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(54) Title: NEW BACILLUS THURINGIENSIS STRAINS AND THEIR INSECTICIDAL PROTEINS		
(57) Abstract		
Four novel <i>Bacillus thuringiensis</i> strains, which are deposited at the BCCM-LMG under accession nos. LMG P-12592, LMG P-12593, LMG P-12594, and LMG P-13493, produce new crystal proteins during sporulation that are toxic to Lepidoptera, more particularly against Noctuidae such as <i>Spodoptera</i> spp. and <i>Agrotis ipsilon</i> , against Pyralidae such as <i>Ostrinia nubilalis</i> , and against Yponomeutidae such as <i>Plutella xylostella</i> , and that are encoded by a novel gene. The crystal proteins contain protoxins, which can yield a toxin as trypsin-digestion product. A plant, the genome of which is transformed with a DNA sequence that comes from either one of the strains and that encodes its respective toxin, is resistant to Lepidoptera. Each strain, itself, or its crystals, crystal proteins, protoxin or toxin can be used as the active ingredient in an insecticidal composition for combatting Lepidoptera.		

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NEW BACILLUS THURINGIENSIS STRAINS AND
THEIR INSECTICIDAL PROTEINS

This invention relates to four novel strains of Bacillus
5 thuringiensis (the "BTS02617A strain", the "BTS02618A strain",
the "BTS02654B strain" and the "BTS02652E strain"), each of
which produces crystallized proteins (the "BTS02617A crystal
proteins", the "BTS02618A crystal proteins", the "BTS02654B
crystal proteins" and the "BTS02652E crystal proteins",
10 respectively) which are packaged in crystals (the "BTS02617A
crystals", the "BTS02618A crystals", the "BTS02654B crystals"
and the "BTS02652E crystals", respectively) during sporulation.
The BTS02617A, BTS02618A, BTS02654B and BTS02652E strains were
deposited under the provisions of the Budapest Treaty at the
15 Belgian Coordinated Collections of Microorganisms - Collection
Laboratorium voor Microbiologie Belgium ("BCCM-LMG"), R.U.G.,
K. Ledeganckstraat 35, B-9000 Gent.

This invention also relates to an insecticide composition
that is active against Lepidoptera and that comprises the
20 BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, as such,
or preferably the BTS02617A, BTS02618A, BTS02654B or BTS02652E
crystals, crystal proteins or the active component(s) thereof
as an active ingredient.

This invention further relates to a gene (the "bBTS02618A
25 gene"), which is present in the genome of the BTS02617A,
BTS02618A, BTS02654B and BTS02652E strains and which encodes
an insecticidal protein (the "BTS02618A protoxin") that is
found in the BTS02617A, BTS02618A, BTS02654B and BTS02652E
crystals. The BTS02618A protoxin is the protein that is
30 produced by the BTS02617A, BTS02618A, BTS02654B and BTS02652E
strains before being packaged into their respective BTS02617A,
BTS02618A, BTS02654B and BTS02652E crystals.

This invention still further relates to a toxin (the
"BTS02618A toxin") which can be obtained (e.g., by trypsin
35 digestion) from the BTS02618A protoxin. The BTS02618A toxin
is an insecticidally active protein which can be liberated from

the BTS02617A crystals, the BTS02618A crystals, the BTS02654B crystals, and the BTS02652E crystals, which are produced by the BTS02617A strain, the BTS02618A strain, the BTS02654B strain and the BTS02652E strain, respectively. This toxin and its
5 protoxin have a high activity against a wide range of lepidopteran insects, particularly against Noctuidae, especially against Spodoptera and Agrotis spp., but also against other important lepidopteran insects such as Pyralidae, particularly the European corn borer, Ostrinia nubilalis, and
10 Yponomeutidae such as Plutella xylostella. This new characteristic of the BTS02618A protoxin and toxin ("(pro)toxin"), i.e., the combination of activity against different economically important Lepidopteran insect families such as Noctuidae, Yponomeutidae and Pyralidae, makes this
15 (pro)toxin an ideally suited compound for combatting a wide range of insect pests by contacting these insects with the (pro)toxin, e.g., by spraying or by expressing the bTS02618A gene in plant-associated bacteria or in plants. The BTS02618A toxin is believed to represent the smallest portion of the
20 BTS02618A protoxin which is insecticidally effective against Lepidoptera.

This invention yet further relates to a chimeric gene that can be used to transform a plant cell and that contains the following operably linked DNA fragments:

- 25 1) a part of the bTS02618A gene (the "insecticidally effective bTS02618A gene part") encoding an insecticidally effective portion of the BTS02618A protoxin, preferably a truncated part of the bTS02618A gene (the "truncated bTS02618A gene") encoding just the BTS02618A toxin;
- 30 2) a promoter suitable for transcription of the insecticidally effective bTS02618A gene part in a plant cell; and
- 3) suitable 3' end transcript formation and polyadenylation signals for expressing the insecticidally
35 effective bTS02618A gene part in a plant cell.

This chimeric gene is hereinafter generally referred to as the "bTS02618A chimeric gene".

This invention also relates to:

1) a cell (the "transformed plant cell") of a plant, such as corn or cotton, the genome of which is transformed with the insecticidally effective bTS02618A gene part, preferably the bTS02618A chimeric gene; and

2) a plant (the "transformed plant") which is regenerated from the transformed plant cell or is produced from the so-regenerated plant and their seeds, the genome of which contains the insecticidally effective bTS02618A gene part, preferably the bTS02618A chimeric gene, and which is resistant to Lepidoptera.

This invention still further relates to :

1) a microbial organism, such as B. thuringiensis or Pseudomonas spp., the genome of which is transformed with all or part of the bTS02618A gene; and

2) a microbial spore, containing a genome which is transformed with all or parts of the bTS02618A gene.

Background of the Invention

20

B. thuringiensis ("Bt") is a Gram-positive bacterium which produces endogenous crystals upon sporulation. The crystals are composed of proteins which are specifically toxic against insect larvae. These crystal proteins and corresponding genes have been classified based on their structure and insecticidal spectrum (Höfte and Whiteley, 1989). The four major classes are Lepidoptera-specific (cryI), Lepidoptera- and Diptera-specific (cryII), Coleoptera-specific (cryIII), and Diptera-specific (cryIV) genes.

30

The fact that conventional submerged fermentation techniques can be used to produce Bt spores on a large scale makes Bt bacteria commercially attractive as a source of insecticidal compositions.

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Gene fragments from some Bt strains, encoding insecticidal proteins, have heretofore been identified and integrated into plant genomes in order to render the plants insect-resistant. However, obtaining expression of such Bt gene fragments in

plants is not a straightforward process. In order to achieve optimal expression of an insecticidal protein in plant cells, it has been found necessary to engineer each Bt gene fragment in a specific way so that it encodes a part of a Bt protoxin that retains substantial toxicity against its target insects (European patent application ("EPA") 86/300,291.1 and 88/402,115.5; U.S. patent application 821,582, filed January 22, 1986).

10 Summary of the Invention

In accordance with this invention, four novel Bt strains, i.e., the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains, are provided. The BTS02617A, BTS02618A, BTS02654B and BTS02652E crystals and crystal proteins, the BTS02618A protoxin and toxin produced by the strains during sporulation, and insecticidally effective portions of the BTS02618A protoxin, as well as equivalents of these crystals, crystal proteins, protoxin, toxin and insecticidally effective protoxin portions, each possess insecticidal activity and can therefore be formulated into insecticidal compositions against Lepidoptera in general, and particularly against Noctuidae, such as Agrotis spp. (cutworms such as Agrotis ipsilon), Mamestra spp. (e.g., the cabbage moth, Mamestra brassica) and Spodoptera spp. (armyworms, such as Spodoptera exigua, Spodoptera frugiperda, Spodoptera littoralis and Spodoptera litura), against Pyralidae (e.g., the European corn borer, Ostrinia nubilalis) and Yponomeutidae (such as Plutella xylostella) which are major pests of various economically important crops, such as corn, cotton and many vegetables such as Brassicas.

Also in accordance with this invention, a plant cell genome is transformed with the insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, or an equivalent thereof such as a modified, synthetic bTS02618A gene. It is preferred that this transformation be carried out with the bTS02618A chimeric gene. The resulting transformed plant cell can be used to produce transformed

plants, seeds of transformed plants and plant cell cultures consisting essentially of the transformed cells. The transformed cells in some or all of the tissues of the transformed plants: 1) contain the insecticidally effective bTS02618A gene part as a stable insert in their genome, and 2) express the insecticidally effective bTS02618A gene part by producing an insecticidally effective portion of its BTS02618A protoxin, preferably its BTS02618A toxin, thereby rendering the plant resistant to Lepidoptera. The transformed plant cells of this invention can also be used to produce, for recovery, such insecticidal Bt proteins.

Further in accordance with this invention, a process is provided for rendering a plant resistant to Lepidoptera by transforming the plant cell genome with the insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, or an equivalent thereof. In this regard, it is preferred that the plant cell be transformed with the bTS02618A chimeric gene.

Yet further in accordance with this invention, there are provided the BTS02618A protoxin, the insecticidally effective portions of such protoxin and the BTS02618A toxin, as well as functional parts of the BTS02618A toxin, as well as the bTS02618A gene, the insecticidally effective bTS02618A gene part, the truncated bTS02618A gene and the chimeric bTS02618A gene, as well as their equivalents.

Also in accordance with this invention, a DNA sequence, either natural or artificial, encoding the BTS02618A protoxin or insecticidally effective portions thereof, such as the toxin, is provided.

Also in accordance with this invention are provided an insecticidal composition against Lepidoptera, particularly Noctuidae, Pyralidae and Yponomeutidae, and a method for controlling Lepidoptera, particularly Noctuidae, Pyralidae and Yponomeutidae, with the insecticidal composition, wherein the insecticidal composition comprises the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, crystals and/or crystal proteins or the BTS02618A protoxin, toxin and/or insecticidally

effective protoxin portions or their equivalents.

Detailed Description of the Invention

5 The BTS02618A protoxin of this invention can be isolated in a conventional manner from the BTS02617A strain, deposited on July, 2 at the BCCM-LMG under accession number **LMG P-12592**, the BTS02618A strain, deposited on July 2, 1992 at the BCCM-LMG under accession number **LMG P-12593**, the BTS02654B strain, deposited on July 2, 1992 at the BCCM-LMG under accession number **LMG P-12594**, or the BTS02652E strain deposited on March 1, 1993 at the BCCM-LMG under accession number **LMG P-13493**. For example, the BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals can be isolated from sporulated cultures of their respective strain (Mahillon and Delcour, 1984), and then, the BTS02618A protoxin can be isolated from the crystals according to the method of Höfte et al. (1986). The protoxins can be used to prepare monoclonal or polyclonal antibodies specific for the protoxin in a conventional manner (Höfte et al., 1988). The BTS02618A toxin can be obtained by protease (e.g., trypsin) digestion of the BTS02618A protoxin.

 The bTS02618A gene can be isolated in a conventional manner. The bTS02618A gene can be identified in the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, using the procedure described in U.S. Patent Application 821,582, filed January 22, 1986, and in EPA 86/300,291.1 and 88/402,115.5 (which are incorporated herein by reference). The bTS02618A gene was identified by: digesting total DNA from one of the above strains with restriction enzymes; size fractionating the DNA fragments, so produced, into DNA fractions of 5 to 10 Kb; ligating these fractions to cloning vectors; screening the E. coli, transformed with the cloning vectors, with a DNA probe that was constructed from a region of the cryIG gene (Smulevitch et al., 1991; Gleave et al., 1992).

35 The term "bTS02618A gene" as used herein includes a DNA sequence encoding the BTS02618A protoxin or toxin or functionally equivalent variants thereof. Indeed, because of

the degeneracy of the genetic code, some amino acid codons can be replaced with others without changing the amino acid sequence of the protein. Furthermore, some amino acids can be substituted by other equivalent amino acids without significantly changing the insecticidal activity of the protein. Also, changes in amino acid composition in regions of the molecule, different from those responsible for binding and toxicity are less likely to cause a difference in insecticidal activity of the protein. Such equivalents of the gene include DNA sequences hybridizing to the DNA sequence of the BTS02618A toxin or protoxin of SEQ ID. No. 4 and encoding a protein with the same insecticidal characteristics as the BTS02618A (pro)toxin, of this invention. In this context, the term "hybridization" refers to conventional hybridization conditions, most preferably stringent hybridization conditions.

The term "functional parts of the BTS02618A toxin" as used herein means any part(s) or domain(s) of the toxin with a specific structure that can be transferred to another (Bt) protein for providing a new hybrid protein with at least one functional characteristic (e.g., the binding and/or toxicity characteristics) of the BTS02618A toxin (Ge et al., 1991). Such parts can form an essential feature of the hybrid Bt protein with the binding and/or toxicity characteristics of the BTS02618A protein. Such a hybrid protein can have an enlarged host range, an improved toxicity and/or can be used in a strategy to prevent insect resistance development (European Patent Publication ("EP") 408 403; Visser et al., 1993).

Alternatively, the 5 to 10 Kb fragments, prepared from total DNA of the BTS02617A or BTS02618A or BTS02654B or BTS02652E strain, can be ligated in suitable expression vectors and transformed in *E. coli*, and the clones can then be screened by conventional colony immunoprobng methods (French et al., 1986) for expression of the toxin with monoclonal or polyclonal antibodies raised against the BTS02618A toxin.

Also, the 5 to 10 Kb fragments, prepared from total DNA of the BTS02617A or BTS02618A or BTS02654B or BTS02652E strain, can be ligated in suitable Bt shuttle vectors (Lereclus et al.,

1992) and transformed in a crystal minus Bt-mutant. The clones are then screened for production of crystals (detected by microscopy) or crystal proteins (detected by SDS-PAGE).

The so-identified bTS02618A gene was sequenced in a conventional manner (Maxam and Gilbert, 1980) to obtain the DNA sequence. Hybridization in Southern blots and sequence comparison indicated that this gene is different from previously described genes encoding protoxins and toxins with activity against Lepidoptera (Höfte and Whiteley, 1989).

An insecticidally effective part of the bTS02618A gene, encoding an insecticidally effective portion of its protoxin, and a truncated part of the gene, encoding just its toxin, can be made in a conventional manner after sequence analysis of the gene. The amino acid sequence of the bTS02618A protoxin and toxin was determined from the DNA sequence of the bTS02618A gene and the truncated bTS02618A gene. By "an insecticidally effective part" or "a part" of the bTS02618A gene is meant a DNA sequence encoding a polypeptide which has fewer amino acids than the bTS02618A protoxin but which is still toxic to Lepidoptera.

In order to express all or an insecticidally effective part of the bTS02618A gene or an equivalent gene in E. coli, in other Bt strains and in plants, suitable restriction sites can be introduced, flanking each gene or gene part. This can be done by site-directed mutagenesis, using well-known procedures (Stanssens et al., 1989; White et al., 1989). In order to obtain improved expression in plants, it may be preferred to modify the codon usage of the bTS02618A gene or insecticidally effective bTS02618A gene part to form an equivalent, modified or artificial gene or gene part in accordance with PCT publications WO 91/16432 and WO 93/09218; EP 0,358,962 and EP 0,359,472. For obtaining enhanced expression in monocot plants such as corn, a monocot intron also can be added to the bTS02618A chimeric gene, and the DNA sequence of the bTS02618A gene part can be further changed in a translationally neutral manner, to modify possibly inhibiting DNA sequences present in the gene part by means of site-

directed intron insertion and/or by introducing changes to the codon usage, e.g., adapting the codon usage to that most preferred by the specific plant (Murray et al., 1989) without changing significantly the encoded amino acid sequence.

5 The insecticidally effective bTS02618A gene part or its equivalent, preferably the bTS02618A chimeric gene, encoding an insecticidally effective portion of the BTS02618A protoxin, can be stably inserted in a conventional manner into the nuclear genome of a single plant cell, and the so-transformed
10 plant cell can be used in a conventional manner to produce a transformed plant that is insect-resistant. In this regard, a disarmed Ti-plasmid, containing the insecticidally effective bTS02618A gene part, in Agrobacterium tumefaciens can be used to transform the plant cell, and thereafter, a transformed
15 plant can be regenerated from the transformed plant cell using the procedures described, for example, in EP 0,116,718, EP 0,270,822, PCT publication WO 84/02,913 and European Patent Application ("EPA") 87/400,544.0 (which are also incorporated herein by reference), and in Gould et al. (1991). Preferred
20 Ti-plasmid vectors each contain the insecticidally effective bTS02618A gene part between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as
25 direct gene transfer (as described, for example in EP 0,233,247), pollen mediated transformation (as described, for example in EP 0,270,356, PCT publication WO 85/01856, and US Patent 4,684,611), plant RNA virus-mediated transformation (as described, for example in EP 0,067,553 and US Patent
30 4,407,956), liposome-mediated transformation (as described, for example in US Patent 4,536,475), and other methods such as the recently described methods for transforming certain lines of corn (Fromm et al., 1990 ; Gordon-Kamm et al., 1990) and rice (Shimamoto et al., 1989; Datta et al., 1990) and the recently
35 described method for transforming monocots generally (PCT publication WO 92/09696).

The resulting transformed plant can be used in a

conventional plant breeding scheme to produce more transformed plants with the same characteristics or to introduce the insecticidally effective bTS02618A gene part in other varieties of the same or related plant species. Seeds, which are obtained from the transformed plants, contain the insecticidally effective bTS02618A gene part as a stable genomic insert. Cells of the transformed plant can be cultured in a conventional manner to produce the insecticidally effective portion of the BTS02618A protoxin, preferably the BTS02618A toxin, which can be recovered for use in conventional insecticide compositions against Lepidoptera (U.S. Patent Application 821,582; EPA 86/300291.1.).

The insecticidally effective bTS02618A gene part, preferably the truncated bTS02618A gene, is inserted in a plant cell genome so that the inserted gene is downstream (i.e., 3') of, and under the control of, a promoter which can direct the expression of the gene part in the plant cell. This is preferably accomplished by inserting the bTS02618A chimeric gene in the plant cell genome. Preferred promoters include: the strong constitutive 35S promoters (the "35S promoters") of the cauliflower mosaic virus of isolates CM 1841 (Gardner et al., 1981), CabbB-S (Franck et al., 1980) and CabbB-JI (Hull and Howell, 1987); and the TR1' promoter and the TR2' promoter (the "TR1' promoter" and "TR2' promoter", respectively) which drive the expression of the 1' and 2' genes, respectively, of the T-DNA (Velten et al., 1984). Alternatively, a promoter can be utilized which is not constitutive but rather is specific for one or more tissues or organs of the plant (e.g., leaves and/or roots) whereby the inserted bTS02618A gene part is expressed only in cells of the specific tissue(s) or organ(s). For example, the insecticidally effective bTS02618A gene part could be selectively expressed in the leaves of a plant (e.g., corn, cotton) by placing the insecticidally effective gene part under the control of a light-inducible promoter such as the promoter of the ribulose-1,5-bisphosphate carboxylase small subunit gene of the plant itself or of another plant such as pea as disclosed in U.S. Patent Application 821,582 and EPA

86/300,291.1. Another alternative is to use a promoter whose expression is inducible (e.g., by temperature or chemical factors).

5 The insecticidally effective bTS02618A gene part is inserted in the plant genome so that the inserted gene part is upstream (i.e., 5') of suitable 3' end transcription regulation signals (i.e., transcript formation and polyadenylation signals). This is preferably accomplished by inserting the bTS02618A chimeric gene in the plant cell genome. Preferred
10 polyadenylation and transcript formation signals include those of the octopine synthase gene (Gielen et al., 1984) and the T-DNA gene 7 (Velten and Schell, 1985), which act as 3'-untranslated DNA sequences in transformed plant cells.

15 The insecticidally effective bTS02618A gene part can optionally be inserted in the plant genome as a hybrid gene (EPA 86/300,291.1; Vaeck et al., 1987) under the control of the same promoter as a selectable marker gene, such as the neo gene (EP 0,242,236) encoding kanamycin resistance, so that the plant expresses a fusion protein.

20 All or part of the bTS02618A gene, encoding an anti-lepidopteran protein, can also be used to transform other bacteria, such as a B. thuringiensis which has insecticidal activity against Lepidoptera or Coleoptera. Thereby, a transformed Bt strain can be produced which is useful for
25 combatting a wide spectrum of lepidopteran and coleopteran insect pests or for combatting additional lepidopteran insect pests. Transformation of bacteria with all or part of the bTS02618A gene, incorporated in a suitable cloning vehicle, can be carried out in a conventional manner, preferably using
30 conventional electroporation techniques as described in Mahillon et al. (1989) and in PCT Patent publication WO 90/06999.

35 The BTS02617A, BTS02618A, BTS02654B or BTS02652E strain also can be transformed with all or an insecticidally effective part of one or more foreign Bt genes such as: the bt18 gene (EP 0,358,557) or another Bt gene coding for an anti-Lepidoptera protein; and the bt109P gene (PCT publication WO 91/16433),

coding for an anti-Coleoptera protein. Thereby, a transformed Bt strain can be produced which is useful for combatting an even greater variety of insect pests (e.g., Coleoptera and/or additional Lepidoptera).

5 Transformation of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain with all or part of a foreign Bt gene, incorporated in a conventional cloning vector, can be carried out in a well known manner, preferably using conventional electroporation techniques (Chassy et al., 1988) or other
10 methods, e.g., as described by Lereclus et al. (1992).

Each of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strains can be fermented by conventional methods (Dulmage, 1981; Bernhard and Utz, 1993) to provide high yields of cells. Under appropriate conditions which are well understood
15 (Dulmage, 1981), the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains each sporulate to produce crystal proteins containing the BTS02168A protoxin in high yields.

An insecticidal, particularly anti-lepidopteran, composition of this invention can be formulated in a
20 conventional manner using the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain or preferably their respective crystals, crystal proteins or the BTS02168A protoxin, toxin or insecticidally effective protoxin portion as an active ingredient, together with suitable carriers, diluents,
25 emulsifiers and/or dispersants (e.g., as described by Bernhard and Utz, 1993). This insecticide composition can be formulated as a wettable powder, pellets, granules or dust or as a liquid formulation with aqueous or non-aqueous solvents as a foam, gel, suspension, concentrate, etc. The concentration of the
30 BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, crystals, crystal proteins, or the BTS02168A protoxin, toxin or insecticidally effective protoxin portions in such a composition will depend upon the nature of the formulation and its intended mode of use. Generally, an insecticide composition
35 of this invention can be used to protect a field for 2 to 4 weeks against Lepidoptera with each application of the composition. For more extended protection (e.g., for a whole

growing season), additional amounts of the composition should be applied periodically.

A method for controlling insects, particularly Lepidoptera, in accordance with this invention preferably comprises applying (e.g., spraying), to a locus (area) to be protected, an insecticidal amount of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain, spores, crystals, crystal proteins or the BTS02168A protoxin, toxin or insecticidally effective protoxin portions, preferably the BTS2168A toxin. The locus to be protected can include, for example, the habitat of the insect pests or growing vegetation or an area where vegetation is to be grown.

To obtain the BTS02618A protoxin or toxin, cells of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain can be grown in a conventional manner on a suitable culture medium and then lysed using conventional means such as enzymatic degradation or detergents or the like. The protoxin can then be separated and purified by standard techniques such as chromatography, extraction, electrophoresis, or the like. The toxin can then be obtained by trypsin digestion of the protoxin.

The BTS02617A, BTS02618A, BTS02654B or BTS02652E cells can also be harvested and then applied intact, either alive or dead, preferably dried, to the locus to be protected. In this regard, it is preferred that a purified BTS02617A, BTS02618A, BTS02654B or BTS02652E strain (either alive or dead) be used, particularly a cell mass that is 90.0 to 99.9 % of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain.

The BTS02617A, BTS02618A, BTS02654B, or BTS02652E cells, crystals or crystal proteins or the BTS02618 protoxin, toxin, or insecticidally effective protoxin portion can be formulated in an insecticidal composition in a variety of ways, using any number of conventional additives, wet or dry, depending upon the particular use. Additives can include wetting agents, detergents, stabilizers, adhering agents, spreading agents and extenders. Examples of such a composition include pastes, dusting powders, wettable powders, granules, baits and aerosol

sprays. Other Bt cells, crystals, crystal proteins, protoxins, toxins, and insecticidally effective protoxin portions and other insecticides, as well as fungicides, biocides, herbicides and fertilizers, can be employed along with the BTS02617A, BTS02618A, BTS02654B or BTS02652E cells, crystals or crystal proteins or the BTS02618 protoxin, toxin or insecticidally effective protoxin portions to provide additional advantages or benefits. Such an insecticidal composition can be prepared in a conventional manner, and the amount of the BTS02617A, BTS02618A, BTS02654B or BTS02652E cells, crystals or crystal proteins or the BTS02618A protoxin, toxin or insecticidally effective protoxin portion employed depends upon a variety of factors, such as the insect pest targeted, the composition used, the type of area to which the composition is to be applied, and the prevailing weather conditions. Generally, the concentration of the BTS02618A protoxin, insecticidally effective protoxin portions or toxin will be at least about 0.1% by weight of the formulation to about 100% by weight of the formulation, more often from about 0.15% to about 0.8% by weight of the formulation.

In practice, some insects can be fed the BTS02618A protoxin, toxin, insecticidally effective protoxin portion or mixtures thereof in the protected area, that is in the area where such protoxin, toxin and/or insecticidally effective protoxin portion has been applied. Alternatively, some insects can be fed intact and alive cells of the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain or transformants thereof, so that the insects ingest some of the strain's protoxin and suffer death or damage.

The following Examples illustrate the invention. The figure and the sequence listing referred to in the Examples are as follows:

Figure 1

Southern blot analysis of AluI-digested total DNA of Bt strain HD127 (lane 1), the BTS02618A strain (lane 2), Bt strain

BTS02459 (containing cryIA(c), 81k, cryIC en cryIE, lane 3), and Bt strain BTS02480E (containing the same genes as HD-127, lane 4), using a mixture of DNA-probes for cryI crystal protein genes, including the cryIG probe (SEQ ID no. 1). Each band
5 corresponds to a particular crystal protein gene. With these probes, the BTS02618A strain is found to contain the cryIA(b) gene and a novel gene, which is the bTS02618A gene, identified by an AluI fragment of approximately 530 bp, hybridizing to the cryIG probe of SEQ ID no. 1. The names of the recognized cryI
10 genes are indicated, as well as the size of some fragments. The bTS02618A gene is indicated with three asterisks; "?" indicates an unknown gene fragment.

Sequence Listing

15 SEQ ID No. 1 - Nucleotide sequence of the DNA probe used to isolate the bTS02618A gene. This probe is derived from part of the cryIG DNA sequence and is complementary to nucleotides 2732-2750 of the DNA sequence described by Smulevitch et al. (1991).

20 SEQ ID No. 2 - The 5' partial nucleotide sequence of the bTS02618A gene, comprising the presumptive translation initiation codon at nucleotide position 195-197.

SEQ ID No. 3 - The 3' partial nucleotide sequence of the bTS02618A gene (N: unknown nucleotide), comprising the
25 presumptive translational stop codon at nucleotide position 1146-1148.

SEQ ID No. 4 - The nucleotide sequence of the bTS02618A gene and the translated amino acid sequence of the BTS02618A protoxin. The open reading frame of the protoxin reaches
30 from nucleotide 668 to nucleotide 4141. The translation initiation codon is at nucleotide position 668-670, the translation stop codon is at nucleotide position 4139-4141.

35 Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA are carried out by the standardized procedures described in Sambrook et al.,

Molecular Cloning - A Laboratory Manual, Second Ed., Cold Spring Harbor Laboratory Press, NY (1989).

Example 1: Characterization of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains.

The BTS02617A, the BTS02618A and the BTS02654B strain were isolated from grain dust sampled in Cadlan, province of Bicol, The Philippines and were deposited at the BCCM-LMG on July 2, 1992 under accession Nos. LMG P-12592, LMG P-12593 and LMG P-12594, respectively. Strain BTS02652E was also isolated from Philippine grain dust, and was deposited at the BCCM-LMG on March, 1, 1993 under accession No. LMG P-13493.

Each strain can be cultivated on conventional standard media, preferably T₃ medium (tryptone 3 g/l, tryptose 2 g/l, yeast extract 1.5 g/l, 5 mg MnCl₂, 0.05 M Na₂PO₄, pH 6.8 and 1.5% agar), preferably at 28°C. For long term storage, it is preferred to mix an equal volume of a spore-crystal suspension with an equal volume of 50% glycerol and store this at -70°C or lyophilize a spore-crystal suspension. For sporulation, growth on T₃ medium is preferred for 48 hours at 28°C, followed by storage at 4°C. During its vegetative phase, each of the strains can also grow under facultative anaerobic conditions, but sporulation only occurs under aerobic conditions.

Sterilization of each strain occurs by autoclave treatment at 120°C (1 bar pressure) for 20 minutes. Such treatment totally inactivates the spores and the BTS02617A, BTS02618A, BTS02654B, and BTS02652E protoxins. UV radiation (254 nm) also inactivates the spores.

After cultivating on Nutrient Agar ("NA", Difco Laboratories, Detroit, MI, USA) for one day, colonies of each of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains form opaque white colonies with irregular edges. Cells of each strain (Gram positive rods of 1.7-2.4 x 5.6-7.7 µm) sporulate after 48 hrs cultivation at 28°C on T₃ agar. The crystal proteins produced during sporulation are packaged in crystals of the BTS02617A, BTS02618A, BTS02654B, and BTS02652E strains.

Quite remarkably, the crystal remains attached to the spore after sporulation.

The Bt serotype of the BTS02617A, BTS02618A, BTS02645B and BTS02652E strains was determined to be serotype tolworthi H9 of all these strains which was determined by conventional serotyping methods as conducted by the WHO Collaborating Center for Entomopathogenic Bacillus.

Example 2 : Insecticidal activity of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains and the BTS02618A protoxin against Noctuidae spp., Yponomeutidae spp. and Pyralidae spp.

Toxicity assays were performed on neonate larvae (for Plutella xylostella, third instar larvae were used) fed on an artificial diet layered with spore-crystal mixtures from one of the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains or the BTS02618A protoxin or toxin. The artificial diet was dispensed in wells of Costar 24-well plates. Formaldehyde was omitted from the diet. 50 μ l of a sample dilution was applied on the surface of the diet and dried in a laminar air flow. For LC₅₀ assays, the dilutions were made in a PBS-BSA buffer, and five dilutions were applied. Two larvae were placed in each well and 24 larvae were used per sample dilution. Dead and living M. brassica, S. frugiperda, H. virescens, O. nubilalis, Plutella xylostella and S. exigua larvae were counted on the fifth day, and dead and living A. ipsilon and S. littoralis larvae were counted on the sixth day. The LC₅₀ and LC₉₅ values (the concentrations required to kill respectively 50% or 95% of the insects tested, expressed in number of spore-crystals/cm² or ng (pro)toxin/cm²) were calculated using Probit-analysis (Finney, 1971), and the results are set forth below.

Spodoptera littoralis

Experiment/Strain	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
Experiment 1				
BTS02618A	2.4	7.7	1.5-3.4	3.2
HD127 ^c	2.5	168	1.2-7.4	1.0
Experiment 2				
BTS02618A	1.1	4	0.8-1.6	3.0
HD127	21.2	133.7	14.4-31.9	2.0

^a 10⁵ spore-crystals per cm²

^b 95 % fiducial limits of LC₅₀ values

^c from the Howard Dulmage collection, housed at the Northern Region Research Center, 1815 North University, Peoria, Ill, USA. The curator is Dr. L.Nakamura.

Experiments with purified BTS02618A protoxin also show a significant toxicity of this protoxin against S. littoralis larvae.

Spodoptera exiqua

1. Crystal/spore mixtures

Experiment/Strain	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
Experiment 1				
BTS02618A	1.4	7.9	0.48-3.9	2.2
HD127	8.2	163.5	5.1-15.7	1.3
Experiment 2				
BTS02618A	1.2	3.56	0.91-1.57	3.5
BTS02617A	0.79	2.12	0.61-1.03	3.81
HD127	3.5	44.2	1.36-11.5*	1.5
Florbac	4.1	53.9	1.5-17.0*	1.47
BTS00170U ^c	5.1	46.5	1.83-24.4*	1.71
Experiment 3				
Javelin ^d	23.12	195.7	14.6-56.7	1.77
Experiment 4				
BTS02618A	1.07	2.91	0.83-1.39	3.8
BTS02617A	0.87	4.7	0.59-1.21	2.22
HD127	4.7	56.9	1.85-18.7*	1.52
Florbac ^e	2.53	48.1	0.79-6.71*	1.29
BTS00170U	1.94	56.3	0.55-5.4*	1.12

^a 10⁵ spore-crystals per cm²^b 95 % fiducial limits of LC₅₀ values, values marked with * are
90 % fiducial limits of LC₅₀ values^c PCT patent publication WO 90/06999^d strain isolated from Javelin® (Sandoz, Lichtstrasse, Basel,
Switzerland)^e strain from Florbac® (Novo Nordisk, Novo Allè, Bagsværd,
Denmark)

2. Toxin/protoxin assays.

ICP		LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
BTS02618A	Protoxin	26.6	100.6	20.9-33.9	2.8
CryIC	Toxin	68.9	313.2	50.5-94.1	2.5
CryID	Toxin	118.6	870.6	82.7-170.0	1.9

^a ng/cm²^b 95 % fiducial limits of LC₅₀ values10 Mamestra brassica

1. Crystal/spore mixtures.

Experiment/Strain	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
HD127	37.8	297.6	17.8-91.1	1.8
BTS02618A	8.6	59.6	6.0-12.2	1.9
BTS02617A	5.2	25.8	3.7-7.1	2.4
BTS02652E	12.9	44.2	9.7-17.2	3.0
BTS02654B	14.2	60.5	10.8-19.9	2.6

20 ^a 10⁵ spore-crystals per cm²^b 95 % fiducial limits of LC₅₀ values

2. Protoxin assays.

ICP		LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
BTS02618A	Protoxin	25.3	125.1	19.3-33.2	2.4
CryIC	Protoxin	22.0	62.9	16.3-29.6	3.6
CryIA(b)	Protoxin	162.4	7169	93.2-283.1	1.0

^a ng/cm²30 ^b 95 % fiducial limits of LC₅₀ values

Agrotis ipsilon

1. Crystal/spore mixtures.

5	<u>Strain</u>	<u>mortality^a</u>	<u>genes^b</u>
	Btgall. ^c	1/20	<u>cryIF</u> , <u>cryIG</u> , <u>cryII</u> , <u>81k</u>
	HD127 ^d	2/20	<u>cryIAa</u> , <u>cryIAb</u> , <u>cryIC</u> , <u>cryID</u> , <u>cryII</u> , <u>81k</u>
	BTS02618A	16/20 ^e	<u>cryIAb</u> , <u>cryII</u> , <u>bTS02618A</u>
10	Buffer	1/20	none

^a number of 1st instar larvae killed after 6 days (10^7 spore-crystals per cm²)

^b genes known to be present in these strains

15 ^c Btgall. as described by Smulevitch et al (1991)

^d HD127 is available at the Howard Dulmage Collection (NRRC, see above)

^e surviving larvae show severe growth-inhibition

20	STRAIN	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
	BTS02618A	84.4	207.9	65.9-109.6	4.2
	HD127	>250			
	BTS02617A	53.4	261.0	27.7-112.3	2.4

25 ^a 10^6 spores/cm²

^b 95 % fiducial limits of LC₅₀ values

2. Toxin/protoxin assay.

30	ICP		LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
	CryIAC	Toxin	>1350			
	BTS02618A	Protoxin	212.2	1973	168.1-267.9	1.7

^a ng/cm²

^b 95 % fiducial limits of LC₅₀ values

Since MacIntosh et al. (1990) described some activity of the CryIAC toxin towards A. ipsilon, purified CryIAC toxin was tested on this insect for comparison but did not cause any significant mortality of A. ipsilon.

5

Heliothis virescens

1. Crystal/spore mixture.

10

Experiment/Strain	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
BTS02617A	1.69	14.99	0.67-2.89	1.73
BTS02618A	2.71	25.4	0.88-6.99	1.69
BTS00170U ^c	15.1	398.7	8.3-41.2	1.15
Dipel ^d	2.99	14.11	1.25-7.76	2.45

15

^a 10³ spore-crystals per cm²

^b 95% fiducial limits of LC₅₀ values

^c PCT patent publication WO 90/06999

^d strain isolated from DipelTM (Abbott Laboratories, North Chicago, Ill., USA)

20

2. Toxin/protoxin assay.

25

ICP		LC ₅₀ ^a	FL _{min-max} ^b	LC ₉₅ ^a	Slope
BTS02618A	Protoxin	31.6	20-50	182.7	2.1
CryIAb	Toxin	7.2	4.9-10.5	169.1	1.2

^a ng/cm²

^b 95 % fiducial limits of LC₅₀ values

Ostrinia nubilalis

1. Crystal/spore mixtures.

Experiment/Strain	LC ₅₀ ^a	LC ₉₅ ^a	FL _{min-max} ^b	Slope
BTS02617A	4.92	12.49	2.45-6.81	4.0
BTS02618A	6.17	39.7	2.93-9.74	2.0
Dipel ^c	>30			

^a 10⁵ spore-crystals per cm²^b 95% fiducial limits of LC₅₀ values^c strain isolated from DipelTM (Abbott Laboratories)

2. Purified protoxin assay

ICP		100 % Mortality ^a
CryIAb	Toxin	1350
CryIB	Toxin	1350
BTS02618A	Protoxin	100

^a concentration at which 100 % mortality was observed (in ng/cm²)

The purified BTS02618A protoxin also showed a significant toxicity to Ostrinia nubilalis larvae, as compared with the CryI toxins that are most active against Ostrinia.

Plutella xylostella

Plutella xylostella larvae also showed significant mortality after application of purified BTS02618A toxin to their artificial diet in several experiments.

Spodoptera frugiperda

Crystal/spore mixtures of a bTS02618A gene-transformed crystal-minus Bt strain (Mahillon et al., 1989) were also found to significantly inhibit larval growth of S. frugiperda larvae in insect feeding trials.

In conclusion, the strains of this invention and the BTS02618A protein of this invention have a strong insecticidal activity against a broad range of insects that are not susceptible to any single currently available Bt protein and have an activity against at least three Spodoptera spp. and against other Noctuidae, such as A. ipsilon, M. brassica and H. virescens, as well as against Pyralidae, such as O. nubilalis and Yponomeutidae such as Plutella xylostella. These results are summarized and compared with results for other CryI genes (Van Frankenhuyzen, 1993) in Table 1 which shows the unique range of insects susceptible to the BTS02618A protein.

Example 3 : Identification of the bTS02618A gene

The bTS02618A gene was identified in the BTS02618A strain by Southern blot analysis (Fig. 1) of AluI digested total DNA of the strain using, as a DNA probe, the DNA sequence of the cryIG gene (Gleave et al., 1992) of SEQ ID No. 1 and using standard hybridization conditions. Partial DNA sequences of the bTS02618A gene, showing its 5' and 3' end portions, are shown in SEQ ID Nos. 2 and 3, respectively, and the full DNA sequence of the bTS02618A gene and the full amino acid sequence of the BTS02618A protein are shown in SEQ ID No. 4.

The partial sequences of SEQ ID Nos. 2 and 3 allow the bTS02618A gene to be recognized in the BTS02617A, BTS02654B and BTS02652E strains and allow the construction of probes to identify and isolate the full gene sequence in these and other Bt strains. The translation initiation codon of the bTS02618A gene is identified at nucleotide position 195-197 in SEQ ID No. 2, corresponding to nucleotide position 668-670 in SEQ ID No. 4. The translation stop codon is identified at nucleotide position 1146-1148 in SEQ ID No. 3, corresponding to nucleotide position

4139-4141 in SEQ ID No. 4.

The bTS02618A gene was also identified in the BTS02617A, BTS02654B and BTS02652E strains by using the DNA sequence of SEQ ID No. 1 as a probe, as well as other DNA probes of conserved DNA fragments in cryI genes.

The full length bTS02618A gene was found to encode a 129.9 kD protoxin. A comparison of the amino acid sequence with other known CryI proteins showed that the C-terminal part (C-terminal of conserved sequence block 5) was homologous with CryIG (88%). The best homology for the N-terminal part (the toxin) was found with the CryIB toxin, but this was found to be less than 50% (homology is expressed as the number of perfect matches divided by the number of amino acids of the longest fragment).

The smallest insecticidal protein is believed to be a 69 kD (615 amino acids) protein stretching from amino acid number 44 to amino acid number 658 in SEQ ID No. 4. A smaller tryptic fragment of 55 kD (494 amino acids), stretching from amino acid number 165 to amino acid number 658 in SEQ ID No. 4, still has insecticidal activity towards S. exigua, but this activity is significantly reduced. Thus, a truncated bTS02618A gene or an equivalent truncated gene preferably encodes the 69 kD protein of the BTS02618A protoxin of SEQ ID No.4 as described above.

Example 4 : Cloning and expression of the bTS02618A gene

In order to isolate the bTS02618A gene, total DNA from the BTS02618A strain was prepared and partially digested with Sau3A. The digested DNA was size fractionated on a sucrose gradient and fragments ranging from 7 Kb to 10 Kb were ligated to the BamH1-digested and BAP-treated cloning vector pUC19 (Yannisch-Perron et al., 1985). Recombinant E.coli clones containing the vector were then screened with the cryIG DNA probe of SEQ ID No. 1 which is described in Example 3, to identify clones containing the bTS02618A gene.

The so-identified DNA fragments were then sequenced according to Maxam and Gilbert (1980). Partial sequences of the bTS02618A gene are shown in SEQ ID Nos. 2 and 3, and a full

sequence of the bTS02618A gene and the BTS02618A protein is shown in SEQ ID No. 4. Based on the DNA sequence analysis, the gene is cut with appropriate restriction enzymes to give the truncated bTS02618A gene encoding the BTS02618A toxin.

5 Expression of the gene in E.coli was induced using standard procedures (Sambrook et al., 1989, supra).

The bTS02618A gene is also introduced by routine procedures into a crystal-minus Bt strain, using Bt plasmids PGI2 or PGI3 (Mahillon and Seurinck 1988; Mahillon et al.,

10 1988).

Example 5: Insertion of the bTS02618A gene and the truncated bTS02618A gene in E. coli and insertion of the truncated bTS02618A gene in plants.

15 In order to express the bTS02618A gene and the truncated bTS02618A gene of Example 4 in E. coli and in plants, different gene cassettes are made in E. coli according to the procedure described in EPA 86/300291.1 and EPA 88/402115.5.

To allow significant expression in plants, cassettes

20 containing a) the truncated gene or b) a hybrid gene that is a fusion of i) the truncated gene and ii) the neo gene are each: inserted between the T-DNA border sequences of intermediate plant expression vectors as described in EPA 86/300291.1; fused to transcript formation and polyadenylation

25 signals in the plant expression vectors; placed under the control of the constitutive promoter from cauliflower mosaic virus driving the 35S3 transcript (Hull and Howell, 1987) or the 2' promoter from the TR-DNA of the octopine Ti-plasmid (Velten et al., 1984); and fused to 3' end transcript formation

30 and polyadenylation signals of the octopine synthase gene (Gielen et al., 1984).

Using standard procedures (Deblaere et al., 1985), the intermediate plant expression vectors, containing the truncated bTS02618A gene, are transferred into the Agrobacterium strain

35 C58C1Rif^R (US Patent Application 821,582; EPA 86/300,291.1) carrying the disarmed Ti-plasmid pGV2260 (Vaeck et al., 1987). Selection for spectinomycin resistance yields cointegrated

plasmids, consisting of pGV2260 and the respective intermediate plant expression vectors. Each of these recombinant Agrobacterium strains is then used to transform different cotton plants so that the truncated bTS02618A gene is
5 contained in, and expressed by, different plant cells.

Example 6: Expression of the truncated bTS02618A gene in plants.

The insecticidal activity against Lepidoptera of the
10 expression products of the truncated bTS02618A gene in leaves of transformed plants, generated from the transformed plant cells of Example 5, is evaluated by recording the growth rate and mortality of Agrotis and Spodoptera spp. larvae fed on these leaves. These results are compared with the growth rate
15 of larvae fed leaves from untransformed plants. Toxicity assays against Agrotis and Spodoptera spp. are performed as described in EP 0,358,557, U.S. Patent Application 821,582 and EPA 86/300,291.1. A significantly higher mortality rate is obtained among larvae fed on leaves of transformed plants containing the
20 truncated bTS02618A gene and the truncated bTS02618A-neo hybrid gene than among larvae fed the leaves of untransformed plants. The transformed plants are also found to resist Ostrinia nubilalis Mamestra brassica, Heliothis virescens and Plutella xylostella attack by their expression of the BTS02618A protein.

Needless to say, this invention is not limited to the
25 BTS02617A strain (BCCM-LMG P-12592), the BTS02618A strain (BCCM-LMG P-12593), the BTS02654B strain (BCCM-LMG P-12594) and the BTS02652E (BCCM-LMG P-13493) strain. Rather, the invention also includes any mutant or variant of the BTS02617A,
30 BTS02618A, BTS02654B, and BTS02652E strain which produces crystals, crystal proteins, protoxin or toxin having substantially the same properties, particularly anti-Lepidoptera properties, quite particularly anti-Noctuidae, anti-Yponomeutidae and anti-Pyralidae properties, especially
35 anti-Spodoptera, anti-Plutella, anti-Ostrinia anti-Mamestra; anti-Heliothis and anti-Agrotis properties, as the respective BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals or

crystal proteins, or the BTS02618A protoxin or toxin. This invention also includes the bTS02618A gene and any insecticidally effective parts thereof, like the truncated bTS02618A gene. In this regard, the term "bTS02618A gene" as
5 used herein means the gene isolated from the BTS02617A, BTS02618A, BTS02654B or BTS02652E strain and hybridizing to the nucleotide sequence of SEQ ID No. 1 and any equivalent gene encoding a protoxin having substantially the same amino acid sequence and insecticidal activity as the BTS02618A protoxin
10 and preferably containing the partial nucleotide sequences shown in SEQ ID Nos. 2 and 3, or the full sequence shown in SEQ ID No. 4.

This invention also is not limited to cotton plants transformed with the truncated bTS02618A gene. It includes any
15 plant, such as tomato, tobacco, rapeseed, alfalfa, sunflower, lettuce, potato, corn, rice, soybean, Brassica species, sugar beet and other legumes and vegetables, transformed with an insecticidally effective part of the bTS02618A gene or an equivalent gene.

Nor is this invention limited to the use of Agrobacterium tumefaciens Ti-plasmids for transforming plant cells with an insecticidally effective bTS02618A gene part. Other known techniques for plant cell transformations, such as by means of liposomes, by electroporation or by vector systems based on
25 plant viruses or pollen, can be used for transforming monocotyledons and dicotyledons with such a gene part.

Furthermore, DNA sequences other than those present naturally in the BTS02617A, BTS02618A, BTS02654B and BTS02652E strains and encoding the BTS02618A protoxin and toxin can be
30 used for transforming plants and bacteria. In this regard, the natural DNA sequence of these genes can be modified by: 1) replacing some codons with others that code either for the same or different, preferably the same, amino acids; 2) deleting or adding some codons; and/or 3) reciprocal recombination as
35 described by Ge et al. (1991); provided that such modifications do not substantially alter the properties, particularly the insecticidal properties, especially anti-lepidoptera

properties, of the encoded, insecticidally effective portions of the BTS02618A protoxin (e.g., toxin). For example, an artificial bTS02618A gene or gene part of this invention, as described above, having a modified codon usage, could be used
5 in certain circumstances instead of a natural insecticidally effective bTS02618A gene part in a bTS02618A chimeric gene of this invention for transforming plants.

Also, other DNA recombinants containing all or part of the bTS02618A gene in association with other foreign DNA,
10 particularly the DNA of vectors suitable for transforming plants and microorganisms other than E. coli, are encompassed by this invention. In this regard, this invention is not limited to the specific plasmids containing the bTS02618A gene, or parts thereof, that were heretofore described, but rather,
15 this invention encompasses any DNA recombinants containing DNA sequences that are their equivalent. Further, the invention relates to all DNA recombinants that include all or part of the bTS02618A gene and that are suitable for transforming microorganisms (e.g., plant associated bacteria such as other
20 Bacillus thuringiensis strains, Bacillus subtilis, Pseudomonas, and Xanthomonas or yeasts such as Streptomyces cerevisiae) under conditions which enable all or part of the gene to be expressed and to be recoverable from said microorganisms or to be transferred to a plant cell.

Table 1.

Activity of CryI proteins towards several lepidopteran insect pests: + and - indicates the presence or absence of insecticidal activity, +/- indicates low activity (according to Van Frankenhuyzen (1993)), NA indicates no data available, the protein BTS02618A is abbreviated as 2618A (data of Van Frankenhuyzen (1993) and this invention (for A. ipsilon and 2618A)).

	2618A	IAb	IAC	IB	IC	IF
<u>S.exigua</u>	+	+/-	-	-	+	+
<u>S.littoralis</u>	+	-	-	-	+	NA
<u>H.virescens</u>	+	+	+	-	+/-	+
<u>A.ipsilon</u>	+	NA	-	NA	NA	NA
<u>O.nubilalis</u>	+	+	+	NA	NA	+
<u>P.xylostella</u>	+	+	+	+	+	NA
<u>M.brassica</u>	+	+	-	-	+	NA

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) NAME: PLANT GENETIC SYSTEMS N.V.
 - (B) STREET: Plateaustraat 22
 - (C) CITY: Gent
 - (E) COUNTRY: Belgium
 - (F) POSTAL CODE (ZIP): 9000
 - (G) TELEPHONE: 32 9 2358454
 - (H) TELEFAX: 32 9 224 06 94
 - (I) TELEX: 11.361 Pgsgen
- (ii) TITLE OF INVENTION: New Bacillus thuringiensis strains and their insecticidal proteins
- (iii) NUMBER OF SEQUENCES: 4
- (iv) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25(EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A)SEQUENCE LENGTH : 19 nucleotides
 - (B)SEQUENCE TYPE : nucleic acid probe
 - (C)STRANDEDNESS : single stranded
 - (D)TOPOLOGY : linear
- (ii) MOLECULE TYPE: synthetic DNA
- (ix) FEATURES: the probe is a part of the coding DNA strand of the cryIG gene, described by Smulevitch et al. (1991).
- (x) PROPERTIES : this probe is used to isolate the bts02618A gene from its containing strain.
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5'-TTCTGTACTATTGATTGTA-3'

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1561 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacillus thuringiensis
- (B) STRAIN: BTS02618A

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (D) OTHER INFORMATION: /function= "contains the translation initiation codon of the bTS02618A gene"

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

AAAAAGAAAT AGGAATAAAT ACTATCCATT TTTTCAAGAA ATATTTTTTTT ATTAGAAAGG	60
AATCTTTCTT ACACGGGAAA ATCCTAAGAT TGAGAGTAAA GATATATATA TATAAATACA	120
ATAAAGAGTT TGTCAGGATT TTTGAAAGAT ATGATATGAA CATGCACTAG ATTTATAGTA	180
TAGGAGGAAA AAGTATGAAT CGAAATAATC AAAATGAATA TGAAATTATT GATGCCCCC	240
ATTGTGGGTG TCCATCAGAT GACGATGTGA GGTATCCTTT GGCAAGTGAC CCAATGCGAG	300
CGTTACAAAA TATGAACTAT AAAGATTACT TACAAATGAC AGATGAGGAC TACACTGATT	360
CTTATATAAA TCCTAGTTTA TCTATTAGTG GTAGAGATGC AGTTCAGACT GCGCTTACTG	420
TTGTTGGGAG AATACTCGGG GCTTTAGGTG TTCCGTTTTC TGGACAAATA GTGAGTTTTT	480
ATCAATTCCT TTAAATACA CTGTGGCCAG TTAATGATAC AGCTATATGG GAAGCTTTCA	540
TGCGACAGGT GGAGGAACCT GTCAATCAAC AAATAACAGA ATTTGCAAGA AATCAGGCAC	600
TTGCAAGATT GCAAGGATTA GGAGACTCTT TTAATGTATA TCAACGTTCC CTTCAAAATT	660
GGTTGGCTGA TCGAAATGAT ACACGAAATT TAAGTGTGTG TCGTGCTCAA TTTATAGCTT	720
TAGACCTTGA TTTTGTTAAT GCTATTCCAT TGTTTGCACT AAATGGACAG CAGGTTCAT	780
TACTGTCAGT ATATGCACAA GCTGTGAATT TACATTTGTT ATTATTAAAA GATGCATCTC	840
TTTTTGGAGA AGGATGGGGA TTCACACAGG GGGAAATTTC CACATATTAT GACCGTCAAT	900
TGGAACCTAAC CGCTAAGTAC ACTAATTACT GTGAACTTG GTATAATACA GGTTTAGATC	960
GTTTAAGAGG AACAAATACT GAAAGTTGGT TAAGATATCA TCAATTCCGT AGAGAAATGA	1020
CTTTAGTGGT ATTAGATGTT GTGGCGCTAT TTCCATATTA TGATGTACGA CTTTATCCAA	1080
CGGGATCAAA CCCACAGCTT ACACGTGAGG TATATACAGA TCCGATTGTA TTTAATCCAC	1140
CAGCTAATGT TGGACTTTGC CGACGTTGGG GTACTAATCC CTATAATACT TTTTCTGAGC	1200
TCGAAATGCT CTTCAATTCG CCACCACATC TTTTGTATAG GCTGAATAGC TTAACAATCA	1260

GCAGTAATCG	ATTTCCAGTT	TCATCTAATT	TTATGGATTA	TTGGTCAGGA	CATACGTTAC	1320
GCCGTAGTTA	TCTGAACGAT	TCAGCAGTAC	AAGAAGATAG	TTATGGCCTA	ATTACAACCA	1380
CAAGAGCAAC	AATTAATCCC	GGAGTTGATG	GAACAAACCG	CATAGAGTCA	ACGGCAGTAG	1440
ATTTTCGTTC	TGCATTGATA	GGTATATATG	GCGTGAATAG	AGCTTCTTTT	GTCCCAGGAG	1500
GCTTGTTTAA	TGGTACGACT	TCTCCTGCTA	ATGGAGGATG	TAGAGATCTC	TATGATACAA	1560
A						1561

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1554 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Bacillus thuringiensis
- (B) STRAIN: BTS02618A

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 1146..1148
- (D) OTHER INFORMATION: /function= "Presumed translational stop codon of bts02618A gene"

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

AAAATTATCC AACATACATT TATCAAAAAG TAGATGCATC GGTGTTAAAG CCTTATACAC	60
GCTATAGACT AGATGGATTT GTGAAGNGTA GTCAAGATTT AGAAATTGAT CTCATCCACC	120
ATCATAAAGT CCATCTTGTA AAAAATGTAC CAGATAATTT AGTATCTGAT ACTTACTCAG	180
ATGGTTCTTG CAGCGGAATC AACCGTTGTG ATGAACAGCA TCAGGTAGAT ATGCAGCTAG	240
ATGCGGAGCA TCATCCAATG GATTGCTGTG AAGCGGCTCA AACACATGAG TTTTCTTCCT	300
ATATTAATAC AGGGGATCTA AATGCAAGTG TAGATCAGGG CATTTGGGTT GTATTAAAAG	360
TTCGAACAAC AGATGGGTAT GCGACGTTAG GAAATCTTGA ATTGGTAGAG GTTGGGCCAT	420
TATCGGGTGA ATCTCTAGAA CGGGAACAAA GAGATAATGC GAAATGGAAT GCAGAGCTAG	480
GAAGAAAACG TGCAGAAATA GATCGTGTGT ATTTAGCTGC GAAACAAGCA ATTAATCATC	540
TGTTTGTAGA CTATCAAGAT CAACAATTAA ATCCAGAAAT TGGGCTAGCA GAAATTAATG	600
AAGCTTCAAA TCTTGTAGAG TCAATTCGG GTGTATATAG TGATACACTA TTACAGATTC	660
CTGGGATTAA CTACGAAATT TACACAGAGT TATCCGATCG CTTACAACAA GCATCGTATC	720
TGTATACGTC TAGAAATGCG GTGCAAAATG GAGACTTTAA CAGTGGTCTA GATAGTTGGA	780
ATACAACTAT GGATGCATCG GTTCAGCAAG ATGGCAATAT GCATTTCTTA GTTCTTTTCGC	840
ATTGGGATGC ACAAGTTTCC CAACAATTGA GAGTAAATCC GAATTGTAAG TATGTCTTAC	900
GTGTGACAGC AAGAAAAGTA GGAGGCGGAG ATGGATACGT CACAATCCGA GATGGCGCTC	960
ATCACCAGA AACTCTTACA TTTAATGCAT GTGACTACGA TGTAATGGT ACGTATGTCA	1020
ATGACAATTC GTATATAACA GAAGAAGTGG TATTCTACCC AGAGACAAAA CATATGTGGG	1080
TAGAGGTGAG TGAATCCGAA GGTTCAATTCT ATATAGACAG TATTGAGTTT ATTGAAACAC	1140
AAGAGTAGAA GAGGGGGATC CTAACGTATA GCAACTATGA GAGGATACTC CGTACAAACA	1200

AAGATTAAAA	AAAGGTAAAA	TGAATAGAAC	CCCCTACTGG	TAGAAGGACC	GATAGGGGGT	1260
TCTTACATGA	AAAAATGTAG	CTGTTTACTA	AGGTGTATAA	AAAACAGCAT	ATCTGATAGA	1320
AAAAAGTGAG	TACCTTATAA	AGAAAGAATT	CCATTCACAG	TTTCGGTATC	ATATAAATAA	1380
TGATAGGGGT	ATCCTTCTTA	TTTACATTAT	TTTTCGCAAT	TATCTCGACG	TTCTTCTTTC	1440
CGCTCACAAT	GATGATGATC	ATGACAACAA	TCGCGTCCAT	AGCGAACTCT	TTCGATATTA	1500
ATAATATCTA	AACTCGTGTA	GCAGTCATTT	CCATTTTTTTT	TGATCCAGTA	AATA	1554

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4344 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 668..4141

(D) OTHER INFORMATION: encompasses the entire sequence of SEQ ID NO 2: from nucleotide position 474 to 2034 in SEQ ID NO 4; also encompasses part of the sequence of SEQ ID NO 3: from nucleotide position 2994 to nucleotide position 4344 in SEQ ID NO 4; SEQ ID NO 3 shows additional nucleotides, located downstream (3') from the sequence shown in SEQ ID NO 4 (nucleotide position 1352 to nucleotide position 1554 in SEQ ID NO 3)

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

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GAATTCGAGC TCGGTACCTT TTCAGTGTAT CGTTTCCCTT CCATCAGGTT TTCAAATTGA      60
AAAGCCGAAT GATTTGAAAC TTGTTTACGA TGTAAGTCAT TTGTCTATGA CGAAAGATAC      120
GTGTAAAAAA CGTATTGAGA TTGATGAATG TGGACAAGTA GAAATTGACT TACAAGTATT      180
AAAGATTAAG GGTGTCCTTT CTTTTATCGG AAATTTCTCT ATTGAACCTA TTCTGTGTGA      240
AAACATGTAT ACAACGGTTG ATAGAGATCC GTCTATTTCC TTAAGTTTCC AAGATACGGT      300
ATATGTGGAC CATATTTTAA AATATAGCGT CCAACAATA CCATATTATG TAATTGATGG      360
TGATCATATT CAAGTACGTG ATTTACAAAT CAACTGATG AAAGAGAATC CGCAATCTGC      420
TCAAGTATCA GGGTTGTTTT GTTTTGTATA TGAGTAAGAA CCGAAGGTTT GTAAAAAAGA      480
AATAGGAATA AATACTATCC ATTTTTTCAA GAAATATTTT TTTATTAGAA AGGAATCTTT      540
CTTACACGGG AAAATCCTAA GATTGAGAGT AAAGATATAT ATATATAAAT ACAATAAAGA      600
GTTTGTGAGG ATTTTGTAAA GATATGATAT GAACATGCAC TAGATTTATA GTATAGGAGG      660
AAAAAGT ATG AAT CGA AAT AAT CAA AAT GAA TAT GAA ATT ATT GAT GCC      709
      Met Asn Arg Asn Asn Gln Asn Glu Tyr Glu Ile Ile Asp Ala
        1              5              10

CCC CAT TGT GGG TGT CCA TCA GAT GAC GAT GTG AGG TAT CCT TTG GCA      757
Pro His Cys Gly Cys Pro Ser Asp Asp Asp Val Arg Tyr Pro Leu Ala
  15              20              25              30

AGT GAC CCA AAT GCA GCG TTA CAA AAT ATG AAC TAT AAA GAT TAC TTA      805
Ser Asp Pro Asn Ala Ala Leu Gln Asn Met Asn Tyr Lys Asp Tyr Leu
      35              40              45

CAA ATG ACA GAT GAG GAC TAC ACT GAT TCT TAT ATA AAT CCT AGT TTA      853
Gln Met Thr Asp Glu Asp Tyr Thr Asp Ser Tyr Ile Asn Pro Ser Leu
      50              55              60

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TCT	ATT	AGT	GGT	AGA	GAT	GCA	GTT	CAG	ACT	GCG	CTT	ACT	GTT	GTT	GGG	901
Ser	Ile	Ser	Gly	Arg	Asp	Ala	Val	Gln	Thr	Ala	Leu	Thr	Val	Val	Gly	
		65					70					75				
AGA	ATA	CTC	GGG	GCT	TTA	GGT	GTT	CCG	TTT	TCT	GGA	CAA	ATA	GTG	AGT	949
Arg	Ile	Leu	Gly	Ala	Leu	Gly	Val	Pro	Phe	Ser	Gly	Gln	Ile	Val	Ser	
	80					85					90					
TTT	TAT	CAA	TTC	CTT	TTA	AAT	ACA	CTG	TGG	CCA	GTT	AAT	GAT	ACA	GCT	997
Phe	Tyr	Gln	Phe	Leu	Leu	Asn	Thr	Leu	Trp	Pro	Val	Asn	Asp	Thr	Ala	
95					100					105					110	
ATA	TGG	GAA	GCT	TTC	ATG	CGA	CAG	GTG	GAG	GAA	CTT	GTC	AAT	CAA	CAA	1045
Ile	Trp	Glu	Ala	Phe	Met	Arg	Gln	Val	Glu	Glu	Leu	Val	Asn	Gln	Gln	
			115					120						125		
ATA	ACA	GAA	TTT	GCA	AGA	AAT	CAG	GCA	CTT	GCA	AGA	TTG	CAA	GGA	TTA	1093
Ile	Thr	Glu	Phe	Ala	Arg	Asn	Gln	Ala	Leu	Ala	Arg	Leu	Gln	Gly	Leu	
			130					135					140			
GGA	GAC	TCT	TTT	AAT	GTA	TAT	CAA	CGT	TCC	CTT	CAA	AAT	TGG	TTG	GCT	1141
Gly	Asp	Ser	Phe	Asn	Val	Tyr	Gln	Arg	Ser	Leu	Gln	Asn	Trp	Leu	Ala	
	145						150					155				
GAT	CGA	AAT	GAT	ACA	CGA	AAT	TTA	AGT	GTT	GTT	CGT	GCT	CAA	TTT	ATA	1189
Asp	Arg	Asn	Asp	Thr	Arg	Asn	Leu	Ser	Val	Val	Arg	Ala	Gln	Phe	Ile	
	160					165					170					
GCT	TTA	GAC	CTT	GAT	TTT	GTT	AAT	GCT	ATT	CCA	TTG	TTT	GCA	GTA	AAT	1237
Ala	Leu	Asp	Leu	Asp	Phe	Val	Asn	Ala	Ile	Pro	Leu	Phe	Ala	Val	Asn	
175					180					185					190	
GGA	CAG	CAG	GTT	CCA	TTA	CTG	TCA	GTA	TAT	GCA	CAA	GCT	GTG	AAT	TTA	1285
Gly	Gln	Gln	Val	Pro	Leu	Leu	Ser	Val	Tyr	Ala	Gln	Ala	Val	Asn	Leu	
				195					200					205		
CAT	TTG	TTA	TTA	TTA	AAA	GAT	GCA	TCT	CTT	TTT	GGA	GAA	GGA	TGG	GGA	1333
His	Leu	Leu	Leu	Leu	Lys	Asp	Ala	Ser	Leu	Phe	Gly	Glu	Gly	Trp	Gly	
			210					215					220			
TTC	ACA	CAG	GGG	GAA	ATT	TCC	ACA	TAT	TAT	GAC	CGT	CAA	TTG	GAA	CTA	1381
Phe	Thr	Gln	Gly	Glu	Ile	Ser	Thr	Tyr	Tyr	Asp	Arg	Gln	Leu	Glu	Leu	
		225					230					235				
ACC	GCT	AAG	TAC	ACT	AAT	TAC	TGT	GAA	ACT	TGG	TAT	AAT	ACA	GGT	TTA	1429
Thr	Ala	Lys	Tyr	Thr	Asn	Tyr	Cys	Glu	Thr	Trp	Tyr	Asn	Thr	Gly	Leu	
	240					245					250					
GAT	CGT	TTA	AGA	GGA	ACA	AAT	ACT	GAA	AGT	TGG	TTA	AGA	TAT	CAT	CAA	1477
Asp	Arg	Leu	Arg	Gly	Thr	Asn	Thr	Glu	Ser	Trp	Leu	Arg	Tyr	His	Gln	
255					260					265					270	
TTC	CGT	AGA	GAA	ATG	ACT	TTA	GTG	GTA	TTA	GAT	GTT	GTG	GCG	CTA	TTT	1525
Phe	Arg	Arg	Glu	Met	Thr	Leu	Val	Val	Leu	Asp	Val	Val	Ala	Leu	Phe	
				275					280					285		
CCA	TAT	TAT	GAT	GTA	CGA	CTT	TAT	CCA	ACG	GGA	TCA	AAC	CCA	CAG	CTT	1573
Pro	Tyr	Tyr	Asp	Val	Arg	Leu	Tyr	Pro	Thr	Gly	Ser	Asn	Pro	Gln	Leu	
			290					295					300			

41

ACA CGT GAG GTA TAT ACA GAT CCG ATT GTA TTT AAT CCA CCA GCT AAT	1621
Thr Arg Glu Val Tyr Thr Asp Pro Ile Val Phe Asn Pro Pro Ala Asn	
305 310 315	
GTT GGA CTT TGC CGA CGT TGG GGT ACT AAT CCC TAT AAT ACT TTT TCT	1669
Val Gly Leu Cys Arg Arg Trp Gly Thr Asn Pro Tyr Asn Thr Phe Ser	
320 325 330	
GAG CTC GAA AAT GCC TTC ATT CGC CCA CCA CAT CTT TTT GAT AGG CTG	1717
Glu Leu Glu Asn Ala Phe Ile Arg Pro Pro His Leu Phe Asp Arg Leu	
335 340 345 350	
AAT AGC TTA ACA ATC AGC AGT AAT CGA TTT CCA GTT TCA TCT AAT TTT	1765
Asn Ser Leu Thr Ile Ser Ser Asn Arg Phe Pro Val Ser Ser Asn Phe	
355 360 365	
ATG GAT TAT TGG TCA GGA CAT ACG TTA CGC CGT AGT TAT CTG AAC GAT	1813
Met Asp Tyr Trp Ser Gly His Thr Leu Arg Arg Ser Tyr Leu Asn Asp	
370 375 380	
TCA GCA GTA CAA GAA GAT AGT TAT GGC CTA ATT ACA ACC ACA AGA GCA	1861
Ser Ala Val Gln Glu Asp Ser Tyr Gly Leu Ile Thr Thr Thr Arg Ala	
385 390 395	
ACA ATT AAT CCC GGA GTT GAT GGA ACA AAC CGC ATA GAG TCA ACG GCA	1909
Thr Ile Asn Pro Gly Val Asp Gly Thr Asn Arg Ile Glu Ser Thr Ala	
400 405 410	
GTA GAT TTT CGT TCT GCA TTG ATA GGT ATA TAT GGC GTG AAT AGA GCT	1957
Val Asp Phe Arg Ser Ala Leu Ile Gly Ile Tyr Gly Val Asn Arg Ala	
415 420 425 430	
TCT TTT GTC CCA GGA GGC TTG TTT AAT GGT ACG ACT TCT CCT GCT AAT	2005
Ser Phe Val Pro Gly Gly Leu Phe Asn Gly Thr Thr Ser Pro Ala Asn	
435 440 445	
GGA GGA TGT AGA GAT CTC TAT GAT ACA AAT GAT GAA TTA CCA CCA GAT	2053
Gly Gly Cys Arg Asp Leu Tyr Asp Thr Asn Asp Glu Leu Pro Pro Asp	
450 455 460	
GAA AGT ACC GGA AGT TCA ACC CAT AGA CTA TCT CAT GTT ACC TTT TTT	2101
Glu Ser Thr Gly Ser Ser Thr His Arg Leu Ser His Val Thr Phe Phe	
465 470 475	
AGC TTT CAA ACT AAT CAG GCT GGA TCT ATA GCT AAT GCA GGA AGT GTA	2149
Ser Phe Gln Thr Asn Gln Ala Gly Ser Ile Ala Asn Ala Gly Ser Val	
480 485 490	
CCT ACT TAT GTT TGG ACC CGT CGT GAT GTG GAC CTT AAT AAT ACG ATT	2197
Pro Thr Tyr Val Trp Thr Arg Arg Asp Val Asp Leu Asn Asn Thr Ile	
495 500 505 510	
ACC CCA AAT AGA ATT ACA CAA TTA CCA TTG GTA AAG GCA TCT GCA CCT	2245
Thr Pro Asn Arg Ile Thr Gln Leu Pro Leu Val Lys Ala Ser Ala Pro	
515 520 525	
GTT TCG GGT ACT ACG GTC TTA AAA GGT CCA GGA TTT ACA GGA GGG GGT	2293
Val Ser Gly Thr Thr Val Leu Lys Gly Pro Gly Phe Thr Gly Gly Gly	
530 535 540	

ATA CTC CGA AGA ACA ACT AAT GGC ACA TTT GGA ACG TTA AGA GTA ACG Ile Leu Arg Arg Thr Thr Asn Gly Thr Phe Gly Thr Leu Arg Val Thr 545 550 555	2341
GTT AAT TCA CCA TTA ACA CAA CAA TAT CGC CTA AGA GTT CGT TTT GCC Val Asn Ser Pro Leu Thr Gln Gln Tyr Arg Leu Arg Val Arg Phe Ala 560 565 570	2389
TCA ACA GGA AAT TTC AGT ATA AGG GTA CTC CGT GGA GGG GTT TCT ATC Ser Thr Gly Asn Phe Ser Ile Arg Val Leu Arg Gly Gly Val Ser Ile 575 580 585 590	2437
GGT GAT GTT AGA TTA GGG AGC ACA ATG AAC AGA GGG CAG GAA CTA ACT Gly Asp Val Arg Leu Gly Ser Thr Met Asn Arg Gly Gln Glu Leu Thr 595 600 605	2485
TAC GAA TCC TTT TTC ACA AGA GAG TTT ACT ACT ACT GGT CCG TTC AAT Tyr Glu Ser Phe Phe Thr Arg Glu Phe Thr Thr Thr Gly Pro Phe Asn 610 615 620	2533
CCG CCT TTT ACA TTT ACA CAA GCT CAA GAG ATT CTA ACA GTG AAT GCA Pro Pro Phe Thr Phe Thr Gln Ala Gln Glu Ile Leu Thr Val Asn Ala 625 630 635	2581
GAA GGT GTT AGC ACC GGT GGT GAA TAT TAT ATA GAT AGA ATT GAA ATT Glu Gly Val Ser Thr Gly Gly Glu Tyr Tyr Ile Asp Arg Ile Glu Ile 640 645 650	2629
GTC CCT GTG AAT CCG GCA CGA GAA GCG GAA GAG GAT TTA GAA GCG GCG Val Pro Val Asn Pro Ala Arg Glu Ala Glu Glu Asp Leu Glu Ala Ala 655 660 665 670	2677
AAG AAA GCG GTG GCG AGC TTG TTT ACA CGT ACA AGG GAC GGA TTA CAG Lys Lys Ala Val Ala Ser Leu Phe Thr Arg Thr Arg Asp Gly Leu Gln 675 680 685	2725
GTA AAT GTG ACA GAT TAT CAA GTG GAC CAA GCG GCA AAT TTA GTG TCA Val Asn Val Thr Asp Tyr Gln Val Asp Gln Ala Ala Asn Leu Val Ser 690 695 700	2773
TGC TTA TCC GAT GAA CAA TAT GGG CAT GAC AAA AAG ATG TTA TTG GAA Cys Leu Ser Asp Glu Gln Tyr Gly His Asp Lys Lys Met Leu Leu Glu 705 710 715	2821
GCG GTA AGA GCG GCA AAA CGC CTC AGC CGC GAA CGC AAC TTA CTT CAA Ala Val Arg Ala Ala Lys Arg Leu Ser Arg Glu Arg Asn Leu Leu Gln 720 725 730	2869
GAT CCA GAT TTT AAT ACA ATC AAT AGT ACA GAA GAG AAT GGC TGG AAG Asp Pro Asp Phe Asn Thr Ile Asn Ser Thr Glu Glu Asn Gly Trp Lys 735 740 745 750	2917
GCA AGT AAC GGT GTT ACT ATT AGC GAG GGC GGT CCA TTC TTT AAA GGT Ala Ser Asn Gly Val Thr Ile Ser Glu Gly Gly Pro Phe Phe Lys Gly 755 760 765	2965
CGT GCA CTT CAG TTA GCA AGC GCA AGA GAA AAT TAT CCA ACA TAC ATT Arg Ala Leu Gln Leu Ala Ser Ala Arg Glu Asn Tyr Pro Thr Tyr Ile 770 775 780	3013

TAT CAA AAA GTA GAT GCA TCG GTG TTA AAG CCT TAT ACA CGC TAT AGA	3061
Tyr Gln Lys Val Asp Ala Ser Val Leu Lys Pro Tyr Thr Arg Tyr Arg	
785 790 795	
CTA GAT GGA TTT GTG AAG AGT AGT CAA GAT TTA GAA ATT GAT CTC ATC	3109
Leu Asp Gly Phe Val Lys Ser Ser Gln Asp Leu Glu Ile Asp Leu Ile	
800 805 810	
CAC CAT CAT AAA GTC CAT CTT GTA AAA AAT GTA CCA GAT AAT TTA GTA	3157
His His His Lys Val His Leu Val Lys Asn Val Pro Asp Asn Leu Val	
815 820 825 830	
TCT GAT ACT TAC TCA GAT GGT TCT TGC AGC GGA ATC AAC CGT TGT GAT	3205
Ser Asp Thr Tyr Ser Asp Gly Ser Cys Ser Gly Ile Asn Arg Cys Asp	
835 840 845	
GAA CAG CAT CAG GTA GAT ATG CAG CTA GAT GCG GAG CAT CAT CCA ATG	3253
Glu Gln His Gln Val Asp Met Gln Leu Asp Ala Glu His His Pro Met	
850 855 860	
GAT TGC TGT GAA GCG GCT CAA ACA CAT GAG TTT TCT TCC TAT ATT AAT	3301
Asp Cys Cys Glu Ala Ala Gln Thr His Glu Phe Ser Ser Tyr Ile Asn	
865 870 875	
ACA GGG GAT CTA AAT GCA AGT GTA GAT CAG GGC ATT TGG GTT GTA TTA	3349
Thr Gly Asp Leu Asn Ala Ser Val Asp Gln Gly Ile Trp Val Val Leu	
880 885 890	
AAA GTT CGA ACA ACA GAT GGG TAT GCG ACG TTA GGA AAT CTT GAA TTG	3397
Lys Val Arg Thr Thr Asp Gly Tyr Ala Thr Leu Gly Asn Leu Glu Leu	
895 900 905 910	
GTA GAG GTT GGG CCA TTA TCG GGT GAA TCT CTA GAA CGG GAA CAA AGA	3445
Val Glu Val Gly Pro Leu Ser Gly Glu Ser Leu Glu Arg Glu Gln Arg	
915 920 925	
GAT AAT GCG AAA TGG AAT GCA GAG CTA GGA AGA AAA CGT GCA GAA ATA	3493
Asp Asn Ala Lys Trp Asn Ala Glu Leu Gly Arg Lys Arg Ala Glu Ile	
930 935 940	
GAT CGT GTG TAT TTA GCT GCG AAA CAA GCA ATT AAT CAT CTG TTT GTA	3541
Asp Arg Val Tyr Leu Ala Ala Lys Gln Ala Ile Asn His Leu Phe Val	
945 950 955	
GAC TAT CAA GAT CAA CAA TTA AAT CCA GAA ATT GGG CTA GCA GAA ATT	3589
Asp Tyr Gln Asp Gln Gln Leu Asn Pro Glu Ile Gly Leu Ala Glu Ile	
960 965 970	
AAT GAA GCT TCA AAT CTT GTA GAG TCA ATT TCG GGT GTA TAT AGT GAT	3637
Asn Glu Ala Ser Asn Leu Val Glu Ser Ile Ser Gly Val Tyr Ser Asp	
975 980 985 990	
ACA CTA TTA CAG ATT CCT GGG ATT AAC TAC GAA ATT TAC ACA GAG TTA	3685
Thr Leu Leu Gln Ile Pro Gly Ile Asn Tyr Glu Ile Tyr Thr Glu Leu	
995 1000 1005	
TCC GAT CGC TTA CAA CAA GCA TCG TAT CTG TAT ACG TCT AGA AAT GCG	3733
Ser Asp Arg Leu Gln Gln Ala Ser Tyr Leu Tyr Thr Ser Arg Asn Ala	
1010 1015 1020	

44

GTG CAA AAT GGA GAC TTT AAC AGT GGT CTA GAT AGT TGG AAT ACA ACT	3781
Val Gln Asn Gly Asp Phe Asn Ser Gly Leu Asp Ser Trp Asn Thr Thr	
1025 1030 1035	
ATG GAT GCA TCG GTT CAG CAA GAT GGC AAT ATG CAT TTC TTA GTT CTT	3829
Met Asp Ala Ser Val Gln Gln Asp Gly Asn Met His Phe Leu Val Leu	
1040 1045 1050	
TCG CAT TGG GAT GCA CAA GTT TCC CAA CAA TTG AGA GTA AAT CCG AAT	3877
Ser His Trp Asp Ala Gln Val Ser Gln Gln Leu Arg Val Asn Pro Asn	
1055 1060 1065 1070	
TGT AAG TAT GTC TTA CGT GTG ACA GCA AGA AAA GTA GGA GGC GGA GAT	3925
Cys Lys Tyr Val Leu Arg Val Thr Ala Arg Lys Val Gly Gly Asp	
1075 1080 1085	
GGA TAC GTC ACA ATC CGA GAT GGC GCT CAT CAC CAA GAA ACT CTT ACA	3973
Gly Tyr Val Thr Ile Arg Asp Gly Ala His His Gln Glu Thr Leu Thr	
1090 1095 1100	
TTT AAT GCA TGT GAC TAC GAT GTA AAT GGT ACG TAT GTC AAT GAC AAT	4021
Phe Asn Ala Cys Asp Tyr Asp Val Asn Gly Thr Tyr Val Asn Asp Asn	
1105 1110 1115	
TCG TAT ATA ACA GAA GAA GTG GTA TTC TAC CCA GAG ACA AAA CAT ATG	4069
Ser Tyr Ile Thr Glu Glu Val Val Phe Tyr Pro Glu Thr Lys His Met	
1120 1125 1130	
TGG GTA GAG GTG AGT GAA TCC GAA GGT TCA TTC TAT ATA GAC AGT ATT	4117
Trp Val Glu Val Ser Glu Ser Glu Gly Ser Phe Tyr Ile Asp Ser Ile	
1135 1140 1145 1150	
GAG TTT ATT GAA ACA CAA GAG TAGAAGAGGG GGATCCTAAC GTATAGCAAC	4168
Glu Phe Ile Glu Thr Gln Glu	
1155	
TATGAGAGGA TACTCCGTAC AAACAAAGAT TAAAAAAGG TAAAATGAAT AGAACCCCT	4228
ACTGGTAGAA GGACCGATAG GGGGTTCTTA CATGAAAAA TGTAGCTGTT TACTAAGGTG	4288
TATAAAAAAC AGCATATCTG ATAGAAAAA GTGAGTACCT TATAAAGAAA GAATTC	4344

Claims

1. The BTS02617A, BTS02618A, BTS02654B or BTS02652E strain or its equivalent.
- 5 2. The BTS02617A, BTS02618A, BTS02654B or BTS02652E crystals or crystal proteins or their equivalents.
3. The BTS02618A protoxin and insecticidally effective protoxin portions particularly the toxin, or the equivalent thereof.
- 10 4. The bTS02618A gene, the insecticidally effective bTS02618A gene part, the truncated bTS02618A gene, the bTS02618A chimeric gene or an equivalent thereof or a hybrid thereof with a selectable marker gene, such as the neo gene, particularly wherein said bTS02618A gene is derived from the BTS02617A, BTS02618A, BTS02654B or
15 BTS02652E strain and hybridizes to the DNA probe of SEQ ID No. 1, especially wherein said bTS02618A gene contains the nucleotide sequences shown in SEQ ID Nos. 2 and 3, more particularly the bTS02618A gene of SEQ ID
20 No. 4.
- 5 . A DNA sequence encoding the BTS02618A protoxin of SEQ ID No.4, preferably insecticidally effective BTS02618A protoxin portions, particularly the BTS02618A toxin.
6. An insecticidal composition, particularly against
25 Lepidoptera, more particularly against Noctuidae, especially Agrotis spp., Spodoptera spp., Plutella spp., Mamestra spp., Heliothis spp. and Ostrinia spp.; said composition comprising an active ingredient selected from the group consisting of: the BTS02617A, BTS02618A,
30 BTS02654B and BTS02652E strains, crystals and crystal proteins, the BTS02618A protoxin and insecticidally effective BTS02618A protoxin portions, particularly the BTS02618A toxin, and equivalents thereof.
7. A transformed microorganism, particularly B.
35 thuringiensis, characterized by the bTS02618A gene, gene part, truncated gene, chimeric gene or hybrid of claim 4.

8. A transformed plant cell, characterized by the insecticidally effective bTS02618A gene part, the truncated bTS02618A gene, the bTS02618A chimeric gene, or a hybrid thereof with a selectable marker gene, such as the neo gene.
9. A plant or a seed thereof comprising a plurality of the plant cells of claim 8.
10. A plant genome containing, integrated therein, the bTS02618A gene part, truncated gene, chimeric gene or hybrid of claim 8.
11. A plant tissue, the cells of which have the plant genome of claim 10.
12. A process for rendering a plant resistant to Lepidoptera, particularly Noctuidae such as Spodoptera spp. and Agrotis spp., Pyralidae such as O. nubilalis, and Yponomeutidae such as Plutella xylostella, characterized by: providing the plant with the plant genome of claim 10.
13. A process for producing plants and reproduction material, such as seeds, of said plants including a heterologous genetic material stably integrated in the genome thereof and capable of being expressed therein in the form of a protein toxic to insects, comprising the non-biological steps of: a) producing transformed plant cells or plant tissue including said heterologous genetic material from starting plant cells or plant tissue not expressing said protein, b) producing regenerated plants or reproduction material of said plants or both from said transformed plant cells or plant tissue including said heterologous genetic material, and c) optionally, biologically replicating said regenerated plants or reproduction material or both; wherein said step of producing said transformed plant cells or plant tissue including said heterologous genetic material is characterized by transforming said starting plant cells or plant tissue with the bTS02618A gene part, truncated gene or hybrid of claim 8 as well

as regulatory elements which are capable of enabling the expression of the gene part, the truncated gene or the hybrid in said plant cells or plant tissue, to cause the stable integration of the gene part, the truncated gene or the hybrid in transformed plant cells or plant tissue, as well as in said plants and reproduction material produced therefrom throughout generations.

14. A process for controlling an insect pest, especially Lepidoptera, particularly Noctuidae, Pyralidae and Yponomeutidae, more particularly Spodoptera spp., Mamestra spp., Heliothis spp., Ostrinia spp., Plutella spp. and Agrotis spp., quite particularly Agrotis ipsilon, Spodoptera exigua, Spodoptera littoralis, Spodoptera frugiperda, Mamestra brassica, Heliothis virescens, Ostrinia nubilalis and Plutella xylostella; said process being characterized by the step of contacting the pest with the BTS02618A protoxin or BTS02618A toxin, preferably the insecticidal composition of claim 6.

- 1 / 1 -

Figure 1