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Short Communication

Microbial insecticides

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Abstract
Insects are the most abundant group of organisms on Earth, and they negatively affect humans in a variety of ways. During the 1940s, a number of chemical insecticides were developed as a means of controlling the proliferation of noxious insect populations. A microbial insecticide can be an organism that either produces a toxic substance that kills an insect species or has the capability of fatally infecting a specific target insect. There are three types of bacterial insecticides like Insecticidal toxin of *Bacillus thuringiensis*, Genetic engineering of *Bt* toxin genes and Baculoviruses as biocontrol agents.

Keywords: Microbial insecticide, Toxin, Genetic engineering,

Introduction
Insects are the most abundant group of organisms on Earth, and they negatively affect humans in a variety of ways:
- They cause massive crop damage,
- They act as vectors of both human and animal diseases.

History of insecticides
During the 1940s, a number of chemical insecticides were developed as a means of controlling the proliferation of noxious insect populations.
- One of these was the chlorinated hydrocarbon DDT (dichlorodiphenyltrichloroethane). DDT proved to be exceptionally effective in killing and controlling many species of pests by attacking the nervous system and muscle tissue of insects.
- Other chlorinated hydrocarbons such as dieldrin, aldrin, chlordane, lindane, and toxophene have since been synthesized and applied on a massive scale.

A microbial insecticide can be an organism that either produces a toxic substance that kills an insect species or has the capability of fatally infecting a specific target insect.
- Insecticidal toxin of *Bacillus thuringiensis*
- Genetic engineering of Bt toxin genes
- Baculoviruses as biocontrol agents

Insecticidal toxin of *Bacillus thuringiensis*
The most studied, most effective, most often utilized microbial insecticides are the toxins synthesized by *Bacillus thuringiensis.*
- Current market for pesticides is $30 billion/year.
- *B. thuringiensis* is a gram positive soil bacterium that produces a toxin or crystal protein (Bt toxin or Cry) that kills certain insects.
- The Bt toxin or Cry is produced when the bacteria sporulates and is present in the parasporal crystal.
- Several different strains and subspecies of *B. thuringiensis* exist and produce different toxins that kill specific insects (Table 1).
- Used as alternative to DDT and organophosphates since the 1920s.
- Bt toxin is used as specific insecticides under trade names such as Dipel and Thuricide.

Target insects for Bt toxin

Cry toxins have specific activities against insect species of the orders Lepidoptera (moths and butterflies), Diptera (flies and mosquitoes), Coleoptera (beetles), Hymenoptera (wasps, bees, ants and sawflies) and nematodes

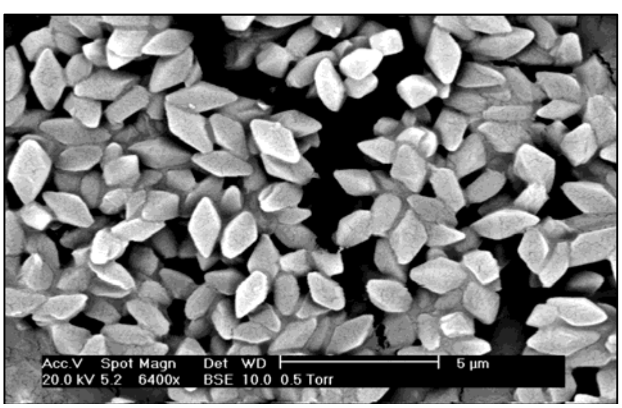
Table 1: Some properties of the insecticidal toxins from various strains of *B. thuringiensis*

Strain/subsp.	Protein size	Target Insects	Cry #
berliner	130-140 kDa	Lepidoptera	CryI
kurstaki KTP, HD1	130-140 kDa	Lepidoptera	CryI
entomocidus 6.01	130-140 kDa	Lepidoptera	CryI
aizawai 7.29	130-140 kDa	Lepidoptera	CryI
aizawai IC 1	135 kDa	Lepidoptera, Diptera	CryII
kurstaki HD-1	71 kDa	Lepidoptera, Diptera	CryII
tenebrionis (sd)	66-73 kDa	Coleoptera	CryIII
morrisoni PG14	125-145 kDa	Diptera	CryIV
israelensis	68 kDa	Diptera	CryIV

Adapted from lereclus *et al*, p. 37-69, intwstle *et al*. (ed.), bacillus thringcusis, an environmental biopesticide: theory and practice (johen wiiley & sons, chichester, united kingdom, 1993

The Cry protein: mode of action:

- The Cry protein is made as an inactive protoxin
- Conversion of the protoxin (e.g., 130 kDa) into the active toxin (e.g., 68 kDa) requires the combination of a slightly alkaline pH (7.5-8) and the action of a specific protease(s) found in the insect gut
- The active toxin binds to protein receptors on the insect gut epithelial cell membrane
- The toxin forms an ion channel between the cell cytoplasm and the external environment, leading to loss of cellular ATP and insect death.



B. thuringienis parasporal crystal composed of Cry1 protoxin protein. Conversion of the 130-kDa protoxin into an active 68-kKa toxin requires an alkaline environment (pH 7.5 to 8) and the action of a specific protease, both of which are found in the insect gut. The activated toxin binds to protein receptors on the insect gut epithelial cells.

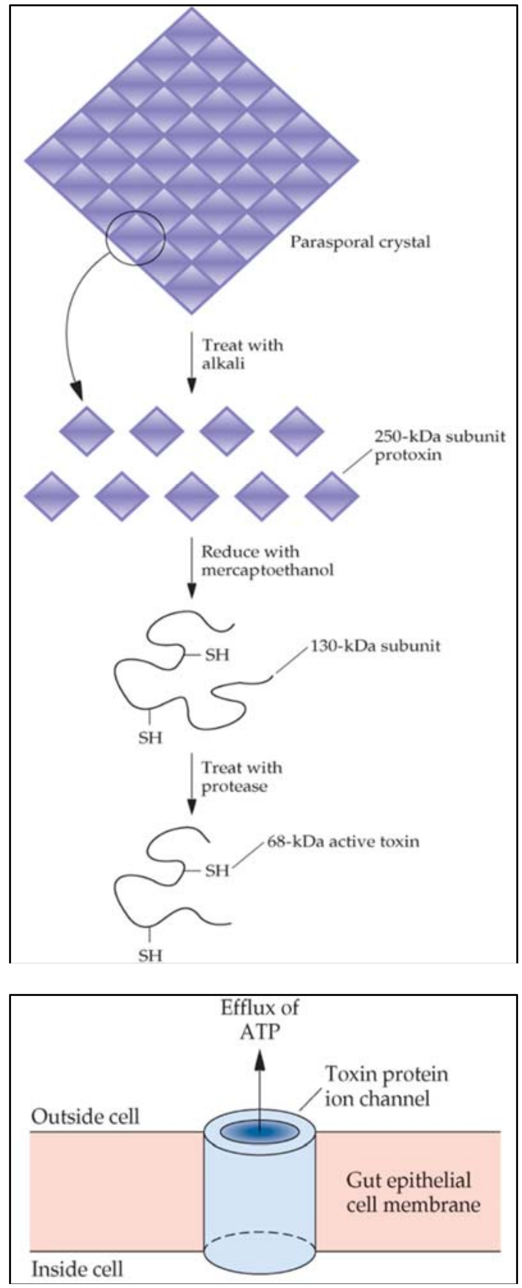


Fig 1: The toxin is inserted in gut epithelial cell membranes of the insect and forms an ion channel between the cell cytoplasm and the external environment, leading to loss of cellular ATP and insect death.

Isolation & genetic engineering of Cry genes

- The Cry (or protoxin) genes are encoded by plasmid DNA, not by chromosomal DNA in *B. thuringiensis*
- Cry genes were expressed in *B. thuringiensis* under the control of the ptet promoter (rather than its sporulation-specific promoter) and provided increase yield
- Constructs have also been produced to enhance toxin action and/or expand its specificity Cry genes were expressed in *B. thuringiensis* under the control of the ptet promoter (rather than its sporulation-specific promoter) and provided increase yield.

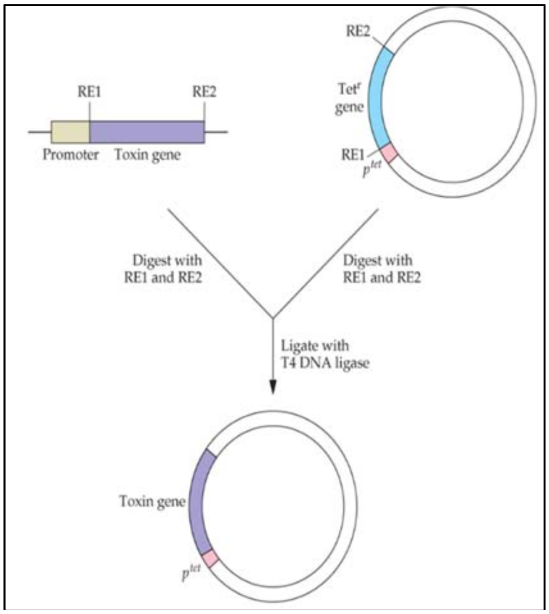


Fig 2: Cry genes were expressed in *B. thuringiensis* under the control of the p^{tet} promoter (rather than its sporulation-specific promoter) and provided increase yield.

(Molecular Biotechnology: Principles and Applications of Recombinant DNA, Fourth Edition Bernard R. Glick, Jack J. Pastemal and Cheryl L. Patten)(American Society for Microbiology 1752 N St. NW, Washington)

Baculoviruses as Biocontrol Agents

- Baculoviruses are rod-shaped, double stranded DNA viruses that can infect and kill a large number of different invertebrate organisms
- Immature (larval) forms of moth species are the most common hosts, but these viruses have also been found infecting sawflies, mosquitoes, and shrimp.
- Baculoviruses have limited host ranges and generally do not allow for insect resistance to develop
- Slow killing of target insects occurs
- In order to speed killing (enhance effectiveness), several genes can be expressed in the baculovirus including diuretic hormone, juvenile hormone esterase, Bt toxin, scorpion toxin, mite toxin, wasp toxin, and a neurotoxin (see Table 2)

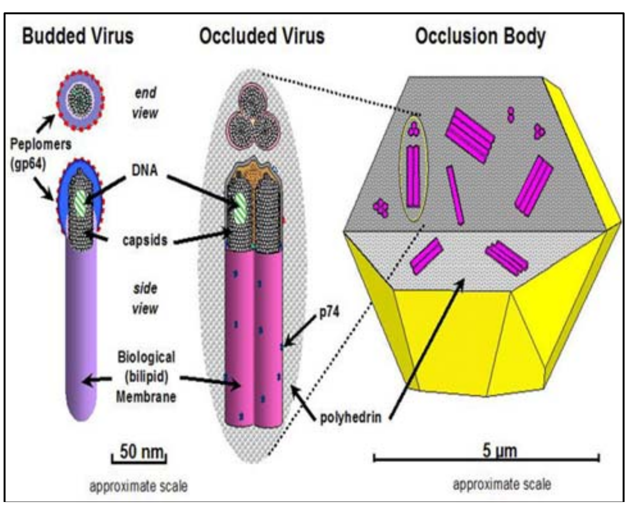


Fig 3: baculovirus multicapsid nucleophedrovirus

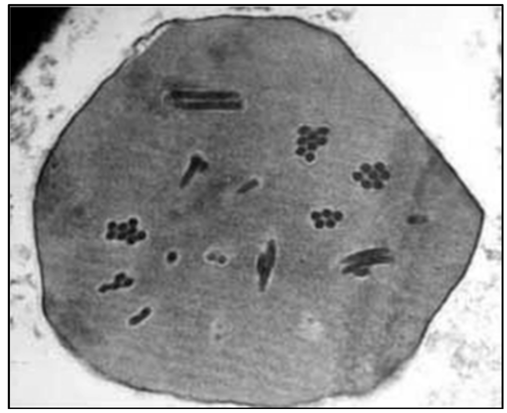


Fig 4: Electron Micrograph of a Baculovirus Occlusion Body

Table 2: Some genes that have been introduced into the baculovirus genome to increase insecticidal activity

Gene	Effect on host insect of introduced gene
Diuretic hormone	Reduced hemolymph volume
Juvenile hormone esterase	Feeding cessation
B. thuringiensis toxin	Feeding cessation
Scorpion toxin	Paralysis
Mite toxin	Paralysis
Wasp toxin	Premature melanization, low weight gain

Baculovirus is also used as an expression system for foreign proteins

“Our new Bac-to-Bac® Baculovirus Expression Systems deliver quick, robust and proven method to produce recombinant baculovirus, expressing your gene of interest in insect cells.”

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