

Baculovirus Cloning, Biopesticides, & Related Products

BTI-47A

In re Application of: Gary W. Blissard
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 6,858,205
Serial No.: 09/925,365
Filed: August 9, 2001
Priority date: August 11, 2000 (Ser. No. 60/224,612)
For: **A GP64NULL BACULOVIRUS PSEUDOTYPED WITH VSV G-PROTEIN**

Status

Issued.

Related Files or Patent Applications

Pending PCT Application (Ser. No. PCT/US01/25047), filed on August 10, 2001.

Summary of Technology

This application discloses a pseudotyped baculovirus comprising a deletion, inactivation or reduction from regulation of a baculovirus envelope protein gene, and is engineered to express an envelope protein from another virus or cell, or another protein or molecule that facilitates entry of said baculovirus into a non-host cell, or provided with a heterologous envelope protein or another protein or molecule that facilitates entry of said baculovirus into a non-host cell by other suitable means. Such baculoviruses can be used to efficiently deliver genes to mammalian cells or organisms, and such genes can be expressed either from the baculovirus genome, or integrated into the mammalian cell genome, and can be used for expression of proteins such that purification of secreted or other protein products does not require removal of contaminating baculovirus particles or baculovirus envelope proteins.

Claim Coverage

The claims generally are directed to a genetically engineered baculovirus, comprising a deletion, inactivation or reduction from regulation of an envelope protein gene of a progenitor baculovirus, from which said engineered baculovirus is derived, wherein said genetically engineered virus is supplied with a heterologous envelope protein or a protein or other molecule that facilitates entry of said engineered baculovirus into a cell that is not normally a host of said progenitor baculovirus, by expression of said heterologous envelope protein in a cell line and subsequent infection of said cell line with said engineered baculovirus, or by further engineering said baculovirus to express said heterologous envelope protein, or by other suitable means.

BTI-39

In re Application of: Robert Granados, Ping Wang
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 6,187,558
Serial No.: 09/103,429
Filed: June 24, 1998
Priority date: NA
For: **INVERTEBRATE INTESTINAL MUCIN cDNA AND RELATED PRODUCTS AND METHODS**

Status

Patent issued February 13, 2001.

Related Files or Patent Applications

PCT Application (Ser. No. PCT/US99/14220), filed on June 23, 1999, entered the National Phase in Canada on June 23, 1999; a continuation-in-part application was filed on April 19, 1999 (BTI-39CIP), incorporating new matter pertaining to additional mucin proteins. The CIP application is on Appeal at the USPTO; the Canadian application is pending.

Summary of Technology

This patent discloses a novel insect intestinal mucin comprising two nearly identical isoforms, IIM14 and IIM22 respectively. These isoforms of the IIM protein have been identified and cloned using *T. ni* larvae. The cDNA and amino acid sequences have been determined and are disclosed. Both IIM isoforms have an approximate molecular mass of 400 kDa. These sequences are useful for the production of transgenic or recombinant vectors including viral, microorganism cell, plant, or animals, wherein the virus, microorganism, cell, plant, or animal is the product of an insertion of a gene expression vector including a DNA that encodes an IIM protein sequence. Also useful is a purified and isolated recombinant DNA sequence comprising a DNA sequence that codes for an IIM protein. The recombinant DNA sequence used can be a cDNA sequence for either IIM isoform IIM14 or IIM22. The invention also provides for the use of the purified amino acid sequences of IIM isoforms IIM14 or IIM22. With this knowledge of the proteinaceous components of the PM, and particularly the mucin-like proteins it will be possible to enhance the effectiveness of bio-engineered pesticides, recombinant viral vectors, enhance the defenses of transgenic plants, or protect insect vectors susceptible to attack by organisms utilizing enhancin or enhancin-like enzymes.

Claim Coverage

The claims generally are directed to an isolated polynucleotide encoding an invertebrate intestinal mucin.

BTI-35

In re Application of: Robert R. Granados and Yoshifumi Hashimoto
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 5,475,090
Serial No.: 07/971,624
Filed: November 4, 1992
Priority date: February 21, 1989 (Ser. No. 07/313,226)
For: **GENE CODED FOR A POLYPEPTIDE WHICH ENHANCES
VIRUS INFECTION OF HOST INSECTS**

Status

Patent issued December 12, 1995.

Related Files or Patent Applications

PCT Application (Ser. No. PCT/US96/13645), filed on August 23, 1996, entered the National Phase in Canada; the Canadian application is pending.

Summary of Technology

This patent discloses baculovirus genes encoding polypeptides termed enhancins. The polypeptides are isolated from the occlusion body of certain baculoviruses, such as *Trichoplusia ni* granulosis virus and *Pseudaletia unipuncta* granulosis virus, Hawaiian strain. The polypeptides have the ability of enhancing the infectivity of baculoviruses and are useful ingredients of pest control compositions.

Claim Coverage

The claims generally are directed to an isolated and purified enhancin found in granulosis viruses obtained from within the vital occlusion body, the enhancin retaining the physical, chemical and biological properties of the enhancin of FIG. 3 or the PuGV DNA of FIG. 6, the enhancin purified by centrifugation and chromatography on a Sephacryl column and displays on a SDS-PAGE analysis no multiple bands and has a disruptive effect on the insect peritrophic membrane proteins and/or interacts with the midgut epithelium in such a manner as to effect the increased absorption, penetration, and uptake of virus by midgut cells with a concomitant increase in host mortality, the percent increase in mortality exceeding 50% when 10 ng of the enhancin per larvae is mixed with *Autographa californica* nuclear polyhedrosis (AcMNPV) inoculum for infection of *Trichoplusia ni* larvae.

BTI-34D1

In re Application of: Robert R. Granados
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 5,011,685
Serial No.: 07/580,083
Filed: September 10, 1990
Priority date: April 6, 1988 (Ser. No. 07/178,259)
For: **BACULOVIRUS PROTEINS AND VIRAL PESTICIDES
CONTAINING SAME**

Status

Patent issued April 30, 1991.

Related Files or Patent Applications

BTI-34 (issued); BTI-34CAN (issued).

Summary of Technology

This patent discloses Nuclear Polyhedrosis Viruses, for example, *Autographa californica* Nuclear Polyhedrosis Virus (AcMNPV), that are useful in the control of lepidopterous larvae, such as the larvae of the cabbage looper *Trichoplusia ni*, and have been found to have enhanced infectivity, when mixed with certain proteins obtained from the granulin fraction of *Trichoplusia ni* Granulosis Virus (TnGV) or *Heliothis armigera* Granulosis Virus (HaGV), and from the polyhedrin fraction of AcMNPV viruses. The proteins from the TnGV granulin fraction have molecular weights of about 101 and about 104 Kda. The enhanced infectivity is correlated to biochemical and structural changes in the *T. ni* peritrophic membrane.

Claim Coverage

The claims generally are directed to viral pesticide comprising a nuclear polyhedrosis virus and a viral factor that enhances infectivity, the factor comprising a baculovirus protein free of occlusion bodies, which protein breaks down the physical structure of the peritrophic membrane of lepidopterous larvae through the degradation of structural glycoproteins.

BTI-32

In re Application of: Robert R. Granados
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 5,717,069
Serial No.: 701846
Filed: August 23, 1996
Priority date: August 24, 1995 (Ser No. 60/002,743)
For: **DNA SEQUENCE CODING FOR ENHANCIN POLYPEPTIDE
WHICH ENHANCES VIRUS INFECTION OF HOST INSECTS**

Status

Patent issued February 10, 1998.

Related Files or Patent Applications

BTI-32AUS (issued); pending National applications in Brazil (32BZL), Canada (32CAN), Europe (32EPO) and Japan (32JPN).

Summary of Technology

This patent discloses an isolated and cloned DNA from a granulosis virus which comprises an amino acid sequence of the vital gene encoding a polypeptide isolated from occlusion bodies of certain baculoviruses and which polypeptide possesses the biological activity of enhancing baculovirus infectivity. Such proteins termed as "enhancins" are found within the viral occlusion body, have a disruptive effect on the insect peritrophic membrane (PM) proteins, and/or interact with the midgut epithelium in such a manner as to permit the increased adsorption, penetration and uptake of virus particles by midgut cells with a concomitant increase in host mortality. Disclosed is a recombinant DNA sequence, which codes for the enhancin protein of the *Helicoverpa armigera* Granulosis Virus.

Claim Coverage

The claims generally are directed to an isolated DNA sequence selected from the group consisting of: a) a DNA sequence which codes for the amino acid sequence of SEQ ID NO. 2; b) a DNA sequence having the sequence of SEQ ID NO. 1; c) a DNA sequence which codes for the residue 1-550 amino acid sequence of SEQ ID NO. 2; and d) a DNA sequence which codes for the residue 551-901 of amino acid sequence of SEQ ID NO. 2.

BTI-31

In re Application of: Gary W. Blissard and Scott C. Monsma
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 5,750,383
Serial No.: 645,863
Filed: May 14, 1996
Priority date: NA
For: **BACULOVIRUS CLONING SYSTEM**

Status

Patent issued May 12, 1998.

Related Files or Patent Applications

None pending.

Summary of Technology

This patent discloses a novel baculovirus cloning system. The new cloning system is a marker-rescue system, using an essential gene, *e.g.*, *gp64*. In this system, a gene essential for viral replication, growth, or propagation in cell culture is removed from or inactivated in the viral genome. Once a null baculovirus is created, it is propagated in a host cell that expresses the essential protein or a functional homolog. For cloning into the baculovirus containing the null-mutation, the virus is used to infect wild type host cells and the same cells are transfected with a plasmid that contains the essential gene, or a functional homolog, linked to a foreign gene under the control of a selected promoter. The baculovirus is "rescued" by the rescue gene linked to the foreign gene and is able to propagate normally and express the foreign gene. The recombinant "rescued" baculovirus can be used for gene expression, biological control or presentation of a foreign protein on the surface of the virus for vaccines and antibody production. As an example of this new cloning system, disclosed are recombinant baculoviruses that contain an insertionally inactivated or deleted *gp64 efp* gene, a gene that encodes a protein essential for viral infectivity and propagation in cell culture and in animals. To generate the virus the GP64 EFP protein was supplied in *trans*, from a stably transfected cell line. Homologous recombination was then used to generate inactivated *gp64 efp* genes in the context of otherwise wild type AcMNPV baculoviruses.

Claim Coverage

The claims generally are directed to a method of cloning DNA into a nuclear polyhedrosis virus.

BTI-15 CIP

In re Application of: Raymond J. St.Leger, Donald W. Roberts, Richard C. Staples
Assignee: Boyce Thompson Institute for Plant Research, Inc.
Patent No.: 5,962,765
Serial No.: 08/382,505
Filed: February 2, 1995
Priority date: August 2, 1991 (Ser. No. 07/739,645)
For: **MOLECULAR CLONING OF A COMPLIMENTARY DNA
SEQUENCE ENCODING A CUTICLE DEGRADING PROTEASE
PRODUCED BY ENTOMOPATHOGENIC FUNGI**

Status

Patent issued October 5, 1999.

Related Files or Patent Applications

None pending.

Summary of Technology

This patent discloses the structure and regulation of the extracellular chymoelastase protease (Pr1) of *Metarhizium anisopliae*, an enzyme involved in the penetration of insect cuticle by *Metarhizium* and other entomopathogenic fungi. The patent discloses the isolation and characterization of a Pr1 cDNA clone with a full-length insert. The genes coding for chymoelastase or slightly altered versions thereof, can be used to transform various organisms (*i.e.*, fungi, viruses, plants, bacteria, *etc.*) such that the transformed organisms are capable of producing chymoelastase in recoverable quantities. Fragments and derivatives of a DNA sequence coding for a chymoelastase could be used to code for a polypeptide having an activity which can: a) bind to insect cuticle; b) enhance signal processing of proteins; c) hydrolyze polypeptides; d) suppress protease expression; or e) be used as a probe to identify homologous genes in organisms. While chymoelastases and Pr1 have been previously isolated, new and novel uses for chymoelastase are disclosed, wherein the chymoelastase is used to selectively degrade protein in the presence of non-protein polymers. A new insecticide is disclosed which comprises a recombinant virus, microorganism, cell, plant or fungi infects, is eaten by or otherwise taken up by, an insect and expresses the enzyme Pr1 within the insect such that Pr1 activates a prophenoloxidase system within the insect.

Claim Coverage

The claims generally are directed to a recombinant virus, microorganism, cell, plant, or fungus, including a DNA sequence that encodes a chymoelastase enzyme, protein Pr1.