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Bacillus thuringiensis: Nature's Smart Solution for Insect Pest Control

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Introduction

Bacillus thuringiensis is a gram-positive soil borne, sporulating bacterium known to produce a wide range of insecticidal crystal inclusions during sporulation. These crystals comprise of one or more proteins called Cry and Cyt toxins and are highly toxic to specific species of invertebrates but harmless to the humans and other vertebrates. These insecticidal proteins are used directly as pesticide formulations and in developing transgenic crops.

Ishiwata first identified the *B. thuringiensis* from *Bombyx mori* in 1902 and it caused severe damage in silk industry in Japan. Beegle and Yamamoto named it as *B. sotto*, which means soft indicating flabby nature of infected larvae. Subsequently, Berliner isolated bacteria from the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) larvae, and it was named as *B. thuringiensis*. *Bt* biopesticides have stimulated microbial control and insect pathology studies, and it has become one of the major insect pathogens used in pest control.

The first *Bt* based biopesticide formulation was Sporeine, which was used for the control of various Lepidoptera. Most of the *Bt* formulations are derived from *Bt* kurstaki HD1 (Biobit, Thuricide and Dipel) and specific strains are used to control pests from different insect

orders *viz*., lepidoptera (kurstaki SA-11, kurstaki SA-12), coleoptera (*Bt* tenebrionis) and diptera (*Bt* israeliensis). *Bt* based insecticides are insect specific, which are harmless to human, birds and other vertebrates. Delfin, Bioasp, Biolep and Spicturin are commercial *Bt* formulations marketed in India. Advancement in biotechnology from 1980's stimulated by, when Schnepf and whiteley first cloned a crystal toxin gene from *Bt subsp Kurstaki* into E. coli, since then, researches were made to find out more infectious strains of *Bt*. There is tremendous resurgence in biological control, through genetic engineering approaches.

Mode of action of Cry proteins in insect

The crystalline proteins release protoxin, when it gets solubilized at high pH in midgut, called δ-endotoxins. The protoxins get activated in midgut by the proteases. The target site for *Bt* toxins is present in the midgut of the insects. Brush border membrane vesicles (BBMV) are one of the primary binding sites for *Bt* toxins in various insect species. After binding to the receptor, the toxin inserts irreversibly into the plasma membrane of the cell leading to lesion formation. Significant positive correlation was observed between the ability to bind BBMV and toxin activity. The toxin part of protein is derived from N-terminal portion and for formation of parasporal inclusion bodies C-terminal portion is important. The 3-domain toxins have been described as pore-forming toxins which induce cell death by forming ionic pores into midgut epithelial cells, only in their target insect species. The *Bt* toxicity lies in the organization of α -helices which is derived from domain I. The toxin induces pore formation in the columnar cell of the apical membrane, which allows rapid fluxes of ions in insect midgut. The pore formation is K+ selective, which is permeable to anions and cations. Cation selective channel formation destroys the membrane potentials in insect midgut which is responsible for necrosis, degeneration of epithelel cells and peritrophic membrane that leads to bacterial septicemia, which occurs after death of the larvae.

Classification of *Bacillus thuringiensis*

The *Bacillus thuringiensis* are classified into serotypes/ subspecies based on their H flagellar antigen determinants. About 323 holotype crystal proteins are documented as toxic to insects of different orders *viz*. Lepidoptera, Coleoptera and Diptera and nematodes (Crickmore *et al.*, 2016). There are currently around 78 primary subgroups of Cry toxins, *i.e*., with different primary ranks in the nomenclature (Cry1, Cry2, Cry3 etc.)

Hofte and Whiteley classified cry proteins into four classes based on their insect specificity and aminoacid sequence homology. They are CryI (Lepidoptera specific), CryII (Dipteran and Lepidopteran specific). CryIII (Coleopteran specific) and CryIV (Dipteran specific). Recently two more classes added they are CryV and CryVI (Nematode specific

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toxins). Crickmore proposed a new method of classification for proteins. Each protein has name which consists of mnemonic Cry with four hierarchical lines consists of numbers, capital letters, lowercase letters and numbers, for example (Cry1Aa1). Hence, the proteins which have less than 45% aminoacid sequence identity been kept in primary level (Cry1, Cry2 etc.). And the proteins which have 78% (Cry4B) and 95% (Cry4Ba) sequence identity constitute the borders for the secondary and tertiary level. This system replaced the old nomenclature that used roman numerals.

Plasmids of large molecular mass were associated with the genes coding for the insecticidal crystal proteins. Many Cry protein genes have been cloned, sequenced, and named as cry and cyt genes. Cry proteins have different shapes such as bipyramidal (Cry1), cuboidal (Cry2), flat rectangular (Cry3A), irregular (Cry3B), spherical (Cry4A and Cry4B) and rhomboidal (Cry 11A). Cry proteins toxic for Lepidopteran insects belongs to the Cry1, Cry2, and Cry9 groups. Cry toxins active against Coleopteran insects are Cry3, Cry7, Cry8, Cry14, Cry18, Cry34 and Cry35. Each of the *Bt* strains can carry one or more crystal toxic genes, and therefore, strains of the organism may synthesize one or more crystal proteins.

Characterization of *Bacillus thuringiensis*

Bacillus thuringiensis was the most terrific biological cause for insect pest control which will affects all important commercial crops. It is toxic to few insect orders like Lepidoptera, Coleoptera, Diptera, Hymenoptera and Mallophaga. Cry toxins are the most important lethal proteins where at least different sequences had found and grouped into seventy-eight family's groups. *Bacillus thuringiensis* produces crystalline inclusions composed of proteins called Delta endotoxins.

Bacillus thuringiensis is a spore forming, aerobic, gram positive and rod-shaped soil bacterium which is widely used in agriculture as a biological insecticide. *Bacillus thuringiensis* has been used in insect pest management for more than hundred years. The most important point about *Bacillus thuringiensis* is that it can produce an insecticidal toxic protein which was more toxic against insect pests. Toxin protein structure inclusion consists of union of many polypeptides with molecular mass between 15-140 KDa. They represent around 30-40% of the total protein after the process of cellular lysis and spore liberation. Those proteins are represented as Cry or Cyt or delta endotoxins which acts as main active ingredients in commercial preparations. The *Bacillus thuringiensis* strain will synthesize one to five or more toxins which were packaged into multiple crystals.

In some cases, acrystalliferous strains are also described. Cry proteins are of high specificity to insect pests and they are safe to the environment and also, they can be considered

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as an alternative to the chemical pesticides. There are some other proteins which were produced by *Bacillus thuringiensis* are VIP (Vegetative Insecticidal Proteins) which were very specific against moth pests. The genetic variety and adaptability of *Bt* strains are highlighted by the fact that numerous isolates from various locales have shown variation in toxicity and protein composition. This variety makes it possible to identify extremely powerful strains for focused pest control.

Toxicity of *Bacillus thuringiensis* Against Lepidopteran Pests

Bt is highly effective against a wide range of lepidopteran pests, including *Helicoverpa armigera*, *Spodoptera* spp., *Plutella xylostella, Earias* Spp., *Achaea janata*, *Opisina arenosella, Tuta absoluta, Pectinophora gossypiella, Chilo partellus*, *Galleria mellonella*, *Manduca sexta, Trichoplusia ni* and *Ephestia kuehniella*. Comparative studies have shown that different *Bt* strains vary in their toxicity, with *Bt kurstaki* often being more potent. The effectiveness of *Bt* is typically measured using LC_{50} and LT_{50} values. Many isolates have demonstrated high mortality rates and significant growth inhibition in target pests. Some newly identified strains exhibit even greater toxicity than standard commercial strains, indicating their potential for future biopesticide development. Application of *Bt* formulations, either alone or in combination with biological control agents such as *Nesidiocoris tenuis*, significantly reduces leaf damage and improves crop yield.

Different application methods, including leaf dipping and spraying have shown comparable efficacy. Mortality rates vary depending on larval stage and concentration, with higher doses resulting in increased mortality, particularly in later instars. Field and laboratory studies have reported mortality rates exceeding 90% with certain *Bt* isolates and commercial formulations such as Dipel and Protecto. Combined use of *Bt* with other biocontrol agents or bioinsecticides like spinosad enhances pest suppression through additive effects.

Cry1Ac is one of the most widely studied *Bt* toxins due to its high efficacy against lepidopteran pests. Various studies have reported its toxicity against species such as *Helicoverpa armigera*, *Spodoptera spp.*, and *Plutella xylostella* and *Tuta absoluta*. The effectiveness of Cry1Ac varies across insect populations and developmental stages. Cry1Ac has been found highly effective against early larval instars, while its efficacy may decrease in later stages. Comparative studies with other Cry proteins, such as Cry2Ab and Cry1Ab indicate differences in toxicity and specificity. While Cry1Ac remains highly effective against certain pests combining multiple toxins can improve pest control and delay resistance development.

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Conclusion

An efficient and environmentally benign biological agent that is frequently used to control insect pests is *Bacillus thuringiensis* (Bt). Without endangering people or other non target creatures, it's extremely specialized Cry and Cyt toxins target insect pests. Major pest groups particularly lepidopterans can be controlled thanks to the variety of *Bt* strains. Its application in transgenic crops and biopesticides has been further expanded by biotechnology advancements. However, for it to be effective over time, appropriate resistance management techniques are necessary. All things considered, *Bt* continues to be an essential part of integrated and sustainable pest management systems.

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