



Bacillus thuringiensis in Pest Management

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Conflict of interests: The author has declared that no conflict of interest exists.

How to cite this article?

Rajadurai, G., Anandakumar, S., Raghu, R., 2023. *Bacillus thuringiensis* in Pest Management. *Plant Health Archives* 1(1), 11-13. DOI: 10.54083/PHA/1.1.2023/11-13.

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Abstract

Bacillus thuringiensis (Bt) is a widely employed pest management biocontrol agent in the agriculture and forestry ecosystem. In nature, this Gram-positive bacterium is found in soil that produces spore-crystal inclusion bodies. This Gram-positive bacterium produces a broad spectrum of insecticidal proteins, which are found to be extremely toxic to different groups of insect pests. Bt toxin genes are very selective and specific to pests; they are not toxic to other than the target organisms such as human, animals, and birds, and they are safer for the environment. The Bt toxin is often employed in organic farming as an insecticide spray to manage insect pests. Additionally, it is one of the sources for the insecticidal genes deployed to genetically modify food crops so that they can naturally impart resistance against numerous insect pests.

Keywords: *Bacillus thuringiensis*, Biopesticides, Cry toxin, Transgenic

Introduction

Bacillus thuringiensis (Bt) is an extensively used agricultural and forestry biocontrol agent for pest management. It is generally a Gram-positive bacterium found mainly in soil that produces spore-crystal inclusion bodies in nature. Across diverse global regions, this microorganism has been consistently detected in a multitude of habitats, encompassing soil ecosystems, deceased insect specimens, insect-abundant surroundings, the foliage of plants, aqueous environments, animal excrement, milling facilities, and stored grain repositories (Gupta *et al.*, 2021). During its vegetative growth phase, this Gram-positive bacterium exudes an expansive repertoire of insecticidal proteins, encompassing both Secreted Insecticidal Proteins (Sip) and Vegetative Insecticidal Proteins (Vip). Likewise, in the stationary phase, this bacterium releases β -exotoxins and crystalline parasporal δ -endotoxins, specifically the cytolytic toxin (Cyt) and the crystal toxin (Cry) (Chattopadhyay and Banerjee, 2018). At relatively low quantities, these crystalline proteins show high insecticidal activity. The genes encoding Bt toxins exhibit a remarkable level of specificity towards targeted pests while concurrently demonstrating negligible harm towards non-target organisms, including human, animals, and avian species. Thus, they offer a safer

alternative for environmental preservation.

In the late nineteenth century, the silk industry in Japan encountered a perplexing phenomenon: the unexpected demise of *Bombyx mori* caterpillars. This puzzling occurrence led to the accidental discovery in 1898 by Japanese researcher Sigetane Ishiwata of spore-forming bacteria, subsequently named *Bacillus sotto* to reflect the phenomenon of "sudden death" that manifested within mere hours of caterpillar ingestion (Ishiwata, 1901). Since it was written in Japanese, the description of "sotto-kin" wasn't attractive to many contemporary insect pathologists. A similar bacterium was found in a dead *Ephestia kuehniella* Mediterranean flour moth in 1909 by German scientist Ernst Berliner in Thuringia, Germany. Berliner was the first to formally describe and name the bacteria *B. thuringiensis*. Since the 1920s, the Bt toxin has been widely employed as an insecticidal spray and is particularly favored in organic farming practices. Furthermore, it stands as a prominent reservoir of insecticidal genes harnessed for genetic modification of food crops, endowing them with naturally imparted resistance against numerous insect pests.

Mode of Action

The mechanism underlying the activity of insecticidal crystal

Article History

RECEIVED on 09th April 2023

RECEIVED in revised form 16th May 2023

ACCEPTED in final form 18th May 2023

proteins (ICPs) encompasses a sequence of intricate steps. This includes the dissolution of crystal proteins, activation of *Bt* toxin through gut proteases, and subsequent recognition of binding sites on the brush border membrane within the midgut region. Subsequently, these processes lead to the formation of pores and eventual lysis of cells, ultimately culminating in the demise of the targeted insects (Figure 1). Regarding their mode of action, the paramount attribute of Cry toxins lies within their exceptional host specificity. This characteristic is attributed to the precise interaction between Cry toxins and surface proteins on larvae's midgut cells microvilli, thereby establishing their distinct binding affinity (Bravo et al., 2011). As previously stated, *B. thuringiensis* produces a number of toxins with insecticidal properties. The toxins produced by *B. thuringiensis* are stomach poisons as contrasted with chemical insecticides, which are usually contact poisons.

Bioinsecticide Products

Commercial formulations of *Bt* have been used for several decades to control lepidopteran insects and, more recently, beetles and flies. Many of these biopesticide bases are crystalline inclusion preparations produced by the wild-type *Bt* strains. The rates are evaluated by biological activity testing, and the bioinsecticide concentration is reported in international units. The potency of these bioinsecticides is modified according to various patterns, such as the quantity needed to completely kill a species' larva in 1 cm² of artificial diets. *Spodoptera* spp. is employed as the reference test insect for a few commercial *Bt* preparations viz., Sandox and Javellin, which indicate the amount of product required per acre.

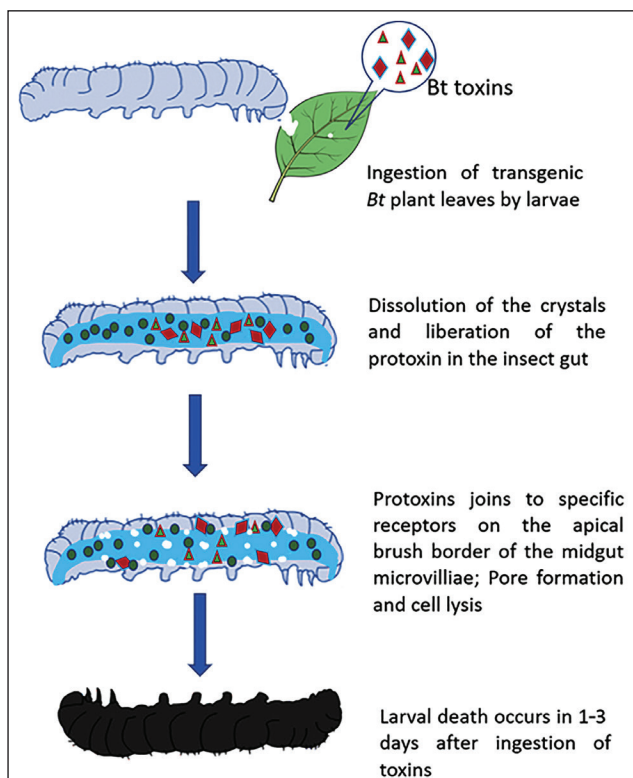


Figure 1: Mode of action of *Bt* toxin

B. thuringiensis strains with various *cry* genes have been identified, and to effectively combat lepidopteran insect pests, biopesticide products derived from two distinct subspecies, namely *kurstaki* and *aizawa*, have been meticulously developed and employed. These tailored solutions exhibit remarkable efficacy in suppressing the population of such pests. A range of commercial formulations, harnessed from these strains, have entered the market as effective solutions for pest management. Prominent among them are Dipel, Javelin, Thuricide, Worm Attack, Caterpillar Killer, and Bactospeine. These meticulously developed products serve as valuable resources in combatting pest infestations and safeguarding agricultural productivity, but many small enterprises sell similar items with different trade names. Similarly, *B.t. var. israelensis (Bti)* exhibits remarkable selectivity and potency against dipteran insects, especially mosquitoes and blackflies, which are transmitters of malaria, onchocercosis, and dengue fever. As a result, *Bti* is used for controlling mosquitoes and black flies. *Bti* is also used in a number of commercial *Bt*-formulated products, including VectoBac[®], TeKnar[®], Bactimos[®] and Skeetal[®]. However, there are some drawbacks to using *Bt*-based biopesticides, including: (i) delayed action, (ii) a limited host range, (iii) limited persistence in the field (due to the impact of solar radiation and temperature), and (iv) they do not reach insects that attack roots or internal plant parts.

Development of Transgenic *Bt* Crops

In the 1980s, several scientists demonstrated that plants may be genetically modified, leading to transgenic *Bt* plants' development. The successful use of *Bt* genes has decreased the population of lepidopterans, which are major pests in various parts of the world, including India. Toxins produced by *Bt* strains, including Cry1Aa, Cry1Ab, Cry1Ac, Cry2a, and Vip3 were mostly used to control lepidopteran pests (Rajadurai et al., 2022). During the mid-1980s, a pioneering endeavor was undertaken to introduce *Bt* genes into tobacco and tomato plants, enabling the production of Cry proteins within plant tissues. This groundbreaking initiative resulted in the development of the first transgenic tomato plants equipped with insect-resistant properties. These plants harbored complete and truncated forms of the gene sourced from *B. thuringiensis* var. *kurstaki* (HD-1 strain), thereby imparting resistance against lepidopteran larvae, particularly the tomato fruit worm and pinworm.

Bollgard and Ingard are the first insect-resistant transgenic cotton lines expressing altered *Cry1Ac* toxin, which were developed and commercialized by Monsanto in 1995. Later, Pioneer Hi-Bred and Dow Agro-Sciences combined to develop a unique transgenic maize line Herculex I expressing the *Cry1F* protein in 2001. In 2002, Monsanto produced the transgenic cotton variety (Bollgard II) expressing two *Bt* toxins, *Cry1Ac* and *Cry2Ab*, and established its effectiveness against various lepidopteran pests. Similarly, the *cry2AX1* gene was synthesized by combining two *Bt* genes *cry2Aa* and *cry2Ac*, and expressed in various agricultural crops to confer resistance against lepidopteran pests (Rajadurai et al., 2018). To date, many transgenic crops have been produced

and evaluated against various insect pests by expressing different *Bt* genes (alone or in combinations). In the year 1994, the authorization for the commercialization and cultivation of genetically modified (GM) crops was granted. In the wake of widespread commercial adoption of GM crops in the United States beginning in 1996, the cultivated area dedicated to such crops experienced a remarkable surge, escalating from 1.7 million hectares in 1994 to a staggering 190.4 million hectares by 2019. The first transgenic crop developed in India is insect-resistant *Bt* brinjal by MAHYCO (Maharashtra Hybrid Seeds Company) in collaboration with two agricultural universities (UAS, Dharwad and TNAU, Coimbatore). In 2002, the first transgenic plant (*Bt* cotton) was commercialized in India by MAHYCO in collaboration with Monsanto. In various nations, 80 million ha of transgenic crops expressing insecticidal *Bt* genes alone, and pyramided with herbicide tolerance genes, are planted.

The first genetically modified food crop permitted for planting in a developing country is insect-resistant *Bt* brinjal (eggplant). That started in 2014, and commercial planting in Bangladesh has benefited both farmers and consumers. Scientists in the Philippines developed *Bt* eggplant using the same technology in native eggplant varieties, and it is anticipated to boost the nation's economy and environment.

Conclusion

The Cry toxins are environmentally safer and an excellent insecticide against various pests; resistance development is relatively rare. It represents a substantial avenue for managing insect pests and advancing transgenic plants possessing resistance against insects. The application of this knowledge has been beneficial since it has reduced the usage of hazardous chemical insecticides and agricultural products. However, the Cry toxins discovered so far do not efficiently control some insect pests. It is necessary to continue studies and research in order to isolate and characterize novel proteins or to use recombinant DNA and protein engineering (site-directed mutagenesis methods) to change

the sequences of *B. thuringiensis cry* genes in order to develop proteins with new and improved toxicity in different pests, which aid in the creation of new biopesticides and genetically modified plants, as well as providing a reservoir of Cry toxins that can be employed if resistance develops.

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