Cry proteins from *Bacillus thuringiensis*: From natural insecticide to biotechnological tool for agricultural pest control

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ABSTRACT

Objective: The Cry protein family from *Bacillus thuringiensis* (Bt) exhibits biocidal activity against various insect orders, with Lepidoptera being among their primary targets. These insects often evolve into major agricultural pests, leading to significant crop losses. Consequently, research efforts have focused on the identification and cloning of the genes encoding these proteins, as well as elucidating their mechanism of action.

Design/Methodology/Approach: Methods for the identification, isolation, and purification of Cry proteins, along with the current state of the art, were explored, described, and discussed by reviewing relevant literature using scientific databases for bibliographic research.

Results: Cry proteins constitute a diverse group of molecules with demostrated activity for combating agricultural insect pests, primarily Lepidoptera. These proteins and their corresponding genes have been widely studied and employed in the genetic transformation of several crop plant species.

Limitations/Implications of the study: Although several models have been proposed to explain the mode of action of Cry proteins, molecular mechanism remain unresolved to fully understand their insecticidal activity.

Findings/Conclusions: Bt encodes multiple groups of proteins with insecticidal activity, among which the Cry protein family is the most extensively studied. The identification of its first members, along with the elucidation of their three-dimensional structure, has been essential for proposing their mechanism of action. This has enabled the targeted control of several Lepidopteran pests in agriculture.

Keywords: Pests, insect, toxins.

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INTRODUCTION

In contemporary agriculture, one of the most pressing challenges is pest management, particularly of insect species that have developed resistance to chemical insecticides. Excessive use of these compounds accelerates resistance, diminishes their efficacy, and

results in significant global economic losses (Walsh *et al.*, 2022; Daraban *et al.*, 2023; Gula, 2023).

Moreover, conventional chemical pesticides generally exhibit a broad spectrum of activity, affecting not only pests but also pollinators, other beneficial insects, animals, and even humans (Devine & Furlong, 2007; Walsh *et al.*, 2022; Daraban *et al.*, 2023). These products also contribute to soil, water, and air pollution. Consequently, global policies are increasingly oriented toward sustainable alternatives to synthetic insecticides (Devine & Furlong, 2007; Jurat-Fuentes *et al.*, 2021; Daraban *et al.*, 2023).

Given this context, it is imperative to design pest-control strategies that minimize environmental impact while preserving biodiversity. Biotechnology has played a pivotal role in this endeavor by providing scientifically grounded solutions based on the use of biological agents with pest-control potential (Agaisse & Lereclus, 1995; Jurat-Fuentes *et al.*, 2021; Pacheco *et al.*, 2023). Compared to chemical pesticides, more favorable alternatives include biochemical control, which employs essential oils, pheromones, hormones, plant metabolites, and enzymes, as well as biological control using microorganisms such as bacteria (*Brevibacillus laterosporus*, *Dickeya dadantii*, *Yersinia* spp., among others), fungi (*Beauveria*, *Metarhizium*, *Paecilomyces*, *Verticillium*, *Trichoderma* and many more), beneficial insects, entomopathogenic nematodes, parasitoids, and viruses such as baculoviruses and entomopoxviruses (Loth *et al.*, 2015; Springer *et al.*, 2018; Glare *et al.*, 2020; Kim *et al.*, 2024; de Sousa *et al.*, 2025; Mitsuhashi, 2025; Ng & Zuharah, 2025).

Nevertheless, several of these strategies face limitations such as reduced biocidal efficacy, restricted specificity, or limited commercial availability, with many still in early research stages. Therefore, the present work focused on analyzing scientific advances related to Bt Cry proteins, which have been extensively studied for insect pest control.

Bt, the Bacterium that Synthesizes Insecticidal Compounds

Bt is a Gram-positive, facultative anaerobic bacterium predominantly found in soil, stagnant water, insects, plants, and other habitats from which it has been isolated (de Maagd *et al.*, 2003). Historical records indicate that it was first isolated at the dawn of the 20th century by the Japanese scientist Shigetane Ishiwata, from silkworms (*Bombyx mori*) that had developed the sotto disease (Roh *et al.*, 2017; Fernández-Chapa *et al.*, 2019; Li *et al.*, 2024). Subsequently, in 1915, Berliner again isolated the bacterium from the flour moth in a storage facility located in Thuringia, Germany, the site from which its name was derived (Valtierra *et al.*, 2020; Li *et al.*, 2024). During the 1950s and 1960s, it was reported that certain *Bt* strains produced intracellular crystal-like inclusions that proved toxic to specific insect orders such as Lepidoptera, Coleoptera, Diptera, Hemiptera and Hymenoptera (Figure 1).

The ability of this bacterium to act selectively on harmful insects without affecting other living organisms made it a valuable tool for biological pest control. In 1938, France introduced a commercial product called Sporine, formulated with *Bt*, intended to eradicate the flour moth (Roh *et al.*, 2017; Li *et al.*, 2024). Since 1961, in the United States of America (USA), the first Bt-based products designed for agricultural use have been marketed (Baum *et al.*, 1999). The potential of this bacterium as an alternative to chemical insecticides

Figure 1. Insecticidal activity of proteins Cry. Cry toxins have insecticidal activity against different orders of insects (Lepidoptera, Diptera, Coleoptera, Hymenoptera and Hemiptera). Adapted from Berry *et al.*, 2025.

stimulated the emergence of multiple research lines, which have since expanded to identify new genes enabling the development of innovative insecticidal products.

At present, numerous *Bt*-derived products are available, which may include spores containing several insecticidal proteins or genetically modified bacteria harboring engineered pesticidal toxins to enhance efficacy (Sanahuja *et al.*, 2011). Some formulations incorporate enzymes that potentiate the insecticidal activity of Cry proteins. These products are applied either as powders or aqueous suspensions sprayed onto plants, allowing insects to ingest the bacterium or its spores through the consumption of plant tissue (Jurat-Fuentes *et al.*, 2021; Sanahuja *et al.*, 2011).

Bt Pesticidal Toxins

Bt pesticidal toxins exhibit insecticidal activity against various Lepidoptera that are major agricultural pests of diverse crops, including: *Spodoptera frugiperda* (fall armyworm, affecting maize), *Trichoplusia ni* (cabbage looper, attacking broccoli and cauliflower), *Manduca sexta* (tobacco hornworm, damaging tobacco, tomato, and pepper), *Plutella xylostella* (diamondback moth, affecting cabbage, radish, watercress, and cauliflower), among many others. In addition, there are Cry proteins toxic to Coleoptera (beetles) and Diptera (mosquitoes), among other insect orders.

In the USA, various *Bt* subspecies are employed to control different insect classes, including strains such as *kurstaki*, *aizawai*, and *galleriae* (Lepidoptera), *morrisoni* biovar *tenebrionis* (Coleoptera), and *israelensis* (Diptera) (Karabörklü *et al.*, 2018).

Furthermore, the use of *Bt* can be integrated as a resource within integrated pest management (IPM) systems, encompassing strategies such as crop rotation, biological control through natural enemies, the use of plant-derived metabolites, and the implementation of appropriate agricultural practices to prevent pest outbreaks. All these

measures aim to minimize reliance on synthetic insecticides, thereby contributing to the production of safer food for human health (Sanahuja *et al.*, 2011). For these reasons, *Bt* continues to be widely used worldwide, not only for agricultural pest control but also for combating disease-vector insects affecting humans, such as mosquitoes, since certain strains produce proteins toxic to them.

Growth and Sporulation

Bt undergoes a vegetative developmental stage during which it reproduces by binary fission. When nutrients become limited, the bacterium initiates a differentiation process that culminates in sporulation. During this phase, it produces spores and proteinaceous parasporal crystals composed mainly of Cry proteins and others, whose presence depends on the strain (Pinos *et al.*, 2021). These crystals can exhibit diverse morphologies, including bipyramidal, cubic, circular, rhomboidal, and amorphous forms (Prieto *et al.*, 1997; Sauka & Benintende, 2008; Li *et al.*, 2024). It is important to emphasize that not all Cry proteins are synthesized during sporulation. For example, Cry3A is produced during the vegetative phase (Agaisse & Lereclus, 1995).

During the vegetative phase, *Bt* undergoes exponential growth in synthetic culture media containing a carbohydrate source such as glucose, a nitrogen source such as peptone or soybean extract, and mineral salts including magnesium, zinc, calcium, and manganese, among others. The optimal pH is approximately 7.0, the optimal growth temperature is 30 °C, and aerobic conditions are required. Upon nutrient depletion, the molecular machinery is activated to initiate sporulation —a process extensively studied in *B. subtilis* (Agaisse & Lereclus, 1995). Throughout sporulation, the cell generates a sporangium that divides into two compartments: the mother cell (where parasporal bodies containing Cry proteins accumulate) and the forespore that ultimately matures into the spore. When environmental conditions become favorable, the spore germinates, and the growth cycle is reinitiated (Lereclus, 2000; Cho & Chung, 2020).

***Bt* and Its Insecticidal Proteins**

Several *Bt* strains have been identified that exhibit toxicity against a wide spectrum of insects and invertebrates, including both agricultural pests and disease vectors such as mosquitoes. This lethality is attributable to the production of insecticidal proteins that form pores in the insect midgut or activate the alternative G-protein mediated apoptotic signalling pathway ultimately leading to death (Jurat-Fuentes *et al.*, 2021; Best *et al.*, 2023; Pacheco *et al.*, 2023).

Bt has emerged as the most widely used bacterium worldwide in the control of insect pests. This success is largely due to the diversity of pesticidal toxins it produces, which display high specificity, strong efficacy, and reduced environmental impact, as previously noted. Importantly, these pesticidal toxins are biodegradable through sunlight and environmental microorganisms, significantly reducing environmental residues (Jurat-Fuentes *et al.*, 2021; Li *et al.*, 2024; Soberón & Bravo, 2025).

In addition, *Bt* synthesizes other bioactive molecules such as cytolytic proteins (Cyt), vegetative insecticidal proteins (Vip), α- and β-exotoxins, enterotoxins, chitinases,

bacteriocins, hemolysins, phospholipases, among others. These compounds can be applied in both pest management and the neutralization of disease vectors, parasites, bacteria, and fungi (Gillis, 2013; Hernández, 2016; Syed *et al.*, 2020; Gupta *et al.*, 2021). Therefore, *Bt* produces a wide array of molecules that are lethal to insects, and their synergistic activity enhances its overall efficiency as an insecticide.

Cry Protein Family

The Cry protein family consists of nearly 800 genes, which, based on amino acid sequence homology, have been classified into 74 families (Cry1-Cry74; Crickmore *et al.*, 2021). Cry genes are predominantly located on plasmids, although some are also chromosomal.

Wild-type *Bt* strains exhibit variability in the number of plasmids; for example, the HD1 strain belonging to the kurstaki subspecies carries 12 plasmids (Faust *et al.*, 1979). One of these plasmids contains the gene encoding Cry1Ab, while another encodes four proteins: Cry1Aa, Cry1Ac, Cry2A, and Cry2B. This capacity to produce multiple toxins accounts for the high efficacy and broad applicability of *Bt* in commercial formulations since its discovery.

Mechanism of Action of Cry Proteins

Cry proteins, which are characterized by a three-domain (3d) structural organization, have been extensively investigated, particularly Cry1Ab. When *Bt* is ingested by susceptible insect larvae, the parasporal crystal inclusions are also consumed. Once released, these crystals are solubilized in the alkaline environment of the insect midgut (Höfte & Whiteley, 1989). Subsequently, the solubilized Cry protoxins require proteolytic activation to exert their function, a process that converts them into active pesticidal toxins (Höfte & Whiteley, 1989).

Model of Action: Receptor Binding and Pore Formation

Pesticidal toxins possess the ability to bind to specific receptors located in the membrane of the intestinal epithelium, such as aminopeptidase-N (APN) and alkaline phosphatase (ALP), both anchored via glycosylphosphatidylinositol (GPI). A significant affinity has also been reported for cadherins. This interaction promotes the oligomerization of 4 to 8 monomers, facilitating the formation of lytic pores in the membranes of midgut epithelial cells. The generation of these pores induces an osmotic imbalance that triggers deregulated ion fluxes, bacterial translocation, septicemia and ultimately leading to cell lysis (Pardo-López *et al.*, 2013; Gómez *et al.*, 2014; Bravo *et al.*, 2023; Soberón & Bravo, 2025).

Experimental approaches such as ligand blotting, surface plasmon resonance, and pulldown assays have demonstrated that the C-terminal region of Cry1Ab binds to APN and ALP but not to cadherins. This suggests that complementary receptors may interact with the region cleaved during protoxin activation, a subject of ongoing investigation (Peña-Cardeña *et al.*, 2018). In addition to the aforementioned receptors, integral membrane proteins belonging to the ATP-binding cassette (ABC) transporter family have also been identified as receptors for Cry pesticidal toxins (Sato, 2024).

Three-Dimensional Structure of Cry Proteins

Cry proteins, with an estimated molecular weight of approximately 130 kDa, are composed of seven domains. During the activation process, however, the protoxin undergoes proteolytic processing, as previously described (Höfte & Whiteley, 1989). In this process, the C-terminal region comprising nearly 500 amino acids —including domains 4 through 7— is cleaved, resulting in an active pesticidal toxin of approximately 60 kDa that retains only three domains. These domains are responsible for receptor binding and pore formation. In laboratory practice, proteolytic processing is typically carried out with trypsin, yielding truncated versions analogous to those isolated from the midguts of susceptible insects (Soberón *et al.*, 2000; Peña-Cardeña *et al.*, 2018).

Elucidation of the three-dimensional configuration of the active portion of Cry1Aa and Cry3A revealed the presence of three structural domains. Consequently, proteins with a similar architecture are referred to as three-domain (3d) Cry proteins (Li *et al.*, Loseva *et al.*, 2001; 1991; Grochulski *et al.*, 1995; Pacheco *et al.*, 2023).

Domain I consists of seven α-helices, six amphipathic helices arranged around a central hydrophobic helix (α5). In contrast, Domain II is composed of two short α-helices and three antiparallel β-sheets terminating in loop regions. Domain III is organized as a β-sandwich structure (Li *et al.*, 1991; Grochulski *et al.*, 1995).

Confirmation of the Three-Domain Structure in Cry Proteins

Subsequent investigations have corroborated this three-domain architecture in other proteins such as Cry3Bb1 (Galitsky *et al.*, 2001), Cry4Ba (Boonserm *et al.*, 2005), Cry4Aa (Boonserm *et al.*, 2006), Cry8Ea1 (Guo *et al.*, 2009), Cry5B (Hui *et al.*, 2012; Li *et al.*, 2022), Cry2Aa (Morse *et al.*, 2001), Cry7Ca1 (Jing *et al.*, 2019), Cry1Da (Wang *et al.*, 2019), and Cry11Aa and Cry11B (Tetreau *et al.*, 2022). Despite sequence diversity, all converge on the three-domain structure. This suggests a conserved folding pattern that supports the hypothesis of an analogous mode of action, the ultimate relevance of which lies in the induction of insect mortality. Achieving a comprehensive understanding of how these proteins function constitutes a critical step toward the development of strategies for insect pest management.

Several approaches —including site-directed mutagenesis, crystallographic and structural analyses, among others— have enabled the proposal of multiple models to explain the mechanism by which these proteins bind to receptors located in the midgut of lepidopterans. Among these are the "umbrella," "buried dragon," "knife," and "folding cane" models, which suggest possible interaction sites with gut receptors, ultimately leading to insect toxicity. An excellent review of these models can be found in Pacheco *et al.*, 2023.

Strategies for Obtaining Insecticidal Proteins from *Bt*

Isolation of New Strains

The most widely used methodology for the detection of novel genes with insecticidal potential relies on the search for *Bt* strains in a free-living state. For this purpose, samples are collected from diverse habitats where entomopathogenic bacteria are likely to proliferate. Sampling sites include soil, foliage, dead insects, water from various sources, and organic

residues from different ecosystems. These samples are subsequently preserved under appropriate laboratory conditions until strain isolation, after which they are subjected to a range of analyses to facilitate identification (Figure 2). These procedures include Gram staining, a variety of biochemical assays, and microscopy for the detection of parasporal bodies (Bravo *et al.*, 1998).

With respect to the molecular identification of isolated strains, amplification is performed using bacterial DNA to target the 16S rRNA gene with universal oligonucleotides via polymerase chain reaction (PCR). The resulting sequences enable taxonomic identification at the genus and species levels (López *et al.*, 2003). Given that the majority of cry genes are typically plasmid-borne, their purification and amplification with universal oligonucleotides is essential. The obtained sequences are analyzed against databases such as the National Center for Biotechnology Information (NCBI), as well as other gene and protein repositories, with the aim of identifying novel genes encoding pesticidal toxins.

Purification of Cry Proteins

Initially, bacterial cultures are grown in liquid media until sporulation occurs. Cry proteins are accumulated within the cell and account for approximately 20-30% of the dry cell mass. In the laboratory, sporulated cultures are harvested by centrifugation to concentrate the bacterial biomass. The pellets are then subjected to sonication to achieve cell lysis and protein release. Fractionation of the cell lysate is performed using sucrose gradients through ultracentrifugation (Soberón *et al.*, 2000). Cry proteins are localized within one of the gradient phases, allowing their separation from the remaining cellular components.

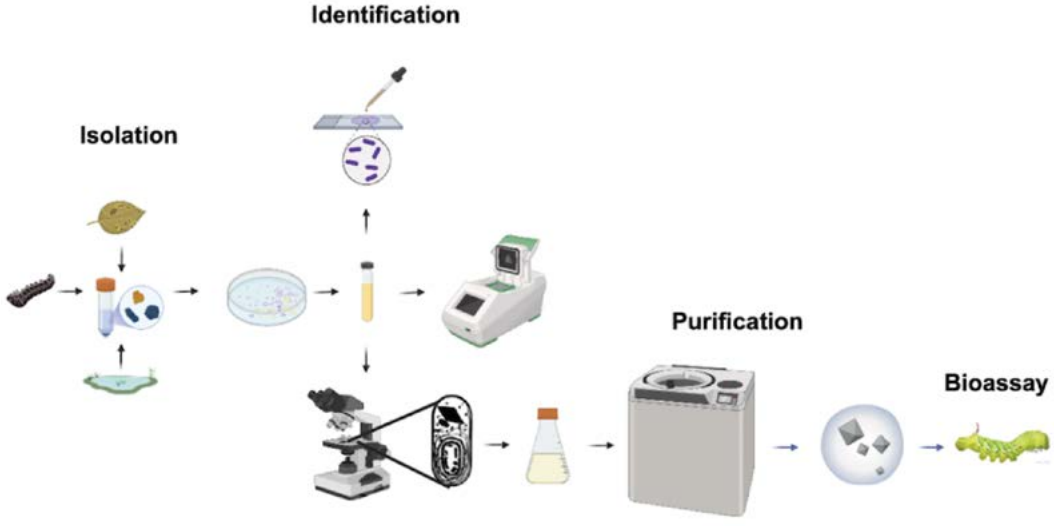

Figure 2. Isolation and identification of bioinsecticidal activity strains. To find new strains, they are isolated from environments where bacteria is likely to be found, such as water, dry leaves, dead insects and soil. The samples are then taken to the laboratory to isolate colonies, which are subjected to various tests such as Gram staining, molecular and microscopic analyses to find bacilli with inclusion bodies, which are then purified and fed to insects to determine their lethality.

The degree of purity is typically assessed using electrophoretic methods, which may be one-dimensional (1D), two-dimensional (2D), or capillary electrophoresis. In most cases, samples still exhibit contamination with other bacterial proteins, necessitating further purification using various chromatographic techniques, including affinity chromatography, ion-exchange chromatography, or size-exclusion chromatography, among others. These methodologies enable the isolation of Cry proteins in quantities and purity levels suitable for subsequent experimental studies.

Detection and Quantification of Cry Proteins

Conventional procedures for protein detection may include immunoassays such as ELISA and Western blot (Bravo *et al.*, 1998; Soberón *et al.*, 2000), both of which rely on antigen-antibody interactions. In the case of Cry proteins, certain assays may yield false positives due to cross-hybridization, an aspect that must be carefully considered. For protein quantification, spectrophotometric techniques such as the Lowry and Bradford assays can be employed. However, some limitations arise: spectrophotometric measurements provide an estimate of the total protein content in a sample rather than quantifying each individual protein present. Accurate determination of the specific Cry proteins within a sample requires highly efficient methodologies capable of precise estimation of each protein. This need has led to the development of reproducible and robust proteomic methods —such as liquid chromatography coupled with mass spectrometry— that allow the relative quantification of each protein in a given sample (Caballero *et al.*, 2020).

Genomics

The advent of next-generation sequencing (NGS) technologies has enabled rapid determination of complete genomic nucleotide arrangements. At present, this procedure is relatively straightforward and cost-effective, allowing accurate elucidation of the nucleotide sequences of genes of interest, whether located on plasmids or chromosomes. This, in turn, facilitates the comprehensive characterization of strains with biotechnological potential (Yamamoto, 2022). Availability of such sequences also allows for the detection of variants and comparative analyses across gene databases. The identification of nucleotide sequences of novel cry genes constitutes essential information used in genetic engineering tools to clone genes into heterologous systems —whether bacteria or plants— with the purpose of enhancing production or synthesizing proteins in more controlled systems (Baum *et al.*, 1999).

Mutagenesis and Protein Engineering

Several tools enable the generation of site-specific mutants for the study of Cry proteins, with site-directed mutagenesis being among the most widely used. Once the DNA sequences are identified, they can be altered through specific mutations incorporated during oligonucleotide design to achieve the desired modification. The oligonucleotides are annealed to the bacterial template DNA and amplified via PCR. The amplified product is then sequenced to confirm the mutation, followed by cloning into expression vectors (Yamamoto, 2022).

Another approach is DNA shuffling, a technique used to restructure and recombine cry genes in order to generate multiple point mutants. This allows researchers to study the effects of discrete amino acid changes. In this method, a gene of interest is amplified and subsequently fragmented using DNase I, generating random fragments that are recombined and re-amplified. Sequences with point mutations differing from the original gene are then selected for cloning (Yamamoto, 2022).

These tools enable modifications such as single amino acid substitutions, facilitating the study of structural changes that affect protein function and the creation of optimized variants to enhance insecticidal efficacy. They have also allowed the generation of diverse mutants that clarify which amino acids are critical for efficient toxin-receptor interactions. Additionally, chimeric proteins —created by exchanging entire domains between different Cry proteins— have been employed to elucidate the roles of specific domains in the mode of action.

In parallel, with experimental approaches, in silico methods such as structural modelling molecular docking, abd computational mutagenesis have been employed to engineer Cry proteins and accelerating the developments of optimized variants.

Bioassays

When a strain with insecticidal potential is identified, one of the most critical evaluations for establishing its biocidal activity is performing bioassays to assess efficacy. This involves maintaining insect colonies under controlled conditions (temperature, humidity, and light) with a diet tailored to the developmental stage of the insects. Rearing insects in the laboratory is labor-intensive and costly but is indispensable for obtaining reliable results regarding protein toxicity. Bioassays allow the direct administration of bacteria or purified proteins for testing in live insects (Soberón *et al.*, 2000; Yamamoto, 2022).

Additionally, maintaining insect colonies facilitates the collection of midgut vesicles, which are essential for *in vitro* experiments that indirectly evaluate pore formation induced by Cry proteins, linking this activity to the observed insecticidal toxicity.

Transgenic Plants with cry Genes

Since the creation of the first transgenic plants in 1983 using *Agrobacterium tumefaciens*, genetic modification of agronomically important plant species has become feasible. This modification involves transferring genes from other species to confer novel traits that provide advantages over their wild-type counterparts (Herrera-Estrella *et al.*, 1983; Sanahuja *et al.*, 2011). In addition to this methodology, biolistics (gene gun technology) was developed, enabling the introduction of genes into plants and is widely used today. Using biolistics, the first genetically modified maize lines were created by incorporating the Cry1AB gene, which is lethal to lepidopterans. This allowed the generation of plants capable of producing their own insecticidal proteins, referred to as *Bt* maize.

This innovation represented a significant step toward the gradual replacement of conventional synthetic pesticides. In 1998, Monsanto introduced the first transgenic maize to the market. These plants constitutively express the *Bt*-encoded Cry1Ab protein, which

is lethal to susceptible lepidopterans that consume plant tissues, thereby enhancing plant protection and growth.

Molecular detection of cry gene expression in plants is typically performed using quantitative reverse transcription PCR (RT-qPCR) to expedite assay processing. This technique also allows relative quantification of cry gene expression and is more efficient than endpoint PCR, which requires longer processing times.

Transgenic plants incorporating *Bt* genes are collectively referred to as *Bt* plants. Currently, several transgenic *Bt* species exist, including maize, cotton, soybean, canola, tomato, potato, and rice, among others (Sanahuja *et al.*, 2011). These plants been widely adopted in several agricultural systems worldwide; more than 50% of the global cultivated area of genetically modified organisms (GMOs) consists of crops expressing cry genes. The implementation of this technology has been associated with reduced reliance on chemical insecticides and agronomic benefits in many production contexts; however, the magnitude of these effects depends on crop species, regional management practices, and regulatory and ecological considerations.

Resistance and Defense Strategies of Pests against Cry Proteins

The progressive resistance of insects to Cry proteins mirrors that observed with synthetic insecticides. Continuous exposure to these toxins exerts selective pressure, promoting the emergence of resistant individuals. Understanding the mechanisms involved is essential to develop strategies that delay resistance and enable the design of more effective pesticidal proteins (Fabrick & Wu, 2023).

Key mechanisms by which insects acquire resistance include:

1. **Weak toxin-receptor interactions**: Site-directed mutagenesis studies on cry genes have demonstrated the critical role of amino acids in receptor binding and pore formation (Pacheco *et al.*, 2023). Amino acid substitutions in the laboratory provide insights into processes leading to insect mortality. Mutations can also occur in genes encoding the receptors themselves, which may disrupt toxin-receptor interactions and hinder toxin action.
2. **Insufficient solubilization and poor protoxin digestion**: Altered expression or reduced activity of midgut proteases can impair protoxin activation, preventing optimal function. Disruptions in the alkaline conditions of the insect midgut can also reduce solubility, limiting pesticidal activity (Pinos *et al.*, 2021).
3. **Sequestration or immobilization of activated toxins**: Upon reaching midgut cells, toxins may be sequestered. Molecules such as esterases and glycolipids have been documented to perform this role (Gunning *et al.*, 2005; Pinos *et al.*, 2021). This process may function as a detoxification mechanism, reducing effective toxin-receptor interactions.
4. **Alteration of membrane components**: At sublethal toxin concentrations, pore formation may occur but without causing severe damage, allowing membrane repair. Endocytic processes, in which the membrane invaginates to form vesicles that are degraded or recycled, facilitate repair (Cytrynska *et al.*, 2016). These

membrane repair mechanisms enhance insect survival and may have evolved over time.

5. **Cumulative selective pressure**: Continuous exposure to a specific pesticidal toxin exerts persistent selection on a population, ultimately promoting resistance development. Resistant insects emerge, complicating pest management and eradication in agricultural areas (Sanahuja *et al.*, 2011).

The simultaneous occurrence of several of these mechanisms in a single insect can generate super-resistant pests, adding an additional layer of complexity to pest control (Wu *et al.*, 2014; Tabashnik *et al.*, 2021; Fabrick & Wu, 2023).

The resistance mechanisms described above are the focus of study in numerous laboratories worldwide, driven by the search for strategies to prevent the emergence of resistant insects. This research enables the optimization of pesticidal toxins to enhance their affinity and specificity, thereby helping to slow the progression of resistance (Fabrick & Wu, 2023).

It is also important to highlight that alternative strategies are being investigated to counteract insect resistance. For example, synergistic combinations of different Cry toxins, or of Cry toxins with other bioactive molecules, are being explored to increase efficacy. However, as noted previously, certain Cry protein receptors —such as APN, ALP, cadherin, and ABC transporters— exhibit variations in their interactions depending on the specific Cry protein studied. Molecular-level investigations are providing insights into how these interactions occur (Pacheco *et al*, 2023; Bravo & Soberón, 2025).

The accumulation of experimental evidence has revealed the complexity of toxin-receptor binding, which is highly specific and dependent on the particular protein involved. Moreover, although numerous potential receptors have been identified, not all interactions result in insect mortality. This underscores the challenges in analyzing resistance mechanisms and designing effective countermeasures, as the full process is not yet completely understood (Sato, 2024).

CONCLUSION

Cry proteins produced by *Bt* were initially identified as insecticidal molecules. This led to their structural and functional characterization, which resulted in advances understanding their mechanism of action at a molecular level, as well as revealing the range of their diversity and laying the foundations for their application as biological insecticides.

Through the study of Cry protein diversity and using *in vivo* and *in silico* experimental strategies, the efforts to improve the insecticidal efficacy of these proteins continue, while also considering the management of insect resistance.

An important applied aspect of Cry proteins has been the generation of transgenic crops to combat specific pests in certain agricultural regions of the planet, where their role as a component of modern agricultural systems is important, particularly outside the centers of origin and diversification of cultivated plants.

Finally, the activity of *Bt* toxins against insects that are vectors of human diseases highlights the relevance of research beyond agricultural applications.

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