



## *Bacillus thuringiensis* (Bt): Clarifying the Genomic Landscape for Precision Pest Management in Agriculture

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### Abstract

The story of *Bt* (*Bacillus thuringiensis*) is presented in detail, covering its discovery in 1901 and its rise to prominence in the worldwide fight against pests. Originating with Shigetane Ishiwata's isolation in 1901 and Ernst Berliner's identification in 1911, *Bt*'s milestones include the 1958 commercialization and 1996 introduction of genetically modified *Bt* crops, covering 1.5 billion hectares by 2022. *Bt*, a dominant force in biocontrol with over 98% of commercialized biopesticides, employs diverse toxins such as Cry, Cyt and Vip families. Its precise insecticidal action, notably Cry proteins' multistep mechanism, targets key pests like Fall Armyworm and Diamondback Moth. *Bt*'s versatile applications extend to combating nematodes and genetic exploration through advanced techniques, including whole genome sequencing. Indigenous *Bt* isolates, exemplified by T405 and T414, showcase robust toxicity. Phylogenetic tree construction unravels the evolutionary pathways of insecticidal crystal proteins, portraying *Bt* as a resilient force in safeguarding agriculture and ecosystems. This review concludes by envisioning the future evolution of *Bt*'s application in agriculture, emphasizing sustainable practices guided by the collaboration between nature and science.

**Keywords:** *Bacillus thuringiensis*, Bioinsecticide proteins, Evolutionary analysis, Genetically modified crops, Genome profiling

### Introduction

The concept of employing microorganisms as biological agents for pest control has long been a foundational principle in the domain of integrated pest management. Over the decades, this method has been greatly improved due to progress in microbiology and biotechnology, establishing itself as a crucial element in sustainable pest control methods. A critical point in this transformation occurred when *Bacillus thuringiensis* (*Bt*) was found in 1901, leading to a groundbreaking period in biological pest management. Initially discovered by Japanese scientist Shigetane Ishiwata, *Bt* was first found in infected silkworm larvae, setting the stage for its future role as a key element in environmentally-friendly pest control methods in farming (Ibrahim et al., 2010). Years of research following this initial discovery led to the development of one of the most effective and

commonly used biological agents in pest control today. The full potential of *Bt* was not realized until later when German microbiologist Ernst Berliner, in 1911, gave a more detailed understanding of the bacterium, leading to its use in agricultural biotechnology (Ibrahim et al., 2010).

In 2022, biotech crops were grown on over 1.5 billion hectares globally, highlighting their crucial role in modern agriculture. By 2019, 72 countries had accepted genetically modified (GM) crops, with 29 growing them and 43 importing them for consumption, livestock feed or industrial processing. Significant cultivation occurred in Latin America, North America, Asia-Pacific, Africa and the European Union in 2019, with 190.40 million hectares planted. Brookes (2022) discovered that the global production of food, feed and fibre has increased by approximately 1 billion tonnes since the introduction of GM crops, while decreasing the

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environmental effect of crop protection by more than 17% since 1996. Furthermore, GM crops have cut carbon emissions by 39.1 billion kg, primarily due to reduced fuel usage, which is comparable to taking 25.9 million automobiles off the road for a year (Dionglay, 2022).

Currently, *Bt* is the most widely used commercial biopesticide, with a market share of over 90% (Jallouli et al., 2020), highlighting its strong presence in biological pest control methods. The multiple-step mode of action of Cry proteins has played a crucial role in managing important agricultural pests like the fall armyworm and diamondback moth. *Bt*, known for its various uses, is also being researched using genomic tools like whole genome sequencing to discover new biopesticidal pathways and improve its effectiveness in controlling nematodes.

Moreover, native *Bt* strains like T405 and T414 have shown considerable insecticidal effectiveness (Ramalakshmi and Udayasuriyan, 2010), indicating the possibility of finding new strains with improved characteristics. Phylogenetic research has enhanced our knowledge of the evolutionary past of insecticidal crystal proteins, showcasing the genetic variety and flexibility that have allowed *Bt* to remain a valuable asset in protecting worldwide agriculture.

This review delves into the various uses of *Bt*, its changes over time, and its increasing importance in sustainable farming methods. The future of *Bt* looks promising with advancements in genetic engineering and bioinformatics, offering better pest management solutions and furthering the collaboration between nature and science for ecological balance.

### Origin of *Bt*

The bacteria *Bacillus thuringiensis* (*Bt*), known for creating insect-killing proteins like Cry and Cyt that only impact insects and not humans or other non-target organisms, has progressed from a scientific interest to a key element of integrated pest management (IPM) (Bravo et al., 2013). Ernst Berliner first discovered *Bt* in a Mediterranean flour moth in 1911, and its commercial application started in France in 1938 with the creation of the first *Bt* pesticide, Sporine, as reported by Berliner (1915). The bacterium was later brought to the United States in 1958 as a biopesticide, greatly improving sustainable pest management techniques (Lambert and Peferoen, 1992).

The adoption of genetically modified (GM) *Bt* crops in 1996 was a significant breakthrough in agriculture, showing how genetically engineered plants can improve crop pest resistance and decrease reliance on traditional chemical pesticides. This breakthrough in genetic modification emphasized the idea of adding external proteins to plants to enhance agricultural efficiency and environmental friendliness (Qaim and Zilberman, 2003; Kleter et al., 2007).

### *Bt*'s Role in Pest Management

*Bt* emerged as an innovation in pest management, well known for its precision and speed. *Bt* is a recognized pioneer in biocontrol worldwide, accounting for more than

98% of commercialized biopesticides. It functions as an environmentally beneficial bioinsecticide. Its pesticides, equipped with  $\delta$ -endotoxin crystals and spores, demonstrate particular toxicity toward their intended insect targets (Lacey et al., 2001; Velivelli et al., 2014).

Different strains like *Bta*, *Bti*, *Btk* and *Bt san diego* have specific toxicity to target insects. Overall, *Bt* is contemplated as an effective and eco-friendly pest management solution (Bravo et al., 2005). In the early days of *Bt* research and development, scientists and companies began experimenting with *Bt* and produced *Bt*-based products. One of these products was named Thuricide. It's interesting to note that Thuricide has survived several industry changes and is currently connected to a product sold by Valent Biosciences. This longevity reflects the enduring importance and effectiveness of *Bt*-based solutions in pest control and agriculture (Steinhaus, 1951).

*Bt* as cost-effective production and versatile application methods, such as conventional spraying and GM *Bt* crops, further enhance its accessibility and affordability in agriculture (Lacey et al., 2015). *Bt* products rely on spore count for standardization, the presence of heat-tolerant exotoxins and low potency due to their basis on subspecies like *B. thuringiensis*. These issues highlighted the need for ongoing improvement in *Bt* insecticides (Beegle and Yamamoto, 1992; Palma et al., 2014) subsp. *kurstaki* HD-1 was found in the isolates of Kurstak and Dulmage (de Barjac and Lemille, 1970). This strain served as the basis for competitive *Bt* products in terms of both performance and cost, widely adopted by *Bt* companies. It excelled in microbial control, especially in forestry (Lewis et al., 1974), contributing significantly to global sales, particularly against pests like the spruce budworm and gypsy moth, accounting for over 60%. Other *Bt* varieties, such as subsp. *tenebrionis*, were also utilized (Schäfer et al., 2023). At first, upland cotton and maize were the *Bt* crops. However, farmers in several nations have been raising *Bt* aubergine (*Solanum melongena* L.) and *Bt* soybean (*Glycines max* L.) in recent years. *Bt* crops are currently being commercially cultivated in more than 29 countries spanning six continents (ISAAA, 2019).

Thuricide, an early *Bt*-based product, remains relevant today. What sets *Bt* apart is its cost-effective production and versatile application methods, including the game-changing advent of genetically modified (GM) *Bt* crops. Between 1996 and 2022, these crops covered a staggering 1.5 billion hectares globally, significantly reducing the need for chemical insecticides in agriculture (Tabashnik et al., 2023).

### Diversity Unleashed: The Arsenal of *Bt* Insecticidal Toxins

The true power of *Bt* lies in its diverse arsenal of insecticidal toxins. Categorized into Cry, Cyt and Vip families, these toxins play a pivotal role in effective pest management (Höfte and Whiteley, 1989). Cry toxins, with approximately 300 variants, target a broad range of insects, originating from crystals. On the other hand, Cyt toxins exhibit cytolytic activity, primarily against dipteran insects. The third family, Vegetative Insecticidal Proteins (Vip), differs by being released by the

vegetative phase of cells. Bacterial pesticidal toxins are classified into sixteen structurally distinct groups, each with unique characteristics. Ongoing research continuously reveals new potentials of *Bt* cells, showcasing the versatility of this natural pest-fighting powerhouse (de Maagd *et al.*, 2003).

Bacterial pesticidal toxins are classified into sixteen structurally distinct groups (Crickmore *et al.*, 2021). Each group has unique structural characteristics. For instance, Cry toxins comprise three domains, with some having a crystallization domain at the C-terminal. Cyt toxins are composed of  $\alpha$ -helices hairpins surrounding a central  $\beta$ -sheet. Gpp toxins are all low molecular weight  $\beta$  structured proteins. Tpp and Mpp are elongated toxic proteins composed mainly of  $\beta$ -strands, resembling the characteristics of specific families. In contrast, App toxins are elongated and mostly composed of  $\alpha$ -helices. While it's been suggested to use the term "pesticidal proteins" instead of "toxins," the latter is used here for consistency and practicality (Crickmore *et al.*, 2021). Recent research

works confirm the new potentials of *B. thuringiensis* cells.

### Cry Proteins Contribute to Insect Resistance Control

*Bt* toxin work through a fascinating multi-step method that reveals their efficiency. These toxic proteins initially form as crystals during bacterial sporulation. When consumed by insect larvae, they break down in their gut and release the protoxin. The mature toxin interacts with cell membranes when it is activated by digestive enzymes, resulting in membrane rupture and disruption of gut cells (Dow and Harvey, 1988; Gill *et al.*, 1992; Lee *et al.*, 1992; Knowles and Dow, 1993; Milne and Kaplan, 1993; Bravo *et al.*, 2007). The N-terminal toxin portion, crucial for toxicity, induces pore formation in the insect midgut. This cascade of events leads to ion fluxes, disrupting membrane potentials, causing necrosis and ultimately resulting in the demise of the targeted larvae (Figure 1). Ongoing research in this domain continues to unveil new aspects of *Bt*'s effectiveness in pest control, shedding light on the intricacies of its action (Salama and Sharaby, 1985; English and Slatin, 1992).

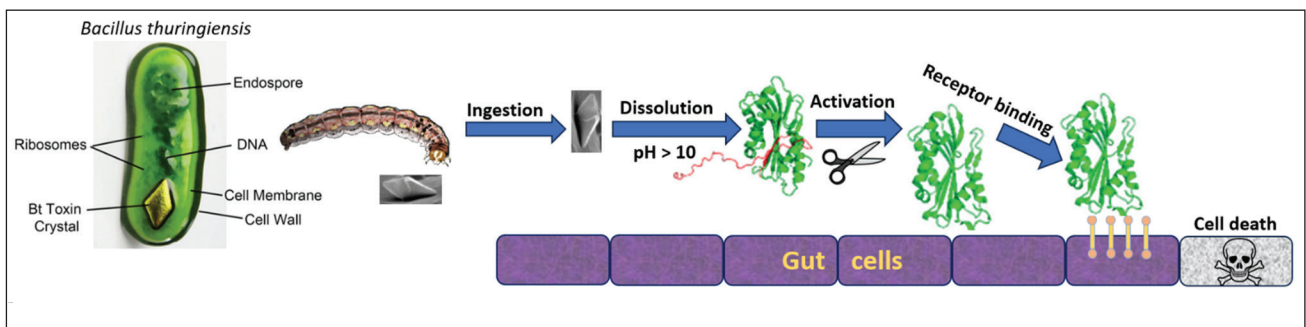


Figure 1: *Bt*'s typical multistep mechanism of action

### *Bt* Attacks the Principal Pests in Agriculture

In the enormous battlefield of agriculture, *Bt* protects crops from major pests that harm crops all over the world.

#### Fall Armyworm (FAW)

Surveys for eight years in Brazil revealed the extensive host range of the FAW, *Spodoptera frugiperda* (J.E. Smith) (FAW) (Noctuidae: Lepidoptera) that have documented by Montezano *et al.* (2018). Approximately 350 distinct species from 76 families were seen to be consumed by FAW, with a predilection for those in the orders Poaceae, Asteraceae and Fabaceae. However, it primarily targeted crops like maize, rice, sugarcane and forage grasses. The presence of sympatric strains of FAW significantly influenced host preferences within specific geographic regions (Rwomushana, 2019; Kenis *et al.*, 2019). Whorl damage assessments revealed that sorghum was the most preferred host among millets, experiencing 60.1% of the damage. Following sorghum, pearl millet had 41.4%, barnyard millet had 22.9% and finger millet had 10.2% of the whorl damage (Monobrullah, 2019). Feeding habits of FAW larvae show that first to 3<sup>rd</sup>-instar larvae consume 2% of the total leaf area over their lifespan. In contrast, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup>-instar larvae voraciously devour 4.7%, 16.3% and 77.2% of the foliage,

respectively, causing extensive de-foliation (Sparks, 1979). Importantly, FAW infestations can lead to substantial yield losses. When maize plants suffered 55 to 70% damage during the mid-to-late whorl stage, the yield loss ranged from 15% to 73% (Hruska and Gould, 1997). FAW have incurred an annual yield decrease of 45% in Ghana and 40% in Zambia, resulting in 6,312 US\$ million in economic losses for 12 African nations (Day *et al.*, 2017). Furthermore, FAW damage in maize crops varied from 26.4 to 55.9%, causing a yield reduction of around 11.57% (Baudron *et al.*, 2019). It has been identified as an invasive pest in several areas, including West and South Sumatra, recently (Herlinda *et al.*, 2022).

In May 2018, FAW was initially identified in the southern region of India (Sharanabasappa *et al.*, 2018; Shylesha *et al.*, 2018). Since then, reports of FAW infestations have surfaced from all around the nation, except Jammu & Kashmir and Himachal Pradesh (Suby *et al.*, 2020), leading to significant economic losses. This widespread distribution of FAW has posed a significant challenge to agriculture in India, impacting crop yields and agricultural livelihoods (Singh *et al.*, 2023). Yield loss of 33% in India caused by FAW, using IoT-based technologies. These findings underscore the significant threat posed by FAW to crops, particularly maize and emphasize the need for effective pest management strategies to mitigate its impact on crop yields and economic



losses (Balla et al., 2019). A current study conducted across various maize-producing blocks in Coimbatore district found whorl infestation levels ranging from 16.0% to 77.7%, with severity scores between 1.3 and 4.3 on a 5-point scale (Srinivasan et al., 2023). Infestations have been reported across various regions: 140,000 hectares in Karnataka, 85,000 hectares in Madhya Pradesh, 59,000 hectares in Rajasthan, 2,000 hectares in Maharashtra, 1,747.9 hectares in Mizoram, 200 hectares in Tamil Nadu and 137 hectares in Andhra Pradesh (Deshmukh et al., 2021).

#### Diamondback Moth (DBM)

DBM *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera) infestations are known to be sporadic, occurring during the growth season and their levels can vary from endemic to severe outbreaks (Al-Ahmad et al., 2009). DBM is a global pest responsible for crop damage that costs more than a million dollars each year to manage (Silva and Furlong, 2012). Larvae are voracious eaters, eating between 62% and 78% of leaves. This limits the number and quality of crops produced and plant development (Gangurde and Wankhede, 2009). The lack of natural enemies and the emergence of pesticide resistance are the main causes of DBM infestations in some nations. Situations involving DBM can have a significant financial impact. For example, a single DBM epidemic in California resulted in damages of more than US\$ 6 million (Cao et al., 2008). In Southeast Asia and India, DBM outbreaks have led to crop losses exceeding 90% and 100%, respectively (Sharma et al., 2017; Marak et al., 2017). In India, DBM infestations have been associated with economic losses of up to 50%, with an estimated annual cost of US\$ 168 million (Srinivasan and Uthamasamy, 2006). Furthermore, DBM has a strong preference for brassica vegetables, causing significant crop losses, of up to 80% worldwide (Javed and Mukhtar, 2017).

#### Tobacco Caterpillar (*S. litura*)

*Spodoptera litura* (Fabricius) (Noctuidae: Lepidoptera), stands out as a significant threat, causing substantial yield losses to many crops. About 112 cultivated plants are infested by this polyphagous pest. The economic losses attributed to *S. litura* infestations can range from 25.8% to 100% (Dhir et al., 1992). This insect pest is very important in India since it damages many crops severely, causing losses in income that can range from 26% to 100% (Dhir et al., 1992; Vijayalakshmi et al., 2016). However, they were newly identified in cocoa plants in India (Madhu et al., 2023). Maintaining the production of this significant winter vegetable in India would require managing and reducing the impact of this insect issue on cabbage agriculture. A thorough examination of the infestation patterns and the degree of damage over time is revealed by the research. Seasonal pest *S. litura* does significant damage during cyclonic weather and strong precipitation following an extended period of dry weather (Thanki et al., 2003). The first infestation occurred in November, with a low average larval population of 0.1 larvae head<sup>-1</sup> and 2% plant infestation. However, the situation escalated significantly, with the maximum plant infestation of 54% observed in January,

accompanied by a larval population of 3.17 larvae head<sup>-1</sup>. These voracious feeders target leaf veins and cut the stems of tender seedlings, earning them the nickname “cutworms.” The damage inflicted by them can lead to staggering losses, ranging from 80% to 100% (CTRI, 2023).

#### Cotton Bollworm (*H. armigera*)

The polyphagous pest *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is well-known for producing significant financial losses on a variety of crops around the globe (Bouslama et al., 2019). It is a serious pest that harms some commercially significant crops, including maize, cotton, okra, pigeon peas, sunflower, tomatoes, sorghum, millet and sunflower (Sharma, 2001). It is the most common species of bollworm that damages Indian cotton, with damage varying from 14% to 56% (Jayaraj, 1990). About half of agricultural insecticides used in China and India are used to manage this pest (Czepak et al., 2013). *H. armigera* exhibits a seasonal infestation in the Vamban region of Tamil Nadu’s east coast plain and hills during the southwest monsoon (Vennila et al., 2020). In chickpea crops, a single *H. armigera* larva can devastate as many as 40 pods, while it is a larva (Sanap and Deshmukh, 1987), resulting in yield losses that can escalate to 400 kg ha<sup>-1</sup> (Rahman, 1990).

#### Eastern Spruce Budworm (*Choristoneura fumiferana*)

Every three to four decades, a moth that is endemic to North America emerges and in recent years, this insect has caused over 25 million acres of trees to lose their leaves. In 2006 alone, it inflicted damage on over 7,500 acres along the St. Lawrence River. According to a survey conducted in 2019, its impact extended to over 24 million acres (Johns et al., 2019). During springtime, larvae commence feeding on fresh foliage, with visible defoliation typically observed by late June once the larvae have completed the majority of their feeding (Oten et al., 2023).

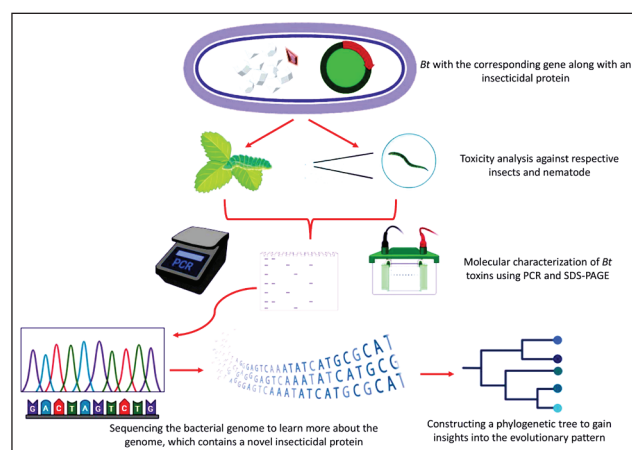


Figure 2: Process for characterizing *Bt* genes and sequencing genomes

#### Toxin Specificity of Insecticidal Protein

The insecticidal protein produced by *Bt* is utilized for pest control in both intensive and extensive farming practices. *Cry1*, *Cry2* and *Vip3* proteins are harmful to agricultural pests including the fall armyworm; of these proteins, *Vip*

exhibits the greatest level of toxicity (Tavares *et al.*, 2021). Even the other key lepidopteran pests DBM (Sarfraz *et al.*, 2005), Tobacco caterpillar (Khurshid *et al.*, 2023) and Cotton Bollworm (Liu *et al.*, 2010) were showing susceptibility to *Cry1*, *Cry2* and *Vip3* toxins. On the other hand, tree crop pests like *Choristoneura fumiferana* can also be controlled using the *Bt* toxin *Cry1Ab* (Lachance *et al.*, 2007).

### Beyond Crop Protection: *Bt*'s Nematode Warfare

Agriculture has long been plagued by plant-parasitic nematodes, like root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* and *Globodera* spp.). Their piercing and sucking feeding behavior causes significant damage to various crops, leading to an estimated global output loss of \$ 125 billion annually (Yu *et al.*, 2015). Some *Bt* strains have the extraordinary capacity to enter worms' digestive tracts, germinate and multiply there (Ruan *et al.*, 2015). Nematicidal characteristics are present in some families of crystal proteins, such as *Cry5*, *Cry6*, *Cry12*, *Cry13*, *Cry14*, *Cry21* and *Cry55* (Yu *et al.*, 2015). In considering this, a potential line of defense against plant-endoparasitic nematodes is provided by plants that express recombinant *Cry* proteins that are active against pests (Li *et al.*, 2008). Several other *Bt* compounds, including thuringiensin, chitinase and metalloproteinase, have displayed nematicidal activities (Sánchez-Soto *et al.*, 2015). Furthermore, genes that encode nematicidal factors, which includes haemolysins, enterotoxins, lantibiotics and proteases, have been found. These factors are predominantly regulated by the transcription regulator PlcR (Ruan *et al.*, 2015). Recent research has extensively tested the nematicidal activities of *Bt* strains against a wide range of nematode species, including animal parasitic nematodes *viz.*, *Ascaris suum*, *Distolabrellus veechi*, *Haemonchus contortus*, *Trichostrongylus* sp. and *Ostertagia circumcincta*, as well as plant parasitic nematodes like *Pratylenchus scribneri*, *Tylenchorhynchus* sp., *Ditylenchus destructor* and *Caenorhabditis elegans* (Guo *et al.*, 2008; Mohammed *et al.*, 2008; Zi-Quan *et al.*, 2008; Khan *et al.*, 2012; Yu *et al.*, 2015; Jouzani *et al.*, 2017; Zhang *et al.*, 2017). Positively, some publications show that *Cry* proteins and spores have a low LC50, which gives optimism for the potential use of *Bt* strains as bio-nematicides in the future.

### Unraveling the Genetic Makeup

#### PCR Screening

To find new *cry* genes in *Bt* isolates, PCR techniques have been applied (Brousseau *et al.*, 1993; Ben-Dov *et al.*, 1997) and introduced specific primers for specific *cry* gene detection. Multiplex PCR was developed and improved to enable the simultaneous detection of multiple *cry* genes (Bourque *et al.*, 1993; Ben-Dov *et al.*, 1999).

Specific *cry* gene sequences in *Bt* strains may be found and identified quickly and sensitively using PCR (Vidal-Quist *et al.*, 2009). It helps to screen concurrent strains according to their pesticidal activity (Juarez-Perez *et al.*, 1997). *Cry* genes are frequently swapped out or merged *via* pyramiding to avoid resistance. Even slight variations in amino acids can

greatly affect *Cry* protein toxicity (Udayasuriyan *et al.*, 1994). Target range, toxicity and insect resistance constraints for *Bt* products need the search for new genes and sensible design approaches based on established *Cry* toxins (Lin *et al.*, 2008). DNA-based techniques have limitations in detecting genes that have already been identified and do not provide information on their expression. On the other hand, proteomic analysis of parasporal crystals offers greater accuracy in determining their presence, as stated by (Chestukhina *et al.*, 1994). For *Bt* strains that were identified in Mexico, the most prevalent gene profiles for *cryI* and *cryIII* were found. With three *cryIA* genes, these strains were the most common, accounting for 48% of the total population. Furthermore, strains with the *cryIB* gene were very common; they made up about 30% of the total sample. Significantly, strains that had the *cryIC* and *cryID* genes showed substantial toxicity towards *S. frugiperda* larvae; however, further information on this toxicity was not given. It was discovered that some strains alone had the *cryID* gene and that these strains were extremely harmful to *S. frugiperda* larvae (Ceron *et al.*, 1995). PCR testing on native *Bt* strains revealed the presence of several important genes. *Cry1Ab*, *Cry1Ac*, *Cry2Aa*, *Cry2Ab* and *Vip3A* were lepidopteran cytotoxic genes found among the identified genes (Karuppaiyan *et al.*, 2022). A thorough PCR investigation reveals that five isolates have *Cry1* and seven isolates have *Cry2*, accordingly, while seven isolates have *Cry1*, *Cry2* and *Vip3* genes present simultaneously (Gothandaraman *et al.*, 2022). PCR analysis of 50 *Bt* isolates indicated an abundance of nematicidal harmful genes, *viz.*, *Cry5*, *Cry6*, *Cry14* and *Cry21* (Ramalakshmi *et al.*, 2020).

Additionally, RAPD-based markers offered an alternative screening method (Hansen *et al.*, 1998). A combination of the *Cry1* and *Cry2* genes was identified (Ben-Dov *et al.*, 1997; Sena *et al.*, 2009) and discovered that 47% of strains carried *Cry1* genes. Jain *et al.* (2012) reported various *cry* gene patterns, with 100% of *Cry1* genes present. (Patel *et al.*, 2013) Different *cry* genes from *Bt* strains were discovered in India. According to Jain *et al.* (2017), *cry* gene profiles varied, with *Cry1* genes being the most common (100%). The genetic variety and occurrence of *cry* genes in *Bt* samples are revealed by these PCR-based investigations (Figure 2).

#### Protein Profiling

SDS-PAGE is a widely used tool to find the different *Cry* proteins present in the crystal mixture preparations from different *Bt* isolates. Among the screening of native *Bt* isolates 12 isolates with 135 kDa (*Cry1*) and 14 isolates with 65 kDa (*Cry2*) bands were identified (Gothandaraman *et al.*, 2022). When Egyptian *Bt* is employed against whiteflies, it reveals the existence of the *Vip3Aa* gene at ~88 kDa, which is then utilized to validate and produce lethal genes (El-Gaied *et al.*, 2020). Two distinct protein bands were identified by the SDS-PAGE protein profiling analysis: one at around 135 kDa and the other at about 65 kDa. The *Cry1* and *Cry2* peptides are represented by these bands, respectively. Interestingly, isolates T429, T434, T437, T438, T444 and T446 exhibited only the *Cry1* protein band at approximately 135

kDa, indicating a different protein profile compared to the aforementioned isolates. In contrast, isolate T441 expressed solely the *Cry2* protein, suggesting further variability in protein composition among the isolates. Additionally, *Vip3* protein was detected in nine isolates, namely T428, T429, T431, T433, T434, T437, T438, T444 and T446, at a molecular weight of approximately 88 kDa (Karupaiyan et al., 2022). Protein bands with molecular weights varying from 26 to 124 kDa were present in *Bt* isolates. Approximately 124 kDa, 90 kDa and 70 kDa bands were detected in 20 isolates, 8 isolates and 12 isolates, respectively. These isolates belong to *Cry1* (~124 kDa), *Cry2* (~70 kDa) and *Vip3* (~90 kDa) (Navya et al., 2021).

The molecular characteristics of proteins produced by different *Bt* isolates, particularly their Cry proteins and their effectiveness against the Coffee berry borer, *Hypothenemus hampei*, were investigated. The study found that *Bt* isolated from coffee farms in Costa Rica produced Cry proteins of different molecular weights. *Cry1* proteins ranged from 130 to 150 kDa, *Cry2* proteins from 65 to 70 kDa, *Cry3* at 75 kDa, *Cry7* and *Cry8* at 130 kDa, *Cry9* between 130-140 kDa, *Cry22* at 76 kDa and *Cry34* and *Cry37* at 14 kDa. These proteins exhibited insecticidal activity against the Coffee berry borer (Arrieta and Espinoza, 2006). Similarly, Ramalakshmi and Udayasuriyan (2010) examined 70 *Bt* strains obtained from Tamil Nadu's Western Ghats using SDS-PAGE. According to their findings, there was protein variation among the 17 strains (24.20%) that showed two main protein bands had molecular weights between 135 and 65 kDa.

Additional research on Cry proteins, having molecular weights between 20 kDa and 160 kDa, has also revealed a variety of electrophoretic patterns by Arrieta and Espinoza (2006), Liu et al. (2007) and Swamy et al. (2013). Certain *Bt* isolates, such as *BtMA-64* and *BtMA-194*, were found to create well-defined bands having molecular weights between 100 kDa and 150 kDa, whereas isolates with lower molecular weights were produced by *BtMA-104*, *BtMA-251*, *BtMA-410* and *BtMA-450* (Lobo et al., 2018). Rajashekhar et al. (2018) revealed a peptide profile with a band ranging in pre-solubilized form from 20 to 245 kDa and in solubilized form from 18 to 110 kDa. Based on their molecular weights, they divided the proteins into three categories: group I (18 to 60 kDa), group II (65 to 105 kDa) and group III (110 to >245 kDa). SDS-PAGE analysis of spore-crystal mixtures of native *Bt* strains revealed a range of molecular weights between 150 and 28 kDa (Reyaz et al., 2017). Using SDS-PAGE analysis, Nair et al. (2018) investigated spore-crystal mixes from *Bt* isolates in Qatar. Research conducted by Fernandez-Luna et al. (2019) concluded that most of the *Bt* isolates generated protein bands of around 25, 40, 75 and/or 120 kDa (Figure 2).

#### Genomic Adventures: Whole Genome Sequencing

Recent leaps in sequencing technologies, particularly Illumina and PacBio strategies, empower scientists to embark on genomic adventures (Cao et al., 2018). WGS is a potent tool for characterizing significant *Bt* strains, offering insights into novel parasporal proteins and identifying genetic variations. This approach allows a thorough examination of

the genetic basis for *Bt*'s pathogenicity and toxicity towards host insects, uncovering hidden secrets within its DNA (Rabha et al., 2023).

#### Genomic Analysis of Indigenous *Bt* Isolates

As a well-known representative of the *Bacillus cereus* genus, *Bt* is the most commonly used biological control agents. A thorough examination of the whole genomes of *Bt* isolates provides important insights into the existence of new genes and plasmids in native strains. The genome analysis enables researchers to gain insights into the genetic makeup and potential functional characteristics of these isolates, contributing to a deeper understanding of their biological control capabilities (Chelliah et al., 2019).

The native *Bt* isolate T405, obtained from Tamil Nadu Agricultural University in India, was subjected to whole-genome sequencing. The results indicated that the 6,673,691 bp genome had 12 plasmids and 563 scaffolds. 6,126 genes were functionally annotated out of 6,174 protein-coding genes, 13 rRNA and 98 tRNA genes found by genome annotation. Notably, T405 contains a large number of virulence factors (immune inhibitors, phospholipases, hydrolases, chitinases, haemolysins, urease subunit genes) and insecticides (*Cry1Ac32*, *Cry1Ab9*, *Cry1Aa6*, *Cry1Ac5*, *Cry1Aa18*, *Cry1Ab8*, *Cry1Ab11*, *Cry2Aa9*, *Cry1Ia40*, *Cry2Aa35*, *cyt*, *Vip3Aa7*, *tpp80Aa*) (Sathyan et al., 2022).

After being sequenced using MiSeq technology, another native *Bt* isolate, T414, which resulted a strong cytotoxicity against *Pectinophora gossypiella*, unveiled a complete genetic landscape consisting of a chromosome and several plasmids, with an entire genome size of 6,493,494 bp. Automatic annotation predicts 152 RNA molecules (rRNAs, tRNAs and ncRNAs) and 6,877 coding sequences. Interestingly, the completed genome is spread among 15 different plasmids and a chromosome, each of which may contribute differently to the pathogenicity and ecological adaptation of the bacteria. Remarkably, the research reveals the existence of plasmid-borne vegetative insecticidal protein gene (*Vip3Aa*) and parasporal crystal genes (*Cry1Aa*, *Cry1Ab*, *Cry1Ac*, *Cry1IaA*, *Cry2Aa*, *Cry2Ab* and *cyt1*). The draft sequence also identifies some virulence factors, including hemolysins, bacteriocins, proteases and chitinases. The location of the Cry, Cyt, or Vip toxins on two distinct plasmid types- referred to in this work as p414A and p414E is one noteworthy finding (Reyaz et al., 2019).

#### Indigenous *Bt* Isolates

The focus shifts to indigenous *Bt* isolates, particularly T405 from Tamil Nadu Agricultural University, India (Sathyan et al., 2022) and T414, exhibiting robust toxicity against *Pectinophora gossypiella* (Reyaz et al., 2019), along with T210, displaying potent toxicity against nematodes (Berryish et al., 2023). Comprehensive whole-genome sequencing reveals the intricate genetic profiles of these isolates, unveiling a plethora of plasmids, insecticidal toxin genes and virulence factors. These thorough analyses significantly enhance our comprehension of the biological control potential inherent in indigenous *Bt* isolates.



### Phylogenetic Tree Construction

Phylogenetics proves to be a potent tool for uncovering the evolution of present-day species (Figure 2). When scientists delve into phylogenetic trees, they acquire a more profound comprehension of species' evolutionary pathways, elucidating both the resemblances and disparities among them (Munjal *et al.*, 2019). Multiple structural domains are present in many proteins from different animals and some of these have shown independent domain development (Baron *et al.*, 1991; Morett and Segovia, 1993). To evaluate the evolutionary relationships between ICPs and each of their functional domains, genetic distances across the Cry sequences were measured using the PROTDIST program from J. Felsenstein's PHYLIP 3.5 evolutionary inference package. For this, the Dayhoff PAM matrix was employed following the previously acquired alignment (Bravo, 1997). Predicting ancestral states is made easier by sequence comparison, which provides a glimpse into the common past of different species. It is vital to understanding the biology of living things to recognize the connections and commonalities between different species. When it comes to sequence comparison, there are two main approaches: alignment-based and alignment-free methods (Chan *et al.*, 2011; Vinga, 2014; Schwartz and Schäffer, 2017). Character-based methods examine each of the sequences at once, concentrating on single character or position at a specific time. Techniques such as maximum parsimony and maximum likelihood fall into this category. These approaches consider variations across the sequence set and are based on probabilistic models, offering an in-depth, probabilistic view of sequence analysis (Alon *et al.*, 2010). Group 1 *Bacilli* encompasses three species: *Bacillus anthracis*, *B. cereus* and *B. thuringiensis*, and these species are closely related genetically. Although they inhabit the same soil environments, they differ in their morphological characteristics. *B. anthracis*, the pathogen responsible for anthrax, is genetically uniform (phylogenetically monomorphic); whereas, *B. cereus* and *B. thuringiensis* show greater genetic variability. An Amplified Fragment Length Polymorphism (AFLP) study reveals the genetic diversity present among *Bacillus* species that do not cause anthrax (Radnedge *et al.*, 2003).

### Conclusion

*Bt* is a game-changer in pest control and agriculture, evolving from a laboratory marvel into a global leader in pest management. Its precision, versatility and eco-friendly approach have propelled *Bt* from its early days as Spore to the widespread adoption of genetically modified *Bt* crops, transforming agriculture worldwide. With its diverse insecticidal toxins and intricate Cry protein mechanisms, *Bt* excels in safeguarding crops and advancing sustainable practices. Beyond agriculture, *Bt* delves into the microscopic realm, tackling plant-parasitic nematodes and unveiling genetic insights through whole genome sequencing. As we look to the future, ongoing research promises breakthroughs and *Bt's* role in agriculture is set to evolve even further. This

fusion of nature and science, embodied by *Bt*, inspires us to embrace a future of sustainable farming with optimism, celebrating the ingenuity and resilience of the natural world. *Bt*, nature's pest warrior, stands as a guiding light toward a future where pest management and environmental harmony go hand in hand.

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