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(54) **PLANTES TRANSFORMEES POUR PRODUIRE DES TOXINES INSECTICIDES DE BACILLUS THURINGIENSIS**

(54) **PLANTS TRANSFORMED TO PRODUCE BACILLUS THURINGIENSIS INSECTICIDAL TOXINS**

(57) De nouvelles souches de Bacillus thuringiensis, qui sont enregistrées auprès du DSM sous les numéros de dépôt 5131 et 5132, produisent pendant la sporulation des protéines cristallines ayant une action toxique sur les coléoptères. Ces protéines cristallines contiennent des protoxines de 74 kDa et 129 kDa, respectivement, qui peuvent produire des toxines de 68 et 66 kDa, respectivement, comme produits de digestion de trypsine. Une plante, dont le génome transformé au moyen d'une séquence d'ADN provenant de l'une des deux souches et codant pour sa toxine respective, résiste aux coléoptères. Chaque souche, ou ses cristaux, ses protéines cristallines, sa protoxine ou sa toxine, peuvent être utilisés comme ingrédient actif dans une composition insecticide contre les coléoptères.

(57) Two new Bacillus thuringiensis strains; which are deposited at the DSM under accession nos. 5131 and 5132, produce crystal proteins during sporulation that are toxic to Coleoptera. The crystal proteins contain 74 kDa and 129 kDa protoxins, respectively, which can yield 68 and 66 kDa toxins, respectively, as trypsin-digestion products. A plant, the genome of which is transformed with a DNA sequence that comes from either one of the strains and that codes for its respective toxin, is resistant to Coleoptera. Each strain, itself, or its crystals, crystal proteins, protoxin or toxin can be used as the active ingredient in an insecticidal composition for combatting Coleoptera.

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(57) Abstract

Two new *Bacillus thuringiensis* strains, which are deposited at the DSM under accession nos. 5131 and 5132, produce crystal proteins during sporulation that are toxic to Coleoptera. The crystal proteins contain 74 kDa and 129 kDa protoxins, respectively, which can yield 68 and 66 kDa toxins, respectively, as trypsin-digestion products. A plant, the genome of which is transformed with a DNA sequence that comes from either one of the strains and that codes for its respective toxin, is resistant to Coleoptera. Each strain, itself, or its crystals, crystal proteins, protoxin or toxin can be used as the active ingredient in an insecticidal composition for combatting Coleoptera.

PLANTS TRANSFORMED TO PRODUCE BACILLUS THURINGIENSIS INSECTICIDAL TOXINS

This invention relates to two new strains of B. thuringiensis (the "BtPGSI208 strain" and the "BtPGSI245 strain"), each of which produces crystallized proteins (the "BtPGSI208 crystal proteins" and the "BtPGSI245 crystal proteins", respectively) which are packaged in crystals (the "BtPGSI208 crystals" and the "BtPGSI245 crystals", respectively) during sporulation. The BtPGSI208 and BtPGSI245 strains were deposited under the provisions of the Budapest Treaty at the Deutsche Sammlung Für Mikroorganismen and Zellkulturen ("DSM"), Mascheroder Weg 1B, D-3300 Braunschweig, Federal Republic of Germany, under accession numbers 5131 and 5132, respectively, on January 19, 1989.

This invention also relates to an insecticide composition that is active against Coleoptera and that comprises the BtPGSI208 or BtPGSI245 strain, as such, or preferably the BtPGSI208 or BtPGSI245 crystals, crystal proteins or the active component(s) thereof as an active ingredient.

This invention further relates to:

1) a DNA sequence (the "btPGSI208 gene"), from the genome of the BtPGSI208 strain, which encodes a 74 kDa protein (the "BtPGSI208 protoxin") that is found in the BtPGSI208 crystals; and

2) A DNA sequence (the "btPGSI245 gene), from the genome of the BtPGSI245 strain, which encodes a 129 kDa protein (the "BtPGSI245 protoxin") that is found in the BtPGSI245 crystals.

The BtPGSI208 and BtPGSI245 protoxins are the proteins that are produced by their respective BtPGSI208 and BtPGSI245 strains before being packaged into their respective

BtPGSI208 and BtPGSI245 crystals.

This invention still further relates to a 68 kDa protein ("the BtPGSI208 toxin") and a 66 kDa protein (the "BtPGSI245 toxin") which can be obtained (e.g., by trypsin digestion) from the BtPGSI208 protoxin and the BtPGSI245 protoxin, respectively. The BtPGSI208 and BtPGSI245 toxins are insecticidally active proteins which can be liberated from the BtPGSI208 crystals and the BtPGSI245 crystals, respectively, produced by the BtPGSI208 strain and the BtPGSI245 strain, respectively, and each toxin has a high activity against Coleoptera. The BtPGSI208 and BtPGSI245 toxins are believed to represent the smallest portions of their respective BtPGSI208 and BtPGSI245 protoxins which are insecticidally effective against Coleoptera.

This invention yet further relates to a chimaeric gene that can be used to transform a plant cell and that contains:

1) a part of the btPGSI208 or btPGSI245 gene (the "insecticidally effective btPGSI208 or btPGSI245 gene part") encoding an insectidicidally effective portion of the respective BtPGSI208 or BtPGSI245 protoxin, preferably a truncated part of the btPGSI208 or btPGSI245 gene (the "truncated btPGSI208 or btPGSI245 gene") encoding just the respective BtPGSI208 or BtPGSI245 toxin;

2) a promoter suitable for transcription of the insecticidally effective btPGSI208 or btPGSI245 gene part in a plant cell; and

3) suitable transcription termination and polyadenylation signals for expressing the insecticidally effective btPGSI208 or btPGSI245 gene part in a plant cell.

This chimaeric gene is hereinafter generally referred to as the "btPGSI208 or btPGSI245 chimaeric gene." Preferably,

the insecticidally effective btPGSI208 or btPGSI245 gene part is present in the btPGSI208 or btPGSI245 chimaeric gene as a hybrid gene comprising a fusion of the truncated btPGSI208 or btPGSI245 gene and a selectable marker gene, such as the neo gene (the "btPGSI208-neo or btPGSI245-neo hybrid gene") encoding a BtPGSI208-NPTII or BtPGSI245-NPTII fusion protein.

This invention also relates to:

1) a cell (the "transformed plant cell") of a plant, such as potato, the genome of which is transformed with the insecticidally effective btPGSI208 or btPGSI245 gene part; and

2) a plant (the "transformed plant") which is regenerated from the transformed plant cell or is produced from the so-regenerated plant, the genome of which contains the insecticidally effective btPGSI208 or btPGSI245 gene part and which is resistant to Coleoptera.

This invention still further relates to a B. thuringiensis ("Bt") strain transformed, preferably by electroporation, with a vector carrying all or part of the btPGSI208 or btPGSI245 gene.

Background of the Invention

B. thuringiensis is a gram-positive bacterium which produces endogenous crystals upon sporulation. The crystals are composed of proteins which are specifically toxic against insect larvae. Three different Bt pathotypes have been described: pathotype A that is active against Lepidoptera, e.g., caterpillars; pathotype B that is active against certain Diptera, e.g., mosquitos and black flies; and pathotype C that is active against Coleoptera, e.g., beetles (Ellar et al, 1986).

A Bt strain, whose crystals are toxic to Coleoptera, has

been described as Bt tenebrionis (US patent 4,766,203; European patent publication 0,149,162), Bt M-7 or Bt San Diego (European patent publication 0,213,818; U.S. patent 4,771,131) and BtS1 (European patent publication 0 305 275).

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.

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. 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 water-soluble part of a Bt protoxin that retains substantial toxicity against its target insects (European patent Publications 0 193 259 and 0 305 275.

Summary of the Invention

In accordance with this invention, the two new Bt strains of pathotype C, i.e., the BtPGSI208 and BtPGSI245 strains, are provided. The BtPGSI208 and BtPGSI245 crystals, crystal proteins, protoxins and toxins, produced by the respective strains during sporulation, as well as insecticidally effective portions of the BtPGSI208 and BtPGSI245 protoxins, each possess insecticidal activity and can therefore be formulated into insecticidal compositions against Coleoptera in general, especially against Agelastica alni, Diabrotica luteola, Haltica tombacina, Anthonomus grandis, Tenebrio molitor, Diabrotica undecimpunctata and

Triboleum castaneum and particularly against the Colorado potato beetle, Leptinotarsa decemlineata, which is a major pest of economically important crops.

Also in accordance with this invention, a plant cell genome is transformed with the insecticidally effective btPGSI208 or btPGSI245 gene part, preferably the truncated btPGSI208 or btPGSI245 gene. It is preferred that this transformation be carried with the btPGSI208 or btPGSI245 chimaeric gene. The resulting transformed plant cell can be used to produce a transformed plant in which the plant cells in some or all of the plant tissues: 1) contain the insecticidally effective btPGSI208 or btPGSI245 gene part as a stable insert in their genome and 2) express the insecticidally effective btPGSI208 or btPGSI245 gene part by producing an insecticidally effective portion of its respective BtPGSI208 or BtPGSI245 protoxin, preferably its respective BtPGSI208 or BtPGSI245 toxin, thereby rendering the plant resistant to Coleoptera. 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 Coleoptera by transforming the plant cell genome with the insecticidally effective btPGSI208 or btPGSI245 gene part, preferably the truncated btPGSI208 or btPGSI245 gene. In this regard, it is preferred that the plant cell be transformed with the btPGSI208 or btPGSI245 chimaeric gene.

Still further in accordance with this invention, there are provided the BtPGSI208 and BtPGSI245 protoxins, the insecticidally effective portions of such protoxins and the BtPGSI208 and BtPGSI245 toxins, as well as the btPGSI208 and btPGSI245 genes, the insecticidally effective btPGSI208 and btPGSI245 gene parts, the truncated btPGSI208 and btPGSI245 genes and the chimaeric btPGSI208 and btPGSI245 genes.

Yet further in accordance with this invention, a Bt strain is transformed, preferably by electroporation, with a vector carrying all or part of the btPGSI208 or btPGSI245 gene encoding all or an insecticidally effective portion of the BtPGSI208 or BtPGSI245 protoxin.

Detailed Description of the Invention

In accordance with this invention, the BtPGSI208 and BtPGSI245 protoxins can be isolated in a conventional manner from, respectively, the BtPGSI208 strain, deposited at the DSM under accession number 5131, and the BtPGSI245 strain, deposited at the DSM under accession number 5132. For example, the BtPGSI208 and BtPGSI245 crystals can be isolated from sporulated cultures of their respective strains (Mahillon and Delcour, 1984), and then, the respective protoxins can be isolated from these crystals according to the method of Höfte et al (1986). The protoxins can be used to prepare monoclonal or polyclonal antibodies specific for these protoxins in a conventional manner (Höfte et al, 1988). The BtPGSI208 toxin can then be obtained by removing (e.g., by trypsin digestion) approximately 57 N-terminal amino acids from the BtPGSI208 protoxin. The BtPGSI245 toxin can be obtained by removing (e.g., by trypsin digestion) approximately 52 N-terminal and approximately 501 C-terminal amino acids from the BtPGSI245 protoxin.

The btPGSI208 and btPGSI245 genes can also be isolated from their respective strains in a conventional manner. For example, the btPGSI208 or btPGSI245 gene can be identified in its respective BtPGSI208 or BtPGSI245 strain, using the procedure described in European patent publications 0 193 259 and 0 305 275.

Preferably, the btPGSI208 and btPGSI245 genes are each identified by: digesting total DNA from their respective BtPGSI208 and BtPGSI245 strains with one or more restriction enzymes; size fractionating the DNA fragments, so produced, into DNA fractions of 5 to 10 Kb; ligating such fractions to cloning vectors; transforming E. coli with the cloning vectors; and screening the clones with a suitable DNA probe. The DNA probe can be constructed: 1) from a highly conserved region of a Bt gene which codes for another crystal protoxin against Coleoptera such as: the bt13 gene described in European patent publication 0 305 275 and by Höfte et al (1987); or 2) on the basis of the N-terminal amino acid sequence of the protoxin encoded by the respective btPGSI208 or btPGSI245 gene, which sequence can be determined by gas-phase sequencing of the immobilized protoxin (European Patent publication 0 305 275).

Alternatively, the 5 to 10 kB fragments, prepared from total DNA of the BtPGSI208 or BtPGSI245 strain, can be ligated in suitable expression vectors and transformed in E. coli, and the clones can then be screened by conventional colony immunoprobing methods (French et al, 1986) for expression of the BtPGSI208 or BtPGSI245 toxin with monoclonal or polyclonal antibodies raised against the toxin.

The so-identifed btPGSI208 and btPGSI245 genes can then each be sequenced in a conventional manner (Maxam and Gilbert, 1980) to obtain the DNA sequences shown in Figs. 1 and 2, respectively. The nucleotide sequences of the btPGSI208 gene and btPGSI245 gene, shown in Figs. 1 and 2, prove that the BtPGSI208 and BtPGSI245 protoxins and toxins are different from previously described protoxins and toxins with activity against Coleoptera (Höfte and Whiteley, 1989).

An insecticidally effective part of each of the sequenced genes, encoding an insecticidally effective

portion of its protoxin, and a truncated part of each of the sequenced genes, encoding just its toxin, can be made in a conventional manner from each gene after the gene has been sequenced. The aminoacid sequences of the BtPGSI208 and BtPGSI245 protoxins and toxins can be determined from the DNA sequences of their respective btPGSI208 and btPGSI245 genes and truncated btPGSI208 and btPGSI245 genes. By "an insecticidally effective part" or "a part" of the btPGSI208 or btPGSI245 gene is meant a DNA sequence encoding a polypeptide which has fewer amino acids then the respective BtPGSI208 or BtPGSI245 protoxin but which is still toxic to Coleoptera. Such a part of the btPGSI208 or btPGSI245 gene can encode a BtPGSI208 or BtPGSI245 protoxin which has been truncated towards at least one trypsin cleavage site of the protoxin (U.S. patent application 821,582; European patent application 86/300291.1).

In order to express all or an insecticidally effective part of the btPGSI208 or btPGSI245 gene in E. coli and in plants, suitable restriction sites are introduced, flanking each gene or gene part. This can be done by site directed mutagenesis, using well-known procedures (Stanssens et al, 1987; Stanssens et al, 1989).

The insecticidally effective btPGSI208 or btPGSI245 gene part, encoding an insecticidally effective portion of its respective BtPGSI208 or BtPGSI245 protoxin, can be stably inserted in a conventional manner into the nuclear genome of a single plant cell, and the so-transformed plant cell be used in a conventional manner to produce a transformed plant that is insect-resistant. In this regard, a disarmed Ti-plasmid, containing the insectidicidally effective btPGSI208 or btPGSI245 gene part, in Agrobacterium tumefaciens can be used to transform the plant cell, and thereafter, a transformed plant can be regenerated from the transformed plant cell using the procedures described, for

example, in European patent publications 0,116,718 and 0,270,822, PCT publication WO 84/02,913 and European patent publication 0 242 246.

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 btPGSI208 or btPGSI245 gene part in other varieties of the same or related plant species. Seeds, which are obtained from the transformed plants, contain the insecticidally effective btPGSI208 or btPGSI245 gene part as a stable genomic insert. Cells of the transformed plant can be cultured in a conventional manner to produce the BtPGSI208 or BtPGSI245 protoxin, preferably the respective toxin, which can be recovered for use in conventional insecticide compositions against Coleoptera (European patent publication 0 193 259).

The insecticidally effective btPGSI208 or btPGSI245 gene part, preferably the truncated btPGSI208 or btPGSI245 gene, is inserted in a plant cell genome so that the inserted part of the 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 btPGSI208 or btPGSI245 chimaeric 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

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constitutive but rather is specific for one or more tissues or organs of the plant (e.g., leaves and/or roots) whereby the inserted btPGSI208 or btPGSI245 gene part is expressed only in cells of the specific tissue(s) or organ(s). For example, the btPGSI208 or btPGSI245 gene part could be selectively expressed in the leaves of a plant (e.g., potato) by placing the 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 European patent publication 0 193 259. Another alternative is to use a promoter whose expression is inducible (e.g., by temperature or chemical factors).

The insecticidally effective btPGSI208 or btPGSI245 gene part is inserted in the plant genome so that the inserted part of the gene is upstream (i.e., 5') of suitable 3' transcription regulation signals (i.e., transcription termination and polyadenylation signals). This is preferably accomplished by inserting the btPGSI208 or btPGSI245 chimaeric gene in the plant cell genome. Preferred polyadenylation and transcription termination 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.

It is preferred that the insecticidally effective btPGSI208 or btPGSI245 gene part be inserted in the plant genome in the same transcriptional unit as, and under the control of, the same promoter as a selectable marker gene. The resulting hybrid btPGSI208 or btPGSI245-marker gene will, thereby, be expressed in a transformed plant as a fusion protein (European patent publication 0 193 259; Vaeck et al, 1987). This

result is preferably accomplished by inserting a btPGSI208 or btPGSI245 chimaeric gene, containing the marker gene, in the plant cell genome. Any conventional marker gene can be utilized, the expression of which can be used to select transformed plant cells. An example of a suitable selectable marker gene is an antibiotic resistance gene such as the neo gene coding for kanamycin resistance (Reiss et al, 1984; European patent publication 0 242 246; European patent publication 0 193 259).

Thereby, the insecticidally effective btPGSI208 or btPGSI245 gene part and the marker gene (e.g., the btPGSI208-neo or btPGSI245-neo hybrid gene) are expressed in a transformed plant as a fusion protein (European patent publication 0 193 259; Vaeck et al, 1987).

All or part of the btPGSI208 and btPGSI245 genes, encoding Coleopteran toxins, can also be used to transform gram-positive 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 combatting both Lepidopteran and Coleopteran insect pests or combatting additional Coleopteran insect pests. Transformation of a bacteria with all or part of the btPGSI208 or btPGSI245 gene, incorporated in a suitable cloning vehicle, can be carried out in a conventional manner, preferably using conventional electroporation techniques as described in PCT patent application PCT/EP89/01539, filed December 11, 1989.

Each of the BtPGSI208 and BtPGSI245 strains can be fermented by conventional methods (Dulmage, 1981) to provide high yields of cells. Under appropriate conditions which are well understood (Dulmage, 1981), the BtPGSI208 and BtPGSI245 strains each sporulate to provide their respective BtPGSI208 and BtPGSI245 crystal proteins in high yields.

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An insecticide composition of this invention can be formulated in a conventional manner using the BtPGSI208 or BtPGSI245 strain or preferably their respective crystals, crystal proteins, protoxin, toxin and/or insecticidally effective portions of their respective protoxin as active ingredient(s), together with suitable carriers, diluents, emulsifiers and/or dispersants. This insecticide composition can be formulated as a wettable powder, pellets, granules or a dust or as a liquid formulation with aqueous or non-aqueous solvents as a foam, gel, suspension, concentrate, etc. The concentration of the BtPGSI208 or BtPGSI245 strain, crystals, crystal proteins, protoxin, toxin and/or protoxin portions in such a composition will depend upon the nature of the formulation and its intended mode of use. Generally, an insecticide composition of this invention can be used to protect a potato field for 2 to 4 weeks against Coleoptera 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.

The following Examples illustrate the invention. The figures, referred to in the Examples, are as follows :

Figure 1 - DNA sequence of the <u>btPGSI208</u> gene. The derived aminoacid sequence of the encoded BtPGSI208 protoxin is presented beneath this sequence. The arrow separates the N-terminal 57 aminoacids from the C-terminal portions encoding the BtPGSI208 toxin. The truncated <u>btPGSI208</u> gene, coding just for the BtPGSI208 toxin, extends from nucleotide position 513 (see arrow) to the TAG termination codon at nucleotide position 2295.

Figure 2 - DNA sequence of the <u>btPGSI245</u> gene. The derived

aminoacid sequence of the encoded BtPGSI245 protoxin is presented beneath this sequence. The arrows delineate the BtPGSI245 toxin between aminoacids 54 and 638 of the BtPGSI245 protoxin. The truncated btPGSI245 gene, coding just for the BtPGSI245 gene, extends from nucleotide position 339 (see arrow) to nucleotide position 2094 (see arrow).

Figure 3 - Total protein patterns by SDS-PAGE of sporulated BtPGSI208 and BtPGSI245 and other Bacillus cultures. Among the comparison strains, B. subt. is Bacillus subtilis, B. cer. is Bacillus cereus, and Bt Darm is Bacillus thuringiensis subsp. darmstadiensis. These comparison strains were obtained from the sources set forth in Table 1, hereinafter. "MW" designates molecular weight markers.

Figure 4A - Protein blotting of total proteins and trypsinized crystal proteins from strains BtS1 and BtPGSI208. Total protein patterns were stained with Indian ink, while crystal proteins were visualized with an antiserum against Bt13 toxin ("anti-CryIIIA"). "HMW" designates molecular weight markers.

Figure 4B - Protein blotting of total proteins and trypsinized crystal proteins from strains BtS1, BtPGSI245 and Bt HD-110. Total protein patterns were probed for their immunoreactivity with an antiserum against Bt13 toxin ("anti-CryIIIA") and an antiserum against Bt2 protoxin ("anti-CryIA(b)"). "LMW" designates molecular weight markers. The comparison strain, HD-110, was Bt HD-110, obtained from Dr. H. Dulmage, Cotton Insect Laboratories, U.S.D.A.,

Brownsville, Texas, U.S.A.

Unless otherwise stated in the Examples, all procedures for making and manipulating recombinant DNA are carried out by the standardized procedures described in Maniatis et al, Molecular Cloning - A laboratory Manual, Cold Spring Harbor Laboratory (1982).

Example 1 : Characterization of the BtPGSI208 and BtPGSI245 strains

The BtPGSI208 strain was isolated from grain dust sampled in Belgium and was deposited at the DSM on January 19, 1989 under accession No. 5131.

The BtPGSI245 strain was isolated from cow dung sampled in the United States and was deposited at the DSM on January 19, 1989 under accession No. 5132.

Each strain can be cultivated on conventional standard media, preferably LB medium (Bacto-tryptone 10 g/l, yeast extract 5 g/l, NaCl 10 g/l and agar 15 g/l), preferably at 28°C. For long term storage , it is preferred to use LB liquid medium containing 50% glycerol at -70°C or lyophilization. For sporulation, the use of T_3 medium (tryptone 3 g/l, tryptose 2 g/l, yeast extract 1.5 g/l, 5 mg $MnCl_2$, 0.05 M Na_2PO_4, pH 6.8 and 1.5% agar) is preferred for 24 hours at 28°C, followed by storage at 4°C. During its vegetative phase, each of the BtPGSI208 and BtPGSI245 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 crystalline BtPGSI208 and BtPGSI245 protoxins. UV radiation (254 nm) inactivates the spores but not the protoxins.

After cultivating on Nutrient Agar ("NA", Difco

Laboratories, Detroit, MI, USA) for one day, colonies of each of the BtPGSI208 and BtPGSI245 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 three days cultivation at 28°C on NA. The crystal proteins produced during sporulation are packaged in flat rhomboid crystals in the BtPGSI208 strain and in bipyramidal crystals in the BtPGSI245 strain.

For the biochemical characterization of the two strains, the following tests were carried out using well known methods as described for example by Sneath et al (1986). Growth was observed in Nutrient Broth ("NB", Difco) supplemented with 2 and 5% NaCl. No growth of the BtPGSI208 strain and only weak growth of the BtPGSI245 strain were observed in the presence of 7% NaCl. Neither strain grew in medium supplemented with 10% NaCl. The BtPGSI208 and BtPGSI245 strains grew well on NA at 20, 28 and 37°C, but not at 4, 10 (although the BtPGSI245 strain grew slowly at this temperature), 50 and 60°C. Both strains grew in NB at pH = 5, pH = 6 and pH = 7 and on NB containing 100 units of lysozyme (Sigma Chemical Company, St Louis, MO, USA) per ml of NB. Growth on NA under anaerobiosis was very weak.

Metabolic characteristics of the two strains were determined using API-20E test strips (API Systems S.A., Montalieu-Vercieu, France). The results of these assays are shown in Table 1, below.

Table 1 Metabolic characteristics of the BtPGSI208 and BtPGSI245 strains as compared with other Bacillus strains (+ = positive reaction ; - = negative reaction ; w = weak reaction ; nd = not determined).

Activity	Bt PGSI 208	Bt PGSI 245	BTS1	BTEN	BDAR	BCER	BSUB
ONPG	-	-	-	-	-	-	+
ADH	-	+	+	+	+	+	-
LDC	-	-	-	-	-	-	-
ODC	-	-	-	-	-	-	-
CIT	-	-	-	-	-	-	-
H2S	-	-	-	-	-	-	-
URE	-	-	-	-	-	-	-
TDA	-	-	-	+	w	+	+
IND	-	-	-	-	-	-	-
VP	-	-	-	w	w	w	+
GEL	-	+	-	+	+	+	+
OX	+	+	+	+	+	+	+
NO2	+	+	+	+	+	+	nd
N2	-	-	-	-	-	-	nd

ONPG = β-galactosidase activity.
ADH = arginine dihydrolase activity.
LDC = lysine decar oxylase activity.
ODC = ornithine decarboxylase activity.
CIT = use of citrate as sole carbon source.
H2S = H_2S formation from thiosulphate.
URE = urease activity.
TDA = tryptophan deaminase activity.
IND = indol formation from tryptophan.
VP = acetoin formation from sodium pyruvate.
GEL = gelatin liquefaction.
OX = oxidase activity.
NO2 = nitrate reduction to nitrite.

N2 = N_2 gas production from nitrate.

BTS1 = Bacillus thuringiensis BtS1 from DSM under accession no. 4288.

BTEN = Bacillus thuringiensis subsp. tenebrionis from DSM under accession no. 2803.

BDAR = Bacillus thuringiensis subsp. darmstadiensis from Institut für Landwirtschaftliche Bacteriologie und Gärungsbiologie der Eidgenössiche Technische Hochschüle, Zürich, Switzerland ("LBG"), under accession no. 4447

BCER = Bacillus cereus from Laboratorium voor Microbiologie, Gent, Belgium ("LMG"), under accession no. 2098.

BSUB = Bacillus subtilis from Agricultural Research Culture Collection, Peoria, Illinois, USA, under accession no. NRRL B-237.

Both strains were found to rapidly decompose casein in skim-milk agar and to deaminate phenylalanine in tests described by Sneath et al (1986).

Acid production from different sugars by the two strains was determined using API-50CHB test strips (API Systems SA). The results are shown in Table 2, below.

Table 2: Acid production by the BtPGSI208 and BtPGSI245 strains as compared with other bacilli (+ = positive reaction; - = negative reaction ; w = weak reaction).

Substrate :	Bt PGSI 208	Bt PGSI 245	BtS1	BTEN	BDAR	BCER	BSUB
Control	-	-	-	-	-	-	-
Glycerol	-	w	+	+	+	+	+
Erythritol	-	-	-	-	-	-	-
D-arabinose	-	-	-	-	-	-	-
L-arabinose	-	-	-	-	-	-	+
Ribose	+	+	+	+	+	+	+
D-Xylose	-	-	-	-	-	-	+
L-Xylose	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-
B Methyl-xyloside	-	-	-	-	-	-	-
Galactose	-	-	-	-	-	-	+
D-Glucose	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+
D-Mannose	-	+	+	+	-	-	+
L-Sorbose	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-
Dulcitol	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	+
Mannitol	-	-	-	-	-	-	+
Sorbitol	-	-	-	-	-	-	+
α-Methyl-D-mannoside	-	-	-	-	-	-	-
α-Methyl-D-glucoside	-	-	-	-	-	-	+
N-Acetylglucosamide	+	+	+	+	+	+	-
Amygdaline	-	-	-	-	-	-	+
Arbutine	+	+	+	+	+	-	+
Esculine	+	+	+	+	+	+	+
Salicine	+	w	-	-	-	-	+
Cellobiose	-	w	-	-	-	-	+
Maltose	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	+
Melibiose	-	-	-	-	-	-	+
Saccharose	+	+	+	+	-	+	+
Trehalose	+	+	+	+	+	+	+
Inuline	-	-	-	-	-	-	+
Melizitose	-	-	-	-	-	-	-
D-Raffinose	-	-	-	-	-	-	+
Starch	+	+	+	+	+	+	+
Glycogen	+	+	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-
B Gentiobiose	-	-	-	-	-	-	-

Table 2 (Continued)

Substrate :	Bt PGSI 208	Bt PGSI 245	BtS1	BTEN	BDAR	BCER	BSUB
D-Turanose	-	-	-	-	-	-	+
D-Lyxose	-	-	-	-	-	-	-
D-Tagatose	-	-	-	-	-	-	-
D-Fucose	-	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-	-
Gluconate	-	-	-	-	-	-	-
2 Ketogluconate	-	-	-	-	-	-	-
5 Ketogluconate	-	-	-	-	-	-	-

Sensitivity of the two strains towards different antibiotics was tested using Oxoid Susceptibility Test Discs on Oxoid Isosensitest agar ("CM471"® of Oxoid Ltd., Basingstoke, Hampshire, England). The results are shown in Table 3, below.

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Table 3: Antibiotic sensitivity as shown by the diameters (in mm) of inhibition zones observed after 24 hours on antibiotic-containing agar, seeded with different bacilli (R= resistant colonies or no growth detected).

Anti-biotic	amount/ disc	Bt PGSI 208	Bt PGSI 245	BtS1	BTEN	BDAR	BCER	BSUB
Chloramphe-nicol	30 ug	25/R	17	19/R	20	22	28	33
Bacitracin	10 i.u	11	10	8	7	14	18	7
Gentamycin	10 ug	26	20	21	20	28	9	25/R
Neomycin	30 ug	24	20/R	13/R	13	26	10	20
Tetracyclin	30 ug	14/R	10	17/R	16/R	10/R	21	22
Carbenicil-lin	100 ug	8	11	0	0	10	0	19
Rifampicin	2 ug	12	13	0	8	8	19	26
Penicillin G	10 i.u	7	8	0	0	0	14/R	14
Streptomycin	10 ug	25/R	15	16	17	20	14	0
Spectinomy-cin	10 ug	0	0	0	0	0	12/R	0
Kanamycin	30 ug	20/R	21/R	0	0	24	15	24
Nalidixic acid	30 ug	25/R	23/R	18/R	25/R	30/R	7	19
Sulphametho-xazole	25 ug	0	0	0	0	0	0	0
Trimethoprim	2.5 ug	0	0	0	0	0	0	26
Ampicillin	10 u	7	9/R	0	0	0	15/R	18

The enzyme spectra of the BtPGSI208 and BtPGSI245 strains were determined using the extended API-ZYM® strips (API Systems S.A.). The results are shown in Table 4, below. Esterase-, peptidase- (AP1, AP2, AP3, AP4, AP5 and AP6 test strips) and osidase-test strips were inoculated with 50 ul cell suspension (10^7 cfu/ml). The osidase reaction was revealed after 4 hours incubation (28°C) with 25 µl 0.1N NaOH. All other reactions were with 25 µl ZYM A and ZYM B reagent (API no. 7048).

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Table 4: Enzymatic spectra of the BtPGSI208 and BtPGSI245 strains as compared to two other Bt strains (0 = no substrate used; 1, 2, 3, 4, 5 = 5, 10, 20, 30 and ≥ 40 nanomoles of substrate hydrolysed respectively).

Substrate	Bt PGSI 208	Bt PGSI 245	BTS1	BDAR
Esterases.				
2-naphtyl-valerate	4	4	2	4
2-naphtyl-caproate	5	5	5	5
2-naphtyl-caprylate	5	5	5	5
2-naphtyl-nonanoate	5	5	4	5
2-naphtyl-caprate	5	3	3	5
2-naphtyl-laurate	2	2	1	2
2-naphtyl-myristate	1	2	1	1
2-naphtyl-palmitate	0	0	1	0
2-naphtyl-stearate	2	1	2	2
Peptidases.				
L-pyrrolidonyl-β-naphtylamide	0	5	5	5
Glycyl-β-naphtylamide	0	0	0	0
L-glutamyl-β-naphtylamide	0	0	0	0
L-leucyl-glycyl-β-naphtylamide	0	1	0	0
L-seryl-L-tyrosyl-β-naphtylamide	4	5	5	5
L-glutamine-β-naphtylamide	1	5	4	5
L-glutamyl-β-naphtylamide	0	3	3	3
Osidases.				
Paranitrophenol-D-galactopyranoside	0	0	0	0
Paranitrophenol-βD-galactopyranoside	0	0	0	0
Paranitrophenol-aD-glucopyranoside	5	5	5	5
Paranitrophenol-βD-glucopyranoside	0	2	0	0
Paranitrophenol-a-maltoside	2	4	3	5
Paranitrophenol-β-maltoside	0	0	0	0
Paranitrophenol-N-acetyl-βD-glucosamidine	3	5	5	5
Paranitrophenol-βD-xylapyranoside	0	0	0	0

Example 2: Characteristics of the BtPGSI208 and BtPGSI245 crystals

The BtPGSI208 and BtPGSI245 strains were grown for 48 to 72 hours at 28°C on T_3 medium. After sporulation, the spores and crystals were harvested in phosphate buffered saline solution ("PBS" from Oxoid Ltd.) by scraping with a Trihalski spatula. The resulting aqueous spore-crystal suspensions were centrifuged, and the pellets were resuspended and incubated overnight in aqueous solutions containing 50mM Na_2CO_3 and 5mM dithiotreitol ("DTT") at pH 10. After centrifugation, the supernatants were recovered containing the respective crystal proteins.

The BtPGSI208 protoxin and toxin, as well as the BtPGSI245 toxin, react only with a polyclonal antiserum raised against the Bt13 toxin as shown in Fig. 4. In contrast, the BtPGSI245 protoxin reacts with polyclonal antisera against the BtS1 or Bt13 toxin (European patent publication 0 305 275) and the Bt2 protoxin (European patent publication 0 193 259) as shown in Fig. 4.

The total protein patterns of the BtPGSI208 and BtPGSI245 strains, compared to other Bacillus strains, are shown in Fig. 3. For this comparison, the crystal proteins of each strain were analyzed on a 12.5% SDS-PAGE gel (Laemmli, 1970) and stained with Coomassie brilliant blue R-250 according to Lambert et al (1987). The crystal proteins were dissolved by exposing the spore-crystal mixtures overnight at 37°C to 50 mM Na_2CO_3, pH 10, 5 mM DTT. Solubilized crystal proteins were digested by adjusting the pH to 9.0 with 0.5 M HCl and by trypsinization (1 µg bovine trypsin/25 µg protein). Trypsin digestion of the BtPGSI208 and BtPGSI245 crystal proteins was performed at 37°C overnight and revealed the presence of tryptic fragments of 68 kDa and 66 kDa, respectively (Fig. 4).

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Immunoblotting experiments, performed according to Peferoen (1988) with polyclonal antisera raised against the Bt13 toxin and the Bt2 protoxin demonstrated, in Figs. 4A and B, that the BtPGSI208 and BtPGSI245 protoxins and toxins are immunologically related to the Bt13 toxin. In addition, the BtPGSI245 protoxin was also shown, in Fig. 4B, to be immunologically related to the Bt2 protoxin. After blotting, the proteins were stained with Indian ink (Sutherland and Skerritt, 1986) to show both immunoreactive and non-immunoreactive proteins (Fig. 4).

Example 3: Insecticidal activity of the BtPGSI208 and BtPGSI245 crystal proteins

As in Example 2, both strains were grown for 48 to 72 hrs at 28°C on T_3 medium. After sporulation, the spores and crystals were harvested in PBS (phosphate buffered saline) with a Trihalski spatula. The resulting spore-crystal suspensions were centrifuged, and the pellets were resuspended and incubated overnight in aqueous Na_2CO_3 and DTT solutions as described in Example 2. After centrifugation, the supernatants were recovered, and their contents of the respective crystal proteins of the two strains were determined.

Potato leaves were dipped in aqueous dilutions of the supernatant solutions and then air dried for two hours. Colorado potato beetle larvae of the second instar were placed on the treated leaves, and mortality of the larvae was measured after three days. These results were compared with the mortality of larvae fed leaves treated with solubilized crystal proteins of Bt HD-1 ("Bt kurstaki Dipel®" from Abbott Laboratories, Abbott Park, North Chicago, Ill., USA) as a control. LC_{50}, expressed as ug of solubilized crystal proteins/ml, was calculated by Probit analysis (Finney, 1971). The results are summarized in Table 5, below.

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Table 5: Comparison of toxicity of solubilized crystal proteins from the BtPGSI208 strain, the BtPGSI245 strain and the BtHD1 strain (control) against larvae of Leptinotarsa decemlineata.

Strain	LC50	FL95min	FL95max	Slope
BtPGSI208	5.0	3.5	7.3	2.4
BtPGSI245	25.1	14.7	43.3	1.5
Control	>500	-	-	-

Example 4: Identification and cloning of the btPGSI208 gene

The BtPGSI208 protoxin from the BtPGSI208 strain was detected by ELISA (Engvall and Pesce, 1978) with a polyclonal antiserum against the Bt13 coleoptera toxin (Höfte et al, 1987). The btPGSI208 gene was identified in the BtPGSI208 strain by preparing total DNA of the BtPGSI208 strain and then digesting the DNA with the restriction enzymes HindIII, EcoRI and ClaI. The so-digested DNA was analyzed by Southern blotting, probing with a nick-translated 2.9 kb HindIII fragment from the genome of the BtS1 strain (European patent application 88/402,115.5) containing the bt13 gene. After hybridization with the probe, the blot was washed under low stringency conditions (2XSSC, 0.1%SDS at 68°C for 2x15 min), showing the presence of the btPGSI208 gene, related to the bt13 gene. The hybridization pattern with the probe also showed that the btPGSI208 gene was clearly different from the bt13 gene.

In order to isolate the btPGSI208 gene, total DNA was prepared from the BtPGSI208 strain. The total DNA preparation was partially digested with Sau3A and was size-fractionated on a sucrose gradient. Fractions containing DNA between 5 kb and 10 kb were ligated to the

BglII-digested and bovine alkaline phosphatase ("BAP")-treated cloning vector pEcoR251 (Deposited at the DSM on July 13, 1988 under accession number DSM 4711). Recombinant E. coli clones containing the vector were then screened with the 2.9 kb HindIII DNA fragment containing the bt13 gene, as a probe, to identify DNA fragments of clones containing the btPGSI208 gene. The so-identified DNA fragments were then sequenced (Fig. 1) according to Maxam and Gilbert (1980).

Based on the analysis of the DNA sequence of the btPGSI208 gene, the gene is cut with an appropriate restriction enzyme to give the truncated btPGSI208 gene, encoding the BtPGSI208 toxin of about 68 kDa.

Example 5: Identification and cloning of the btPGSI245 gene

The BtPGSI245 protoxin from the BtPGSI245 strain was detected by ELISA (Engvall and Pesce, 1978) with a polyclonal antiserum directed against the Bt13 coleoptera toxin. Colony hybridization of the BtPGSI245 strain and Southern blotting of the BtPGSI245 total DNA, that had been probed with the 2.9 kb HindIII DNA fragment containing the bt13 gene and washed under the previously described low stringency conditions, revealed no hybridizing DNA.

In order to isolate the btPGSI245 gene, total DNA from the BtPGSI245 strain was prepared and partially digested with Sau3A. The digested DNA was size fractionated on a sucrose gradient and fragments ranging from 5 kb to 10 kb were ligated to the BamHI-digested and BAP-treated cloning vector pUC18 (Yannisch-Perron et al, 1985). Recombinant clones containing the vector were then screened by colony immunoprobing (French et al, 1986) with an antiserum against the Bt13 toxin. Positive colonies were purified, and total protein preparations were again analyzed by immunoblotting with the antiserum against the Bt13 toxin.

DNA fragments, containing the BtPGSI245 gene, from clones expressing the BtPGSI245 protoxin, were then sequenced (Fig. 2) according to Maxam and Gilbert (1980).

Based on the analysis of the DNA sequence of the btPGSI245 gene, the gene is cut with an appropriate restriction enzyme to give the truncated btPGSI245 gene, encoding the BtPGSI245 toxin of about 66 kDa.

Example 6: Construction of a btPGSI208-neo hybrid gene and a btPGSI245-neo hybrid gene

Following the procedure of European patent publications 0 305 275 and 0 193 259, the truncated btPGSI208 and btPGSI245 genes from Examples 4 and 5, respectively, are each fused to the neo gene to form the corresponding hybrid gene.

Example 7: Insertion of the btPGSI208 and btPGSI245 genes, the truncated btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes in E. coli and insertion of the truncated btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes in potato plants

The btPGSI208 and btPGSI245 genes and the truncated btPGSI208 and btPGSI245 genes from Examples 4 and 5 and the btPGSI208-neo and btPGSI245-neo hybrid genes from Example 6 are each inserted into, and expressed by, different E. coli in a conventional manner, using E. coli expression vectors as described by Botterman and Zabeau (1987). In order to express these genes in E. coli and in plants, different gene cassettes are made. In this regard, a BamHI restriction site is introduced at the ATG initiation codon of the btPGSI208 gene by site directed mutagenesis (Stanssens et al, 1988; Stanssens et al, 1989). The 4th nucleotide of the btPGSI208 gene is changed from an A to a

G, yielding a unique BamHI site. According to the "Kozak rules" (Kozak, 1986), this mutation should also optimize the translation initiation in plant cells. In this way, the AAT codon (second codon) coding for Asn is changed into a GAT codon coding for Asp. Similarly, a NcoI is introduced at the ATG translation initiation codon of the btPGSI245 gene.

The insecticidal activity, against Colorado potato beetle second instar larvae, of the BtPGSI208 protoxin produced in E. coli (transformed with the btPGSI208 gene) is given in Table 6, below. Toxicity is expressed as 50% lethal concentration in ug/ml, followed by the 95% confidence interval and the slope of the probit line.

Table 6

LC50(ug/ml)	slope
1.0 (0.6-1.8)	2.3

The toxicity of the BtPGSI208 protoxin produced in E. coli was about 5 times greater than the toxicity of the BtPGSI208 protoxin produced by the BtPGSI208 strain. Trypsin digestion of the BtPGSI208 protoxin, produced in E. coli, yielded the BtPGSI208 toxin which also was active against Colorado potato beetle.

Using the procedures described European patent publications 0 193 259 and 0 305 275, the truncated btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes are isolated and are cloned into the intermediate T-DNA vector, pGSH160 (Deblaere et al, 1988), between the vector's T-DNA terminal border repeat sequences. To provide major expression in plants, the

hybrid genes and truncated genes are placed under the control of the strong constitutive promoters, Cabb-JI 35S promotor (Hull and Howell, 1987) or the TR2' promoter (Velten et al, 1984), and are fused to the transcription termination 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 btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes, are transferred into the Agrobacterium strain C 58 C1 Rif^R (European patent publication 0 193 259) 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 potato plants (Solanum tuberosum) so that the truncated btPGSI208 gene, the truncated btPGSI245 gene, the btPGSI208-neo hybrid gene and the btPGSI245-neo hybrid gene are contained in, and expressed by, different potato plant cells.

Example 8: Expression of the truncated btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes in potato plants

The insecticidal activity against Coleoptera of the expression products of the truncated btPGSI208 and btPGSI245 genes and the btPGSI208-neo and btPGSI245-neo hybrid genes in leaves of transformed potato plants, generated from the transformed potato plant cells of Example 7, is evaluated by recording the growth rate and mortality of Leptinotarsa decemlineata larvae fed on these leaves. These results are compared with the growth rate of

larvae fed leaves from untransformed potato plants. Toxicity assays are performed as described in European Patent publication 0 305 275 and European patent publication 0 193 259.

A significantly higher mortality rate is obtained among larvae fed on leaves of transformed potato plants containing the truncated btPGSI208 gene, the truncated btPGSI245 gene, the btPGSI208-neo hybrid gene or the btPGSI245-neo hybrid gene than among larvae fed the leaves of untransformed plants.

Example 9: Transformation of a Bt strain with the endogenous btPGSI208 gene

In order to enhance its insecticidal spectrum, the BtS174A strain (Mahillon et al, 1989) is electroporated with plasmid pAMbt21R1. pAMbt21R1 is obtained by cloning the 5.4 kb HpaI fragment of plasmid pJL21 into the EcoRV site of the tetracycline resistance gene of the shuttle vector pAM401 (Wirth et al, 1987). Plasmid pJL21 is obtained by cloning a 6kb fragment, containing the btPGSI208 gene, from the BtPGSI208 strain into the BglII site of plasmid pEcoR251 (DSM accession no. 4711).

The electroporation procedure is carried out as described in PCT patent application no. PCT/EP89/01539. The BtS174A strain, transformed with pAMbt21R1, retains its insecticidal activity against Lepidoptera and demonstrates a newly acquired insecticidal activity against Coleoptera due to expression of the btPGSI208 gene in the strain.

Needless to say, this invention is not limited to the BtPGSI208 (DSM 5131) strain and the BtPGSI245 (DSM 5132) strain. Rather, the invention also includes any mutant or variant of the BtPGSI208 or BtPGSI245 strain which produces crystals, crystal proteins, protoxin or toxin having substantially the same properties as the BtPGSI208 or

B

BtPGSI245 crystals, crystal proteins, protoxin or toxin. In this regard, variants of the BtPGSI208 and BtPGSI245 strains include variants whose total protein pattern is substantially the same as the protein pattern of either the BtPGSI208 strain or the BtPGSI245 strain as shown in Fig. 3.

This invention also is not limited to potato plants transformed with the truncated btPGSI208 or btPGSI245 gene. It includes any plant, such as tomato, tobacco, rapeseed, alfalfa, sunflowers, cotton, corn, soybeans, brassicas, sugar beets and other vegetables, transformed with an insecticidally effective part of the btPGSI208 or btPGSI245 gene.

Nor is this invention limited to the use of Agrobacterium tumefaciens Ti-plasmids for transforming plant cells with an insecticidally effective btPGSI208 or btPGSI245 gene part. Other known techniques for plant cell transformations, such as by means of liposomes, by electroporation or by vector systems based on plant viruses or pollen, can be used for transforming monocotyledons and dicotyledons with such a gene part.

Furthermore, DNA sequences other than those shown in Fig. 1 for the btPGSI208 gene and the truncated btPGSI208 gene and in Fig. 2 for the btPGSI245 gene and the truncated btPGSI245 gene can be used for transforming plants and bacteria. In this regard, the DNA sequence of Fig. 1 or 2 can be modified by: 1) replacing some codons with others that code either for the same amino acids or for other amino acids; and/or 2) deleting or adding some codons; provided that such modifications do not substantially alter the properties of the encoded, insecticidally effective portion of the BtPGSI208 or BtPGSI245 protoxin or toxin.

Also, other DNA recombinants containing the aforementioned DNA sequences in association with other

foreign DNA, 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 btPGSI208 and btPGSI245 genes, or parts thereof, that were heretofore described, but rather, this invention encompasses any DNA recombinants containing a DNA sequence that is their equivalent. Further, the invention relates to all DNA recombinants that include all or part of either the btPGSI208 gene or the btPGSI245 gene and that are suitable for transforming microorganisms (e.g., plant associated bacteria such as 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.

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CLAIMS

1. A protein comprising the amino acid sequence of Figure 2 or an insecticidally effective portion thereof comprising the amino acid sequence of Figure 2 from amino acid position 53 to amino acid position 637.

2. A protein comprising the amino acid sequence of Figure 2 from amino acid position 1 to amino acid position 637.

3. A protein comprising the amino acid sequence of Figure 2 from amino acid position 53 to amino acid position 637.

4. A DNA encoding the protein of any one of Claims 1 to 3.

5. A DNA comprising the DNA of Figure 2 from nucleotide position 184 to nucleotide position 2094 or the DNA of Figure 2 from nucleotide position 184 to nucleotide position 3597.

6. A chimeric gene, comprising the DNA of Claim 4 under the control of a promoter suitable for transcription in plant cells.

7. A transformed plant cell, comprising the chimeric gene of Claim 6.

8. A process for rendering a plant resistant to Coleoptera comprising: transforming the plant with the chimeric gene of Claim 6.

9. A process for producing plants resistant to Coleoptera, comprising: stably transforming plant cells with the chimeric gene of Claim 6, regenerating transformed plants from said plant cells and biologically replicating said plants.

10. A transformed microorganism comprising the DNA of Claim 4 or 5.

11. The transformed microorganism of Claim 10 which is *B. thuringiensis*.

12. The protein of any one of Claims 1 to 3 further comprising the amino acid sequence of a selectable marker.

13. An isolated BtPGSI245 strain with DSM deposit number 5132.

14. A crystal-spore mixture of the strain of Claim 13.

15. An insecticidal composition with activity against Coleoptera, which comprises the strain of Claim 13 or the crystal-spore mixture of Claim 14.

16. A process for controlling a Coleopteran insect pest comprising: contacting said pest with the insecticidal composition of Claim 15.

17. The process of Claim 16, wherein the Coleopteran pest is *Leptinotarsa decemlineata*.

18. An insecticdal composition having activity against Coleoptera, which comprises the protein of any one of Claims 1 to 3.

19. An isolated BtPGSI208 strain with DSM deposit number 5131.

20. An insecticidal composition comprising a crystal-spore mixture of the strain of Claim 19.

FIGURE 1

```
                10         20         30         40         50
TTATCTACAT TTAATCATCT CCATTATAAA TATAATATCT AAACACATTG

                60         70         80         90        100
TTGCAAGAGA ATTAATACTA CTTTGATATT TTTAATATAC TTAACCTAAT

               110        120        130        140        150
GTATTGTTAA GTTAATATAG AAATATACCT ATATTAACAA GGAATTTATT

               160        170        180        190        200
AAAAATAATT TTGTATACTT TTCATTGTAA TAACATGATT TTTAAAACAA

               210        220        230        240        250
AAAAGTGTAT AAACAACTTA TCAGGAAGGG GGGGATGCGC AAAGAATAAA

               260        270        280        290        300
AAGAGAATGC TTATAATGTT CAATGGTTTT ATAGGAAGGC ATTTTATCAG

               310        320        330        340   >
GTAGAAAGTT ATGTATTATG ATAAGAATGG GAGGAAGAAA A ATG
                                                   MET

                350        359        368        377
AAT CCA AAC AAT CGA AGT GAA TAT GAT ACG ATA AAG GTT
Asn Pro Asn Asn Arg Ser Glu Tyr Asp Thr Ile Lys Val

386        395        404        413        422
ACA CCT AAC AGT GAA TTG CCA ACT AAC CAT AAT CAA TAT
Thr Pro Asn Ser Glu Leu Pro Thr Asn His Asn Gln Tyr

           431        440        449        458
CCT TTA GCT GAC AAT CCA AAT TCG ACA CTA GAA GAA TTA
Pro Leu Ala Asp Asn Pro Asn Ser Thr Leu Glu Glu Leu
```

FIGURE 1 (continued 1)

```
    467         476         485         494
AAT TAT AAA GAA TTT TTA AGA ATG ACT GCA GAC AAT TCT
Asn Tyr Lys Glu Phe Leu Arg MET Thr Ala Asp Asn Ser

503         512         521         530         539
ACG GAA GTG CTA GAC AGC TCT ACA GTA AAA GAT GCA GTT
Thr Glu Val Leu Asp Ser Ser Thr Val Lys Asp Ala Val

        548         557         566         575
GGG ACA GGA ATT TCT GTT GTA GGA CAG ATT TTA GGT GTT
Gly Thr Gly Ile Ser Val Val Gly Gln Ile Leu Gly Val

    584         593         602         611
GTA GGG GTT CCA TTT GCT GGG GCG CTC ACT TCA TTT TAT
Val Gly Val Pro Phe Ala Gly Ala Leu Thr Ser Phe Tyr

620         629         638         647         656
CAA TCA TTT CTT AAC GCT ATA TGG CCA AGT GAT GCT GAC
Gln Ser Phe Leu Asn Ala Ile Trp Pro Ser Asp Ala Asp

        665         674         683         692
CCA TGG AAG GCT TTT ATG GCA CAA GTG GAA GTA CTG ATA
Pro Trp Lys Ala Phe MET Ala Gln Val Glu Val Leu Ile

    701         710         719         728
GAT AAG AAA ATA GAG GAG TAT GCT AAA AGT AAA GCT CTT
Asp Lys Lys Ile Glu Glu Tyr Ala Lys Ser Lys Ala Leu

737         746         755         764         773
GCA GAG TTA CAG GGT CTT CAA AAT AAT TTT GAA GAT TAT
Ala Glu Leu Gln Gly Leu Gln Asn Asn Phe Glu Asp Tyr
```

FIGURE 1 (continued 2)

```
       782        791        800        809
GTA AAT GCG TTG GAT TCC TGG AAG AAA GCG CCT GTA AAT
Val Asn Ala Leu Asp Ser Trp Lys Lys Ala Pro Val Asn

   818        827        836        845
TTA CGA AGT CGA AGA AGC CAA GAT CGA ATA AGA GAA CTT
Leu Arg Ser Arg Arg Ser Gln Asp Arg Ile Arg Glu Leu

854        863        872        881        890
TTT TCT CAA GCA GAA AGC CAT TTT CGT AAT TCC ATG CCG
Phe Ser Gln Ala Glu Ser His Phe Arg Asn Ser MET Pro

       899        908        917        926
TCA TTT GCG GTT TCC AAA TTC GAA GTT CTG TTT CTA CCA
Ser Phe Ala Val Ser Lys Phe Glu Val Leu Phe Leu Pro

   935        944        953        962
ACA TAT GCA CAA GCT GCA AAT ACA CAT TTA TTG CTA TTA
Thr Tyr Ala Gln Ala Ala Asn Thr His Leu Leu Leu Leu

971        980        989        998        1007
AAA GAT GCT CAA GTT TTT GGA GAA GAA TGG GGA TAT TCT
Lys Asp Ala Gln Val Phe Gly Glu Glu Trp Gly Tyr Ser

      1016       1025       1034       1043
TCA GAA GAT ATT GCT GAA TTT TAT CAA AGA CAA TTA AAA
Ser Glu Asp Ile Ala Glu Phe Tyr Gln Arg Gln Leu Lys

  1052       1061       1070       1079
CTT ACG CAA CAA TAC ACT GAC CAT TGT GTC AAT TGG TAT
Leu Thr Gln Gln Tyr Thr Asp His Cys Val Asn Trp Tyr
```

FIGURE 1 (continued 3)

```
1088        1097        1106        1115        1124
 AAT GTT GGA TTA AAT AGT TTA AGA GGT TCA ACT TAT GAT
 Asn Val Gly Leu Asn Ser Leu Arg Gly Ser Thr Tyr Asp

       1133        1142        1151        1160
 GCA TGG GTC AAA TTT AAC CGT TTT CGC AGA GAA ATG ACA
 Ala Trp Val Lys Phe Asn Arg Phe Arg Arg Glu MET Thr

   1169        1178        1187        1196
 TTA ACT GTA TTA GAT CTA ATT GTA TTA TTC CCA TTT TAT
 Leu Thr Val Leu Asp Leu Ile Val Leu Phe Pro Phe Tyr

1205        1214        1223        1232        1241
 GAT GTT CGG TTA TAC TCA AAA GGA GTT AAA ACA GAA CTA
 Asp Val Arg Leu Tyr Ser Lys Gly Val Lys Thr Glu Leu

       1250        1259        1268        1277
 ACA AGA GAC ATT TTT ACA GAT CCA ATT TTT ACA CTC AAT
 Thr Arg Asp Ile Phe Thr Asp Pro Ile Phe Thr Leu Asn

   1286        1295        1304        1313
 GCT CTT CAA GAG TAT GGA CCA ACT TTT TCG AGT ATA GAA
 Ala Leu Gln Glu Tyr Gly Pro Thr Phe Ser Ser Ile Glu

1322        1331        1340        1349        1358
 AAC TCT ATT CGA AAA CCT CAT TTA TTT GAT TAT TTG CGT
 Asn Ser Ile Arg Lys Pro His Leu Phe Asp Tyr Leu Arg

       1367        1376        1385        1394
 GGG ATT GAA TTT CAT ACG CGT CTT CGA CCT GGT TAC TCT
 Gly Ile Glu Phe His Thr Arg Leu Arg Pro Gly Tyr Ser
```

FIGURE 1 (continued 4)

```
  1403        1412        1421        1430
GGG AAA GAT TCT TTC AAT TAT TGG TCT GGT AAT TAT GTA
Gly Lys Asp Ser Phe Asn Tyr Trp Ser Gly Asn Tyr Val

1439        1448        1457        1466        1475
 GAA ACT AGA CCT AGT ATA GGA TCT AAT GAT ACA ATC ACT
 Glu Thr Arg Pro Ser Ile Gly Ser Asn Asp Thr Ile Thr

       1484        1493        1502        1511
 TCC CCA TTT TAT GGA GAT AAA TCT ATT GAA CCT ATA CAA
 Ser Pro Phe Tyr Gly Asp Lys Ser Ile Glu Pro Ile Gln

  1520        1529        1538        1547
 AAG CTA AGC TTT GAT GGA CAA AAA GTT TAT CGA ACT ATA
 Lys Leu Ser Phe Asp Gly Gln Lys Val Tyr Arg Thr Ile

1556        1565        1574        1583        1592
 GCT AAT ACA GAC ATA GCG GCT TTT CCG GAT GGC AAG ATA
 Ala Asn Thr Asp Ile Ala Ala Phe Pro Asp Gly Lys Ile

       1601        1610        1619        1628
 TAT TTT GGT GTT ACG AAA GTT GAT TTT AGT CAA TAT GAT
 Tyr Phe Gly Val Thr Lys Val Asp Phe Ser Gln Tyr Asp

  1637        1646        1655        1664
 GAT CAA AAA AAT GAA ACT AGT ACA CAA ACA TAT GAT TCA
 Asp Gln Lys Asn Glu Thr Ser Thr Gln Thr Tyr Asp Ser

1673        1682        1691        1700        1709
 AAA AGA TAC AAT GGC TAT TTA GGT GCA CAG GAT TCT ATC
 Lys Arg Tyr Asn Gly Tyr Leu Gly Ala Gln Asp Ser Ile
```

FIGURE 1 (continued 5)

```
      1718        1727        1736        1745
GAC CAA TTA CCA CCA GAA ACA ACA GAT GAA CCA CTT GAA
Asp Gln Leu Pro Pro Glu Thr Thr Asp Glu Pro Leu Glu

   1754        1763        1772        1781
AAA GCA TAT AGT CAT CAG CTT AAT TAC GCA GAA TGT TTC
Lys Ala Tyr Ser His Gln Leu Asn Tyr Ala Glu Cys Phe

1790        1799        1808        1817        1826
TTA ATG CAG GAC CGT CGT GGA ACA ATT CCA TTT TTT ACT
Leu MET Gln Asp Arg Arg Gly Thr Ile Pro Phe Phe Thr

      1835        1844        1853        1862
TGG ACA CAT AGA AGT GTA GAC TTT TTT AAT ACA ATT GAT
Trp Thr His Arg Ser Val Asp Phe Phe Asn Thr Ile Asp

   1871        1880        1889        1898
GCT GAA AAA ATT ACT CAA CTT CCA GTA GTG AAA GCA TAT
Ala Glu Lys Ile Thr Gln Leu Pro Val Val Lys Ala Tyr

1907        1916        1925        1934        1943
GCC TTG TCT TCA GGC GCT TCC ATT ATT GAA GGT CCA GGA
Ala Leu Ser Ser Gly Ala Ser Ile Ile Glu Gly Pro Gly

      1952        1961        1970        1979
TTC ACA GGA GGA AAT TTA CTA TTC CTA AAA GAA TCT AGT
Phe Thr Gly Gly Asn Leu Leu Phe Leu Lys Glu Ser Ser

   1988        1997        2006        2015
AAT TCA ATT GCT AAA TTT AAA GTT ACC TTA AAT TCA GCA
Asn Ser Ile Ala Lys Phe Lys Val Thr Leu Asn Ser Ala
```

FIGURE 1 (continued 6)

```
2024        2033        2042        2051        2060
 GCC TTG TTA CAA CGA TAT CGC GTA AGA ATA CGC TAT GCT
 Ala Leu Leu Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala

            2069        2078        2087        2096
 TCA ACC ACT AAC CTA CGA CTT TTC GTG CAA AAT TCA AAC
 Ser Thr Thr Asn Leu Arg Leu Phe Val Gln Asn Ser Asn

    2105        2114        2123        2132
 AAT GAT TTT CTT GTC ATC TAC ATT AAT AAA ACT ATG AAT
 Asn Asp Phe Leu Val Ile Tyr Ile Asn Lys Thr MET Asn

2141        2150        2159        2168        2177
 ATA GAT GGT GAT TTA ACA TAT CAA ACA TTT GAT TTC GCA
 Ile Asp Gly Asp Leu Thr Tyr Gln Thr Phe Asp Phe Ala

            2186        2195        2204        2213
 ACT AGT AAT TCT AAT ATG GGA TTC TCT GGT GAT ACA AAT
 Thr Ser Asn Ser Asn MET Gly Phe Ser Gly Asp Thr Asn

    2222        2231        2240        2249
 GAC TTT ATA ATA GGA GCA GAA TCT TTC GTT TCT AAT GAA
 Asp Phe Ile Ile Gly Ala Glu Ser Phe Val Ser Asn Glu

2258        2267        2276        2285        2294
 AAA ATC TAT ATA GAT AAG ATA GAA TTT ATC CCA GTA CAA
 Lys Ile Tyr Ile Asp Lys Ile Glu Phe Ile Pro Val Gln

 <          2307        2317        2327        2337        2347
 TAG CAAGTGAATT TTGGAATATA GGGCGATGGT CAAAATGAAA GGATAAGAAG
```

FIGURE 1 (continued 7)

```
      2357       2367       2377       2387       2397
GTGAATTTTG ATGGTTAGGA AAGATTCTTT TAACAAAAGC AACATGGAAA

      2407       2417
AGTATACAGT ACAAATGGGT ACCGAGCT
```

FIGURE 2

```
          10         20         30         40         50
TTTGGATTGT GAGCATGTAC AGGTTTGTGA TTTACAAGCA AAACCAATCT

          60         70         80         90         100
GCGAAGATTG TTGTCATTTT ATAAAGGTAA CAGGATATTT TCAAATTTGT

        110        120        130        140        150
ACCGATTAAA TAAAAAATAT TTAGATTAAC ACTGTTGTTT TTTACAACTA

        160        170        180     >  189
TCCGTATGGA CAAATTTAAC AAGGAGTGAA AAT ATG AAT TTA
                                       MET Asn Leu

    198        207        216        225
AAT AAT TTA GAT GGA TAT GAA GAT AGT AAT AGA ACA TTA
Asn Asn Leu Asp Gly Tyr Glu Asp Ser Asn Arg Thr Leu

234        243        252        261        270
AAT AAT TCT CTC AAT TAT CCT ACT CAA AAA GCA TTA TCA
Asn Asn Ser Leu Asn Tyr Pro Thr Gln Lys Ala Leu Ser

        279        288        297        306
CCA TCA TTA AAG AAT ATG AAC TAC CAG GAT TTT TTA TCT
Pro Ser Leu Lys Asn MET Asn Tyr Gln Asp Phe Leu Ser

    315        324        333        342
ATA ACT GAG AGG GAA CAA CCT GAA GCA CTC GCT AGT GGT
Ile Thr Glu Arg Glu Gln Pro Glu Ala Leu Ala Ser Gly

351        360        369        378        387
AAT ACA GCT ATT AAT ACT GTA GTT AGT GTT ACG GGG GCT
Asn Thr Ala Ile Asn Thr Val Val Ser Val Thr Gly Ala
```

FIGURE 2 (continued 1)

```
          396         405         414         423
ACA CTA AGT GCG TTA GGT GTC CCA GGT GCA AGT TTT ATC
Thr Leu Ser Ala Leu Gly Val Pro Gly Ala Ser Phe Ile

     432         441         450         459
ACT AAC TTT TAC CTG AAA ATT GCA GGC CTT TTA TGG CCA
Thr Asn Phe Tyr Leu Lys Ile Ala Gly Leu Leu Trp Pro

468         477         486         495         504
GAA AAT GGA AAA ATT TGG GAT GAA TTT ATG ACA GAA GTA
Glu Asn Gly Lys Ile Trp Asp Glu Phe MET Thr Glu Val

          513         522         531         540
GAA GCA CTT ATT GAT CAA AAA ATA GAA GAA TAT GTA AGA
Glu Ala Leu Ile Asp Gln Lys Ile Glu Glu Tyr Val Arg

     549         558         567         576
AAT AAA GCG ATT GCA GAA TTA GAT GGA TTA GGA TCA GCC
Asn Lys Ala Ile Ala Glu Leu Asp Gly Leu Gly Ser Ala

585         594         603         612         621
TTA GAT AAA TAT CAA AAA GCA CTT GCA GAT TGG CTG GGC
Leu Asp Lys Tyr Gln Lys Ala Leu Ala Asp Trp Leu Gly

          630         639         648         657
AAA CAA GAT GAT CCA GAA GCT ATA CTT TCT GTG GCA ACT
Lys Gln Asp Asp Pro Glu Ala Ile Leu Ser Val Ala Thr

     666         675         684         693
GAA TTT CGT ATA ATA GAT TCT CTT TTT GAA TTT AGT ATG
Glu Phe Arg Ile Ile Asp Ser Leu Phe Glu Phe Ser MET
```

FIGURE 2 (continued 2)

```
702         711         720         729         738
CCT TCA TTT AAG GTT ACT GGA TAT GAA ATA CCA TTA CTA
Pro Ser Phe Lys Val Thr Gly Tyr Glu Ile Pro Leu Leu

        747         756         765         774
ACA GTT TAC GCA CAA GCG GCA AAC CTT CAT CTA GCT TTA
Thr Val Tyr Ala Gln Ala Ala Asn Leu His Leu Ala Leu

    783         792         801         810
TTA AGA GAT TCT ACT CTT TAT GGA GAT AAA TGG GGA TTC
Leu Arg Asp Ser Thr Leu Tyr Gly Asp Lys Trp Gly Phe

819         828         837         846         855
ACT CAG AAC AAC ATT GAG GAA AAT TAT AAT CGT CAA AAG
Thr Gln Asn Asn Ile Glu Glu Asn Tyr Asn Arg Gln Lys

        864         873         882         891
AAA CGC ATT TCT GAA TAT TCA GAC CAT TGC ACC AAG TGG
Lys Arg Ile Ser Glu Tyr Ser Asp His Cys Thr Lys Trp

    900         909         918         927
TAT AAT AGT GGT CTT AGC AGA TTG AAC GGT TCC ACT TAT
Tyr Asn Ser Gly Leu Ser Arg Leu Asn Gly Ser Thr Tyr

936         945         954         963         972
GAA CAA TGG ATA AAT TAT AAT CGT TTT CGT AGA GAA ATG
Glu Gln Trp Ile Asn Tyr Asn Arg Phe Arg Arg Glu MET

        981         990         999        1008
ATA TTA ATG GCA TTA GAT CTT GTC GCT GTA TTT CCT TTT
Ile Leu MET Ala Leu Asp Leu Val Ala Val Phe Pro Phe
```

FIGURE 2 (continued 3)

```
   1017       1026       1035       1044
CAT GAC CCT CGA AGG TAT TCA ATG GAA ACA AGT ACG CAG
His Asp Pro Arg Arg Tyr Ser MET Glu Thr Ser Thr Gln

1053       1062       1071       1080       1089
TTA ACG AGA GAA GTG TAT ACC GAT CCA GTT AGC TTG TCA
Leu Thr Arg Glu Val Tyr Thr Asp Pro Val Ser Leu Ser

      1098       1107       1116       1125
ATT AGC AAT CCA GAT ATA GGT CCA AGT TTT TCT CAG ATG
Ile Ser Asn Pro Asp Ile Gly Pro Ser Phe Ser Gln MET

   1134       1143       1152       1161
GAA AAT ACT GCA ATT AGA ACA CCA CAC CTT GTT GAT TAT
Glu Asn Thr Ala Ile Arg Thr Pro His Leu Val Asp Tyr

1170       1179       1188       1197       1206
TTA GAT GAG CTT TAT ATA TAT ACA TCA AAA TAT AAA GCA
Leu Asp Glu Leu Tyr Ile Tyr Thr Ser Lys Tyr Lys Ala

      1215       1224       1233       1242
TTT TCA CAT GAG ATT CAA CCA GAC CTA TTT TAT TGG AGT
Phe Ser His Glu Ile Gln Pro Asp Leu Phe Tyr Trp Ser

   1251       1260       1269       1278
GCA CAT AAG GTT AGC TTT AAA AAA TCG GAG CAA TCC AAT
Ala His Lys Val Ser Phe Lys Lys Ser Glu Gln Ser Asn

1287       1296       1305       1314       1323
TTA TAT ACA ACA GGC ATA TAT GGT AAA ACA AGT GGA TAT
Leu Tyr Thr Thr Gly Ile Tyr Gly Lys Thr Ser Gly Tyr
```

FIGURE 2 (continued 4)

```
      1332        1341        1350        1359
ATT TCA TCA GGG GCA TAT TCA TTT CAT GGG AAT GAT ATC
Ile Ser Ser Gly Ala Tyr Ser Phe His Gly Asn Asp Ile

   1368        1377        1386        1395
TAT AGA ACA TTA GCA GCT CCA TCA GTT GTA GTT TAT CCG
Tyr Arg Thr Leu Ala Ala Pro Ser Val Val Val Tyr Pro

1404        1413        1422        1431        1440
TAT ACT CAG AAT TAT GGT GTC GAG CAA GTT GAG TTT TAC
Tyr Thr Gln Asn Tyr Gly Val Glu Gln Val Glu Phe Tyr

      1449        1458        1467        1476
GGT GTA AAA GGG CAT GTA CAT TAT AGA GGA GAT AAC AAA
Gly Val Lys Gly His Val His Tyr Arg Gly Asp Asn Lys

   1485        1494        1503        1512
TAT GAT CTG ACG TAT GAT TCT ATT GAT CAA TTA CCC CCA
Tyr Asp Leu Thr Tyr Asp Ser Ile Asp Gln Leu Pro Pro

1521        1530        1539        1548        1557
GAC GGA GAA CCA ATA CAC GAA AAA TAC ACT CAT CGA TTA
Asp Gly Glu Pro Ile His Glu Lys Tyr Thr His Arg Leu

      1566        1575        1584        1593
TGT CAT GCT ACA GCT ATA TTT AAA TCA ACT CCG GAT TAT
Cys His Ala Thr Ala Ile Phe Lys Ser Thr Pro Asp Tyr

   1602        1611        1620        1629
GAT AAT GCT ACT ATC CCG ATC TTT TCT TGG ACG CAT AGA
Asp Asn Ala Thr Ile Pro Ile Phe Ser Trp Thr His Arg
```

FIGURE 2 (continued 5)

```
1638        1647        1656        1665        1674
 AGT GCG GAG TAT TAC AAT AGA ATC TAT CCA AAC AAA ATC
 Ser Ala Glu Tyr Tyr Asn Arg Ile Tyr Pro Asn Lys Ile

     1683        1692        1701        1710
 ACA AAA ATT CCA GCT GTA AAA ATG TAT AAA CTA GAT GAT
 Thr Lys Ile Pro Ala Val Lys MET Tyr Lys Leu Asp Asp

    1719        1728        1737        1746
 CCA TCT ACA GTT GTC AAA GGG CCT GGA TTT ACA GGT GGA
 Pro Ser Thr Val Val Lys Gly Pro Gly Phe Thr Gly Gly

1755        1764        1773        1782        1791
 GAT TTA GTT AAG AGA GGG AGT ACT GGT TAT ATA GGA GAT
 Asp Leu Val Lys Arg Gly Ser Thr Gly Tyr Ile Gly Asp

     1800        1809        1818        1827
 ATA AAG GCT ACC GTA AAC TCT CCA CTT TCT CAA AAA TAT
 Ile Lys Ala Thr Val Asn Ser Pro Leu Ser Gln Lys Tyr

    1836        1845        1854        1863
 CGT GTT AGA GTT CGA TAC GCT ACT AAT GTT TCT GGA CAA
 Arg Val Arg Val Arg Tyr Ala Thr Asn Val Ser Gly Gln

1872        1881        1890        1899        1908
 TTC AAC GTG TAT ATT AAT GAT AAA ATA ACG CTT CAA ACA
 Phe Asn Val Tyr Ile Asn Asp Lys Ile Thr Leu Gln Thr

     1917        1926        1935        1944
 AAG TTT CAA AAT ACT GTA GAA ACA ATA GGT GAA GGA AAA
 Lys Phe Gln Asn Thr Val Glu Thr Ile Gly Glu Gly Lys
```

FIGURE 2 (continued 6)

```
   1953        1962        1971        1980
GAT TTA ACC TAT GGT TCA TTT GGA TAT ATA GAA TAT TCT
Asp Leu Thr Tyr Gly Ser Phe Gly Tyr Ile Glu Tyr Ser

1989        1998        2007        2016        2025
ACG ACC ATT CAA TTT CCG GAT GAG CAT CCA AAA ATC ACT
Thr Thr Ile Gln Phe Pro Asp Glu His Pro Lys Ile Thr

      2034        2043        2052        2061
CTT CAT TTA AGC GAT TTG AGT AAC AAT TCA TCA TTT TAT
Leu His Leu Ser Asp Leu Ser Asn Asn Ser Ser Phe Tyr

   2070        2079        2088        2097
GTA GAT TCA ATC GAA TTT ATC CCT GTA GAT GTA AAT TAT
Val Asp Ser Ile Glu Phe Ile Pro Val Asp Val Asn Tyr

2106        2115        2124        2133        2142
GCT GAA AAA GAA AAA CTA GAA AAA GCA CAG AAA GCC GTG
Ala Glu Lys Glu Lys Leu Glu Lys Ala Gln Lys Ala Val

      2151        2160        2169        2178
AAT ACC TTG TTT ACA GAG GGA AGA AAT GCA CTC CAA AAA
Asn Thr Leu Phe Thr Glu Gly Arg Asn Ala Leu Gln Lys

   2187        2196        2205        2214
GAC GTG ACA GAT TAT AAA GTG GAC CAG GTT TCA ATT TTA
Asp Val Thr Asp Tyr Lys Val Asp Gln Val Ser Ile Leu

2223        2232        2241        2250        2259
GTG GAT TGT ATA TCA GGG GAT TTA TAT CCC AAT GAG AAA
Val Asp Cys Ile Ser Gly Asp Leu Tyr Pro Asn Glu Lys
```

FIGURE 2 (continued 7)

```
      2268        2277        2286        2295
CGC GAA CTA CAA AAT CTA GTC AAA TAC GCA AAA CGT TTG
Arg Glu Leu Gln Asn Leu Val Lys Tyr Ala Lys Arg Leu

   2304        2313        2322        2331
AGC TAT TCC CGT AAT TTA CTT CTA GAT CCA ACA TTC GAT
Ser Tyr Ser Arg Asn Leu Leu Leu Asp Pro Thr Phe Asp

2340        2349        2358        2367        2376
TCT ATT AAT TCA TCT GAG GAG AAT GGT TGG TAT GGA AGT
Ser Ile Asn Ser Ser Glu Glu Asn Gly Trp Tyr Gly Ser

      2385        2394        2403        2412
AAT GGT ATT GTG ATT GGA AAT GGG GAT TTT GTA TTC AAA
Asn Gly Ile Val Ile Gly Asn Gly Asp Phe Val Phe Lys

   2421        2430        2439        2448
GGT AAC TAT TTA ATT TTT TCA GGT ACC AAT GAT ACA CAA
Gly Asn Tyr Leu Ile Phe Ser Gly Thr Asn Asp Thr Gln

2457        2466        2475        2484        2493
TAT CCA ACA TAT CTC TAC CAA AAA ATA GAT GAA TCC AAA
Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys

      2502        2511        2520        2529
CTC AAA GAA TAT ACA CGC TAT AAA CTG AAA GGT TTT ATC
Leu Lys Glu Tyr Thr Arg Tyr Lys Leu Lys Gly Phe Ile

   2538        2547        2556        2565
GAA AGT AGT CAG GAT TTA GAA GCT TAT GTG ATT CGC TAT
Glu Ser Ser Gln Asp Leu Glu Ala Tyr Val Ile Arg Tyr
```

FIGURE 2 (continued 8)

```
2574        2583        2592        2601        2610
 GAT GCA AAA CAT AGA ACA TTG GAT GTT TCT GAT AAT CTA
 Asp Ala Lys His Arg Thr Leu Asp Val Ser Asp Asn Leu

       2619        2628        2637        2646
 TTA CCA GAT ATT CTC CCT GAG AAT ACA TGT GGA GAA CCA
 Leu Pro Asp Ile Leu Pro Glu Asn Thr Cys Gly Glu Pro

    2655        2664        2673        2682
 AAT CGC TGC GCG GCA CAA CAA TAC CTG GAT GAA AAT CCA
 Asn Arg Cys Ala Ala Gln Gln Tyr Leu Asp Glu Asn Pro

2691        2700        2709        2718        2727
 AGT CCA GAA TGT AGT TCG ATG CAA GAT GGA ATT TTG TCT
 Ser Pro Glu Cys Ser Ser MET Gln Asp Gly Ile Leu Ser

       2736        2745        2754        2763
 GAT TCG CAT TCA TTT TCT CTT AAT ATA GAT ACA GGT TCT
 Asp Ser His Ser Phe Ser Leu Asn Ile Asp Thr Gly Ser

    2772        2781        2790        2799
 ATC AAT CAC AAT GAG AAT TTA GGA ATT TGG GTG TTG TTT
 Ile Asn His Asn Glu Asn Leu Gly Ile Trp Val Leu Phe

2808        2817        2826        2835        2844
 AAA ATT TCG ACA TTA GAA GGA TAT GCG AAA TTT GGA AAT
 Lys Ile Ser Thr Leu Glu Gly Tyr Ala Lys Phe Gly Asn

       2853        2862        2871        2880
 CTA GAA GTG ATT GAA GAT GGC CCA GTT ATT GGA GAA GCA
 Leu Glu Val Ile Glu Asp Gly Pro Val Ile Gly Glu Ala
```

FIGURE 2 (continued 9)

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2889       2898       2907       2916
TTA GCC CGT GTG AAA CGC CAA GAA ACG AAG TGG AGA AAC
Leu Ala Arg Val Lys Arg Gln Glu Thr Lys Trp Arg Asn

2925       2934       2943       2952       2961
AAG TTA GCC CAA CTG ACA ACG GAA ACA CAA GCG ATT TAT
Lys Leu Ala Gln Leu Thr Thr Glu Thr Gln Ala Ile Tyr

      2970       2979       2988       2997
ACA CGA GCA AAA CAA GCG CTG GAT AAT CTT TTT GCG AAT
Thr Arg Ala Lys Gln Ala Leu Asp Asn Leu Phe Ala Asn

   3006       3015       3024       3033
GCA CAA GAC TCT CAC TTA AAA AGA GAT GTT ACA TTT GCG
Ala Gln Asp Ser His Leu Lys Arg Asp Val Thr Phe Ala

3042       3051       3060       3069       3078
GAA ATT GCG GCT GCA AGA AAG ATT GTC CAA TCA ATA CGC
Glu Ile Ala Ala Ala Arg Lys Ile Val Gln Ser Ile Arg

      3087       3096       3105       3114
GAA GCG TAT ATG TCA TGG TTA TCT GTT GTT CCA GGT GTA
Glu Ala Tyr MET Ser Trp Leu Ser Val Val Pro Gly Val

   3123       3132       3141       3150
AAT CAC CCT ATT TTT ACA GAG TTA AGT GGG CGA GTA CAA
Asn His Pro Ile Phe Thr Glu Leu Ser Gly Arg Val Gln

3159       3168       3177       3186       3195
CGA GCA TTT CAA TTA TAT GAT GTA CGA AAT GTT GTG CGT
Arg Ala Phe Gln Leu Tyr Asp Val Arg Asn Val Val Arg
```

FIGURE 2 (continued 10)

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      3204        3213        3222        3231
AAT GGT CGA TTC CTC AAT GGC TTA TCC GAT TGG ATT GTA
Asn Gly Arg Phe Leu Asn Gly Leu Ser Asp Trp Ile Val

  3240        3249        3258        3267
ACA TCT GAC GTA AAG GTA CAA GAA GAA AAT GGG AAT AAC
Thr Ser Asp Val Lys Val Gln Glu Glu Asn Gly Asn Asn

3276        3285        3294        3303        3312
GTA TTA GTT CTT AAC AAT TGG GAT GCA CAA GTA TTA CAA
Val Leu Val Leu Asn Asn Trp Asp Ala Gln Val Leu Gln

      3321        3330        3339        3348
AAC GTA AAA CTC TAT CAA GAC CGT GGG TAT ATC TTA CAT
Asn Val Lys Leu Tyr Gln Asp Arg Gly Tyr Ile Leu His

   3357        3366        3375        3384
GTA ACA GCG CGC AAG ATA GGA ATT GGG GAA GGA TAT ATA
Val Thr Ala Arg Lys Ile Gly Ile Gly Glu Gly Tyr Ile

3393        3402        3411        3420        3429
ACG ATT ACG GAT GAA GAA GGG CAT ACA GAT CAA TTG AGA
Thr Ile Thr Asp Glu Glu Gly His Thr Asp Gln Leu Arg

      3438        3447        3456        3465
TTT ACT GCA TGT GAA GAG ATT GAT GCA TCT AAT GCG TTT
Phe Thr Ala Cys Glu Glu Ile Asp Ala Ser Asn Ala Phe

   3474        3483        3492        3501
ATA TCC GGT TAT ATT ACA AAA GAA CTG GAA TTC TTC CCA
Ile Ser Gly Tyr Ile Thr Lys Glu Leu Glu Phe Phe Pro
```

FIGURE 2 (continued 11)

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3510        3519        3528        3537        3546
 GAT ACA GAG AAA GTG CAT ATA GAA ATA GGC GAA ACA GAA
 Asp Thr Glu Lys Val His Ile Glu Ile Gly Glu Thr Glu

      3555        3564        3573        3582
GGA ATA TTC CTG GTA GAA AGT ATA GAG TTA TTT TTG ATG
Gly Ile Phe Leu Val Glu Ser Ile Glu Leu Phe Leu MET

   3591        3600 <  .  3610        3620        3630
GAA GAG CTA TGT TAA TAGGGAGATT ATTCAACAAA TATTTGTTTG
Glu Glu Leu Cys  .

      3640        3650        3660        3670        3680
ATTCAAAATA AAATAAAATG CATACAATCC TCTTTATCAG ACGGTATTTC

      3690        3700        3710        3720        3730
TAATAATTAT AAATATAGGT TGAAAGTTAA AAAATAAAAA CACGCTATTC

      3740        3750        3760        3770        3780
CCATTACTAG AAGGAGGGAG TAACGTGTTT TTTCATGAGT AAAAAAACAA

      3790        3800        3810        3820        3830
TTAGCTATAT TTATCTATTC TCTATAGAAG AAGCGGATTG ATAAGAACCG

      3840        3850        3860        3870        3880
TAAGTGACAG GAATAGCATT TATATCTTAT AGTGCAAGTC CAAACAAATG

      3890        3900        3910        3920        3930
AGGGTAGTAG AGTGACAAAA ACGCTTGAAG TTTTCCAAAA AAGAAATCAA

      3940        3950        3960        3970        3980
GTACAAATTG AAATTAGTAC AACAAATGTT ATTTCTTTAG TAGAACGTAT
```

FIGURE 2 (continued 12)

3990 4000
AGAATTATTA TGTTTGGAAG ATGA

Total protein pattern of
sporulated B.t.s by SDS-PAGE

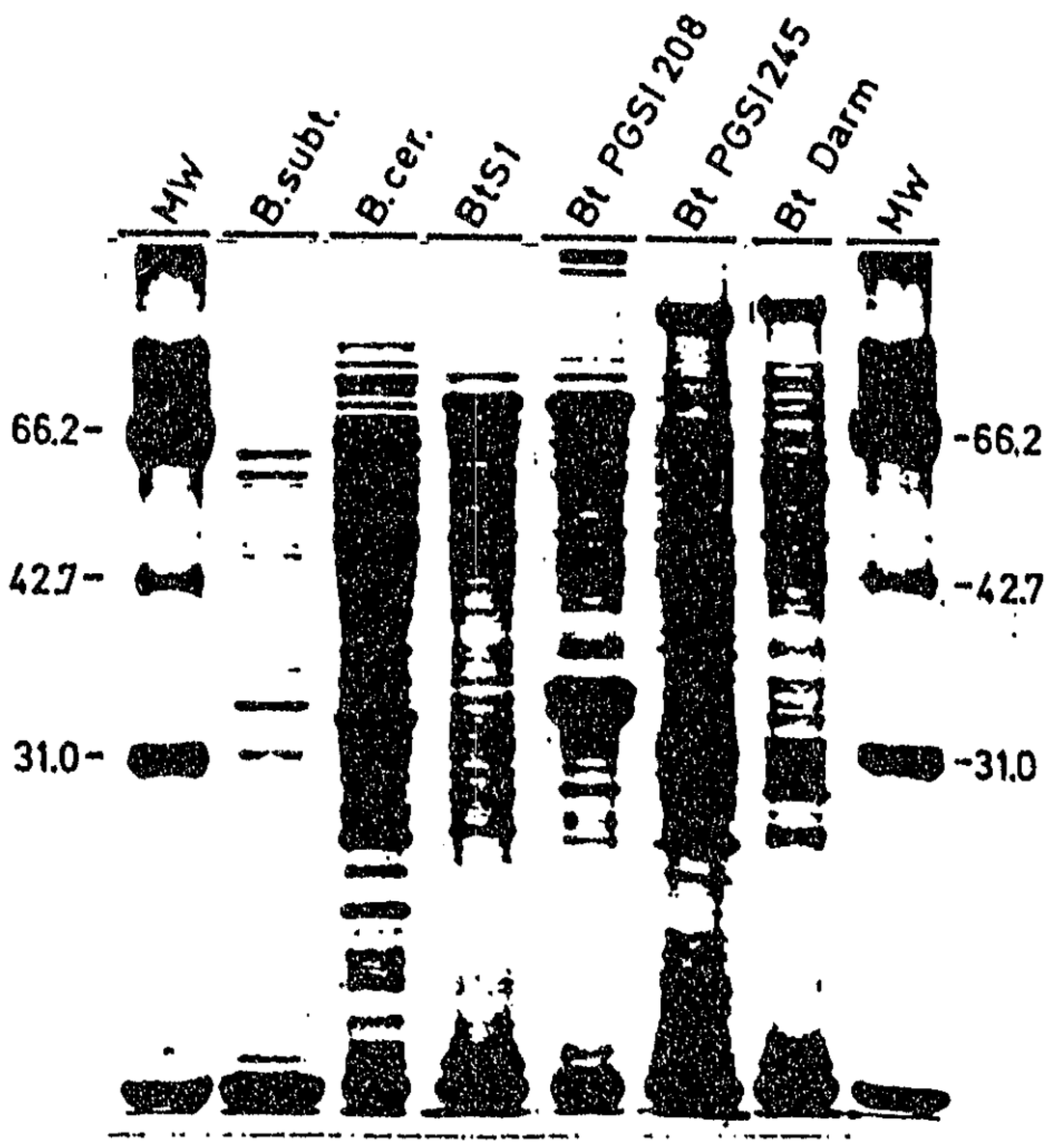

Figure 3

Protein blotting of BtS1 and PGSI208 proteins and trypsinized crystal proteins

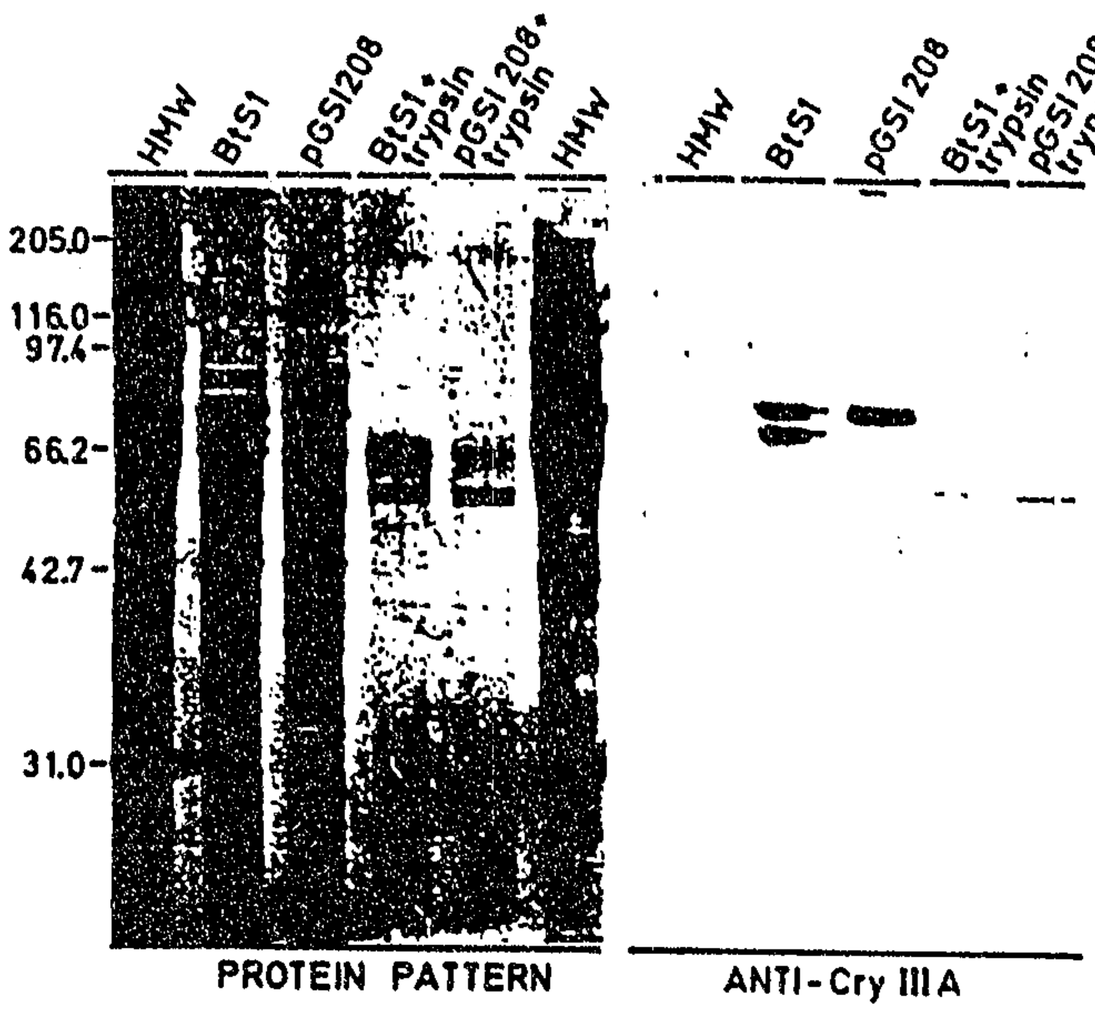

Figure 4a

Protein blotting of HD110, PGSI245 and BtS1 solubilized and trypsinized crystal proteins

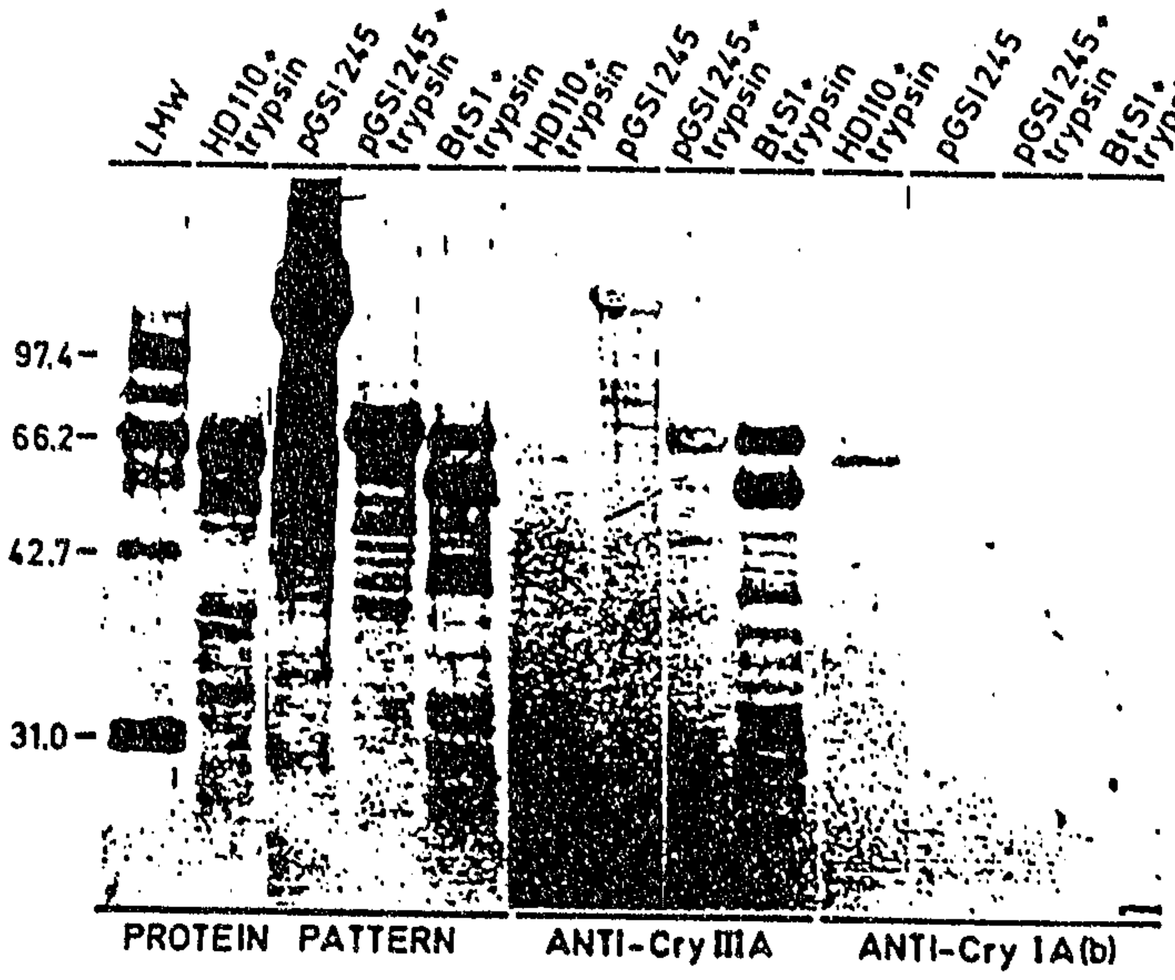

Figure 4b