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Entomopathogenic Bacteria in Biological Control of Insect Pests

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Naturally occurring entomo pathogens are important regulatory factors in insect populations. The comparison of entomo pathogens with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost. In addition to efficacy, the advantages of use of microbial control agents are numerous. These include safety for humans and other non-target organisms, reduction of pesticide residues in food, preservation of other natural enemies, and increased biodiversity in managed ecosystems.

Introduction

Bacteria are relative simple unicellular microorganism that lack internal organelles such as a nucleus and mitrochondria and which reproduce by binary fission. Majority of the bacteria currently are spore forming members of the bacterial family Bacillaceae and also Enterobacteraceae. In Bacillaceae the genus *Bacillus* is most important. The insect pathogenic *Bacilli* occur in healthy and diseased insects, but also occur in and can be isolated from many other habitats including insect frays, soil, plants, granaries and aquatic environments. Over 90 species of naturally occurring, insect-specific (entomopathogenic) bacteria have been isolated from insects, plants, and the soil, but only a few have been studied intensively. Much attention has been given to *Bacillus thuringiensis*, a species that has been developed as a microbial insecticide and bacterial was first time isolated by Berliner in 1915 in Germany from the diseased larvae of the Mediterranean flour moth, *Ephestia kuhniella*. Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects.

There are two major types of bacteria that are used in insect control:

1. Those that cause fatal infection diseases. *E.g. Bacillus popilliae* Dutky, a bacterium that infects and kills Coleopteran larvae, particularly soil-inhibiting members of the family Scarabaeidae.
2. Those that kill insect primarily through the action of insecticidal toxins. *e.g.* B.t, a species that produces toxins- protein, endotoxins and nucleotide exotoxins-capable of killing insects whether or not they directly associated with the bacterium.

Mode of Action

B. Thuringiensis produces a proteinaceous paraporal crystalline inclusion during sporulation. Upon ingestion by insects, this crystalline inclusion is solubilized in the midgut, releasing

proteins called delta–endotoxin. These proteins are activated by midgut proteases and the activated toxins diffuse through the peritrophic membrane of midgut causing a disruption in membrane integrity and ultimately leasing to lethal epticemia and death.

The Bt protein in commercial formulations is effective when eaten by insects with a specific (usually alkaline) gut pH and the specific gut membrane structures required to bind the toxin. When ingested by a susceptible insect, the protein toxin damages the gut lining, leading to gut paralysis. Affected insects stop feeding and die from the combined effects of starvation and tissue damage. *Bt* spores do not usually spread to other insects or cause disease outbreaks on their own as occurs with many pathogens.

Bt genes have been transferred into other microorganisms to produce more active formulations, some of which are commercially available. Additionally, researchers have genetically engineered varieties of several plant species to express the *Bt* toxin as part of the plant's normal development. This has led to the production of "insect-resistant" Bt-transformed lines of tobacco, cotton, corn, tomatoes, potatoes, and others. The following bacteria that are used as biological control agents of insect are:

1	*B. thuningiensis var. kurstaki*	Lepidoptera
2	*B. t. var. israeliensis*	Diptera
3	*B.t. var. tenebrionis*	Coleoptera
4	*B. popilliae*	Scarabaeid beetles
5	*B. sphericus*	Mosquitoes

Bacillus thuringiensis Berliner is a complex of bacterial subspecies all of which are characterized by the production of a parasporal body during sporulation. This parasporal body contains one or more proteins in crystalline form and many of these are highly to certain species of insects. In the insecticidal isolates, the toxin are known as Endotoxins and often occur in the parasporal body as protoxic that after ingestion are activated by proteolysis in the gut. The activated toxins destroy midgut epithelial cells, killing sensitive insects within a day or two of ingestion. In insect species only moderately sensitive to the toxins, such as Spodoptera species, the spore contributes to the activity of the bacterium. A primary reason for the success of *Bt* is that it s easily grown on a large scale, in simple, cheap, readily available media.

The most widely used Bt is the HD1 isolates of *B. thuringiensis* subsp. *Kurstaki*, an isolate that produced four major endotoxin proteins packaged into the crystalline parasporal body and is used in formulation against lepidopterous pests. Another successful *Bt* is the ONR60A isolate of *B. thuringiensis* subsp. *Israelensis* which is highly toxic to the larvae of many mosquito and blackfly species. This isolate also produces a parasporal body that contains four major endotoxins.

Symptoms

The first sign of poisoning in insect is paralysis of the gut and mouthparts, leading to a cessation of feeding. Subsequent to the onset of gut paralysis, there is a swelling and destruction of microvillus of the midgut which leads to disruption of ion and glucose separation of midgut cells

from the basement membrane and brusting of separated cells in the midgut lumen. Disruption of midgut structure and function lead to ion and pH imbalance in the haemolymph, total body paralysis and death.

Classification of insecticidal protein from *B. thuringiensis*

Host	Protein
Lepidoptera	Cry 1A ato A c, Cry 1B a, C a, C b, D a, Cry 9A a, Cry 2 A b
Coleoptera	Cry 3A a, B a, Cry 7 A a, Cry 3 ca
Diptera	Cry 4 A a, B a, Cry 10 A a
Coleoptera/ Lepidoptera	Cry 116

Commercially available *B. thuringiensis* products with target pest and Crop in India

Trade name	Target Pest	Crop	Manufacture
Halt	Diamond back moth, Lepidopterous caterpillars	Cabbage	Wockhard Ltd.
Biolet	Cotton bollworms	Cotton	Biotech International Ltd., New Delhi
Bioasap	Cotton bollworms, tobacco caterpillar	Cotton	Biotech International Ltd., New Delhi
Delfin WG	Cotton bollworms	Cotton, castor	M/s Sandoz India Ltd. Bombay
Dipel 8 L	Cotton bollworms, tobacco caterpillar	Cotton, tomato, okra, brinjal	Lupin Agrochemicals Pvt. Ltd. Bombay
Spicturin	American bollworm, Leaf folder, tobacco caterpillar, Diamond back moth	Cotton, rice, cabbage, cauliflower, chillies	Tuticorin Alkali chemicals and Fertilizers Ltd., Chennai
Biobit	Lepidopterous caterpillar	Several	Rallis India Ltd., Bangalore

Biology and Use of *Bacillus poilliae* for Scarb Control

The milky disease of Scrabs caused by *B. popilliae*, the term milky disease is derived from the opaque white colour that characterizes diseased larvae and results from the accumulation of sporulation bacteria in the haemolymph. The disease is initiated when grubs feeding on the roots of grasses to other plant ingest the bacterial spores. The spore germinates in the midgut and vegetative cell invade the midgut epithelium where they grow and reproduce, changing in form as they progress toward invasion of hemocoel. After passing through the basement membrane of the midgut, the bacteria colonize the haemolymph over a period of several weeks and sporulate, reaching population of 10^8cells per milliliter. The disease is fatal, providing that the larvae ingest

a sufficient number of spores early in their development. Dead larvae in essence become foci of spores that can serve as a source of infection for up to 30years.

Biology and Use of *Bacillus spaericus* for Mosquito Control

Three isolates *i.e.*1593 from Indonesia, 2297 from Srilanka, and 2362 from Nigeria, of which first two were obtained from soil and water sample at mosquito breeding sites, whereas 1593 was isolated from a dead adult black fly. 2362 is the most toxic to the widest ranges of mosquito species. Its target of action is the midgut epithelium. The toxins bind to microvilli, causing hypertrophy and lysis of cell. MTX toxins are produced in addition to binary toxins during vegetative growth but binary toxins are the most active and important.

Biology and use of *Serratia entomophila* for Scrab Control

A novel bacterium named *Serratia entomophila* causes disease in the grass grub, *Costelystra zealandica* an important pest, an important pest of pastures in New Zealand and has been developed as a biological control agent for this pest. This bacterium adheres to the chitinous region of the foregut, where it grows extensively, eventually leading the larvae to develop an amber colour and resulting death.

Conclusion

Bt is the most important and widely used entomopathogenic biological agent. The most common species are *B. thuningiensis var. kurstaki, B.t. var. israeliensis, B.t. var. tenebrionis, B. popilliae, B. sphericus. Bt* genes have been transferred into other microorganisms to produce more active formulations or led to the production of "insect-resistant" transgenic lines of tobacco, cotton, corn, tomatoes, potatoes, and others.

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