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Bacillus thuringiensis: Nature's Insecticide and Its Mechanism of Action

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Abstract:

Bacillus thuringiensis (Bt) is a naturally occurring, spore-forming, gram-positive bacterium with significant insecticidal properties, widely used in integrated pest management (IPM) due to its specificity and environmental safety. Bt produces crystalline proteins that, when ingested by target insects, disrupt the gut epithelium, leading to gut paralysis, starvation, and eventual death. This targeted action allows Bt to control specific pests, such as caterpillars and mosquito larvae, without harming non-target organisms like beneficial insects and pollinators. The development of genetically modified crops incorporating Bt genes has further expanded its use in agriculture, reducing reliance on chemical insecticides. However, concerns about insect resistance necessitate careful management strategies, including the use of non-Bt refuges. This article explores the mechanisms of Bt's insecticidal activity, the various insect responses, and the importance of sustainable practices to prolong Bt's efficacy in pest control.

Keywords: *Bacillus thuringiensis*, crystalline proteins, Beneficial insects, Bt genes

Introduction:

Bacillus thuringiensis or Bt is a naturally occurring rod-shaped, spore-forming aerobic, gram-positive bacterium that is found throughout most areas of the world. It can be found in soils on leaves/needles and other common environmental situations. When bacteria produce spores, they also produce unique crystalline proteins. When eaten these natural proteins are toxic to certain insects, but not to human beings, birds, or other animals.

Sporeforming:*Bacillus* spp. *Paenibacillus* spp., and *Clostridium* spp

Non-spore-forming: *Pseudomonas*, *Serratia*, *Yersinia*, *Photorhabdus*, and *Xenorhabdus*

Insecticidal Activity:

The insecticidal activity of the bacterium is due to three proteins, crystal (Cry) cytolytic (Cyt), and vegetative insecticidal protein (Vip) produced during the bacterial infection on the larvae.

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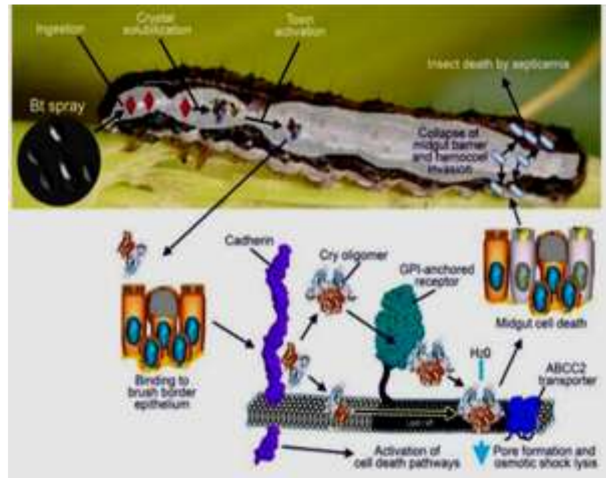
These proteins are accumulated in the crystalline inclusion bodies sporulation (Cry proteins, Cyt proteins) and bacterial growth (Vip proteins)

MODE OF ACTION:

Bacillus thuringiensis produces a **parasporal inclusion**, a protein crystal body during sporulation. A huge number of related crystal proteins are known and more than one protein type can co-assemble in one crystal. Many distinct crystal protein **(Cry) genes** have been described. The gut epithelium is the primary target tissue for Bt delta-endotoxin action. The crystal proteins exert their effect on the host by causing lysis of midgut epithelial cells, which leads to **gut paralysis.**

The insect stops feeding and, if it does not recover, eventually dies. Upon ingestion, the crystals dissolve in the alkaline environment of the midgut and then the protoxin is proteolytically processed to produce the actual toxin. Activation of actual toxin usually involves the removal of a small number of Nterminal amino acid residues along with the cleavage of the C-terminal half (Gill *et al.*, 1992). After that the activated toxin binds to specific receptors present on the membranes of the host insect epithelial midgut cells and induces the formation of pores in the membrane of midgut epithelial cells.

This is followed by permeability of cell membrane increase which finally leads to **cell lysis**, **disruption of gut integrity** and eventually to the death of the insect from **starvation or septicemia** (Adang 1985; Gill *et al.*, 1992; Bauer, 1995).



Mode of action of Bt, an Entomopathogenic bacteria, adopted from Glare *et al.*, 2017 Basic and applied research: entomopathogenic bacteria

Toxin Activation and Binding:

1. **Midgut enzymes activate Monomeric toxin along GPI anchored and cadherin receptors (midgut epithelial membrane)**
2. **Proteolytic action eliminates Alpha helical structures in domain 1**
3. **Oligomerization of toxin leads ton formation of pore, rupturing of midgut epithelial cells and finally death of larva**

In *Manduca sexta*, Cry 1Ab, Cry 1Aa, and Cry 1Ac proteins bind to cadherin-like receptors on midgut epithelial membranes, leading to conformational changes and proteolytic cleavage. The resulting oligomeric toxins exhibit a higher affinity for aminopeptidase receptors compared to their monomeric forms, facilitating pore formation and insect mortality.

Unlike caterpillars, the response in mosquitoes is different where upon ingestion of *Bt* subsp. *israelensis* delta-endotoxin, the mosquito larva is killed within 20-30 min. *Paenibacillus popilliae* is commonly used against Japanese beetle larvae and known to cause the milky spore disease. Although *Serratia* is not registered for use in the USA, a species is registered for use against a pasture insect in New Zealand.

Insect Responses to Bt Toxins:

Type I response

Midgut paralysis occurs within a few minutes after delta-endotoxin is ingested. Include cessation of feeding, increase in hemolymph pH, vomiting, diarrhea, and sluggishness. General paralysis and septicemia occur in 24-48 hours resulting in the death of the insect.

Examples of insects that show Type I response include silkworm, tomato hornworm, and tobacco hornworm

Type II response

Midgut paralysis occurs within a few minutes after the ingestion of delta-endotoxin, but there will be no general paralysis. Septicemia occurs within 24-72 hours

Type III response :

Midgut paralysis occurs after delta-endotoxin is ingested followed by cessation of feeding. Insect may move actively as there will be no general paralysis. Mortality occurs in 48-96 hours. Higher mortality occurs if spores are ingested. Insect examples include Mediterranean flour moth, corn earworm, gypsy moth, spruce budworm.

Type IV response

Insects are naturally resistant to infection and older instars are less susceptible than the younger ones. Midgut paralysis occurs after delta-endotoxin is ingested followed by cessation of feeding. Insect may move actively as there will be no general paralysis. Mortality occurs in 72-96 or more hours. Higher mortality occurs if spores are ingested. Cutworms and armyworms are examples for this category

Infection:

Bacterial infections in insects can be broadly classified as bacteremia, septicemia, and toxemia.

Bacteremia : occurs when the bacteria multiply in the insect's haemolymph without the production of toxins. This situation occurs in the case of bacterial symbionts and rarely occurs with bacterial pathogens.

Septicemia occurs most frequently with pathogenic bacteria, which invade the haemocoel, multiply, produce toxins, and kill the insect.

Toxemia occurs when the bacteria produce toxins and the bacteria are usually confined to the gut lumen, as in the case of brachytosis of the tent caterpillar. Pathogenic bacteria, upon ingestion by a susceptible insect, multiply and produce toxins in the midgut lumen. The insect loses its appetite, becomes diarrheic, discharges watery feces, and may vomit. The invasion of the bacteria into the haemocoel results in septicemia and death of the insect. The bacteria, in general, are extracellular pathogens except for the pathogenic rickettsia and mollicutes

Targeted Insect Control:

Different strains of *Bacillus thuringiensis* (Bt) are highly specific, with *Bt kurstaki* targeting caterpillars and *Bt israelensis* targeting mosquito larvae. This specificity makes Bt play role in integrated pest management (IPM), reducing the need for broad-spectrum synthetic insecticides.

Environmental Safety and Sustainability:

Bt is environmentally friendly, non-polluting, and safe for non-target organisms, including beneficial insects. Its use in pest management reduces the reliance on chemical insecticides, promoting sustainable agriculture.

Genetically Modified Organisms (GMOs):

Bt genes have been engineered into crops like corn, cotton, and potatoes, allowing them to produce Bt proteins. This has increased crop protection but raised concerns about insect resistance and impacts on non-target species.

Resistance Management:

To prevent resistance, strategies like planting non-Bt refuges are essential. These practices help maintain susceptible insect populations, prolonging Bt's effectiveness in pest control.

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