictus</u> , rice leafhopper
	Soybean	
		<u>Myzus persicae</u> , green peach aphid <u>Empoasca fabae</u> , potato leafhopper
30	Barley	<u>Schizaphis graminum</u> , greenbug
	Oil Seed Rape	
		<u>Brevicoryne brassicae</u> , cabbage aphid



TABLE 4

Hemiptera (Bugs)

5	Maize	<u>Blissus leucopterus leucopterus</u> , chinch bug
	Sorghum	<u>Blissus leucopterus leucopterus</u> , chinch bug
10	Cotton	<u>Lygus lineolaris</u> , tarnished plant bug
	Rice	<u>Blissus leucopterus leucopterus</u> , chinch bug <u>Acrosternum hilare</u> , green stink bug
15	Soybean	<u>Acrosternum hilare</u> , green stink bug
20	Barley	<u>Blissus leucopterus leucopterus</u> , chinch bug <u>Acrosternum hilare</u> , green stink bug <u>Euschistus servus</u> , brown stink bug

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TABLE 5

Orthoptera (Grasshoppers, Crickets, and Cockroaches)

Maize	
5	<u>Melanoplus femurrubrum</u> , redlegged grasshopper <u>Melanoplus sanguinipes</u> , migratory grasshopper
Wheat	
10	<u>Melanoplus femurrubrum</u> , redlegged grasshopper <u>Melanoplus differentialis</u> , differential grasshopper <u>Melanoplus sanguinipes</u> , migratory grasshopper
Cotton	
15	<u>Melanoplus femurrubrum</u> , redlegged grasshopper <u>Melanoplus differentialis</u> , differential grasshopper
Soybean	
20	<u>Melanoplus femurrubrum</u> , redlegged grasshopper <u>Melanoplus differentialis</u> , differential grasshopper
Structural/Household	
	<u>Periplaneta americana</u> , American cockroach <u>Blattella germanica</u> , German cockroach <u>Blatta orientalis</u> , oriental cockroach

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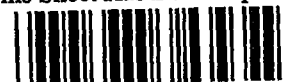


TABLE 6

Diptera (Flies and Mosquitoes)

Maize	
5	<u>Hylemya platura</u> , seedcorn maggot <u>Agromyza parvicornis</u> , corn blotch leafminer
Sorghum	
10	<u>Contarinia sorghicola</u> , sorghum midge
Wheat	
	<u>Mayetiola destructor</u> , Hessian fly <u>Sitodiplosis mosellana</u> , wheat midge <u>Meromyza americana</u> , wheat stem maggot
15	<u>Hylemya coarctata</u> , wheat bulb fly
Sunflower	
	<u>Neolasioptera murtfeldtiana</u> , sunflower seed midge
Soybean	
20	<u>Hylemya platura</u> , seedcorn maggot
Barley	
25	<u>Hylemya platura</u> , seedcorn maggot <u>Mayetiola destructor</u> , Hessian fly
Insects attacking humans and animals and disease carriers	
	<u>Aedes aegypti</u> , yellowfever mosquito <u>Aedes albopictus</u> , forest day mosquito
30	<u>Phlebotomus papatasi</u> , sand fly <u>Musca domestica</u> , house fly <u>Tabanus atratus</u> , black horse fly <u>Cochliomyia hominivorax</u> , screwworm fly

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TABLE 7

Thysanoptera (Thrips)

5	Maize	
	<u>Anaphothrips obscurus</u> , grass thrips	
	Wheat	
	<u>Frankliniella fusca</u> , tobacco thrips	
10	Cotton	
	<u>Thrips tabaci</u> , onion thrips	
	<u>Frankliniella fusca</u> , tobacco thrips	
15	Soybean	
	<u>Sericothrips variabilis</u> , soybean thrips	
	<u>Thrips tabaci</u> , onion thrips	

20

TABLE 8

Hymenoptera (Sawflies, Ants, Wasps, etc.)

25	Maize	
	<u>Solenopsis milesta</u> , thief ant	
	Wheat	
	<u>Cephus cinctus</u> , wheat stem sawfly	

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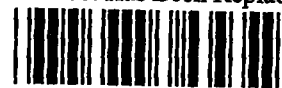


TABLE 9

Other Orders and Representative Species

- 5 Dermaptera (Earwigs)
Forficula auricularia, European earwig
- Isoptera (Termites)
Reticulitermes flavipes, eastern subterranean termite
- 10 Mallophaga (Chewing Lice)
Cuculotogaster heterographa, chicken head louse
Bovicola bovis, cattle biting louse
- 15 Anoplura (Sucking Lice)
Pediculus humanus, head and body louse
- Siphonaptera (Fleas)
Ctenocephalides felis, cat flea



TABLE 10

Acari (Mites and Ticks)

5	Maize	<u>Tetranychus urticae</u> , twospotted spider mite
10	Sorghum	<u>Tetranychus cinnabarinus</u> , carmine spider mite <u>Tetranychus urticae</u> , twospotted spider mite
	Wheat	<u>Aceria tulipae</u> , wheat curl mite
15	Cotton	<u>Tetranychus cinnabarinus</u> , carmine spider mite <u>Tetranychus urticae</u> , twospotted spider mite
20	Soybean	<u>Tetranychus turkestanii</u> , strawberry spider mite <u>Tetranychus urticae</u> , twospotted spider mite
	Barley	<u>Petrobia latens</u> , brown wheat mite
25	Important human and animal <u>Acari</u>	<u>Dermacentor variabilis</u> , American dog tick <u>Argas persicus</u> , fowl tick <u>Dermatophagoides farinae</u> , American house dust mite <u>Dermatophagoides pteronyssinus</u> , European house dust mite
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Now that it has been recognized that pesticidal proteins can be isolated from the vegetative growth phase of Bacillus, other strains can be isolated by standard techniques and tested for activity against particular plant and non-plant pests. Generally Bacillus strains can be isolated from any environmental sample, including soil, plant, insect, grain elevator dust, and other sample material, etc., by methods known in the art. See, for example, Travers et al. (1987) Appl. Environ. Microbiol. 53:1263-1266; Saleh et al. (1969) Can J. Microbiol. 15:1101-1104; DeLucca et al. (1981) Can J. Microbiol. 27:865-870; and Norris, et al. (1981) "The genera Bacillus and Sporolactobacillus," In Starr et al. (eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Vol. II, Springer-Verlog Berlin Heidelberg.

After isolation, strains can be tested for pesticidal activity during vegetative growth. In this manner, new pesticidal proteins and strains can be identified.

Such Bacillus microorganisms which find use in the invention include Bacillus cereus and Bacillus thuringiensis, as well as those Bacillus species listed in Table 11.



TABLE 11

List of Bacillus species

Morphological Group 1		Unassigned Strains	
5	<u>B. megaterium</u>	Subgroup A	
	<u>B. cereus*</u>		<u>B. apiarus*</u>
	<u>B. cereus var. mycoides</u>	35	<u>B. filicollonicus</u>
	<u>B. thuringiensis*</u>		<u>B. thiaminolyticus</u>
	<u>B. licheniformis</u>		<u>B. alcalophilus</u>
10	<u>B. subtilis*</u>	Subgroup B	
	<u>B. pumilus</u>		<u>B. cirroflagellus</u>
	<u>B. firmus*</u>	40	<u>B. chitinosporus</u>
	<u>B. coagulans</u>		<u>B. lentus</u>
15	Morphological Group 2	Subgroup C	
	<u>B. polymyxa</u>		<u>B. badius</u>
	<u>B. macerans</u>	45	<u>B. aneurinolyticus</u>
	<u>B. circulans</u>		<u>B. macroides</u>
	<u>B. stearothermophilus</u>		<u>B. freundenreichii</u>
20	<u>B. alvei*</u>	Subgroup D	
	<u>B. laterosporus*</u>		<u>B. pantothenicus</u>
	<u>B. brevis</u>	50	<u>B. epiphytus</u>
	<u>B. pulvifaciens</u>	Subgroup E1	
	<u>B. popilliae*</u>		<u>B. aminovorans</u>
25	<u>B. lentimorbus*</u>	55	<u>B. globisporus</u>
	<u>B. larvae*</u>		<u>B. insolitus</u>
	Morphological Group 3		<u>B. psychrophilus</u>
	<u>B. sphaericus*</u>	Subgroup E2	
30	<u>B. pasteurii</u>		<u>B. psychrosaccharolyticus</u>
		60	<u>B. macquariensis</u>

*=Those Bacillus strains that have been previously found in insects

65 Grouping according to Parry, J.M. et al. (1983) Color Atlas of Bacillus species, Wolfe Medical Publications, London.



In accordance with the present invention, the pesticidal proteins produced during vegetative growth can be isolated from Bacillus. In one embodiment, insecticidal proteins produced during vegetative growth, herein after referred to as VIP's (Vegetative Insecticidal Protein), can be isolated. Methods for protein isolation are known in the art. Generally, proteins
5 can be purified by conventional chromatography, including gel-filtration, ion-exchange, and immunoaffinity chromatography, by high-performance liquid chromatography, such as reversed-phase high-performance liquid chromatography, ion-exchange high-performance liquid chromatography, size-exclusion high-performance liquid chromatography, high-performance chromatofocusing and hydrophobic interaction chromatography, etc., by electrophoretic
10 separation, such as one-dimensional gel electrophoresis, two-dimensional gel electrophoresis, etc. Such methods are known in the art. See for example Current Protocols in Molecular Biology, Vols. 1 and 2, Ausubel et al. (eds.), John Wiley & Sons, NY (1988). Additionally, antibodies can be prepared against substantially pure preparations of the protein. See, for example, Radka et al. (1983) J. Immunol. 128:2804; and Radka et al. (1984) Immunogenetics
15 19:63. Any combination of methods may be utilized to purify protein having pesticidal properties. As the protocol is being formulated, pesticidal activity is determined after each purification step.

Such purification steps will result in a substantially purified protein fraction. By "substantially purified" or "substantially pure" is intended protein which is substantially free of
20 any compound normally associated with the protein in its natural state. "Substantially pure" preparations of protein can be assessed by the absence of other detectable protein bands following SDS-PAGE as determined visually or by densitometry scanning. Alternatively, the absence of other amino-terminal sequences or N-terminal residues in a purified preparation can indicate the level of purity. Purity can be verified by rechromatography of "pure" preparations
25 showing the absence of other peaks by ion exchange, reverse phase or capillary electrophoresis.



The terms "substantially pure" or "substantially purified" are not meant to exclude artificial or synthetic mixtures of the proteins with other compounds. The terms are also not meant to exclude the presence of minor impurities which do not interfere with the biological activity of the protein, and which may be present, for example, due to incomplete purification.

5 Some proteins are single polypeptide chains while many proteins consist of more than one polypeptide chain. Once purified protein is isolated, the protein, or the polypeptides of which it is comprised, can be characterized and sequenced by standard methods known in the art. For example, the purified protein, or the polypeptides of which it is comprised, may be fragmented as with cyanogen bromide, or with proteases such as papain, chymotrypsin, trypsin, 10 lysyl-C endopeptidase, etc. (Oike et al. (1982) J. Biol. Chem. 257:9751-9758; Liu et al. (1983) Int. J. Pept. Protein Res. 21:209-215). The resulting peptides are separated, preferably by HPLC, or by resolution of gels and electroblotting onto PVDF membranes, and subjected to amino acid sequencing. To accomplish this task, the peptides are preferably analyzed by automated sequencers. It is recognized that N-terminal, C-terminal, or internal amino acid 15 sequences can be determined. From the amino acid sequence of the purified protein, a nucleotide sequence can be synthesized which can be used as a probe to aid in the isolation of the gene encoding the pesticidal protein.

It is recognized that the proteins will vary in molecular weight, component peptides, activity against particular pests, and in other characteristics. However, by the methods set forth 20 herein, proteins active against a variety of pests may be isolated and characterized.

Once the purified protein has been isolated and characterized it is recognized that it may be altered in various ways including amino acid substitutions, deletions, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants of the pesticidal proteins can be prepared by mutations in the DNA. Such variants will 25 possess the desired pesticidal activity. Obviously, the mutations that will be made in the DNA



encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

5 In this manner, the present invention encompasses the pesticidal proteins as well as components and fragments thereof. That is, it is recognized that component polypeptides or fragments of the proteins may be produced which retain pesticidal activity. These fragments include truncated sequences, as well as N-terminal, C-terminal, internal and internally deleted amino acid sequences of the proteins.

10 Most deletions, insertions, and substitutions of the protein sequence are not expected to produce radical changes in the characteristics of the pesticidal protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays.

15 The proteins or other component polypeptides described herein may be used alone or in combination. That is, several proteins may be used to control different insect pests. Additionally, certain of the proteins of the invention enhance the activity of the pesticidal proteins. These proteins are referred to herein as "auxiliary proteins." While the mechanism of action is not entirely certain, when the auxiliary protein and the pesticidal protein of interest are together, the insecticidal properties of the pesticidal protein are enhanced several fold.

20 The pesticidal proteins of the present invention may vary in molecular weight, having component polypeptides at least a molecular weight of 30 kDa or greater, preferably about 50 kDa or greater.

25 The auxiliary proteins of the invention may vary in molecular weight, having at least a molecular weight of about 15 kDa or greater, preferably about 20 kDa or greater. The auxiliary proteins themselves may have component polypeptides.



It is possible that the pesticidal protein and the auxiliary protein may be components of a multimeric, pesticidal protein. Such a pesticidal protein which includes the auxiliary proteins as one or more of its component polypeptides may vary in molecular weight, having at least a molecular weight of 50 kDa up to at least 200 kDa, preferably about 100 kDa to 150 kDa.

5 An auxiliary protein may be used in combination with the pesticidal proteins of the invention to enhance activity. To determine whether the auxiliary protein will affect activity, the pesticidal protein can be expressed alone and in combination with the auxiliary protein and the respective activities compared in feeding assays for increased pesticidal activity.

10 It may be beneficial to screen strains for potential pesticidal activity by testing activity of the strain alone and in combination with the auxiliary protein. In some instances the auxiliary protein with the native proteins of the strains yields pesticidal activity where none is seen in the absence of the auxiliary protein.

The auxiliary protein can be modified, as described above, by various methods known in the art. Therefore, for purposes of the invention, the term "Vegetative Insecticidal Protein" 15 (VIP) encompasses those proteins produced during vegetative growth which alone or in combination can be used for pesticidal activity. This includes pesticidal proteins, auxiliary proteins and those proteins which demonstrate activity only in the presence of the auxiliary protein or the polypeptide components of these proteins.

20 It is recognized that there are alternative methods available to obtain the nucleotide and amino acid sequences of the present proteins. For example, to obtain the nucleotide sequence encoding the pesticidal protein, cosmid clones, which express the pesticidal protein, can be isolated from a genomic library. From larger active cosmid clones, smaller subclones can be made and tested for activity. In this manner, clones which express an active pesticidal protein can be sequenced to determine the nucleotide sequence of the gene. Then, an amino acid 25 sequence can be deduced for the protein. For general molecular methods, see, for example,



Molecular Cloning, A Laboratory Manual, Second Edition, Vols. 1-3, Sambrook et al. (eds.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989), and the references cited therein.

The present invention also encompasses nucleotide sequences from organisms other than
 5 Bacillus, where the nucleotide sequences are isolatable by hybridization with the Bacillus nucleotide sequences of the invention. Such nucleotide sequences can be tested for pesticidal activity. The invention also encompasses the proteins encoded by the nucleotide sequences. Furthermore, the invention encompasses proteins obtained from organisms other than Bacillus wherein the protein cross-reacts with antibodies raised against the proteins of the invention.
 10 Again the isolated proteins can be assayed for pesticidal activity by the methods disclosed herein.

Once the nucleotide sequences encoding the pesticidal proteins of the invention have been isolated, they can be manipulated and used to express the protein in a variety of hosts including other organisms, including microorganisms and plants.

15 The pesticidal genes of the invention can be optimized for enhanced expression in plants. See, for example U.S. Application Serial No. 07/951,715; EPA 0359472; EPA 0385962; WO 91/16432; Perlak et al (1991) Proc. Natl. Acad. Sci. USA 88:3324-3328; and Murray et al (1989) Nucleic Acids Research 17: 477-498. In this manner, the genes can be synthesized utilizing plant preferred codons. That is the preferred codon for a particular host is the single
 20 codon which most frequently encodes that amino acid in that host. The maize preferred codon, for example, for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is found in Murray et al. (1989), Nucleic Acids Research 17:477-498, the disclosure of which is incorporated herein by reference. Synthetic genes could also be made based on the distribution of codons a particular host uses for
 25 a particular amino acid.



In this manner, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

In like manner, the nucleotide sequences can be optimized for expression in any
5 microorganism. For Bacillus preferred codon usage, see, for example US Patent No. 5,024,837 and Johansen et al (1988) Gene 65:293-304.

Methodologies for the construction of plant expression cassettes as well as the introduction of foreign DNA into plants are described in the art. Such expression cassettes may include promoters, terminators, enhancers, leader sequences, introns and other regulatory
10 sequences operably linked to the pesticidal protein coding sequence.

Generally, for the introduction of foreign DNA into plants Ti plasmid vectors have been utilized for the delivery of foreign DNA as well as direct DNA uptake, liposomes, electroporation, micro-injection, and the use of microprojectiles. Such methods had been published in the art. See, for example, Guerche et al., (1987) Plant Science 52:111-116;
15 Neuhauser et al., (1987) Theor. Appl. Genet. 75:30-36; Klein et al., (1987) Nature 327: 70-73; Howell et al., (1980) Science 208:1265; Horsch et al., (1985) Science 227: 1229-1231; DeBlock et al., (1989) Plant Physiology 91:694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988); and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press, Inc. (1989). See also US patent Application Serial
20 No. 08/008,374 herein incorporated by reference. See also, EPA 0193259 and EPA 0451878A1. It is understood that the method of transformation will depend upon the plant cell to be transformed.

It is further recognized that the components of the expression cassette may be modified to increase expression. For example, truncated sequences, nucleotide substitutions or other
25 modifications may be employed. See, for example Perlak et al. (1991) Proc. Natl. Acad. Sci.



USA 88:3324-3328; Murray et al. (1989) Nucleic Acids Research 17:477-498; and WO 91/16432.

The construct may also include any other necessary regulators such as terminators, (Guerineau et al., (1991), Mol. Gen. Genet., 226:141-144; Proudfoot, (1991), Cell, 5 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; Joshi et al., (1987), Nucleic Acid Res., 15:9627-9639); plant translational consensus sequences (Joshi, C.P., (1987), Nucleic Acids Research, 15:6643-6653), introns (Luehrsen and Walbot, (1991), Mol. Gen. Genet., 225:81-93) and the like, operably linked to the 10 nucleotide sequence. It may be beneficial to include 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translational leaders are known in the art and include:

- Picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein, O., Fuerst, T.R., and Moss, B. (1989) PNAS USA 86:6126-6130);
- 15 Potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison et al., (1986); MDMV leader (Maize Dwarf Mosaic Virus); Virology, 154:9-20), and
- Human immunoglobulin heavy-chain binding protein (BiP), (Macejak, D.G., and Sarnow, P., (1991), Nature, 353:90-94;
- Untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4),
- 20 (Jobling, S.A., and Gehrke, L., (1987), Nature, 325:622-625;
- Tobacco mosaic virus leader (TMV), (Gallie, D.R. et al., (1989), Molecular Biology of RNA, pages 237-256; and
- Maize Chlorotic Mottle Virus leader (MCMV) (Lommel, S.A. et al., (1991), Virology, 81:382-385. See also, Della-Cioppa et al., (1987), Plant Physiology, 84:965-968.



A plant terminator may be utilized in the expression cassette. See, Rosenberg et al., (1987), Gene, 56:125; Guerinneau et al., (1991), Mol. Gen. Genet., 226:141-144; 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
 5 Acids Res., 17:7891-7903; Joshi et al., (1987), Nucleic Acid Res., 15:9627-9639.

For tissue specific expression, the nucleotide sequences of the invention can be operably linked to tissue specific promoters. See, for example, US Application Serial No. 07/951,715 herein incorporated by reference.

It is recognized that the genes encoding the pesticidal proteins can be used to transform
 10 insect pathogenic organisms. Such organisms include Baculoviruses, fungi, protozoa, bacteria and nematodes.

The Bacillus strains of the invention may be used for protecting agricultural crops and products from pests. Alternatively, a gene encoding the pesticide may be introduced via a suitable vector into a microbial host, and said host applied to the environment or plants or
 15 animals. Microorganism hosts may be selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplana) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for
 20 improved protection of the pesticide from environmental degradation and inactivation.

Such microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., Pseudomonas, Erwinia, Serratia, Klebsiella,
Xanthomonas, Streptomyces, Rhizobium, Rhodopseudomonas, Methylius, Agrobacterium,
Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, and Alcaligenes; fungi,
 25 particularly yeast, e.g., Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces,



Rhodotorula, and Aureobasidium. Of particular interest are such phytosphere bacterial species as Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacteria, Rhodopseudomonas spheroides, Xanthomonas campestris, Rhizobium melioli, Alcaligenes entrophus, Clavibacter xyli and Azotobacter vinlandii; and phytosphere yeast

5 species such as Rhodotorula rubra, R. glutinis, R. marina, R. aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces rosues, S. odoris, Kluyveromyces veronae, and Aureobasidium pollulans. Of particular interest are the pigmented microorganisms.

A number of ways are available for introducing a gene expressing the pesticidal protein

10 into the microorganism host under conditions which allow for stable maintenance and expression of the gene. For example, expression cassettes can be constructed which include the DNA constructs of interest operably linked with the transcriptional and translational regulatory signals for expression of the DNA constructs, 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

15 the host, whereby integration or stable maintenance will occur.

Transcriptional and translational regulatory signals include but are not limited to promoter, transcriptional initiation start site, operators, activators, enhancers, other regulatory elements, ribosomal binding sites, an initiation codon, termination signals, and the like. See, for example, US Patent 5,039,523; US Patent No. 4,853,331; EPO 0480762A2; Sambrook et al.

20 supra; Molecular Cloning, a Laboratory Manual, Maniatis et al. (eds) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982); Advanced Bacterial Genetics, Davis et al. (eds.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1980); and the references cited therein.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the

25 activity of the toxin in the cell when the then treated cell is applied to the environment of the



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 a 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 protein gene into the host, availability of expression systems, efficiency of expression, stability of the protein 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.

General methods for employing the strains of the invention in pesticide control or in engineering other organisms as pesticidal agents are known in the art. See, for example US Patent No. 5,039,523 and EP 0480762A2.

The Bacillus strains of the invention or the microorganisms which have been genetically altered to contain the 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 pesticides are produced by introducing a heterologous gene into a cellular host. Expression of the heterologous gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. 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.

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 both fertilizers or micronutrient donors or other preparations that influence plant growth. They can also be selective herbicides, insecticides, fungicides, bactericides, 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.

5 Preferred methods of applying an active ingredient of the present invention or an agrochemical composition of the present invention which contains at least one of the pesticidal proteins produced by the bacterial strains of the present invention are 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.

10 In one embodiment of the invention a Bacillus cereus microorganism has been isolated which is capable of killing Diabrotica virgifera virgifera, and Diabrotica longicornis barberi. The novel B. cereus strain AB78 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, IL 61604, USA and given Accession No. NRRL B-21058.

15 A protein has been substantially purified from the B. cereus strain. Purification of the protein has been verified by SDS-PAGE and biological activity. The protein has a molecular weight of about 60 to about 100 kDa, particularly about 70 to about 90 kDa, more particularly about 80 kDa.

Amino-terminal sequencing has revealed the N-terminal amino-acid sequence to be:

20 NH₂-Lys-Arg-Glu-Ile-Asp-Glu-Asp-Thr-Asp-Thr-Asx-Gly-Asp-Ser-Ile-Pro- (SEQ ID NO:8)
where Asx represents either Asp or Asn. The entire amino acid sequence is given in SEQ ID NO:7.

An oligonucleotide probe for the region of the gene encoding amino acids 3-9 of the NH₂-terminus has been generated. The probe was synthesized based on the codon usage of a



Bacillus thuringensis (Bt) δ -endotoxin gene. The nucleotide sequence of the oligonucleotide probe used for Southern hybridizations was as follows:

5'- GAA ATT GAT CAA GAT ACN GAT -3' (SEQ ID NO:9)

where N represents any base.

5 In addition, the DNA probe for the Bc AB78 VIP-1 gene described herein, permits the screening of any Bacillus strain or other organisms to determine whether the VIP-1 gene (or related gene) is naturally present or whether a particular transformed organism includes the VIP-1 gene.

10 The invention now being generally described, the same will be better understood by reference to the following detailed examples that are provided for the purpose of illustration and are not to be considered limiting of the invention unless so specified.



EXPERIMENTALExample 1. AB78 Isolation and Characterization

Bacillus cereus strain AB78 was isolated as a plate contaminant in the laboratory on T3 media (per liter: 3 g tryptone, 2 g tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate (pH 6.8), and 0.005 g $MnCl_2$; Travers, R.S. 1983). AB78 gave significant activity against western corn rootworm. Antibiotic activity against gram-positive Bacillus spp. was also demonstrated (Table 12).

Table 12

Antibiotic activity of AB78 culture supernatant

<u>Bacteria tested</u>	<u>Zone of inhibition(cm)</u>	
	<u>AB78</u>	<u>Streptomycin</u>
<u>E. coli</u>	0.0	3.0
<u>B. megaterium</u>	1.1	2.2
<u>B. mycoides</u>	1.3	2.1
<u>B. cereus</u> CB	1.0	2.0
<u>B. cereus</u> 11950	1.3	2.1
<u>B. cereus</u> 14579	1.0	2.4
<u>B. cereus</u> AB78	0.0	2.2
<u>Bt</u> var. <u>isrealensis</u>	1.1	2.2
<u>Bt</u> var. <u>tenebrionis</u>	0.9	2.3

Morphological characteristics of AB78 are as follows:

Vegetative rods straight, 3.1-5.0 mm long and 0.5-2.0 mm wide. Cells with rounded ends, single in short chains. Single subterminal, cylindrical-oval, endospore formed per cell. No



parasporal crystal formed. Colonies opaque, erose, lobate and flat. No pigments produced.

Cells motile. Flagella present.

Growth characteristics of AB78 are as follows:

Facultative anaerobe with optimum growth temperature of 21-30°C. Will grow at 15,
5 20, 25, 30 and 37°C. Will not grow above 40°C. Grows in 5-7% NaCl.

Table 13 provides the biochemical profile of AB78.

Table 13

Biochemical characteristics of B. cereus strain AB78.

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Acid from L-arabinose	-	Methylene blue reoxidized	+
Gas from L-arabinose	-	Nitrate reduced	+
Acid from D-xylose	-	NO ₃ reduced to NO ₂	+
Gas from D-xylose	-	VP	+
Acid from D-glucose	+	H ₂ O ₂ decomposed	+
Gas from D-glucose	-	Indole	-
Acid from lactose	-	Tyrosine decomposed	+
Gas from lactose	-	Dihydroxyacetone	-
Acid from sucrose	-	Litmus milk acid	-
Gas from sucrose	-	Litmus milk coagulated	-
Acid from D-mannitol	-	- Litmus milk alkaline	-
Gas from D-mannitol	-	Litmus milk peptonized	-
Propionate utilization	+	+ Litmus milk reduced	-
Citrate utilization	+	Casein hydrolyzed	+
Hippurate hydrolysis	w	Starch hydrolyzed	+
Methylene blue reduced	+	Gelatin liquidified	+
		Lecithinase produced	w

w= weak reaction



Example 2. Bacterial Culture

A subculture of Bc strain AB78 was used to inoculate the following medium, known as TB broth:

5	Tryptone	12	g/l
	Yeast Extract	24	g/l
	Glycerol	4	ml/l
	KH ₂ PO ₄	2.1	g/l
10	K ₂ HPO ₄	14.7	g/l
	pH 7.4		

The potassium phosphate was added to the autoclaved broth after cooling. Flasks were incubated at 30°C on a rotary shaker at 250 rpm for 24 h.-36 h.

15 The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

During vegetative growth, usually 24-36 h. after starting the culture, AB78 bacteria were centrifuged from the culture supernatant. The culture supernatant containing the active protein was used in bioassays.

20

Example 3. Insect Bioassays

B. cereus strain AB78 was tested against various insects as described below.

25 Western, Northern and Southern corn rootworm, Diabrotica virgifera virgifera, D. longcornis barberi and D. undecempunctata howardi, respectively:, dilutions were made of AB78 culture supernatant grown 24-36 h., mixed with molten artificial diet (Marrone et al.



(1985) J. of Economic Entomology 78:290-293) and allowed to solidify. Solidified diet was cut and placed in dishes. Neonate larvae were placed on the diet and held at 30°C. Mortality was recorded after 6 days.

E. coli clone bioassay: E. coli was grown overnight in L-Amp100 at 37°C. Ten ml
5 culture was sonicated 3X for 20 sec each. 500 ml of sonicated culture was added to molten western corn rootworm diet.

Colorado potato beetle Leptinotarsa decemlineata:-dilutions in Triton X-100 (to give final concentration of 0.1% TX-100) were made of AB78 culture supernatant grown 24-36 h. Five cm² potato leaf pieces were dipped into these dilutions, air dried, and placed on moistened
10 filter paper in plastic dishes. Neonate larvae were placed on the leaf pieces and held at 30°C. Mortality was recorded after 3-5 days.

Yellow mealworm, Tenebrio molitor:- dilutions were made of AB78 culture supernatant grown 24-36 h., mixed with molten artificial diet (Bioserv #F9240) and allowed to solidify. Solidified diet was cut and placed in plastic dishes. Neonate larvae were placed on the
15 diet and held at 30°C. Mortality was recorded after 6-8 days.

European corn borer, black cutworm, tobacco budworm, tobacco hornworm and beet armyworm; Ostrinia nubilalis, Agrotis ipsilon, Heliothis virescens, Manduca sexta and Spodoptera exigua, respectively: -dilutions, in TX-100 (to give final concentration of 0.1% TX-100), were made of AB78 culture supernatant grown 24-36 hrs. 100 ml was pipetted onto
20 the surface of 18 cm² of solidified artificial diet (Bioserv #F9240) and allowed to air dry. Neonate larvae were then placed onto the surface of the diet and held at 30°C. Mortality was recorded after 3-6 days.

Northern house mosquito, Culex pipiens:-dilutions were made of AB78 culture supernatant grown 24-36 h. 100 ml was pipetted into 10 ml water in a 30 ml plastic cup. Third



instar larvae were added to the water and held at room temperature. Mortality was recorded after 24-48 hours. The spectrum of entomocidal activity of AB78 is given in Table 14.

Table 14

Activity of AB78 culture supernatant against various insect species

Insect species tested to date	Order	Activity
Western corn rootworm (<u>Diabrotica virgifera</u> <u>virgifera</u>)	Col	+++
Northern corn rootworm (<u>Diabrotica longicornis</u> <u>barberi</u>)	Col	+++
Southern corn rootworm (<u>Diabrotica undecimpunctata</u> <u>howardi</u>)	Col	-
Colorado potato beetle (<u>Leptinotarsa decemlineata</u>)	Col	-
Yellow mealworm (<u>Tenebrio molitor</u>)	Col	-
European corn borer (<u>Ostrinia nubilalis</u>)	Lep	-
Tobacco budworm (<u>Heliothis virescens</u>)	Lep	-
Tobacco hornworm (<u>Manduca sexta</u>)	Lep	-
Beet armyworm (<u>Spodoptera exigua</u>)	Lep	-
Black cutworm (<u>Agrotis ipsilon</u>)	Lep	-
Northern house mosquito (<u>Culex pipiens</u>)	Dip	-



The newly discovered B. cereus strain AB78 showed a significantly different spectrum of insecticidal activity as compared to known coleopteran active δ -endotoxins from Bt. In particular, AB78 showed more selective activity against beetles than known coleopteran-active Bt strains in that it was specifically active to Diabrotica spp. More specifically, it was most active against D. virgifera virgifera and D. longicornis barberi but not D. undecimpunctata howardi.

A number of Bacillus strains were bioassayed for activity during vegetative growth (Table 15) against western corn rootworm. The results demonstrate that AB78 is unique in that activity against western corn rootworm is not a general phenomenon.

Table 15

Activity of culture supernatants from various Bacillus spp. against western corn rootworm

<u>Bacillus strain</u>	<u>Percent WCRW mortality</u>
<u>B. cereus</u> AB78 (Bat.1)	100
<u>B. cereus</u> AB78 (Bat.2)	100
<u>B. cereus</u> (Carolina Bio.)	12
<u>B. cereus</u> ATCC 11950	12
<u>B. cereus</u> ATCC 14579	8
<u>B. mycoides</u> (Carolina Bio.)	30
<u>B. popilliae</u>	28
<u>B. thuringiensis</u> HD135	41
<u>B. thuringiensis</u> HD191	9
<u>B. thuringiensis</u> GC91	4
<u>B. thuringiensis israelensis</u>	24
Water Control	4



Specific activity of AB78 against western corn rootworm is provided in Table 16.

Table 16

Activity of AB78 culture supernatant against neonate western corn rootworm

Culture supernatant concentration (μ l/ml)	Percent WCRW mortality
100	100
25	87
10	80
5	40
2.5	20
1	6
0	0

The LC_{50} was calculated to be 6.2 μ l of culture supernatant per ml of western corn rootworm diet.

Example 4. Isolation and Purification of Corn Rootworm Active Protein from AB78.

Culture media free of cells and debris was made to 70% saturation by the addition of solid ammonium sulfate i.e. (472 g/L). Dissolution was at room temperature followed by cooling in an ice bath and centrifugation at 10,000 x g for thirty minutes to pellet out the precipitated proteins.

The supernatant was discarded and the pellet was dissolved in 1/10 the original volume with 20 mM TRIS-HCl at pH 7.5.

The dissolved pellet was desalted either by dialysis in 20 mM TRIS HCl pH 7.5, or passing through a desalting column.



The desalted material was titrated to pH 3.5 with 20 mM sodium citrate pH 2.5. Following a thirty minute room temperature incubation the solution was centrifuged at 3000 X g for ten minutes. The supernatant at this stage contained the greatest amount of active protein.

Following neutralization of the pH to 7.0 the supernatant was applied to a Mono-Q,
5 anion exchange, column equilibrated with 20 mM TRIS pH 7.5 at a flow rate of 300 mL/min. The column was developed with a stepwise and linear gradient employing 400 mM NaCl in 20 mM TRIS pH 7.5.

Bioassay of the column fractions and SDS-PAGE analysis were used to confirm the active fractions. SDS-PAGE analysis identified the biologically active protein as having a
10 molecular weight in the range of 80 kDa.

Example 5. Sequence Analysis of the Corn Rootworm Active Protein

The 80 kDa protein isolated by SDS-PAGE was transferred to PVDF membrane and was
15 subjected to amino-terminal sequencing as performed by repetitive Edman cycles on the ABI 470 pulsed-liquid sequencer. Transfer was carried out in 10 mM CAPS buffer with 10% methanol pH 11.0 as follows:

Incubation of the gel following electrophoresis was done in transfer buffer for five minutes.

20 ProBlott PVDF membrane was wetted with 100% MeOH briefly then equilibrated in transfer buffer.

The sandwich was arranged between foam sponges and filter paper squares with the configuration of Cathode-Gel-Membrane-Anode.

Transfer was performed at 70 V constant voltage for 1 hour.



Following transfer the membrane was rinsed with water and stained for two minutes with 0.25% Coomassie Blue R-250 in 50% MeOH.

Destaining was done with several rinses with 50% MeOH 40% water 10% acetic acid.

Following destaining the membrane was air dried prior to excision of the bands for
 5 sequence analysis. A BlottCartridge and appropriate cycles were utilized to achieve maximum efficiency and yield. Data analysis was performed using the model 610 Sequence Analysis software for identifying and quantifying the PTH-amino acid derivatives for each sequential cycle.

The N-terminal sequence was determined to be:

10 NH₂-Lys-Arg-Glu-Ile-Asp-Glu-Asp-Thr-Asp-Thr-Asx-Gly-Asp-Ser-Ile-Pro- (SEQ ID NO:8)
 where Asx represents Asp or Asn.

Example 6. Construction of DNA Probe

An oligonucleotide probe for the region of the gene encoding amino acids 3-9 of the
 15 N-terminal sequence (Example 5) was generated. The probe was synthesized based on the codon usage of a Bacillus thuringensis (Bt) δ -endotoxin gene. The nucleotide sequence

5'- GAA ATT GAT CAA GAT ACN GAT -3' (SEQ ID NO:9)

was used as a probe in Southern hybridizations. The oligonucleotide was synthesized using standard procedures and equipment.

20

Example 7. Isoelectric Point Determination of the Corn Rootworm Active Protein

Purified protein from step 5 of the purification process was analyzed on a 3-9 pI isoelectric focusing gel using the Phastgel electrophoresis system (Pharmacia). Standard



operating procedures for the unit were followed for both the separation and silver staining development procedures. The pI was approximated at about 4.9.

Example 8. PCR Data On AB78

PCR analysis (See, for example US patent Application Serial No. 08/008,006; and, Carozzi et al. (1991) Appl. Environ. Microbiol. 57(11):3057-3061, herein incorporated by reference.) was used to verify that the B. cereus strain AB78 did not contain any insecticidal crystal protein genes of B. thuringensis or B. sphaericus (Table 17).

Table 17

Bacillus insecticidal crystal protein gene primers tested by PCR against AB78 DNA.

Primers Tested	Product Produced
2 sets specific for CryIIIA	Negative
CryIIIB	Negative
2 sets specific for CryIA	Negative
CryIA(a)	Negative
CryIA(b) specific	Negative
CryIB	Negative
CryIC specific	Negative
CryIE specific	Negative
2 sets specific for <u>B. sphaericus</u>	Negative
2 sets specific for CryIV	Negative
<u>Bacillus</u> control (PI-PLC)	Positive



Example 9. Cosmid Cloning of Total DNA from B. cereus Strain AB78

The VIP-1 gene was cloned from total DNA prepared from strain AB78 as follows:

5

Isolation of AB78 DNA was as follows:

1. Grow bacteria in 10 ml L-broth overnight. (Use 50 ml sterile centrifuge tube)
2. Add 25 ml of fresh L-broth and ampicillin (30 mg/ml).
3. Grow cells 2-6 h. at 30°C with shaking.
- 10 4. Spin cells in a 50 ml polypropylene orange cap tube in IEC benchtop clinical centrifuge at 3/4 speed.
5. Resuspend cell pellet in 10 ml TES.
6. Add 30 mg lysozyme and incubate 2 hrs at 37°C.
7. Add 200 ml 20% SDS and 400 ml Proteinase K (20 mg/ml). Incubate at 37°C.
- 15 8. Add 200 ml fresh Proteinase K. Incubate 1 hr. at 55°C. Add 5 ml TES (TES = 50 mM Tris pH 8.0, 100mM EDTA, 15 mM NaCl) to make 15 ml final volume.
9. Phenol extract twice (10 ml phenol, spin at room temperature at 3/4 speed in an IEC benchtop clinical centrifuge). Transfer supernatant (top) to a clean tube with a wide bore pipet.
- 20 10. Extract once with 1:1 vol. phenol:chloroform/isoamyl alcohol (24:1 ratio).
11. Precipitate DNA with an equal volume of cold isopropanol; Centrifuge to pellet DNA.
12. Resuspend pellet in 5 ml TE.
13. Precipitate DNA with 0.5 ml 3M NaOAc pH 5.2 and 11 ml 95% ethanol. Place at -20°C for 2 h.

This Sheet Has Been Replaced



14. "Hook" DNA from tube with a plastic loop, transfer to a microfuge tube, spin, pipet off excess ethanol, dry in vacuo.
15. Resuspend in 0.5 ml TE. Incubate 90 min. at 65°C to help get DNA back into solution.
16. Determine concentration using standard procedures.

5

This Sheet Has Been Replaced



Cosmid Cloning of AB78

All procedures, unless indicated otherwise, were performed according to Stratagene Protocol, Supercos 1 Instruction Manual, Cat. No. 251301.

Generally, the steps were as follows:

- 5 A. Sau 3A Partial Digestion of the AB78 DNA.
- B. Preparation of Vector DNA
- C. Ligation and packaging of DNA
- D. Titering the cosmid library
 - 10 1. Start a culture of HB101 cells by placing 50 ml of an overnight culture in
 5 mls of TB with 0.2% maltose. Incubate 3.5 hrs. at 37°C.
 2. Spin out cells and resuspend in 0.5 mls 10 mM MgSO₄.
 3. Add together:
 - 100 ml cells
 - 100 ml diluted packaging mixture
 - 15 100 ml 10 mM MgSO₄
 - 30 ml TB
 4. Adsorb at room temperature for 30 minutes with no shaking.
 5. Add 1 ml TB and mix gently. Incubate 30 minutes at 37°C.
 - 20 6. Plate 200 ml onto L-amp plates. Incubate at 37°C overnight.

At least 400 cosmid clones were screened for activity against western corn rootworm as described in Example 3. DNA from 5 active clones and 5 non-active clones were used in Southern hybridizations. Results demonstrated that hybridization using the above described oligonucleotide probe correlated with western corn rootworm activity (Table 18).

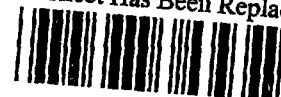


Cosmid clones P3-12 and P5-4 have been deposited with the Agricultural Research Service Patent Culture Collection (NRRL) and given accession nos. B-21061 and B-21059 respectively.

Table 18

Activity of AB78 cosmid clones against western corn rootworm.

Clone	Mean percent mortality (N=4)
Clones which hybridize with probe	
P1-73	47
P1-83	64
P2-2	69
P3-12	85
P5-4	97
Clones which do not hybridize with probe	
P1-2	5
P3-8	4
P3-9	12
P3-18	0
P4-6	9



Example 10. Identification of a 6 kb region active against western corn rootworm.

DNA from P3-12 was partially digested with restriction enzyme *Sau* 3A, and ligated into the *E. coli* vector pUC19 and transformed into *E. coli*. A DNA probe specific for the 80 kDa protein was synthesized by PCR amplification of a portion of P3-12 DNA. The oligonucleotides MK113 and MK117, which hybridize to portions of VIP-1, were synthesized using the partial amino acid sequence of the 80 kDa protein. Plasmid subclones were identified by colony hybridization to the PCR probe, and tested for activity against western corn rootworm. One such clone, PL2, hybridizes to the PCR fragment, and is active against western corn rootworm by the assay previously described.

A 6 kb *Cla* I restriction fragment from PL2 was cloned into the *Sma* I site of the *E. coli*-*Bacillus* shuttle vector pHT 3101 (Lereclus, D. *et al*, 1989, FEMS Microbiology Letters 60:211-218) to yield pCIB6201. This construct confers anti-western corn rootworm activity upon both *Bacillus* and *E. coli* strains, in either orientation. pCIB6022 contains this same 6 kb *Cla* I fragment in pBluescript SK(+) (Stratagene), produces equivalent VIP-1 protein (by western blot), and is also active against western corn rootworm.

The nucleotide sequence of pCIP6022 was determined by the dideoxy termination method of Sanger *et al.*, Proc. Natl. Acad. Sci. USA, 74:5463-5467 (1977), using PRISM Ready Reaction Dye Deoxy Terminator Cycle Sequencing Kits and PRISM Sequenase® Terminator Double-Stranded DNA Sequencing Kit and analyzed on ABI 373 automatic sequencer. The sequence is given in SEQ ID NO:1. pCIB6022 was deposited with the Agricultural Research Service, Patent Culture Collection, (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given the Accession No. NRRL B-21222.



Example 11. Functional dissection of the VIP-1 DNA region.

To confirm that the VIP-1 open reading frame (ORF) is necessary for insecticidal activity a translational frameshift mutation was created in the gene. The restriction enzyme Bgl II recognizes a unique site located 1758 bp into the coding region of VIP-1. pCIB6201 was digested with Bgl II, and the single-stranded ends filled-in with DNA polymerase (Klenow fragment) and dNTPS. The plasmid was re-ligated and transformed into *E. coli*. The resulting plasmid, pCIB6203, contains a four nucleotide insertion in the coding region of VIP-1.

pCIB6203 does not confer insecticidal activity, confirming that VIP-1 is an essential component of western corn rootworm activity.

To further define the region necessary to encode VIP-1, subclones of the VIP-1 and VIP-2 (auxiliary protein) region were constructed and tested for their ability to complement the mutation in pCIB6203. pCIB6023 contains the 3.7kb Xba I-EcoRV fragment in pBluescript SK(+) (Stratagene). Western blot analysis indicates that pCIB6023 produces VIP-1 protein of equal size and quantity as clones PL2 and pCIB 6022. pCIB6023 contains the entire gene for the 80kd protein. pCIB6023 was deposited with the Agricultural Research Service, Patent Culture Collection, (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given the Accession No. NRRL B-21223.

pCIB6023 shows some western corn rootworm activity. However, the level of activity is less than the activity of pCIB6022. A mixture of cells containing pCIB6203 (VIP-1-mutated, and VIP-2) and cells containing pCIB6023(only VIP-1) shows high activity against western corn rootworm. Thus, pCIB6023 must produce functional VIP-1 gene product, and pCIB6203 must produce a functional VIP-2 gene product. These results suggest a requirement for additional gene product(s) from the VIP-2 region, in combination with VIP-1, to confer maximal western corn rootworm activity. See Table 19.



TABLE 19
Characterization of pCIB 6022

Construct(s) tested	Activity vs WCRV
	+++
	+
	-
	+++

Boxed regions represent the extent of VIP-1. Light shading indicates the regions encoding the 80 kDa peptide observed in *Bacillus*. Dark shading represents the N-terminal amino acids predicted by the DNA sequence of VIP-1. Large "X" represents the location of the frameshift mutation introduced into VIP-1. Arrows represent constructs transcribed by the beta-galactosidase promoter. Restriction sites: C - *Cla* I; X - *Xba* I; S - *Sca* I; RI - *Eco* RI; B - *Bgl* II; RV - *Eco* RV.



Example 12. AB78 Antibody Production

Antibody production was initiated in 2 Lewis rats to allow for both the possibility of moving to production of hybridoma cell lines and also to produce enough serum for limited screening of cDNA library. Another factor was the very limited amount of antigen available and
5 the fact that it could only be produced to purity by PAGE and subsequent electrotransfer to nitrocellulose.

Due to the limited availability of antigen on nitrocellulose, the nitrocellulose was emulsified in DMSO and injected into the hind footpads of the animals to elicit B-cell production in the popliteal lymph nodes just upstream. A strong reacting serum was produced
10 by western analysis within the first production bleed. Several subsequent injections and bleeds produced enough serum to accomplish all of the screening required.

Hybridoma production with one of the rats was then initiated. The popliteal lymph node was excised, macerated, and the resulting cells fused with mouse myeloma P3x63Ag8.653. Subsequent cell screening was accomplished as described below. Four initial wells were
15 selected which gave the highest emulsified antigen reaction to be moved to limited dilution cloning. An additional 10 wells were chosen for expansion and cryopreservation.

Procedure to Emulsify AB78 on nitrocellulose in DMSO for ELISA screening:

After electrotransfer of AB78 samples run on PAGE to nitrocellulose, the reversible stain Ponceaus is used to visualize all protein transferred. The band corresponding to AB78
20 toxin, previously identified and N-terminal sequenced, is identified and excised from nitrocellulose. Each band is approximately 1mmx5mm in size to minimize the amount of nitrocellulose emulsified. A single band is placed in a microfuge tube with 250ul of DMSO and macerated using a plastic pestle (Kontes, Vineland, NJ). To aid in emulsification, the DMSO mixture is heated for 2-3 minutes at 37°C-45°C. Some further maceration might be necessary
25 following heating; however, all of the nitrocellulose should be emulsified. Once the AB78 is

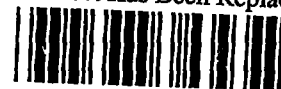


emulsified, the sample is placed on ice. In preparation for microtiter plate coating with the emulsified antigen, the sample must be diluted in borate buffered saline as follows: 1:5, 1:10, 1:15, 1:20, 1:30, 1:50, 1:100, and 0. The coating antigen must be prepared fresh immediately prior to use.

5 ELISA protocol:

1. Coat with AB78/DMSO in BBS. Incubate overnight at 4°C.
2. Wash plate 3X with 1X ELISA wash buffer.
3. Block (1% BSA & 0.05% Tween 20 in PBS) for 30 minutes at Room Temperature.
- 10 4. Wash plate 3X with 1X ELISA wash buffer.
5. Add Rat Serum. Incubate 1.5 hours at 37°C.
6. Wash plate 3X with 1X ELISA wash buffer.
7. Add Goat anti-Rat at a conc. of 2ug/ml in ELISA diluent. Incubate 1 hr. at 37°C.
8. Wash plate 3X with 1X ELISA wash buffer.
- 15 9. Add Rabbit anti-Goat Alkaline Phosphatase at 2ug/ml in ELISA diluent.
Incubate 1Hr. at 37°C.
10. Wash 3X with 1X ELISA wash buffer.
11. Add Substrate. Incubate 30 minutes at Room Temperature.
- 20 12. Stop with 3N NaOH after 30 minutes.

This Sheet Has Been Replaced



Example 13. Activation of insecticidal activity of non-active Bt strains with AP78 VIP clones.

Adding pCIB6203 together with culture supernatant from a Bt strain GC91 produces 100% mortality in Diabrotica virgifera virgifera. Neither pCIB6203 nor GC91 is active on

5 Diabrotica virgifera virgifera by itself. Data are shown below:

Test material	Percent <u>Diabrotica</u> mortality
pCIB6203	0
GC91	16
pCIB6203 + GC91	100
Control	0

Example 14. Isolation and Biological Activity of B.cereus AB81.

10

A second B. cereus strain, designated AB81, was isolated from grain bin dust samples by standard methodologies. A subculture of AB81 was grown and prepared for bioassay as described in Example 2. Biological activity was evaluated as described in Example 3. The results are as follows:

15

Insect species tested	Percent Mortality
<u>Ostrinia nubilalis</u>	0
<u>Agrotis ipsilon</u>	0
<u>Diabrotica virgifera virgifera</u>	55

20

25



Example 15. Isolation and Biological Activity of *B. thuringiensis* AB6.

A *B. thuringiensis* strain, designated AB6, was isolated from grain bin dust samples by standard methods known in the art. A subculture of AB6 was grown and prepared for bioassay as described in Example 2. Half of the sample was autoclaved 15 minutes to test for the presence of β -exotoxin.

Biological activity was evaluated as described in Example 3. The results are as follows:

Insect species tested	Percent Mortality
<i>Ostrinia nubilalis</i>	0
<i>Agrotis ipsilon</i>	100
<i>Agrotis ipsilon</i> (autoclaved sample)	0
<i>Diabrotica virgifera virgifera</i>	0

Strain AB6 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, USA, and given Accession No. NRRL B-21060.

Example 16. Isolation and Biological characterization of *B. thuringiensis* AB88.

A Bt strain, designated AB88, was isolated from grain bin dust samples by standard methodologies. A subculture of AB88 was grown and prepared for bioassay as described in Example 2. Half of the sample was autoclaved 15 minutes to test for the presence of β -exotoxin. Biological activity was evaluated against a number of insect species as described in Example 3. The results are as follows:



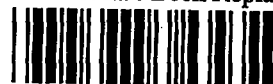
Insect species tested	Order	Percent mortality of culture supernatant	
		Non-autoclaved	Autoclaved
<u>Agrotis ipsilon</u>	Lepidoptera	100	5
<u>Ostrinia nubilalis</u>	Lepidoptera	100	0
<u>Spodoptera</u>			
<u>frugiperda</u>	Lepidoptera	100	4
<u>Helicoverpa zea</u>	Lepidoptera	100	12
<u>Heliothis virescens</u>	Lepidoptera	100	12
<u>Leptinotarsa</u>			
<u>decemlineata</u>	Coleoptera	0	0
<u>Diabrotica virgifera</u>			
<u>virgifera</u>	Coleoptera	0	5

Delta-endotoxin crystals were purified from strain AB88 by standard methodologies. No activity from pure crystals was observed when bioassayed against Agrotis ipsilon.

5 **Example 17. Purification of VIPs from Strain AB88:**

Bacterial liquid culture was grown overnight at 30°C in TB media. Cells were spun out and the supernatant kept. Proteins were precipitated with ammonium sulfate (70% saturation), centrifuged and the pellet kept. The pellet was resuspended in the original volume of 20 mM Tris pH 7.5 and dialyzed against the same buffer. AB88 dialysate was more turbid than
10 comparable material from AB78. AB88 proteins have been separated by several different methods following clarification including isoelectric focusing (Rotofor, BioRad, Hercules, CA), precipitation at pH 4.5, ion-exchange chromatography, size exclusion chromatography and ultrafiltration.

European Corn Borer-active protein remained in the pellet obtained by pH 4.5
15 precipitation of dialysate. When preparative IEF was done on the dialysate using pH 3-10 ampholytes, ECB insecticidal activity was found in all fractions with pH of 7 or greater. SDS-PAGE of these fractions showed protein bands of MW ~60 kDa and ~80 kDa. The 60 kDa and 80 kDa bands were separated by anion exchange HPLC on a Poros-Q column (PerSeptive



Biosystems, Cambridge, MA). N-terminal sequence was obtained from two fractions containing proteins of slightly differing MW, but both of approximately 60 kDa in size. The sequences obtained were similar to each other and to some δ -endotoxins.

anion exchange fraction 23 (smaller): xEPFVSAxxxQxxx (SEQ ID NO:10)

5 anion exchange fraction 28 (larger): xEYENVEPFVSAx (SEQ ID NO:11)

When the (active) pH 4.5 pellet was further separated by anion exchange on a Poros-Q column, activity was found only in fractions with a major band of ~60 kDa.

Black Cutworm-active protein also remained in the pellet when AB88 dialysate was brought down to pH 4.5. In preparative IEF using pH 3-10 ampholytes, activity was not found
10 in the ECB-active IEF fractions; instead, it was highest in a fraction of pH 4.5-5.0. Its major components have molecular weights of ~35 and ~80 kDa.

The pH 4.5 pellet was separated by anion exchange HPLC to yield fractions containing only the 35 kDa material and fractions containing both 35 kDa and 80 kDa bands.

15 **Example 18. Characterization of AB88 VIP.**

Fractions containing the various lepidopteran active vegetative proteins were generated as described in Example 17. Analysis of active fractions demonstrates that different VIP's are responsible for the different lepidopteran species activity.

20

The Agrotis ipsilon activity is due to an 80 kDa and or a 35 kDa protein either delivered singly or in combination. These proteins are not related to any δ -endotoxins from Bt as evidenced by the lack of sequence homology of known Bt δ -endotoxin sequences. Also, these proteins are not found in the AB88 δ -endotoxin crystal. N-terminal sequences of the major δ -



endotoxin proteins were compared with the N-terminal sequences of the 80 kDa and 35 kDa VIP and reveal no sequence homology. A summary of the results follows:

<u>Agrotis</u> VIP N-terminal sequences	N-terminal sequence of major δ -endotoxin proteins
	130 kDa MDNNPNINE (SEQ ID NO:14)
80 kDa MNKNNTKLPTRALP (SEQ ID NO:12)	80 kDa MDNNPNINE (SEQ ID NO:15)
	60 kDa MNVLNSGRTTI (SEQ ID NO:16)
35 kDa ALSENTGKDGGYTVP (SEQ ID NO:13)	

- 5 The Ostrinia nubilalis activity is due to a 60 kDa VIP and the Spodoptera frugiperda activity is due to a VIP of unknown size.

Bacillus thuringiensis strain AB88 has been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University
10 Street, Peoria, Illinois 61604, USA and given the Accession No. NRRL B-21225.

Example 19. Isolation and Biological Activity of Other Bacillus sp.

Other Bacillus species have been isolated which produce proteins with insecticidal
15 activity during vegetative growth. These strains were isolated from environmental samples by standard methodologies. Isolates were prepared for bioassay and assayed as described in Examples 2 and 3 respectively. Isolates which produced insecticidal proteins during vegetative growth with activity against Agrotis ipsilon in the bioassay are tabulated below.

<u>Bacillus</u> isolate	Presence of δ -endotoxin crystal	Percent mortality
AB6	+	100
AB53	-	80
AB88	+	100
AB195	-	60
AB211	-	70
AB217	-	83
AB272	-	80
AB279	-	70
AB289	+	100
AB292	+	80
AB294	-	100
AB300	-	80
AB359	-	100

Isolates AB289, AB294 and AB359 have been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North
 5 University Street, Peoria IL 61604, USA and given the Accession Numbers NRRL B-21227, NRRL B-21229, and NRRL B-21226 respectively.

Bacillus isolates which produce insecticidal proteins during vegetative growth with activity against Diabrotica virgifera virgifera are tabulated below.

10

<u>Bacillus</u> isolate	Presence of δ -endotoxin crystal	Percent mortality
AB52	-	50
AB59	-	71
AB68	+	60
AB78	-	100
AB122	-	57
AB218	-	64
AB256	-	64



Isolates AB59 and AB256 have been deposited in the Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria Illinois 61604, USA, and given the Accession Numbers NRRL B-21228 and B-21230, respectively.

5

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

10

The following deposits have been made at Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A.:

15

1. E. coli PL2 Accession No. NRRL B-21221

2. E. coli pCIB 6022 Accession No. NRRL B-21222

3. E. coli pCIB 6023 Accession No. NRRL B-21223

4. Bacillus thuringiensis HD73-78VIP Accession No. NRRL B-21224

5. Bacillus thuringiensis AB88 Accession No. NRRL B-21225

20

6. Bacillus thuringiensis AB359 Accession No. NRRL B-21226

7. Bacillus thuringiensis AB289 Accession No. NRRL B-21227

8. Bacillus sp. AB59 Accession No. NRRL B-21228

9. Bacillus sp. AB294 Accession No. NRRL B-21229

10. Bacillus sp. AB256 Accession No. NRRL B-21230

25

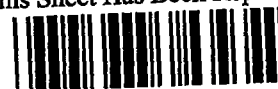
11. E. coli P5-4 Accession No. NRRL B-21059



- | | |
|---------------------------------------|----------------------------|
| 12. <u>E. coli</u> P3-12 | Accession No. NRRL B-21061 |
| 13. <u>Bacillus cereus</u> AB78 | Accession No. NRRL B-21058 |
| 14. <u>Bacillus thuringiensis</u> AB6 | Accession No. NRRL B-21060 |

5

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.



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(F) POSTAL CODE (ZIP): 27713

- (ii) TITLE OF INVENTION: Novel Pesticidal Proteins and Strains
(iii) NUMBER OF SEQUENCES: 18
(iv) COMPUTER READABLE FORM:
 (A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: Patent In Release #1.0, Version #1.25 (EPO)
(vi) PRIOR APPLICATION DATA:
 (A) APPLICATION NUMBER: US 08/037,057
 (B) FILING DATE: 25-MAR-1993

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 6106 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Bacillus cereus
 (B) STRAIN: AB78
 (C) INDIVIDUAL ISOLATE: NRRL B-21058
(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1082..1810
 (D) OTHER INFORMATION: /product= "VIP-2"
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(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1925..2470
 (D) OTHER INFORMATION: /product= "VIP-2"
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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Leu	Ser	Ser	Glu	Arg	Leu	Ala	Ala	Phe	Gly	Ser	Arg	Lys	Ile	Ile	Leu	
110						115					120					
CGA	TTA	CAA	GTT	CCG	AAA	GGA	AGT	ACG	GGT	GCG	TAT	TTA	AGT	GCC	ATT	2344
Arg	Leu	Gln	Val	Pro	Lys	Gly	Ser	Thr	Gly	Ala	Tyr	Leu	Ser	Ala	Ile	
125					130				135						140	
GGT	GGA	TTT	GCA	AGT	GAA	AAA	GAG	ATC	CTA	CTT	GAT	AAA	GAT	AGT	AAA	2392
Gly	Gly	Phe	Ala	Ser	Glu	Lys	Glu	Ile	Leu	Leu	Asp	Lys	Asp	Ser	Lys	
				145					150					155		
TAT	CAT	ATT	GAT	AAA	GTA	ACA	GAG	GTA	ATT	ATT	AAA	GGT	GTT	AAG	CGA	2440
Tyr	His	Ile	Asp	Lys	Val	Thr	Glu	Val	Ile	Ile	Lys	Gly	Val	Lys	Arg	
			160					165						170		
TAT	GTA	GTG	GAT	GCA	ACA	TTA	TTA	ACA	AAT	TAAGGAGATG	AAAAATATGA					2490
Tyr	Val	Val	Asp	Ala	Thr	Leu	Leu	Thr	Asn							
		175						180								
AGAAAAAGTT	AGCAAGTGTT	GTAACGTGTA	CGTTATTAGC	TCCTATGTTT	TTGAATGGAA											2550
ATGTGAATGC	TGTTTACGCA	GACAGCAAAA	CAAATCAAAT	TTCTACAACA	CAGAAAAATC											2610
AACAGAAAGA	GATGGACCGA	AAAGGATTAC	TTGGGTATTA	TTTCAAAGGA	AAAGATTTTA											2670
GTAATCTTAC	TATGTTTGCA	CCGACACGTG	ATAGTACTCT	TATTTATGAT	CAACAAACAG											2730
CAAATAAACT	ATTAGATAAA	AAACAACAAG	AATATCAGTC	TATTCGTTGG	ATTGGTTTGA											2790
TTCAGAGTAA	AGAAACGGGA	GATTTACAT	TTAACTTATC	TGAGGATGAA	CAGGCAATTA											2850
TAGAAATCAA	TGGGAAAATT	ATTTCTAATA	AAGGGAAAGA	AAAGCAAGTT	GTCCATTTAG											2910
AAAAAGGAAA	ATTAGTTCCA	ATCAAAATAG	AGTATCAATC	AGATACAAAA	TTTAATATTG											2970
ACAGTAAAC	ATTTAAGAA	CTTAAATTAT	TTAAATAGA	TAGTCAAAAC	CAACCCAGC											3030
AAGTCCAGCA	AGATGAAGTG	AGAAATCCTG	AATTTAACAA	GAAAGAATCA	CAGGAATTCT											3090
TAGCGAAACC	ATCGAAAATA	AATCTTTTCA	CTCAAMAAAT	GAAAAGGGAA	ATTGATGAAG											3150
ACACGGATAC	GGATGGGGAC	TCTATTCCTG	ACCTTTGGGA	AGAAAATGGG	TATACGATTC											3210
AMAATAGAAT	CGCTGTAAAG	TGGGACGATT	CTCTAGCAAG	TAAAGGGTAT	ACGAAATTTG											3270
TTTCAAATCC	ACTAGAAAGT	CACACAGTTG	GTGATCCTTA	TACAGATTAT	GAAAAGGCAG											3330
CAAGAGATCT	AGATTTGTCA	AATGCAAAGG	AAACGTTTAA	CCCATTGGTA	GCTGCTTTTC											3390
CAAGTGTGAA	TGTTAGTATG	GAAAAGGTGA	TATTATCACC	AAATGAAAAT	TTATCCAATA											3450
GTGTAGAGTC	TCATTCATCC	ACGAATTGGT	CTTATACAAA	TACAGAAGGT	GCTTCTGTTG											3510
AAGCGGGGAT	TGGACCAAAA	GGTATTTCTG	TCGGAGTTAG	CGTAAACTAT	CAACACTCTG											3570
AAACAGTTGC	ACAAGAATGG	GGAACATCTA	CAGGAAATAC	TTCGCAATTC	AATACGGCTT											3630
CAGCGGGATA	TTTAAATGCA	AATGTTTCGAT	ATAACAATGT	AGGAACTGGT	GCCATCTACG											3690
ATGTAAAACC	TACAACAAGT	TTTGTATTAA	ATAACGATAC	TATCGCAACT	ATTACGGCGA											3750



AATCTAATTC	TACAGCCTTA	AATATATCTC	CTGGAGAAAG	TTACCCGAAA	AAAGGACAAA	3810
ATGGAATCGC	AATAACATCA	ATGGATGATT	TTAATTCCCA	TCCGATTACA	TTAAATAAAA	3870
AACAAGTAGA	TAATCTGCTA	AATAATAAAC	CTATGATGTT	GGAAACAAAC	CAAACAGATG	3930
GTGTTTATAA	GATAAAAGAT	ACACATGGAA	ATATAGTAAC	TGGCGGAGAA	TGGAATGGTG	3990
TCATACAACA	AATCAAGGCT	AAAACAGCGT	CTATTATTGT	GGATGATGGG	GAACGTGTAG	4050
CAGAAAAACG	TGTAGCGGCA	AAAGATTATG	AAAATCCAGA	AGATAAAACA	CCGTCTTTAA	4110
CTTTAAAAGA	TGCCCTGAAG	CTTTCATATC	CAGATGAAAT	AAAAGAAATA	GAGGGATTAT	4170
TATATTATAA	AAACAAACCG	ATATACGAAT	CGAGCGTTAT	GACTTACTTA	GATGAAAATA	4230
CAGCAAAGA	AGTGACCAA	CAATTAAATG	ATACCACTGG	GAAATTTAAA	GATGTAAGTC	4290
ATTTATATGA	TGTAAACTG	ACTCCAAAA	TGAATGTTAC	AATCAAATTG	TCTATACTTT	4350
ATGATAATGC	TGAGTCTAAT	GATAACTCAA	TTGGTAAATG	GACAAACACA	AATATTGTTT	4410
CAGGTGGAAA	TAACGGAAAA	AAACAATATT	CTTCTAATAA	TCCGGATGCT	AATTTGACAT	4470
TAAATACAGA	TGCTCAAGAA	AAATTAAATA	AAAATCGTGA	CTATTATATA	AGTTTATATA	4530
TGAAGTCAGA	AAAAAACACA	CAATGTGAGA	TTACTATAGA	TGGGGAGATT	TATCCGATCA	4590
CTACAAAAAC	AGTGAATGTG	AATAAAGACA	ATTACAAAAG	ATTAGATATT	ATAGCTCATA	4650
ATATAAAAAG	TAATCCAATT	TCTTCACTTC	ATATTAAAAC	GAATGATGAA	ATAACTTTAT	4710
TTTGGGATGA	TATTTCTATA	ACAGATGTAG	CATCAATAAA	ACCGGAAAAT	TTAACAGATT	4770
CAGAAATTAA	ACAGATTTAT	AGTAGGTATG	GTATTAAGTT	AGAAGATGGA	ATCCTTATTG	4830
ATAAAAAAGG	TGGGATTCAT	TATGGTGAAT	TTATTAATGA	AGCTAGTTTT	AATATTGAAC	4890
CATTGCCAAA	TTATGTGACC	AAATATGAAG	TTACTTATAG	TAGTGAGTTA	GGACCAAACG	4950
TGAGTGACAC	ACTTGAAAGT	GATAAAATTT	ACAAGGATGG	GACAATTAAA	TTTGATTTTA	5010
CCAAATATAG	TAAAAATGAA	CAAGGATTAT	TTTATGACAG	TGGATTAAAT	TGGGACTTTA	5070
AAATTAATGC	TATTACTTAT	GATGGTAAAG	AGATGAATGT	TTTTCATAGA	TATAATAAAT	5130
AGTTATTATA	TCTATGAAGC	TGGTGCTAAA	GATAGTGTA	AAGTTAATAT	ACTGTAGGAT	5190
TGTAATAAAA	GTAATGGAAT	TGATATCGTA	CTTTGGAGTG	GGGGATACTT	TGTAAATAGT	5250
TCTATCAGAA	ACATTAGACT	AAGAAAAGTT	ACTACCCCCA	CTTGAAAATG	AAGATTCAAC	5310
TGATTACAAA	CAACCTGTTA	AATATTATAA	GGTTTTAACA	AAATATTAAA	CTCTTTATGT	5370
TAATACTGTA	ATATAAAGAG	TTTAATTGTA	TTCAAATGAA	GCTTTCCAC	AAAATTAGAC	5430
TGATTATCTA	ATGAAATAAT	CAGTCTAATT	TTGTAGAACA	GGTCTGGTAT	TATTGTACGT	5490
GGTCACTAAA	AGATATCTAA	TATTATTGGG	CAAGGCGTTC	CATGATTGAA	TCCTCGAATG	5550



TCTTGCCCTT TTCATTTATT TAAGAAGGAT TGTGGAGAAA TTATGGTTTA GATAATGAAG 5610
 AAAGACTTCA CTTCTAATTT TTGATGTTAA ATAAATCAAA ATTTGGCGAT TCACATTGTT 5670
 TAATCCACTG ATAAACATA CTGGAGTGTT CTTAAAAAAT CAGCTTTTTT CTTTATAAAA 5730
 TTTTGCTTAG CGTACGAAAT TCGTGTTTTG TTGGTGGGAC CCCATGCCCCA TCAACTTAAG 5790
 AGTAAATTAG TAATGAACCT TCGTTCATCT GGATTAAAAT AACCTCAAAT TAGGACATGT 5850
 TTTTAAAAAT AAGCAGACCA AATAAGCCTA GAATAGGTAT CATTTTAA AATTATGCTG 5910
 CTTTCTTTTG TTTTCCAAAT CCATTATACT CATAAGCAAC ACCCATAATG TCAAAGACTG 5970
 TTTTGTCTC ATATCGATAA GCTTGATATC GAATTCCTGC AGCCCGGGGG ATCCACTAGT 6030
 TCTAGAGCGG CCGCCACCGC GGTGGAGCTC CAGCTTTTGT TCCCTTAGT GAGGGTTAAG 6090
 TTCGAGCTTG TCGTGG 6106

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 243 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Lys Arg Met Glu Gly Lys Leu Phe Met Val Ser Lys Lys Leu Gln
 1 5 10 15
 Val Val Thr Lys Thr Val Leu Leu Ser Thr Val Phe Ser Ile Ser Leu
 20 25 30
 Leu Asn Asn Glu Val Ile Lys Ala Glu Gln Leu Asn Ile Asn Ser Gln
 35 40 45
 Ser Lys Tyr Thr Asn Leu Gln Asn Leu Lys Ile Thr Asp Lys Val Glu
 50 55 60
 Asp Phe Lys Glu Asp Lys Glu Lys Ala Lys Glu Trp Gly Lys Glu Lys
 65 70 75 80
 Glu Lys Glu Trp Lys Leu Thr Ala Thr Glu Lys Gly Lys Met Asn Asn
 85 90 95
 Phe Leu Asp Asn Lys Asn Asp Ile Xaa Thr Asn Tyr Lys Glu Ile Thr
 100 105 110
 Phe Ser Met Ala Gly Ser Phe Glu Asp Glu Ile Lys Asp Leu Lys Glu
 115 120 125
 Ile Asp Lys Met Phe Asp Lys Thr Asn Leu Ser Asn Ser Ile Ile Thr
 130 135 140



Tyr Lys Asn Val Glu Pro Thr Thr Ile Gly Phe Asn Lys Ser Leu Thr
 145 150 155 160
 Glu Gly Asn Thr Ile Asn Ser Asp Ala Met Ala Gln Phe Lys Glu Gln
 165 170 175
 Phe Leu Asp Arg Asp Ile Lys Phe Asp Ser Tyr Leu Asp Thr His Leu
 180 185 190
 Thr Ala Gln Gln Val Ser Ser Lys Glu Arg Val Ile Leu Lys Val Thr
 195 200 205
 Val Pro Ser Gly Lys Gly Ser Thr Thr Pro Thr Lys Ala Gly Val Ile
 210 215 220
 Leu Asn Asn Ser Glu Tyr Lys Met Leu Ile Asp Asn Gly Tyr Met Val
 225 230 235 240
 His Val Asp

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 182 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Met Lys Asn Tyr Glu Glu Trp Ala Lys Asp Leu Thr Asp Ser Gln Arg
 1 5 10 15
 Glu Ala Leu Asp Gly Tyr Ala Arg Gln Asp Tyr Lys Glu Ile Asn Asn
 20 25 30
 Tyr Leu Arg Asn Gln Gly Gly Ser Gly Asn Glu Lys Leu Asp Ala Gln
 35 40 45
 Ile Lys Asn Ile Ser Asp Ala Leu Gly Lys Lys Pro Ile Pro Glu Asn
 50 55 60
 Ile Thr Val Tyr Arg Trp Cys Gly Met Pro Glu Phe Gly Tyr Gln Ile
 65 70 75 80
 Ser Asp Pro Leu Pro Ser Leu Lys Asp Phe Glu Glu Gln Phe Leu Asn
 85 90 95
 Thr Ile Lys Glu Asp Lys Gly Tyr Met Ser Thr Ser Leu Ser Ser Glu
 100 105 110
 Arg Leu Ala Ala Phe Gly Ser Arg Lys Ile Ile Leu Arg Leu Gln Val
 115 120 125
 Pro Lys Gly Ser Thr Gly Ala Tyr Leu Ser Ala Ile Gly Gly Phe Ala
 130 135 140



Ser Glu Lys Glu Ile Leu Leu Asp Lys Asp Ser Lys Tyr His Ile Asp
 145 150 155 160
 Lys Val Thr Glu Val Ile Ile Lys Gly Val Lys Arg Tyr Val Val Asp
 165 170 175
 Ala Thr Leu Leu Thr Asn
 180

(2) INFORMATION FOR SEQ ID NO: 4:

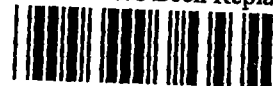
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2655 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Bacillus cereus*
 - (B) STRAIN: AB78
 - (C) INDIVIDUAL ISOLATE: NRRL B-21058
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..2652
 - (C) IDENTIFICATION METHOD: experimental
 - (D) OTHER INFORMATION: /product= "100 kDa protein VIP-1"
 /evidence= EXPERIMENTAL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

ATG AAA AAT ATG AAG AAA AAG TTA GCA AGT GTT GTA ACG TGT ACG TTA	48
Met Lys Asn Met Lys Lys Lys Leu Ala Ser Val Val Thr Cys Thr Leu	
1 5 10 15	
TTA GCT CCT ATG TTT TTG AAT GGA AAT GTG AAT GCT GTT TAC GCA GAC	96
Leu Ala Pro Met Phe Leu Asn Gly Asn Val Asn Ala Val Tyr Ala Asp	
20 25 30	
AGC AAA ACA AAT CAA ATT TCT ACA ACA CAG AAA AAT CAA CAG AAA GAG	144
Ser Lys Thr Asn Gln Ile Ser Thr Thr Gln Lys Asn Gln Gln Lys Glu	
35 40 45	
ATG GAC CGA AAA GGA TTA CTT GGG TAT TAT TTC AAA GGA AAA GAT TTT	192
Met Asp Arg Lys Gly Leu Leu Gly Tyr Tyr Phe Lys Gly Lys Asp Phe	
50 55 60	
AGT AAT CTT ACT ATG TTT GCA CCG ACA CGT GAT AGT ACT CTT ATT TAT	240
Ser Asn Leu Thr Met Phe Ala Pro Thr Arg Asp Ser Thr Leu Ile Tyr	
65 70 75 80	
GAT CAA CAA ACA GCA AAT AAA CTA TTA GAT AAA AAA CAA CAA GAA TAT	288
Asp Gln Gln Thr Ala Asn Lys Leu Leu Asp Lys Lys Gln Gln Glu Tyr	



85										90										95										
CAG	TCT	ATT	CGT	TGG	ATT	GGT	TTG	ATT	CAG	AGT	AAA	GAA	ACG	GGA	GAT	336														
Gln	Ser	Ile	Arg	Trp	Ile	Gly	Leu	Ile	Gln	Ser	Lys	Glu	Thr	Gly	Asp															
			100					105					110																	
TTC	ACA	TTT	AAC	TTA	TCT	GAG	GAT	GAA	CAG	GCA	ATT	ATA	GAA	ATC	AAT	384														
Phe	Thr	Phe	Asn	Leu	Ser	Glu	Asp	Glu	Gln	Ala	Ile	Ile	Glu	Ile	Asn															
		115					120					125																		
GGG	AAA	ATT	ATT	TCT	AAT	AAA	GGG	AAA	GAA	AAG	CAA	GTT	GTC	CAT	TTA	432														
Gly	Lys	Ile	Ile	Ser	Asn	Lys	Gly	Lys	Glu	Lys	Gln	Val	Val	His	Leu															
		130				135					140																			
GAA	AAA	GGA	AAA	TTA	GTT	CCA	ATC	AAA	ATA	GAG	TAT	CAA	TCA	GAT	ACA	480														
Glu	Lys	Gly	Lys	Leu	Val	Pro	Ile	Lys	Ile	Glu	Tyr	Gln	Ser	Asp	Thr															
		145			150					155					160															
AAA	TTT	AAT	ATT	GAC	AGT	AAA	ACA	TTT	AAA	GAA	CTT	AAA	TTA	TTT	AAA	528														
Lys	Phe	Asn	Ile	Asp	Ser	Lys	Thr	Phe	Lys	Glu	Leu	Lys	Leu	Phe	Lys															
				165					170					175																
ATA	GAT	AGT	CAA	AAC	CAA	CCC	CAG	CAA	GTC	CAG	CAA	GAT	GAA	CTG	AGA	576														
Ile	Asp	Ser	Gln	Asn	Gln	Pro	Gln	Gln	Val	Gln	Gln	Asp	Glu	Leu	Arg															
			180				185						190																	
AAT	CCT	GAA	TTT	AAC	AAG	AAA	GAA	TCA	CAG	GAA	TTC	TTA	GCG	AAA	CCA	624														
Asn	Pro	Glu	Phe	Asn	Lys	Lys	Glu	Ser	Gln	Glu	Phe	Leu	Ala	Lys	Pro															
		195					200					205																		
TCG	AAA	ATA	AAT	CTT	TTC	ACT	CAA	MAA	ATG	AAA	AGG	GAA	ATT	GAT	GAA	672														
Ser	Lys	Ile	Asn	Leu	Phe	Thr	Gln	Xaa	Met	Lys	Arg	Glu	Ile	Asp	Glu															
		210				215					220																			
GAC	ACG	GAT	ACG	GAT	GGG	GAC	TCT	ATT	CCT	GAC	CTT	TGG	GAA	GAA	AAT	720														
Asp	Thr	Asp	Thr	Asp	Gly	Asp	Ser	Ile	Pro	Asp	Leu	Trp	Glu	Glu	Asn															
		225			230				235						240															
GGG	TAT	ACG	ATT	CAM	AAT	AGA	ATC	GCT	GTA	AAG	TGG	GAC	GAT	TCT	CTA	768														
Gly	Tyr	Thr	Ile	Xaa	Asn	Arg	Ile	Ala	Val	Lys	Trp	Asp	Asp	Ser	Leu															
				245					250					255																
GCA	AGT	AAA	GGG	TAT	ACG	AAA	TTT	GTT	TCA	AAT	CCA	CTA	GAA	AGT	CAC	816														
Ala	Ser	Lys	Gly	Tyr	Thr	Lys	Phe	Val	Ser	Asn	Pro	Leu	Glu	Ser	His															
			260				265						270																	
ACA	GTT	GGT	GAT	CCT	TAT	ACA	GAT	TAT	GAA	AAG	GCA	GCA	AGA	GAT	CTA	864														
Thr	Val	Gly	Asp	Pro	Tyr	Thr	Asp	Tyr	Glu	Lys	Ala	Ala	Arg	Asp	Leu															
		275					280					285																		
GAT	TTG	TCA	AAT	GCA	AAG	GAA	ACG	TTT	AAC	CCA	TTG	GTA	GCT	GCT	TTT	912														
Asp	Leu	Ser	Asn	Ala	Lys	Glu	Thr	Phe	Asn	Pro	Leu	Val	Ala	Ala	Phe															
		290				295				300																				
CCA	AGT	GTG	AAT	GTT	AGT	ATG	GAA	AAG	GTG	ATA	TTA	TCA	CCA	AAT	GAA	960														
Pro	Ser	Val	Asn	Val	Ser	Met	Glu	Lys	Val	Ile	Leu	Ser	Pro	Asn	Glu															
		305			310				315						320															
AAT	TTA	TCC	AAT	AGT	GTA	GAG	TCT	CAT	TCA	TCC	ACG	AAT	TGG	TCT	TAT	1008														
Asn	Leu	Ser	Asn	Ser	Val	Glu	Ser	His	Ser	Ser	Thr	Asn	Trp	Ser	Tyr															





				565				570				575				
GTT Val	ATG Met	ACT Thr	TAC Tyr 580	TTA Leu	GAT Asp	GAA Glu	AAT Asn 585	ACA Thr 585	GCA Ala	AAA Lys	GAA Glu	GTG Val	ACC Thr 590	AAA Lys	CAA Gln	1776
TTA Leu	AAT Asn 595	GAT Asp	ACC Thr	ACT Thr	GGG Gly	AAA Lys 600	TTT Phe 600	AAA Lys	GAT Asp	GTA Val	AGT Ser	CAT His 605	TTA Leu	TAT Tyr	GAT Asp	1824
GTA Val 610	AAA Lys	CTG Leu	ACT Thr	CCA Pro	AAA Lys	ATG Met 615	AAT Asn 615	GTT Val	ACA Thr	ATC Ile	AAA Lys 620	TTG Leu	TCT Ser	ATA Ile	CTT Leu	1872
TAT Tyr 625	GAT Asp	AAT Asn	GCT Ala	GAG Glu	TCT Ser 630	AAT Asn	GAT Asp	AAC Asn	TCA Ser	ATT Ile 635	GGT Gly	AAA Lys	TGG Trp	ACA Thr	AAC Asn 640	1920
ACA Thr	AAT Asn	ATT Ile	GTT Val	TCA Ser 645	GGT Gly	GGA Gly	AAT Asn	AAC Asn	GGA Gly 650	AAA Lys	AAA Lys	CAA Gln	TAT Tyr	TCT Ser 655	TCT Ser	1968
AAT Asn	AAT Asn	CCG Pro	GAT Asp 660	GCT Ala	AAT Asn	TTG Leu	ACA Thr	TTA Leu 665	AAT Asn	ACA Thr	GAT Asp	GCT Ala	CAA Gln 670	GAA Glu	AAA Lys	2016
TTA Leu	AAT Asn 675	AAA Lys	AAT Asn	CGT Arg	GAC Asp	TAT Tyr	TAT Tyr 680	ATA Ile	AGT Ser	TTA Leu	TAT Tyr	ATG Met 685	AAG Lys	TCA Ser	GAA Glu	2064
AAA Lys 690	AAC Asn	ACA Thr	CAA Gln	TGT Cys	GAG Glu	ATT Ile 695	ACT Thr	ATA Ile	GAT Asp	GGG Gly	GAG Glu 700	ATT Ile	TAT Tyr	CCG Pro	ATC Ile	2112
ACT Thr 705	ACA Thr	AAA Lys	ACA Thr	GTG Val	AAT Asn 710	GTG Val	AAT Asn	AAA Lys	GAC Asp 715	AAT Asn	TAC Tyr 715	AAA Lys	AGA Arg	TTA Leu	GAT Asp 720	2160
ATT Ile	ATA Ile	GCT Ala	CAT His	AAT Asn 725	ATA Ile	AAA Lys	AGT Ser	AAT Asn	CCA Pro 730	ATT Ile	TCT Ser	TCA Ser	CTT Leu	CAT His 735	ATT Ile	2208
AAA Lys	ACG Thr	AAT Asn	GAT Asp 740	GAA Glu	ATA Ile	ACT Thr	TTA Leu	TTT Phe 745	TGG Trp	GAT Asp	GAT Asp	ATT Ile	TCT Ser 750	ATA Ile	ACA Thr	2256
GAT Asp	GTA Val 755	GCA Ala	TCA Ser	ATA Ile	AAA Lys	CCG Pro	GAA Glu 760	AAT Asn	TTA Leu	ACA Thr	GAT Asp	TCA Ser 765	GAA Glu	ATT Ile	AAA Lys	2304
CAG Gln 770	ATT Ile	TAT Tyr	AGT Ser	AGG Arg	TAT Tyr	GGT Gly 775	ATT Ile	AAG Lys	TTA Leu	GAA Glu	GAT Asp 780	GGA Gly	ATC Ile	CTT Leu	ATT Ile	2352
GAT Asp 785	AAA Lys	AAA Lys	GGT Gly	GGG Gly	ATT Ile 790	CAT His	TAT Tyr	GGT Gly	GAA Glu 795	TTT Phe 795	ATT Ile	AAT Asn	GAA Glu	GCT Ala	AGT Ser 800	2400
TTT Phe	AAT Asn	ATT Ile	GAA Glu	CCA Pro	TTG Leu	CCA Pro	AAT Asn	TAT Tyr	GTG Val	ACC Thr	AAA Lys	TAT Tyr	GAA Glu	GTT Val	ACT Thr	2448



805	810	815	
TAT AGT AGT GAG TTA GGA CCA AAC GTG AGT GAC ACA CTT GAA AGT GAT			2496
Tyr Ser Ser Glu Leu Gly Pro Asn Val Ser Asp Thr Leu Glu Ser Asp			
820	825	830	
AAA ATT TAC AAG GAT GGG ACA ATT AAA TTT GAT TTT ACC AAA TAT AGT			2544
Lys Ile Tyr Lys Asp Gly Thr Ile Lys Phe Asp Phe Thr Lys Tyr Ser			
835	840	845	
AAA AAT GAA CAA GGA TTA TTT TAT GAC AGT GGA TTA AAT TGG GAC TTT			2592
Lys Asn Glu Gln Gly Leu Phe Tyr Asp Ser Gly Leu Asn Trp Asp Phe			
850	855	860	
AAA ATT AAT GCT ATT ACT TAT GAT GGT AAA GAG ATG AAT GTT TTT CAT			2640
Lys Ile Asn Ala Ile Thr Tyr Asp Gly Lys Glu Met Asn Val Phe His			
865	870	875	880
AGA TAT AAT AAA TAG			2655
Arg Tyr Asn Lys			

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 884 amino acids

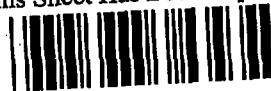
(B) TYPE: amino acid

(D) TOPOLOGY: linear

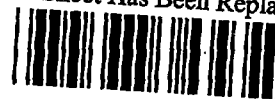
(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met	Lys	Asn	Met	Lys	Lys	Lys	Leu	Ala	Ser	Val	Val	Thr	Cys	Thr	Leu
1				5					10					15	
Leu	Ala	Pro	Met	Phe	Leu	Asn	Gly	Asn	Val	Asn	Ala	Val	Tyr	Ala	Asp
	20						25						30		
Ser	Lys	Thr	Asn	Gln	Ile	Ser	Thr	Thr	Gln	Lys	Asn	Gln	Gln	Lys	Glu
	35					40						45			
Met	Asp	Arg	Lys	Gly	Leu	Leu	Gly	Tyr	Tyr	Phe	Lys	Gly	Lys	Asp	Phe
50					55					60					
Ser	Asn	Leu	Thr	Met	Phe	Ala	Pro	Thr	Arg	Asp	Ser	Thr	Leu	Ile	Tyr
65				70					75					80	
Asp	Gln	Gln	Thr	Ala	Asn	Lys	Leu	Leu	Asp	Lys	Lys	Gln	Gln	Glu	Tyr
			85					90						95	
Gln	Ser	Ile	Arg	Trp	Ile	Gly	Leu	Ile	Gln	Ser	Lys	Glu	Thr	Gly	Asp
	100					105							110		
Phe	Thr	Phe	Asn	Leu	Ser	Glu	Asp	Glu	Gln	Ala	Ile	Ile	Glu	Ile	Asn
	115					120						125			
Gly	Lys	Ile	Ile	Ser	Asn	Lys	Gly	Lys	Glu	Lys	Gln	Val	Val	His	Leu
130					135						140				



Glu Lys Gly Lys Leu Val Pro Ile Lys Ile Glu Tyr Gln Ser Asp Thr
 145 150 155 160
 Lys Phe Asn Ile Asp Ser Lys Thr Phe Lys Glu Leu Lys Leu Phe Lys
 165 170 175
 Ile Asp Ser Gln Asn Gln Pro Gln Gln Val Gln Gln Asp Glu Leu Arg
 180 185 190
 Asn Pro Glu Phe Asn Lys Lys Glu Ser Gln Glu Phe Leu Ala Lys Pro
 195 200 205
 Ser Lys Ile Asn Leu Phe Thr Gln Xaa Met Lys Arg Glu Ile Asp Glu
 210 215 220
 Asp Thr Asp Thr Asp Gly Asp Ser Ile Pro Asp Leu Trp Glu Glu Asn
 225 230 235 240
 Gly Tyr Thr Ile Xaa Asn Arg Ile Ala Val Lys Trp Asp Asp Ser Leu
 245 250 255
 Ala Ser Lys Gly Tyr Thr Lys Phe Val Ser Asn Pro Leu Glu Ser His
 260 265 270
 Thr Val Gly Asp Pro Tyr Thr Asp Tyr Glu Lys Ala Ala Arg Asp Leu
 275 280 285
 Asp Leu Ser Asn Ala Lys Glu Thr Phe Asn Pro Leu Val Ala Ala Phe
 290 295 300
 Pro Ser Val Asn Val Ser Met Glu Lys Val Ile Leu Ser Pro Asn Glu
 305 310 315 320
 Asn Leu Ser Asn Ser Val Glu Ser His Ser Ser Thr Asn Trp Ser Tyr
 325 330 335
 Thr Asn Thr Glu Gly Ala Ser Val Glu Ala Gly Ile Gly Pro Lys Gly
 340 345 350
 Ile Ser Phe Gly Val Ser Val Asn Tyr Gln His Ser Glu Thr Val Ala
 355 360 365
 Gln Glu Trp Gly Thr Ser Thr Gly Asn Thr Ser Gln Phe Asn Thr Ala
 370 375 380
 Ser Ala Gly Tyr Leu Asn Ala Asn Val Arg Tyr Asn Asn Val Gly Thr
 385 390 395 400
 Gly Ala Ile Tyr Asp Val Lys Pro Thr Thr Ser Phe Val Leu Asn Asn
 405 410 415
 Asp Thr Ile Ala Thr Ile Thr Ala Lys Ser Asn Ser Thr Ala Leu Asn
 420 425 430
 Ile Ser Pro Gly Glu Ser Tyr Pro Lys Lys Gly Gln Asn Gly Ile Ala
 435 440 445
 Ile Thr Ser Met Asp Asp Phe Asn Ser His Pro Ile Thr Leu Asn Lys
 450 455 460



Lys Gln Val Asp Asn Leu Leu Asn Asn Lys Pro Met Met Leu Glu Thr
 465 470 475 480
 Asn Gln Thr Asp Gly Val Tyr Lys Ile Lys Asp Thr His Gly Asn Ile
 485 490 495
 Val Thr Gly Gly Glu Trp Asn Gly Val Ile Gln Gln Ile Lys Ala Lys
 500 505 510
 Thr Ala Ser Ile Ile Val Asp Asp Gly Glu Arg Val Ala Glu Lys Arg
 515 520 525
 Val Ala Ala Lys Asp Tyr Glu Asn Pro Glu Asp Lys Thr Pro Ser Leu
 530 535 540
 Thr Leu Lys Asp Ala Leu Lys Leu Ser Tyr Pro Asp Glu Ile Lys Glu
 545 550 555 560
 Ile Glu Gly Leu Leu Tyr Tyr Lys Asn Lys Pro Ile Tyr Glu Ser Ser
 565 570 575
 Val Met Thr Tyr Leu Asp Glu Asn Thr Ala Lys Glu Val Thr Lys Gln
 580 585 590
 Leu Asn Asp Thr Thr Gly Lys Phe Lys Asp Val Ser His Leu Tyr Asp
 595 600 605
 Val Lys Leu Thr Pro Lys Met Asn Val Thr Ile Lys Leu Ser Ile Leu
 610 615 620
 Tyr Asp Asn Ala Glu Ser Asn Asp Asn Ser Ile Gly Lys Trp Thr Asn
 625 630 635 640
 Thr Asn Ile Val Ser Gly Gly Asn Asn Gly Lys Lys Gln Tyr Ser Ser
 645 650 655
 Asn Asn Pro Asp Ala Asn Leu Thr Leu Asn Thr Asp Ala Gln Glu Lys
 660 665 670
 Leu Asn Lys Asn Arg Asp Tyr Tyr Ile Ser Leu Tyr Met Lys Ser Glu
 675 680 685
 Lys Asn Thr Gln Cys Glu Ile Thr Ile Asp Gly Glu Ile Tyr Pro Ile
 690 695 700
 Thr Thr Lys Thr Val Asn Val Asn Lys Asp Asn Tyr Lys Arg Leu Asp
 705 710 715 720
 Ile Ile Ala His Asn Ile Lys Ser Asn Pro Ile Ser Ser Leu His Ile
 725 730 735
 Lys Thr Asn Asp Glu Ile Thr Leu Phe Trp Asp Asp Ile Ser Ile Thr
 740 745 750
 Asp Val Ala Ser Ile Lys Pro Glu Asn Leu Thr Asp Ser Glu Ile Lys
 755 760 765
 Gln Ile Tyr Ser Arg Tyr Gly Ile Lys Leu Glu Asp Gly Ile Leu Ile
 770 775 780



Asp Lys Lys Gly Gly Ile His Tyr Gly Glu Phe Ile Asn Glu Ala Ser
 785 790 795 800
 Phe Asn Ile Glu Pro Leu Pro Asn Tyr Val Thr Lys Tyr Glu Val Thr
 805 810 815
 Tyr Ser Ser Glu Leu Gly Pro Asn Val Ser Asp Thr Leu Glu Ser Asp
 820 825 830
 Lys Ile Tyr Lys Asp Gly Thr Ile Lys Phe Asp Phe Thr Lys Tyr Ser
 835 840 845
 Lys Asn Glu Gln Gly Leu Phe Tyr Asp Ser Gly Leu Asn Trp Asp Phe
 850 855 860
 Lys Ile Asn Ala Ile Thr Tyr Asp Gly Lys Glu Met Asn Val Phe His
 865 870 875 880
 Arg Tyr Asn Lys

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2004 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus cereus*
- (B) STRAIN: AB78
- (C) INDIVIDUAL ISOLATE: NRRL B-21058

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..2001
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /product= "80 kDa protein VIP-1"
/evidence= EXPERIMENTAL

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATG AAA AGG GAA ATT GAT GAA GAC ACG GAT ACG GAT GGG GAC TCT ATT	48
Met Lys Arg Glu Ile Asp Glu Asp Thr Asp Thr Asp Gly Asp Ser Ile	
1 5 10 15	
CCT GAC CTT TGG GAA GAA AAT GGG TAT ACG ATT CAM AAT AGA ATC GCT	96
Pro Asp Leu Trp Glu Asn Gly Tyr Thr Ile Xaa Asn Arg Ile Ala	
20 25 30	
GTA AAG TGG GAC GAT TCT CTA GCA AGT AAA GGG TAT ACG AAA TTT GTT	144



Val	Lys	Trp	Asp	Asp	Ser	Leu	Ala	Ser	Lys	Gly	Tyr	Thr	Lys	Phe	Val	
		35					40					45				
TCA	AAT	CCA	CTA	GAA	AGT	CAC	ACA	GTT	GGT	GAT	CCT	TAT	ACA	GAT	TAT	192
Ser	Asn	Pro	Leu	Glu	Ser	His	Thr	Val	Gly	Asp	Pro	Tyr	Thr	Asp	Tyr	
	50					55					60					
GAA	AAG	GCA	GCA	AGA	GAT	CTA	GAT	TTG	TCA	AAT	GCA	AAG	GAA	ACG	TTT	240
Glu	Lys	Ala	Ala	Arg	Asp	Leu	Asp	Leu	Ser	Asn	Ala	Lys	Glu	Thr	Phe	
	65				70					75					80	
AAC	CCA	TTG	GTA	GCT	GCT	TTT	CCA	AGT	GTG	AAT	GTT	AGT	ATG	GAA	AAG	288
Asn	Pro	Leu	Val	Ala	Ala	Phe	Pro	Ser	Val	Asn	Val	Ser	Met	Glu	Lys	
				85					90					95		
GTG	ATA	TTA	TCA	CCA	AAT	GAA	AAT	TTA	TCC	AAT	AGT	GTA	GAG	TCT	CAT	336
Val	Ile	Leu	Ser	Pro	Asn	Glu	Asn	Leu	Ser	Asn	Ser	Val	Glu	Ser	His	
			100					105					110			
TCA	TCC	ACG	AAT	TGG	TCT	TAT	ACA	AAT	ACA	GAA	GGT	GCT	TCT	GTT	GAA	384
Ser	Ser	Thr	Asn	Trp	Ser	Tyr	Thr	Asn	Thr	Glu	Gly	Ala	Ser	Val	Glu	
			115				120					125				
GCG	GGG	ATT	GGA	CCA	AAA	GGT	ATT	TCG	TTC	GGA	GTT	AGC	GTA	AAC	TAT	432
Ala	Gly	Ile	Gly	Pro	Lys	Gly	Ile	Ser	Phe	Gly	Val	Ser	Val	Asn	Tyr	
	130					135					140					
CAA	CAC	TCT	GAA	ACA	GTT	GCA	CAA	GAA	TGG	GGA	ACA	TCT	ACA	GGA	AAT	480
Gln	His	Ser	Glu	Thr	Val	Ala	Gln	Glu	Trp	Gly	Thr	Ser	Thr	Gly	Asn	
	145				150					155					160	
ACT	TCG	CAA	TTC	AAT	ACG	GCT	TCA	GCG	GGA	TAT	TTA	AAT	GCA	AAT	GTT	528
Thr	Ser	Gln	Phe	Asn	Thr	Ala	Ser	Ala	Gly	Tyr	Leu	Asn	Ala	Asn	Val	
				165					170					175		
CGA	TAT	AAC	AAT	GTA	GGA	ACT	GGT	GCC	ATC	TAC	GAT	GTA	AAA	CCT	ACA	576
Arg	Tyr	Asn	Asn	Val	Gly	Thr	Gly	Ala	Ile	Tyr	Asp	Val	Lys	Pro	Thr	
				180				185					190			
ACA	AGT	TTT	GTA	TTA	AAT	AAC	GAT	ACT	ATC	GCA	ACT	ATT	ACG	GCG	AAA	624
Thr	Ser	Phe	Val	Leu	Asn	Asn	Asp	Thr	Ile	Ala	Thr	Ile	Thr	Ala	Lys	
		195					200					205				
TCT	AAT	TCT	ACA	GCC	TTA	AAT	ATA	TCT	CCT	GGA	GAA	AGT	TAC	CCG	AAA	672
Ser	Asn	Ser	Thr	Ala	Leu	Asn	Ile	Ser	Pro	Gly	Glu	Ser	Tyr	Pro	Lys	
	210					215					220					
AAA	GGA	CAA	AAT	GGA	ATC	GCA	ATA	ACA	TCA	ATG	GAT	GAT	TTT	AAT	TCC	720
Lys	Gly	Gln	Asn	Gly	Ile	Ala	Ile	Thr	Ser	Met	Asp	Asp	Phe	Asn	Ser	
	225				230					235					240	
CAT	CCG	ATT	ACA	TTA	AAT	AAA	AAA	CAA	GTA	GAT	AAT	CTG	CTA	AAT	AAT	768
His	Pro	Ile	Thr	Leu	Asn	Lys	Lys	Gln	Val	Asp	Asn	Leu	Leu	Asn	Asn	
				245					250					255		
AAA	CCT	ATG	ATG	TTG	GAA	ACA	AAC	CAA	ACA	GAT	GGT	GTT	TAT	AAG	ATA	816
Lys	Pro	Met	Met	Leu	Glu	Thr	Asn	Gln	Thr	Asp	Gly	Val	Tyr	Lys	Ile	
			260					265					270			
AAA	GAT	ACA	CAT	GGA	AAT	ATA	GTA	ACT	GGC	GGA	GAA	TGG	AAT	GGT	GTC	864



Lys	Asp	Thr	His	Gly	Asn	Ile	Val	Thr	Gly	Gly	Glu	Trp	Asn	Gly	Val		
		275					280					285					
ATA	CAA	CAA	ATC	AAG	GCT	AAA	ACA	GCG	TCT	ATT	ATT	GTG	GAT	GAT	GGG	912	
Ile	Gln	Gln	Ile	Lys	Ala	Lys	Thr	Ala	Ser	Ile	Ile	Val	Asp	Asp	Gly		
	290					295					300						
GAA	CGT	GTA	GCA	GAA	AAA	CGT	GTA	GCG	GCA	AAA	GAT	TAT	GAA	AAT	CCA	960	
Glu	Arg	Val	Ala	Glu	Lys	Arg	Val	Ala	Ala	Lys	Asp	Tyr	Glu	Asn	Pro		
305					310					315					320		
GAA	GAT	AAA	ACA	CCG	TCT	TTA	ACT	TTA	AAA	GAT	GCC	CTG	AAG	CTT	TCA	1008	
Glu	Asp	Lys	Thr	Pro	Ser	Leu	Thr	Leu	Lys	Asp	Ala	Leu	Lys	Leu	Ser		
				325					330					335			
TAT	CCA	GAT	GAA	ATA	AAA	GAA	ATA	GAG	GGA	TTA	TTA	TAT	TAT	AAA	AAC	1056	
Tyr	Pro	Asp	Glu	Ile	Lys	Glu	Ile	Glu	Gly	Leu	Leu	Tyr	Tyr	Lys	Asn		
			340					345					350				
AAA	CCG	ATA	TAC	GAA	TCG	AGC	GTT	ATG	ACT	TAC	TTA	GAT	GAA	AAT	ACA	1104	
Lys	Pro	Ile	Tyr	Glu	Ser	Ser	Val	Met	Thr	Tyr	Leu	Asp	Glu	Asn	Thr		
		355					360					365					
GCA	AAA	GAA	GTG	ACC	AAA	CAA	TTA	AAT	GAT	ACC	ACT	GGG	AAA	TTT	AAA	1152	
Ala	Lys	Glu	Val	Thr	Lys	Gln	Leu	Asn	Asp	Thr	Thr	Gly	Lys	Phe	Lys		
	370					375					380						
GAT	GTA	AGT	CAT	TTA	TAT	GAT	GTA	AAA	CTG	ACT	CCA	AAA	ATG	AAT	GTT	1200	
Asp	Val	Ser	His	Leu	Tyr	Asp	Val	Lys	Leu	Thr	Pro	Lys	Met	Asn	Val		
385					390				395						400		
ACA	ATC	AAA	TTG	TCT	ATA	CTT	TAT	GAT	AAT	GCT	GAG	TCT	AAT	GAT	AAC	1248	
Thr	Ile	Lys	Leu	Ser	Ile	Leu	Tyr	Asp	Asn	Ala	Glu	Ser	Asn	Asp	Asn		
				405					410					415			
TCA	ATT	GGT	AAA	TGG	ACA	AAC	ACA	AAT	ATT	GTT	TCA	GGT	GGA	AAT	AAC	1296	
Ser	Ile	Gly	Lys	Trp	Thr	Asn	Thr	Asn	Ile	Val	Ser	Gly	Gly	Asn	Asn		
			420					425					430				
GGA	AAA	AAA	CAA	TAT	TCT	TCT	AAT	AAT	CCG	GAT	GCT	AAT	TTG	ACA	TTA	1344	
Gly	Lys	Lys	Gln	Tyr	Ser	Ser	Asn	Asn	Pro	Asp	Ala	Asn	Leu	Thr	Leu		
		435					440					445					
AAT	ACA	GAT	GCT	CAA	GAA	AAA	TTA	AAT	AAA	AAT	CGT	GAC	TAT	TAT	ATA	1392	
Asn	Thr	Asp	Ala	Gln	Glu	Lys	Leu	Asn	Lys	Asn	Arg	Asp	Tyr	Tyr	Ile		
	450					455					460						
AGT	TTA	TAT	ATG	AAG	TCA	GAA	AAA	AAC	ACA	CAA	TGT	GAG	ATT	ACT	ATA	1440	
Ser	Leu	Tyr	Met	Lys	Ser	Glu	Lys	Asn	Thr	Gln	Cys	Glu	Ile	Thr	Ile		
465					470					475					480		
GAT	GGG	GAG	ATT	TAT	CCG	ATC	ACT	ACA	AAA	ACA	GTG	AAT	GTG	AAT	AAA	1488	
Asp	Gly	Glu	Ile	Tyr	Pro	Ile	Thr	Thr	Lys	Thr	Val	Asn	Val	Asn	Lys		
				485					490					495			
GAC	AAT	TAC	AAA	AGA	TTA	GAT	ATT	ATA	GCT	CAT	AAT	ATA	AAA	AGT	AAT	1536	
Asp	Asn	Tyr	Lys	Arg	Leu	Asp	Ile	Ile	Ala	His	Asn	Ile	Lys	Ser	Asn		
			500					505					510				
CCA	ATT	TCT	TCA	CTT	CAT	ATT	AAA	ACG	AAT	GAT	GAA	ATA	ACT	TTA	TTT	1584	



Pro	Ile	Ser	Ser	Leu	His	Ile	Lys	Thr	Asn	Asp	Glu	Ile	Thr	Leu	Phe		
	515						520					525					
TGG	GAT	GAT	ATT	TCT	ATA	ACA	GAT	GTA	GCA	TCA	ATA	AAA	CCG	GAA	AAT	1632	
Trp	Asp	Asp	Ile	Ser	Ile	Thr	Asp	Val	Ala	Ser	Ile	Lys	Pro	Glu	Asn		
	530						535					540					
TTA	ACA	GAT	TCA	GAA	ATT	AAA	CAG	ATT	TAT	AGT	AGG	TAT	GGT	ATT	AAG	1680	
Leu	Thr	Asp	Ser	Glu	Ile	Lys	Gln	Ile	Tyr	Ser	Arg	Tyr	Gly	Ile	Lys		
	545						550				555				560		
TTA	GAA	GAT	GGA	ATC	CTT	ATT	GAT	AAA	AAA	GGT	GGG	ATT	CAT	TAT	GGT	1728	
Leu	Glu	Asp	Gly	Ile	Leu	Ile	Asp	Lys	Lys	Gly	Gly	Ile	His	Tyr	Gly		
				565						570					575		
GAA	TTT	ATT	AAT	GAA	GCT	AGT	TTT	AAT	ATT	GAA	CCA	TTG	CCA	AAT	TAT	1776	
Glu	Phe	Ile	Asn	Glu	Ala	Ser	Phe	Asn	Ile	Glu	Pro	Leu	Pro	Asn	Tyr		
			580					585					590				
GTG	ACC	AAA	TAT	GAA	GTT	ACT	TAT	AGT	AGT	GAG	TTA	GGA	CCA	AAC	GTG	1824	
Val	Thr	Lys	Tyr	Glu	Val	Thr	Tyr	Ser	Ser	Glu	Leu	Gly	Pro	Asn	Val		
		595					600					605					
AGT	GAC	ACA	CTT	GAA	AGT	GAT	AAA	ATT	TAC	AAG	GAT	GGG	ACA	ATT	AAA	1872	
Ser	Asp	Thr	Leu	Glu	Ser	Asp	Lys	Ile	Tyr	Lys	Asp	Gly	Thr	Ile	Lys		
	610						615				620						
TTT	GAT	TTT	ACC	AAA	TAT	AGT	AAA	AAT	GAA	CAA	GGA	TTA	TTT	TAT	GAC	1920	
Phe	Asp	Phe	Thr	Lys	Tyr	Ser	Lys	Asn	Glu	Gln	Gly	Leu	Phe	Tyr	Asp		
	625					630				635					640		
AGT	GGA	TTA	AAT	TGG	GAC	TTT	AAA	ATT	AAT	GCT	ATT	ACT	TAT	GAT	GGT	1968	
Ser	Gly	Leu	Asn	Trp	Asp	Phe	Lys	Ile	Asn	Ala	Ile	Thr	Tyr	Asp	Gly		
				645					650						655		
AAA	GAG	ATG	AAT	GTT	TTT	CAT	AGA	TAT	AAT	AAA	TAG					2004	
Lys	Glu	Met	Asn	Val	Phe	His	Arg	Tyr	Asn	Lys							
			660						665								

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 667 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Lys	Arg	Glu	Ile	Asp	Glu	Asp	Thr	Asp	Thr	Asp	Gly	Asp	Ser	Ile		
1				5					10					15			
Pro	Asp	Leu	Trp	Glu	Glu	Asn	Gly	Tyr	Thr	Ile	Xaa	Asn	Arg	Ile	Ala		
		20						25					30				
Val	Lys	Trp	Asp	Asp	Ser	Leu	Ala	Ser	Lys	Gly	Tyr	Thr	Lys	Phe	Val		
		35					40					45					



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



Ala Lys Glu Val Thr Lys Gln Leu Asn Asp Thr Thr Gly Lys Phe Lys
 370 375 380
 Asp Val Ser His Leu Tyr Asp Val Lys Leu Thr Pro Lys Met Asn Val
 385 390 395 400
 Thr Ile Lys Leu Ser Ile Leu Tyr Asp Asn Ala Glu Ser Asn Asp Asn
 405 410 415
 Ser Ile Gly Lys Trp Thr Asn Thr Asn Ile Val Ser Gly Gly Asn Asn
 420 425 430
 Gly Lys Lys Gln Tyr Ser Ser Asn Asn Pro Asp Ala Asn Leu Thr Leu
 435 440 445
 Asn Thr Asp Ala Gln Glu Lys Leu Asn Lys Asn Arg Asp Tyr Tyr Ile
 450 455 460
 Ser Leu Tyr Met Lys Ser Glu Lys Asn Thr Gln Cys Glu Ile Thr Ile
 465 470 475 480
 Asp Gly Glu Ile Tyr Pro Ile Thr Thr Lys Thr Val Asn Val Asn Lys
 485 490 495
 Asp Asn Tyr Lys Arg Leu Asp Ile Ile Ala His Asn Ile Lys Ser Asn
 500 505 510
 Pro Ile Ser Ser Leu His Ile Lys Thr Asn Asp Glu Ile Thr Leu Phe
 515 520 525
 Trp Asp Asp Ile Ser Ile Thr Asp Val Ala Ser Ile Lys Pro Glu Asn
 530 535 540
 Leu Thr Asp Ser Glu Ile Lys Gln Ile Tyr Ser Arg Tyr Gly Ile Lys
 545 550 555 560
 Leu Glu Asp Gly Ile Leu Ile Asp Lys Lys Gly Gly Ile His Tyr Gly
 565 570 575
 Glu Phe Ile Asn Glu Ala Ser Phe Asn Ile Glu Pro Leu Pro Asn Tyr
 580 585 590
 Val Thr Lys Tyr Glu Val Thr Tyr Ser Ser Glu Leu Gly Pro Asn Val
 595 600 605
 Ser Asp Thr Leu Glu Ser Asp Lys Ile Tyr Lys Asp Gly Thr Ile Lys
 610 615 620
 Phe Asp Phe Thr Lys Tyr Ser Lys Asn Glu Gln Gly Leu Phe Tyr Asp
 625 630 635 640
 Ser Gly Leu Asn Trp Asp Phe Lys Ile Asn Ala Ile Thr Tyr Asp Gly
 645 650 655
 Lys Glu Met Asn Val Phe His Arg Tyr Asn Lys
 660 665

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:



- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacillus cereus
- (B) STRAIN: AB78
- (C) INDIVIDUAL ISOLATE: NRRL B-21058

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1..16
- (D) OTHER INFORMATION: /note= "N-terminal sequence of protein purified from strain AB78"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Lys	Arg	Glu	Ile	Asp	Glu	Asp	Thr	Asp	Thr	Asx	Gly	Asp	Ser	Ile	Pro
1					5				10					15	

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 1..21
- (D) OTHER INFORMATION: /note= "Oligonucleotide probe based on amino acids 3 to 9 of SEQ ID NO:8, using codon usage of Bacillus thuringiensis"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

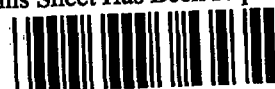
GAAATTGATC AAGATACNGA T

21

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 amino acids



- (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: *Bacillus thuringiensis*
 - (B) STRAIN: AB88
- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION: 1..14
 - (D) OTHER INFORMATION: /note= "N-terminal amino acid sequence of protein known as anion exchange fraction 23 (smaller)"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
- Xaa Glu Pro Phe Val Ser Ala Xaa Xaa Xaa Gln Xaa Xaa Xaa
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 11:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: *Bacillus thuringiensis*
 - (B) STRAIN: AB88
- (ix) FEATURE:
- (A) NAME/KEY: Peptide
 - (B) LOCATION: 1..13
 - (D) OTHER INFORMATION: /note= "N-terminal amino acid sequence of protein known as anion exchange fraction 23 (larger)"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
- Xaa Glu Tyr Glu Asn Val Glu Pro Phe Val Ser Ala Xaa
1 5 10
- (2) INFORMATION FOR SEQ ID NO: 12:





- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Bacillus thuringiensis*
- (ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION: 1..9
 (D) OTHER INFORMATION: /note= "N-terminal sequence of a
 130 kDa delta-endotoxin"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
- Met Asp Asn Asn Pro Asn Ile Asn Glu
1 5
- (2) INFORMATION FOR SEQ ID NO: 15:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION: 1..9
 (D) OTHER INFORMATION: /note= "N-terminal sequence of 80
 kDa delta-endotoxin"
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:
- Met Asp Asn Asn Pro Asn Ile Asn Glu
1 5
- (2) INFORMATION FOR SEQ ID NO: 16:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11 amino acids
 (B) TYPE: amino acid



(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Bacillus thuringiensis*

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..11

(D) OTHER INFORMATION: /note= "N-terminal sequence from 60 kDa delta-endotoxin"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Met Asn Val Leu Asn Ser Gly Arg Thr Thr Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2655 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

ATGAAGAACA TGAAGAAGAA GCTGGCCAGC GTGGTGACCT GCACCCTGCT GGCCCCCATG	60
TTCCTGAACG GCAACGTGAA CGCCGTGTAC GCCGACAGCA AGACCAACCA GATCAGCACC	120
ACCCAGAAGA ACCAGCAGAA GGAGATGGAC CGCAAGGGCC TGCTGGGCTA CTACTTCAAG	180
GGCAAGGACT TCAGCAACCT GACCATGTTT GCCCCACGC GTGACAGCAC CCTGATCTAC	240
GACCAGCAGA CCGCCAACAA GCTGCTGGAC AAGAAGCAGC AGGAGTACCA GAGCATCCGC	300
TGGATCGGCC TGATCCAGAG CAAGGAGACC GGCGACTTCA CCTTCAACCT GAGCGAGGAC	360
GAGCAGGCCA TCATCGAGAT CAACGGCAAG ATCATCAGCA ACAAGGGCAA GGAGAAGCAG	420
GTGGTGACCC TGGAGAAGGG CAAGCTGGTG CCCATCAAGA TCGAGTACCA GAGCGACACC	480
AAGTTCAACA TCGACAGCAA GACCTTCAAG GAGCTGAAGC TTTTCAAGAT CGACAGCCAG	540



AACCAGCCCC	AGCAGGTGCA	GCAGGACGAG	CTGCGCAACC	CCGAGTTCAA	CAAGAAGGAG	600
AGCCAGGAGT	TCCTGGCCAA	GCCCAGCAAG	ATCAACCTGT	TCACCCAGCA	GATGAAGCGC	660
GAGATCGACG	AGGACACCGA	CACCGACGGC	GACAGCATCC	CCGACCTGTG	GGAGGAGAAC	720
GGCTACACCA	TCCAGAACCG	CATCGCCGTG	AAGTGGGACG	ACAGCCTGGC	TAGCAAGGGC	780
TACACCAAGT	TCGTGAGCAA	CCCCCTGGAG	AGCCACACCG	TGGGCGACCC	CTACACCGAC	840
TACGAGAAGG	CCGCCCCGGA	CCTGGACCTG	AGCAACGCCA	AGGAGACCTT	CAACCCCCTG	900
GTGGCCGCCT	TCCCCAGCGT	GAACGTGAGC	ATGGAGAAGG	TGATCCTGAG	CCCCAACGAG	960
AACCTGAGCA	ACAGCGTGGA	GAGCCACTCG	AGCACCAACT	GGAGCTACAC	CAACACCGAG	1020
GGCGCCAGCG	TGGAGGCCGG	CATCGGTCCC	AAGGGCATCA	GCTTCGGCGT	GAGCGTGAAC	1080
TACCAGCACA	GCGAGACCGT	GGCCCAGGAG	TGGGGCACCA	GCACCGGCAA	CACCAGCCAG	1140
TTCAACACCG	CCAGCGCCGG	CTACCTGAAC	GCCAACGTGC	GCTACAACAA	CGTGGGCACC	1200
GGCGCCATCT	ACGACGTGAA	GCCCACCACC	AGCTTCGTGC	TGAACAACGA	CACCATCGCC	1260
ACCATCACCG	CCAAGTCGAA	TTCCACCGCC	CTGAACATCA	GCCCCGGCGA	GAGCTACCCC	1320
AAGAAGGGCC	AGAACGGCAT	CGCCATCACC	AGCATGGACG	ACTTCAACAG	CCACCCCATC	1380
ACCCTGAACA	AGAAGCAGGT	GGACAACCTG	CTGAACAACA	AGCCCATGAT	GCTGGAGACC	1440
AACCAGACCG	ACGGCGTCTA	CAAGATCAAG	GACACCCACG	GCAACATCGT	GACCGGCGGC	1500
GAGTGGAACG	GCGTGATCCA	GCAGATCAAG	GCCAAGACCG	CCAGCATCAT	CGTCGACGAC	1560
GGCGAGCGCG	TGGCCGAGAA	GCGCGTGGCC	GCCAAGGACT	ACGAGAACCC	CGAGGACAAG	1620
ACCCCCAGCC	TGACCCTGAA	GGACGCCCTG	AAGCTGAGCT	ACCCCGACGA	GATCAAGGAG	1680
ATCGAGGGCC	TGCTGTACTA	CAAGAACAAG	CCCATCTACG	AGAGCAGCGT	GATGACCTAT	1740
CTAGACGAGA	ACACCGCCAA	GGAGGTGACC	AAGCAGCTGA	ACGACACCAC	CGGCAAGTTC	1800
AAGGACGTGA	GCCACCTGTA	CGACGTGAAG	CTGACCCCCA	AGATGAACGT	GACCATCAAG	1860
CTGAGCATCC	TGTACGACAA	CGCCGAGAGC	AACGACAACA	GCATCGGCAA	GTGGACCAAC	1920
ACCAACATCG	TGAGCGGCGG	CAACAACGGC	AAGAAGCAGT	ACAGCAGCAA	CAACCCCGAC	1980
GCCAACCTGA	CCCTGAACAC	CGACGCCCAG	GAGAAGCTGA	ACAAGAACCG	CGACTACTAC	2040
ATCAGCCTGT	ACATGAAGAG	CGAGAAGAAC	ACCCAGTGCG	AGATCACCAT	CGACGGCGAG	2100
ATATACCCCA	TCACCACCAA	GACCGTGAAC	GTGAACAAGG	ACAACCTACAA	GCGCCTGGAC	2160
ATCATCGCCC	ACAACATCAA	GAGCAACCCC	ATCAGCAGCC	TGCACATCAA	GACCAACGAC	2220
GAGATCACCC	TGTTCTGGGA	CGACATATCG	ATTACCGACG	TCGCCAGCAT	CAAGCCCGAG	2280
AACCTGACCG	ACAGCGAGAT	CAAGCAGATA	TACAGTCGCT	ACGGCATCAA	GCTGGAGGAC	2340



GGCATCCTGA TCGACAAGAA GGGCGGCATC CACTACGGCG AGTTCATCAA CGAGGCCAGC	2400
TTCAACATCG AGCCCCTGCA GAACTACGTG ACCAAGTACG AGGTGACCTA CAGCAGCGAG	2460
CTGGGCCCCA ACGTGAGCGA CACCCTGGAG AGCGACAAGA TTTACAAGGA CGGCACCATC	2520
AAGTTCGACT TCACCAAGTA CAGCAAGAAC GAGCAGGGCC TGTTCTACGA CAGCGGCCTG	2580
AACTGGGACT TCAAGATCAA CGCCATCACC TACGACGGCA AGGAGATGAA CGTGTTCCAC	2640
CGCTACAACA AGTAG	2655

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2010 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

GGATCCATGA AGCGCGAGAT CGACGAGGAC ACCGACACCG ACGGCGACAG CATCCCCGAC	60
CTGTGGGAGG AGAACGGCTA CACCATCCAG AACCGCATCG CCGTGAAGTG GGACGACAGC	120
CTGGCTAGCA AGGGCTACAC CAAGTTCGTG AGCAACCCCC TGGAGAGCCA CACCGTGGGC	180
GACCCCTACA CCGACTACGA GAAGGCCGCC CGCGACCTGG ACCTGAGCAA CGCCAAGGAG	240
ACCTTCAACC CCCTGGTGGC CGCCTTCCCC AGCGTGAACG TGAGCATGGA GAAGGTGATC	300
CTGAGCCCCA ACGAGAACCT GAGCAACAGC GTGGAGAGCC ACTCGAGCAC CAACTGGAGC	360
TACACCAACA CCGAGGGCGC CAGCGTGGAG GCCGGCATCG GTCCCAAGGG CATCAGCTTC	420
GGCGTGAGCG TGAACTACCA GCACAGCGAG ACCGTGGCCC AGGAGTGGGG CACCAGCACC	480
GGCAACACCA GCCAGTTCAA CACCGCCAGC GCCGGCTACC TGAACGCCAA CGTGCGCTAC	540
AACAACGTGG GCACCGGCGC CATCTACGAC GTGAAGCCCA CCACCAGCTT CGTGCTGAAC	600
AACGACACCA TCGCCACCAT CACCGCCAAG TCGAATTCCA CCGCCCTGAA CATCAGCCCC	660
GGCGAGAGCT ACCCCAAGAA GGGCCAGAAC GGCATCGCCA TCACCAGCAT GGACGACTTC	720
AACAGCCACC CCATCACCCCT GAACAAGAAG CAGGTGGACA ACCTGCTGAA CAACAAGCCC	780
ATGATGCTGG AGACCAACCA GACCGACGGC GTCTACAAGA TCAAGGACAC CCACGGCAAC	840
ATCGTGACCG GCGGCGAGTG GAACGGCGTG ATCCAGCAGA TCAAGGCCAA GACCGCCAGC	900



ATCATCGTCG	ACGACGGCGA	GCGCGTGGCC	GAGAAGCGCG	TGGCCGCCAA	GGACTACGAG	960
AACCCCGAGG	ACAAGACCCC	CAGCCTGACC	CTGAAGGACG	CCCTGAAGCT	GAGCTACCCC	1020
GACGAGATCA	AGGAGATCGA	GGGCCTGCTG	TACTACAAGA	ACAAGCCCAT	CTACGAGAGC	1080
AGCGTGATGA	CCTATCTAGA	CGAGAACACC	GCCAAGGAGG	TGACCAAGCA	GCTGAACGAC	1140
ACCACCGGCA	AGTTCAAGGA	CGTGAGCCAC	CTGTACGACG	TGAAGCTGAC	CCCCAAGATG	1200
AACGTGACCA	TCAAGCTGAG	CATCCTGTAC	GACAACGCCG	AGAGCAACGA	CAACAGCATC	1260
GGCAAGTGGA	CCAACACCAA	CATCGTGAGC	GGCGGCAACA	ACGGCAAGAA	GCAGTACAGC	1320
AGCAACAACC	CCGACGCCAA	CCTGACCCTG	AACACCGACG	CCCAGGAGAA	GCTGAACAAG	1380
AACCGCGACT	ACTACATCAG	CCTGTACATG	AAGAGCGAGA	AGAACACCCA	GTGCGAGATC	1440
ACCATCGACG	GCGAGATATA	CCCCATCACC	ACCAAGACCG	TGAACGTGAA	CAAGGACAAC	1500
TACAAGCGCC	TGGACATCAT	CGCCCACAAC	ATCAAGAGCA	ACCCCATCAG	CAGCCTGCAC	1560
ATCAAGACCA	ACGACGAGAT	CACCCTGTTC	TGGGACGACA	TATCGATTAC	CGACGTCGCC	1620
AGCATCAAGC	CCGAGAACCT	GACCGACAGC	GAGATCAAGC	AGATATACAG	TCGCTACGGC	1680
ATCAAGCTGG	AGGACGGCAT	CCTGATCGAC	AAGAAGGGCG	GCATCCACTA	CGGCGAGTTC	1740
ATCAACGAGG	CCAGCTTCAA	CATCGAGCCC	CTGCAGAACT	ACGTGACCAA	GTACGAGGTG	1800
ACCTACAGCA	GCGAGCTGGG	CCCCAACGTG	AGCGACACCC	TGGAGAGCGA	CAAGATTTAC	1860
AAGGACGGCA	CCATCAAGTT	CGACTTCACC	AAGTACAGCA	AGAACGAGCA	GGGCCTGTTC	1920
TACGACAGCG	GCCTGAACTG	GGACTTCAAG	ATCAACGCCA	TCACCTACGA	CGGCAAGGAG	1980
ATGAACGTGT	TCCACCGCTA	CAACAAGTAG				2010

This Sheet Has Been Replaced



INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>26</u>, line <u>12-14</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 18 March 1993 (18.03.93)	Accession Number NRRL B-21058
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description
on page 41, line 1-3

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☐

Name of depositary institution **Agricultural Research Culture
Collection (NRRL)
International Depositary Authority**

Address of depositary institution (including postal code and country)
**1815 N. University Street
Peoria, IL 61604
USA**

Date of deposit
18 March 1993 (18.03.93)

Accession Number
NRRL B-21059

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

We request the Expert Solution where available

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

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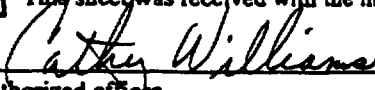
S-19506/A/CGC 1695

International application No.

PCT/US 94/03131

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>48</u> , line <u>18-20</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 18 March 1993 (18.03.93)	Accession Number NRRL B-21060
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
 We request the Expert Solution where available 	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit") 	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>41</u>, line <u>1-3</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 18 March 1993 (18.03.93)	Accession Number NRRL B-21061
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>42</u> , line <u>8-10</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 09 March 1994 (09.03.94)	Accession Number NRRL B-21221
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>42</u> , line <u>21-24</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 09 March 1994 (09.03.94)	Accession Number NRRL B-21222
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐ We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>43</u> , line <u>15-18</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 09 March 1994 (09.03.94)	Accession Number NRRL B-21223
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐ We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description
on page 53, line 18

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☐

Name of depositary institution **Agricultural Research Culture Collection (NRRL)
International Depositary Authority**

Address of depositary institution (including postal code and country)

**1815 N. University Street
Peoria, IL 61604
USA**

Date of deposit

09 March 1994 (09.03.94)

Accession Number

NRRL B-21224

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description
on page 51, line 8-10

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☐

Name of depositary institution **Agricultural Research Culture Collection (NRRL)
International Depository Authority**

Address of depositary institution (including postal code and country)

**1815 N. University Street
Peoria, IL 61604
USA**

Date of deposit

09 March 1994 (09.03.94)

Accession Number

NRRL B-21225

C. ADDITIONAL INDICATIONS (leave blank if not applicable)

This information is continued on an additional sheet ☐

We request the Expert Solution where available

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")

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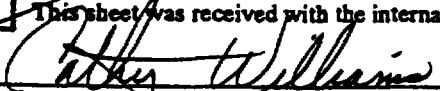
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International application No.

PCT/US 94/03131

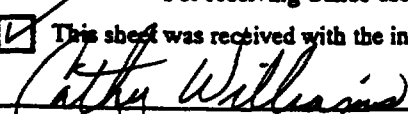
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 09 March 1994 (09.03.94)	Accession Number NRRL B-21226
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐ We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable) The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depositary Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
Date of deposit 09 March 1994 (09.03.94)	Accession Number NRRL B-21227
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐	
We request the Expert Solution where available	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
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INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐	
Name of depositary institution Agricultural Research Culture Collection (NRRL) International Depository Authority	
Address of depositary institution (including postal code and country) 1815 N. University Street Peoria, IL 61604 USA	
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International application No.

94/03131

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>52</u> , line <u>3-6</u>	
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International Application No.

PCT/US 94/03131

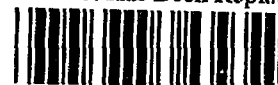
INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>53</u> , line <u>1-4</u>	
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What is claimed is:

1. A substantially purified Bacillus strain which produces a pesticidal protein during vegetative growth.
- 5 2. The Bacillus strain of claim 1 wherein said Bacillus is selected from a Bacillus species listed in Table 11.
3. The Bacillus strain of claim 1 wherein said protein is capable of killing pests selected from insects, fungi, bacteria, nematodes, mites, ticks, protozoan pathogens, animal parasites, and the like.
- 10 4. The Bacillus strain of claim 3, wherein said protein is capable of killing insects selected from orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Mallophaga, Anoplura, Siphonaptera, or Trichoptera.
5. The Bacillus strain of claim 4, wherein said coleopteran species is a Diabrotica.
- 15 6. The Bacillus strain of claim 5, wherein said Diabrotica is Diabrotica virgifera virgifera or Diabrotica longicornis barberi.
7. The Bacillus strain of claim 4, wherein said lepidopteran species is an Agrotis.
8. The Bacillus strain of claim 7, wherein said Agrotis is Agrotis ipsilon.
9. The Bacillus strain of claim 2, wherein said Bacillus is Bacillus cereus.
- 20 10. The Bacillus strain of claim 9, wherein said Bacillus cereus is Bacillus cereus having Accession No. NRRL B-21058.
11. The Bacillus strain of claim 2, wherein said Bacillus is Bacillus thuringensis.
12. The Bacillus strain of claim 11, wherein said Bacillus thuringensis is Bacillus thuringensis having Accession No. NRRL B-21060.



13. The *Bacillus* strain of claim 2, wherein said protein has a molecular weight of 30 kDa or greater.
14. The *Bacillus* strain of claim 13, wherein said protein has a molecular weight of about 60 to about 100 kDa.
- 5 15. The *Bacillus* strain of claim 14, wherein said protein has a molecular weight of about 80 kDa.
16. The *Bacillus* strain of claim 15, wherein said protein has the sequence given in SEQ. ID. NO:7.
17. The *Bacillus* strain of claim 14, wherein said protein has a molecular weight of about 100
10 kDa.
18. The *Bacillus* strain of claim 17, wherein said protein has the sequence given in SEQ. ID. NO:5.
19. A substantially pure pesticidal protein isolatable during the vegetative growth phase of *Bacillus* spp. or analogs and active fragments thereof.
- 15 20. The pesticidal protein of claim 19 wherein said *Bacillus* is selected from a *Bacillus* species listed in Table 11.
21. The pesticidal protein of claim 20, wherein said insects are selected from orders Coleoptera, Diptera, Hymenoptera, Lepidoptera, Homoptera, Hemiptera, Orthoptera, Thysanoptera, Dermaptera, Isoptera, Mallophaga, Anoplura, Siphonaptera, or
20 Trichoptera.
22. The pesticidal protein of claim 21, wherein said coleopteran species is a *Diabrotica*.
23. The pesticidal protein of claim 22, wherein said *Diabrotica* is *Diabrotica virgifera virgifera* or *Diabrotica longicornis barberi*.
24. The pesticidal protein of claim 21, wherein said lepidopteran species is an *Agrotis*.
- 25 25. The pesticidal protein of claim 24, wherein said *Agrotis* is *Agrotis ipsilon*.



26. The pesticidal protein of claim 19, wherein said Bacillus is Bacillus cereus.
27. The pesticidal protein of claim 26, wherein said Bacillus cereus is Bacillus cereus having Accession No. B-21058.
28. The pesticidal protein of claim 19, wherein said Bacillus is Bacillus thuringensis.
- 5 29. The pesticidal protein of claim 28, wherein said Bacillus thuringensis is Bacillus thuringensis selected from Accession Numbers NRRL B-21060, NRRL B-21224, NRRL B-21225, NRRL B-21226 and NRRL B-21227.
30. The pesticidal protein of claim 19, wherein said protein has a molecular weight of 30 kDa or greater.
- 10 31. The pesticidal protein of claim 30, wherein said protein has a molecular weight of about 60 to about 100 kDa.
32. The pesticidal protein of claim 31, wherein said protein has a molecular weight of about 80 kDa.
33. The pesticidal protein of claim 32, wherein said protein has the sequence given in SEQ
15 ID NO:7.
34. The pesticidal protein of claim 31, wherein said protein has the sequence given in SEQ ID NO:5.
35. The pesticidal protein of claim 19, wherein said protein comprises an N-terminal sequence as set forth in SEQ ID NOS:10 or 11.
- 20 36. A substantially pure nucleotide sequence which encodes the protein of claim 19.
37. A substantially pure nucleotide sequence which encodes the protein of claim 33.
38. A substantially pure nucleotide sequence which encodes the protein of claim 34.
39. A substantially pure nucleotide sequence which encodes the protein of claim 35.
40. The nucleotide sequence of claim 36, wherein said sequence has been optimized for
25 expression in a plant.



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41. The nucleotide sequence of claim 40, wherein said plant is selected from maize, soybean, cotton, wheat, sunflower, tomato, potato, and oilseed rape.
42. The nucleotide sequence of claim 37, wherein said sequence has been optimized for expression in a plant.
- 5 43. The nucleotide sequence of claim 42, wherein said plant is selected from maize, soybean, cotton, wheat, sunflower, tomato, potato, and oilseed rape.
44. The nucleotide sequence of claim 38, wherein said sequence has been optimized for expression in a plant.
45. The nucleotide sequence of claim 44, wherein said sequence is set forth in SEQ ID NO:
10 18.
46. The nucleotide sequence of claim 39, wherein said sequence has been optimized for expression in a plant.
47. The nucleotide sequence of claim 46, wherein said sequence is set forth in SEQ ID NO:
17.
- 15 48. The nucleotide sequence of claim 36, wherein said sequence has been optimized for expression in a microorganism.
49. The nucleotide sequence of claim 48, wherein said microorganism is selected from Bacillus, Pseudomonas, Saccharomyces, Clavibacter, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Agrobacterium, insect pathogenic viruses, fungi,
20 protozoans and nematodes.
50. A plant which has been stably transformed with the nucleotide sequence of any one of claims 36-47
51. The plant of claim 48, wherein said plant is a maize plant.
52. The nucleotide sequence of claim 36, wherein said sequence is essentially the sequence
25 of E. coli clone P5-4 having Accession No. NRRL B-21059.



53. The nucleotide sequence of claim 36, wherein said sequence is essentially the sequence of E. coli clone P3-12 having Accession No. NRRL B-21061.
54. The nucleotide sequence of claim 36, wherein said sequence is contained in E. coli clone pCIB 6022 having Accession No. NRRL B-21222.
- 5 55. The nucleotide sequence of claim 54 wherein said sequence is given as VIP-1 in SEQ ID NO:1.
56. An auxiliary protein which enhances the pesticidal activity of a pesticidal protein.
57. The auxiliary protein of claim 56 wherein said pesticidal protein is from Bacillus.
58. The auxiliary protein of claim 57 wherein said pesticidal protein is from B. cereus.
- 10 59. The auxiliary protein of claim 58 wherein said pesticidal protein is from strain AB78.
60. The auxiliary protein of claim 56 wherein said auxiliary protein is from Bacillus.
61. The auxiliary protein of claim 60 wherein said auxiliary protein is from B. cereus.
62. The auxiliary protein of claim 61 wherein said auxiliary protein is from strain AB78.
63. A substantially pure nucleotide sequence which encodes the auxiliary protein of any one of claims 56, 60, 61, and 62.
- 15 64. The nucleotide sequence of claim 63 wherein said sequence is contained in E. coli clone pCIB6022 having Accession No. NRRL B-21222.
65. The Bacillus strain of claim 1 wherein said strain is AB88 having Accession No. NRRL B-21225.
- 20 66. The Bacillus strain of claim 1 wherein said strain is AB289 having Accession No. NRRL B-21227.
67. The Bacillus strain of claim 1 wherein said strain is AB294 having Accession No. NRRL B-21229.
68. The Bacillus strain of claim 1 wherein said strain is AB359 having Accession No. NRRL B-21226.
- 25



69. The Bacillus strain of claim 1 wherein said strain is AB59 having Accession No. NRRL B-21228.
70. The Bacillus strain of claim 1 wherein said strain is AB256 having Accession No. NRRL B-21230.

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