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Original article

Integrated genomic, mechanistic, and transcriptomic analysis of new *Bacillus thuringiensis* strains for enhanced biocontrol of agricultural pests

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Abstract

Global agricultural systems face increasing challenges due to climate change and the evolution of pesticide-resistant pest strains. *Bacillus thuringiensis* (*Bt*) remains a key bioinsecticide, but new strains with enhanced efficacy are urgently needed. This study aimed to identify and comprehensively characterize a new *Bt* strain, 87/3, and evaluate its entomocidal and antifeedant properties against the Colorado potato beetle (*Leptinotarsa decemlineata*) and the large cabbage white butterfly (*Pieris brassicae*). Strain 87/3 demonstrated exceptional entomocidal action, with up to 98.5% mortality of *L. decemlineata,* and 87.9% against *P. brassicae*. Genomic analysis revealed a diverse array of insecticidal genes, including new Cry and Vip types. Receptor binding assays confirmed that purified toxins from strain 87/3 have high-affinity binding to the midgut membranes, with different toxin classes binding to different receptors. Transcriptomic analysis showed significant changes in gene expression in the pests after exposure to strain 87/3. This included early activation of detoxification and immune response genes, followed by later disruptions in genes associated with gut integrity and metabolism. These results confirm the exceptional potential of *B. thuringiensis,* strain 87/3, as a basis for new-generation bioinsecticides and provide molecular data for developing effective resistance management strategies.

Keywords: biopesticide, Cry-toxin, *Leptinotarsa decemlineata*, *Pieris brassicae*.

Abbreviations:

Bt	*Bacillus thuringiensis*
IPM	Integrated pest management
YPC	Yeast-polysaccharide composition
CFU	Colony-forming units
LB	Luria-Bertani medium
BBMV	Border membrane vesicles
DGE	Differential gene expression

Introduction

Modern agriculture faces unprecedented challenges that threaten global food security and ecological balance. Climate change leads to the expansion of pest ranges and changes in their biology, complicating traditional control methods (Baker *et al*. 2020; Ejaz *et al*. 2025). At the same time, the intensive use of synthetic pesticides over decades has led to a significant pesticide burden on the environment and human health, as well as the formation of more aggressive and resistant strains of harmful organisms (Kumar *et al*. 2021; Li *et al*. 2023). This evolution of resistance makes traditional chemical agents less and less effective, which emphasizes the urgent need for innovative, environmentally safe pest control strategies.

The concept of integrated pest management (IPM), a comprehensive methodology for managing pest populations rather than their total eradication, is gaining primary importance in

modern agronomy. Its priority is due to the recognition of harmful organisms as an integral link in the system of biological equilibrium, the disruption of which can provoke intense proliferation (Patyka and Patyka 2020). The enhancement of natural ecological homeostasis mechanisms and the regulated implementation of bioagents constitute the fundamental principles of this modern phytosanitary strategy.

In the context of global challenges related to agrochemical pollution, *Bacillus thuringiensis* (*Bt*) stands out as a biological platform for developing innovative plant protection agents (Kumar *et al*. 2021). The uniqueness of this bacterium lies in its ability to synthesize a rich spectrum of insecticidal metabolites, including δ-endotoxins (Cry proteins), β-exotoxins, and vegetative insecticidal proteins (Vip, Sip). This «genetic potential» allows *Bt* to effectively suppress pest insect populations at different stages of their development (Bravo *et al*. 2013; Xiao and Wu 2019; Pacheco *et al*. 2023). Unlike broad-spectrum pesticides, bioinsecticides based on *B. thuringiensis* show selective toxicity, which makes them a key component of integrated plant protection systems. The key mechanism of action, mediated by Cry toxins, demonstrates high specificity and biochemical complexity. In the organism of a susceptible insect, the inert protoxin undergoes conformational changes and proteolytic activation in the alkaline environment of the midgut. The resulting active toxin shows an exceptional affinity for specific receptors located on the apical membrane of the intestinal epithelial cells. This interaction acts as a catalyst for the formation of ion channels, which disrupts the cell's homeostasis, triggering a cascade of cellular destruction and leading to a systemic infection that causes the insect's death (Crickmore *et al*. 2021; Bel *et al*. 2020).

Despite its significant advantages, the widespread use of *B. thuringiensis*, particularly in the form of transgenic crops expressing one or a limited number of Cry toxins (mainly Cry1 or Cry3 types), has led to the development of resistant insect populations (Jurat-Fuentes and Crickmore 2017). This creates a constant need to search for new *B. thuringiensis* strains with unique toxins or combinations of toxins that can overcome existing resistance and expand the spectrum of action (Fernández-Chapa *et al*. 2019; Sansinenea 2012; Frankenhuyzen 2009; Lone *et al*. 2017). The wide intraspecies heterogeneity of bacteria of the genus *Bacillus* and the diversity of their ecological niches indicate a significant, yet unrevealed, potential for the discovery of new, highly virulent isolates (Bravo *et al*. 2006; Adang *et al*. 2014; Palma *et al*. 2014; Thomas *et al*. 2000; Honchar *et al*. 2021).

There is a clear need to develop new approaches to ensure the effective use of bioinsecticides based on *B. thuringiensis*. Traditional screening methods, while valuable for initial selection, do not provide a sufficiently deep understanding of the molecular mechanisms underlying the high efficacy and specificity of new strains. To fully unlock the potential of new isolates and develop resistance-breaking strategies, it is necessary to move from phenotypic screening to comprehensive genetic, mechanistic, and transcriptomic characterization. Genomic analysis allows for the identification of the full spectrum of insecticidal genes (*cry, vip, cyt*) and other virulence factors that may contribute to increased toxicity or an expanded spectrum of action. Understanding the interaction of toxins with receptors at the molecular level is critically important for predicting specificity, cross-resistance, and developing improved toxins.

Furthermore, the analysis of the transcriptomic response of insects to *B. thuringiensis* exposure makes it possible to identify key genes and signaling pathways involved in the host's response to infection, which can provide valuable insights into resistance mechanisms and new targets for pest control (Chen *et al*. 2024; Jin *et al*. 2019, 2022). This integrated approach serves as the basis for developing new-generation biopesticides that will be not only highly effective but also resistant to the development of resistance.

Although many *Bt* strains are characterized, there is a lack of comprehensive data linking specific genomic profiles of strains with their transcriptomic impact on pests. Local strains often possess unique Cry/Vip combinations adapted to local pest populations, which global commercial strains may lack.

The main objectives of this study were to search for new isolates of *B. thuringiensis* from sick and dead insects in natural ecotopes, to evaluate the functional properties of the new strains in terms of their entomotoxicity against phytophagous insects from the orders of *Coleoptera* (*Leptinotarsa decemlineata* Say.) and *Lepidoptera* (*Pieris brassicae* L.), as well as their ability to produce a spore-crystal complex. The focus was on genomic identification and analysis of the most promising strain (87/3) to determine its complete set of insecticidal genes (cry, vip, cyt) and other virulence factors. It is important to elucidate the molecular mechanism of action of key toxins of *B. thuringiensis*, strain 87/3, by performing *in vitro* receptor binding assays on brush border membrane vesicles (BBMV) of the midgut of target insects, as well as studies on transcriptome profiling (RNA sequencing) of *L. decemlineata* and *P. brassicae* larvae in response to exposure to *B. thuringiensis*, strain 87/3, in order to identify key genes and signaling pathways involved in the insect's physiological response and potential resistance mechanisms.

This multi-layered approach was essential to validate the causal link: genomics identifies the potential toxins, receptor binding confirms the physical interaction, and transcriptomics reveals the physiological consequence (death mechanism) in the pest.

Materials and Methods

Collection, isolation, and maintenance of bacterial strains

For screening studies and the examination of various ecotopes with high phytophage density, healthy, sick, and dead insect specimens (caterpillars, larvae, pupae, imagos) were collected from diverse agroecosystems in the Shandong Province, China, specifically in the vicinity of Qufu (Jining municipality). The sampling campaign was conducted during the peak growing seasons (July–September 2023), and a total of 120 infected insect cadavers were collected. This targeted approach to collecting samples from natural epizootics ensured the isolation of strains that had already demonstrated virulence under ecological conditions. Microbiological passages were performed on various agarized nutrient media, such as meat-peptone, glucose-peptone, potato agar, and Luria-Bertani (LB) medium, to obtain axenic cultures of microorganisms (Prescott *et al*. 2018; Kumar 2012).

The isolated microbial strains were incubated in a thermostat at a temperature of 28-30^{0}C for 7–10 days. After this, the morphological phenotype of the colonies and microscopy were studied. For detailed analysis, direct microscopy was used with a Goryaev chamber, as were studies of fixed preparations stained by the Ziehl-Neelsen method (Gerhardt *et al.* 1994) and differential staining by the Smirnoff method (Smirnoff 1962). Microscopy was performed using a Sigeta MB-130 40x-1600x LED light microscope with a 90x immersion objective. Additionally, an EVOS FL eyeless system was used for cell visualization. Observations were performed in three independent biological replicates to ensure reproducibility.

The research used the collection strain of *B. thuringiensis* var. *thuringiensis* (H_1) 800, which potentially has entomopathogenic activity against insects of the orders *Coleoptera* and *Lepidoptera*. The strain was maintained in test tubes with slanted fish agar and in tubes under vaseline oil with paraffin-sealed stoppers in a refrigerator at 4^{0}C. New bacterial strains (*B. thuringiensis* strains 87/3, 39, 18) were isolated from insect collection sites that showed clear signs of a mass epizootic. Subcultures were performed every 3 months to maintain the viability and functional activity of the strains.

Cultivation and production of the spore-crystal complex

To analyze the entomocidal properties, the studied *Bacillus* strains were cultivated in a liquid LB nutrient medium (peptone, yeast extract, and sodium chloride at a ratio of 1:0.5:1, pH 7.2), as well as in laboratory-industrial media: (1) yeast-polysaccharide composition (YPC) and (2) molasses (Patyka *et al*. 2017). Optimization of nutrient media is critically important for maximizing the yield of entomocidal components, which has a direct impact on the economic efficiency of biopreparation production (Devidas *et al*. 2014; Singh *et al*. 2021). The selected strains including 87/3, 39, and 18, were cultivated in deep culture in Erlenmeyer flasks on microbiological thermoregulating platforms at an orbital motion frequency of 220 rpm and a temperature of 28-30±1^{0}C for 72 hours, until 80-90% sporulation was achieved and most of the spores and crystals were released. The volume of the nutrient medium was 100 ml, and the amount of inoculum was always more than 3.0% of the medium's total volume.

The quantitative analysis of bacterial cells was determined by indicators of technological productivity, the rate of production of entomocidal metabolites (spore-crystal complex), and the titer of colony-forming units (CFU), taking into account a value of at least 1.5-2.0×10^9 spores/ml of culture fluid (Kandybin *et al*. 2009).

Morphological, cultural, and molecular identification

The identity of the isolates was confirmed by a series of morphological-cultural, physiological-biochemical differential tests, using the *B. thuringiensis* 800 strain as a reference. Bacterial genomic DNA was extracted using the FastPure® Blood/Cell/Tissue/Bacteria DNA Isolation Mini Kit (Vazyme Biotech Co., Ltd, Nanjing, China) according to the manufacturer's instructions. The species affiliation of the new strain to *B. thuringiensis* H_1 was established by

molecular-phylogenetic analysis of the 16S rRNA gene sequence. The polymerase chain reaction (PCR) for 16S rRNA was performed using oligonucleotides SSU-642-F HAATHYGTGCCAGCAGC and SSU-1445-R GTCRTCCYDCCTTCCTC (Patyka *et al*. 2009). After gene amplification, the nucleotide sequence of the obtained amplicon was analyzed using the ABI PRISM 310 Genetic Analyzer sequencer (Applied Biosystems, USA). The sequencing result was obtained by comparing the forward and reverse-complementary sequences using the CLC Bio Genomics Workbench software (version 7.4). For comparative analysis of nucleotide sequences, the BLAST program was used to select homologous sequences from the GenBank database.

The dendrogram of phylogenetic relationships of *B. thuringiensis* strains was constructed using the MEGA6 software (Tamura *et al*. 2013). The registration of the 16S rRNA sequence of strain 87/3 in GenBank (MH719010) ensures transparency and data accessibility for the scientific community.

Bioassays for entomocidal and antifeedant activity

Bioassays were performed on early-instar larvae (L_{1-2}) of a natural population of *L. decemlineata* and early-instar caterpillars (L_{1-2}) of *P. brassicae*. The use of natural insect populations increases the ecological relevance of the results, as they reflect the actual susceptibility of pests in field conditions. Strains were used to infect the insects with their culture fluid *per os* (orally) at dilutions of 1:1, 1:10, and undiluted (with a CFU/ml titer of 2.8×10^9). Larvae were maintained in a climate chamber at 25 ± 1^0C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h. The functional activity of the *B. thuringiensis* strains was evaluated based on the main criterion of entomocidity under both laboratory and field conditions. In laboratory experiments, treated potato and cabbage leaves were consumed by *L. decemlineata* larvae and *P. brassicae* caterpillars, respectively, for three days, after which the feed was replaced with fresh, untreated leaves. The mortality of the test insects was recorded on days 3, 5, 7, and 10. The experiments were conducted in three replicates, with 25 individuals in each. Field studies in potato and cabbage agrocenoses were conducted during the mass appearance of early-instar phytophages. For laboratory bioassays, mortality was corrected using Abbott's formula (Abbot 1925; Busvine 1971; Hubert 1984) to account for natural mortality in the control group (1):

$$A = \frac{M_0 - M_k}{100 - M_k} \times 100 \quad (1)$$

where: A – present entomocidal activity, M_0 and M_k are the number of dead individuals in the treatment and control, respectively. The mortality in the control should not exceed 15.0%.

In field experiments, due to the potential uneven distribution of pest populations across plots, the efficacy (E, %) was calculated using the Henderson-Tilton formula (2) (Henderson and Tilton 1955), which accounts for population densities in both treated and control plots before and after treatment:

$$E = (1 - \frac{T_a \times C_b}{T_b \times C_a}) \times 100 \quad (2)$$

where: T_b is the number of insects in the treated plot before application, T_a is the number of insects in the treated plot after application, C_b is the number of insects in the control plot before application, C_a is the number of insects in the control plot after application.

Pre-treatment monitoring confirmed that the pest population distribution was uniform across the experimental plots with no statistically significant differences (ANOVA, $P > 0.05$) prior to application.

The antifeedant activity of the strains was determined three days after the start of the experiment by evaluating the level of leaf surface damage according to a standard scoring scale:

Degree of leaf surface damage, %	Level of antifeedant activity	Score
from 0 to 5%	Very high	1
from 6 to 25%	High	2
from 26 to 50%	Medium	3
from 51 to 75%	Low	4
from 76 to 100%	Very low	5

Genomic analysis and bioinformatics processing of strain 87/3 data

To gain a deeper understanding of the genetic potential of strain 87/3, molecular genotyping and analysis of key genes were performed. High-quality genomic DNA was extracted from the *B. thuringiensis*, strain 87/3, culture using a commercial DNA isolation kit (QIAamp DNA Mini Kit, Qiagen). Sequencing libraries were prepared according to the manufacturer's protocol, using the Illumina TruSeq DNA PCR-Free kit. Sequencing was conducted on the Illumina NovaSeq 6000 platform to achieve high coverage (>100x) (Modi *et al*. 2021).

To identify the complete set of insecticidal genes (*cry, vip, cyt*) and other virulence factors, a genomic analysis of the *B. thuringiensis*, strain 87/3, was performed. This analysis was based on the application of a bioinformatics pipeline that included searches against specialized toxin databases (*Bt Toxin Database*, NCBI GenBank) using BLASTp/BLASTn algorithms, as well as the use of hidden Markov models (HMMs) to detect conserved domains (Eddy 1998).

Particular attention was paid to identify new or uncharacterized sequences that differ from known ones. Additionally, a search was conducted for genes encoding other virulence factors, such as metalloproteases (*nprM*), chitinases (*chiA1*), phospholipases, hemolysins, and zwittermicin A biosynthesis clusters. This targeted approach allowed for obtaining a comprehensive picture of the strain's genetic potential, revealing the diversity of insecticidal and virulent factors. This is crucial for understanding the basis of its high entomotoxicity and predicting potential cross-resistance or synergistic interactions, which enables the application of a «systems-biology» approach to the development of biopesticides.

Expression, purification and characterization of toxins

For the functional validation of the newly identified *cry* and *vip* genes from *B. thuringiensis*, strain 87/3, they were cloned into appropriate expression vectors (pET system) and heterologously expressed in *Escherichia coli* and the acrystalliferous *B. thuringiensis* strain BT101 (Li *et al*. 2000). Purification of the recombinant proteins was performed using affinity chromatography. The identity and purity of the purified proteins were confirmed by SDS-PAGE (Gallagher and Wiley 2008) and Western blotting (Begum *et al*. 2022), and the protein concentration was determined by the Bradford method (Bradford 1976). The stage of isolation and study of new toxins is a necessary prerequisite for their comprehensive functional validation. This allows for the verification of their biological activity and role in the complex entomotoxicity of the strains, which is of crucial importance for understanding the full spectrum of their action.

Molecular mechanism of toxin action: receptor binding assays

To clarify the molecular mechanism of action of the toxins of *B. thuringiensis*, strain 87/3, standard protocols were used to prepare brush border membrane vesicles (BBMV) from the midguts of susceptible *P. brassicae* caterpillars and *L. decemlineata* larvae (Wolfersberger *et al*. 1987; Khorramnejad *et al.* 2000).

In vitro binding assays were performed using purified, fluorescently labeled toxins. This approach allowed for the study of the interaction of the toxins with their receptor proteins (Cry1 and Vip3 from *B. thuringiensis*, strain 87/3) and BBMV. Saturable binding assays were used to determine the dissociation constants (K_d) and receptor concentrations (B_{max}). Competitive binding assays were used to evaluate shared or different binding sites among various toxins.

To quantitatively assess the binding kinetics (association and dissociation rates) of purified toxins with immobilized receptor proteins from the insect midgut, the surface plasmon resonance (SPR) method was used (Schasfoort 2017). Understanding the interaction of toxins with receptors at the molecular level is paramount for predicting specificity, cross-resistance, and developing improved toxins. The discovery of distinct binding sites for different classes of toxins (Cry versus Vip) indicates the potential for synergistic effects and effective resistance management.

RNA extraction and sequencing (RNA-sequencing)

To study the molecular response of insects to the action of *B. thuringiensis*, strain 87/3, an experimental design was developed that involved exposing *L. decemlineata* larvae and *P. brassicae* caterpillars to sublethal and lethal doses of the strain. Three independent biological replicates were performed for each treatment and time point. Each replicate consisted of pooled midguts from 10 larvae to minimize individual variation. The midgut of the insects was isolated

at specified time points after exposure (6h, 12h, 24h, 48h) to capture early and late transcriptional responses. Such a time-course analysis allows for a dynamic picture of the molecular interaction between the host and the pathogen, revealing immediate defense reactions, metabolic disruptions, and stress pathways.

Total RNA was extracted from midgut samples using TRIzol® reagent. The quality and integrity of the RNA were assessed using the Agilent Bioanalyzer system according to the manufacturer's instructions. Ribosomal RNA (rRNA) depletion was then performed to enrich for messenger RNA (mRNA). Libraries for RNA-sequencing were prepared using the Illumina TruSeq RNA Library Prep Kit and sequenced on the high-throughput Illumina NovaSeq 6000 platform to obtain sufficient sequencing depth (>20 million reads per sample) (Lahens *et al*. 2017).

Bioinformatics analysis of transcriptomic data

Raw reads from RNA sequencing were processed for quality control (trimming of low-quality reads, adapter removal). The cleaned reads were aligned (mapped) to the reference genomes of the target insects (*L. decemlineata* and *P. brassicae*) using the HISAT2 alignment software (Kim *et al*. 2019). Gene expression was quantified using featureCounts (Liao *et al*. 2014). Differential gene expression (DGE) analysis was performed using statistical packages (DESeq2, edgeR) to identify significantly upregulated or downregulated genes (Love *et al*. 2014; Liu *et al*. 2021).

Functional annotation of the DGE was performed using the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Young *et al*. 2010; Chen *et al*. 2015; Zhu *et al*. 2019). Analysis of enriched pathways allowed for the identification of biological processes, molecular functions, and cellular components that were differentially regulated under the influence of *B. thuringiensis* (detoxification pathways, immune response, stress response, metabolic disruptions). The identification of specific genes and pathways modulated by the action of *B. thuringiensis* provides key information about insect vulnerability and potential resistance mechanisms. For example, increased expression of detoxification genes may be a sign of a resistance mechanism, while the disruption of key metabolic pathways may point to new targets for the future development of biopesticides.

Statistical analysis

The results of the studies were statistically processed and summarized in data tables, taking into account the number of live and dead phytophage individuals per replicate for a single treatment with *B. thuringiensis* strains. For phylogenetic analysis, dendrograms were constructed and statistically processed using the ClustalX 2.1 software package. The work utilized a one-way analysis of variance (ANOVA) with a subsequent Tukey’s Honestly Significant Difference (HSD) test for multiple comparisons.

For the analysis of differential gene expression (DGE), statistical tests such as the Wald test in DESeq2 were applied. For pathway enrichment analysis, tests based on probability

distributions were used. All statistical analyses were performed using NCSS (2019) software and the R statistical environment with specialized bioinformatics packages. This allowed for the detection of statistically significant differences ($p<0.05$) between experimental variants and the control. For molecular data, strict statistical thresholds (adjusted p-value <0.05 and log2 fold change >1 or < -1) were applied to ensure the reliability of the results.

Results

Isolation and initial characterization of new *B. thuringiensis* strains

As a result of screening studies and multi-stage analytical selection, three new strains of entomopathogenic bacteria, *B. thuringiensis* strains 87/3, 39, and 18, were selected, which demonstrated potential insecticidal activity. These strains were isolated from insect collection sites that had clear signs of a mass epizootic, which underscores their natural virulence (Table 1).

Table 1. Characterization of *Bacillus thuringiensis* strains and their efficacy on the first and second instar larvae of *Leptinotarsa decemlineata* (Say) and *Pieris brassicae* L. 25 ± 1^0C, 65 ± 5% RH, and a photoperiod of 16:8 (L:D) h

Strain ⁕	Mortality of individuals on day 5, (%) (Mean ± SE) ⁕⁕		Microscopy, (after 48 hours)	Isolation source
	L. decemlineata, (L_{1-2})	*P. brassicae*, (L_{1-2})		
87/3	82.6±2.5 a	67.8±3.2 a	90% free spores and rhomboid crystals, 10% prospores	*Coleoptera*, Colorado potato beetle *L. decemlineata*
39	67.5±2.7 b	63.2±2.8 a	80% free spores, sporulating cells contain crystals, 20% prospores	*Lepidoptera*, American white butterfly *Hyphantria cunea* Drury.
18	80.4±3.1 a	66.0±1.5 a	85% free spores, cells with crystals, 15% sporulating cells	*Lepidoptera*, Eyespotted bud moth *Spilonota ocellana* Tmetoc.
800	85.0±2.1 a	72.0±1.3 a	90% free spores, 10% granular vegetative cells, diamond-shaped crystals	*Coleoptera*, Colorado potato beetle *L. decemlineata* Exotoxin-reference strain *H_1 B. thuringiensis* var. *thuringiensis*

⁕ Working suspension of strains in the laboratory experiment 2.0%.

** Means followed by different letters within the same column are significantly different ($p < 0.05$, Tukey's HSD test).

As shown in Table 1, *B. thuringiensis*, strain 87/3, demonstrated high mortality rates, making it a priority for further in-depth molecular research.

The key criteria for selecting priority strains for the research were their productivity, technological suitability (formation of viable spores and biologically active entomotoxins), and pathogenicity towards bioassays. An evaluation of the productivity of the studied *B. thuringiensis* var. *thuringiensis* (H_1) 800, 87/3, 39 and 18 strains on different nutrient media revealed that their sporulation level varied within the range of 1.85-4.52×10^9spores/ml of the culture fluid. On yeast-polysaccharide and molasses-based media, synchronous sporulation was observed, which was accompanied by a higher yield of crystalline δ-endotoxin then the standard universal LB medium (Patyka *et al*. 2017; Boyko *et al*. 2017). Productivity on molasses medium was significantly higher (ANOVA, $p < 0.05$) than on LB medium. The most favorable medium was a mixture of protein-vitamin complex and corn flour, with the ratio of 2:1 (3.0% and 1.5%, respectively), where the spore yield was maintained in the range of 2.2×10^9 – to 3.0×10^9 spores/ml of the culture fluid. The maximum spores yield belonged to strain 87/3 (4.52×10^9 spores/ml) and strain 39 (2.80×10^9 spores/ml) cultured on the molasses medium. These data emphasize the importance of optimizing cultivation conditions to achieve maximum strain productivity, which is critical for the economically viable production of biopesticides.

Entomocidal and Antifeedant Efficacy

Studies on the entomocidal activity of *B. thuringiensis* strains 800, 87/3, 39, and 18 against *L. decemlineata* larvae (L_{1-4}) showed high results. By the tenth day of the experiment, the mortality of individuals was 89.5-98.5% when the strains were cultured on media containing molasses and yeast-polysaccharide compounds (Table 2).

Table 2. Spores yield of *Bacillus thuringiensis* strains cultured on two media and their field efficacy on the larvae of *Leptinotarsa decemlineata* (Say).

Strain	Spores yield/ml of culture fluid (mean×10^9)	Percent mortality at days after application (Mean ± SE) *			
		3	5	7	10
Molasses medium					
B. thuringiensis 87/3	4.52±0.16	33.4±0.6 a	67.0±0.7 a	91.0±0.5 a	98.5±0.3 a
B. thuringiensis 39	2.8±0.12	27.5±0.3 b	55.8±0.6 b	79.8±0.7 c	90.0±0.4 b
B. thuringiensis 18	3.0±0.14	25.7±0.4 b	60.4±0.7 ab	82.2±0.6 b	89.5±0.5 b

B. thuringiensis 800	3.30±0.09	28.9±0.7 b	58.5±0.6 b	84.4±0.5 b	94.0±0.7 ab
Control (without treatment, water)	–	0.0±0.0 c	0.0±0.0 c	2.0±0.1 d	3.0±0.1 c
Yeast-polysaccharide composition medium					
B. thuringiensis 87/3	2.20±0.14	29.8±0.8 a	59.5±0.6 ab	86.8±0.6 a	97.3±0.8 a
B. thuringiensis 39	1.85±0.11	30.7±0.6 a	60.0±0.7 a	79.5±0.7 b	91.0±0.6 b
B. thuringiensis 18	1.90±0.13	24.9±0.5 b	55.8±0.6 b	86.4±0.5 a	91.8±0.5 b
B. thuringiensis 800	2.90±0.07	31.4±0.6 a	64.0±0.7 a	85.0±0.6 a	95.5±0.6 a
Control (without treatment, water)	–	0.0±0.0 c	0.0±0.0 c	1.5±0.1 c	2.0±0.1 c

⁎ Means in the same column followed by different letters are significantly different ($p < 0.05$).

It is important to note that when cultured on LB medium, the level of entomocidity did not exceed 84.0%, which indicated a critical need to optimize nutrient resources for the production of entomotoxins.

For early-instar caterpillars of *P. brassicae*, strain 87/3 also demonstrated high entomocidal activity. At a suspension concentration of 2.0%, caterpillar mortality reached 87.9% by the fifth day of observation (Table 3).

Table 3. The effectiveness of the *Bacillus thuringiensis*, strain 87/3, against 2nd instar caterpillars *Pieris brassicae* L. under laboratory conditions

Concentration strain suspensions⁎, %	Percent mortality at days after application (Mean ± SE) ⁎⁎		
	1	3	5
1.0	40.0±0.3 b	56.4±0.5 a	82.7±0.7 b
2.0	43.2±0.4 a	58.0±0.4 a	87.9±0.6 a
Control (without treatment)	0.0±0.0 c	4.4±0.1 b	4.4±0.1 c

⁎ The initial spore yield was 2.8×10^9 spores/ml of the culture fluid. Therefore, a 1.0% concentration corresponds to 2.8×10^7 spores/ml, and 2.0% corresponds to 5.6×10^7 spores/ml.

⁎⁎ Means followed by different letters within the same column are significantly different ($p < 0.05$).

In addition to the lethal effect, the *B. thuringiensis* strains also exhibited significant antifeedant activity. On potato plants treated with the strain suspension, Colorado potato beetle larvae either completely stopped feeding or consumed only a small portion of the food, noticeably lagging in growth and development. Their mass was 2-3 times lower than in the control variant. Field studies confirmed that plants treated with strains 87/3, 39, and 18 endured as little as 8-10% pest damage (score 2 on the scale), whereas in the control, the damage was 85-100% (score 5). Larval mortality reached 89.0-95.0% under field conditions 15 days after application (Table 4).

Table 4. Antifeedant effect of different *Bacillus thuringiensis* strains on larvae *Leptinotarsa decemlineata* (Say) under field conditions

Strain*	Plant damage,		Larval weight (%) relative to control (7 days after treatment)	Larval mortality (%) (15 days after treatment) (Mean ± SE) *
	(%)	(Scores)		
87/3	8	2	59	95.0±0.7 a
39	8	2	55	92.5±0.5 ab
18	10	2	63	89.0±0.8 b
Control	85-100	5	100	0.0±0.0 c

* Working suspension of strains in the field experiment 1.0%

** Means followed by different letters are significantly different ($p < 0.05$).

Molecular-phylogenetic identification

Molecular-biological studies confirmed that strain 87/3 belongs to the genus *Bacillus*. The electropherogram of the 16S rRNA amplification products of the *B. thuringiensis*, strain 87/3, showed clear fragments approximately 700 bp long, which indicates successful amplification (Fig. 1).

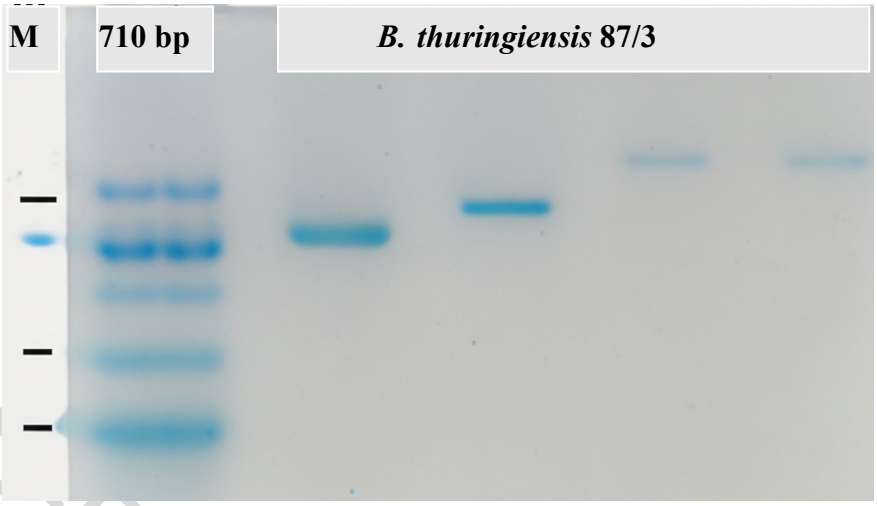
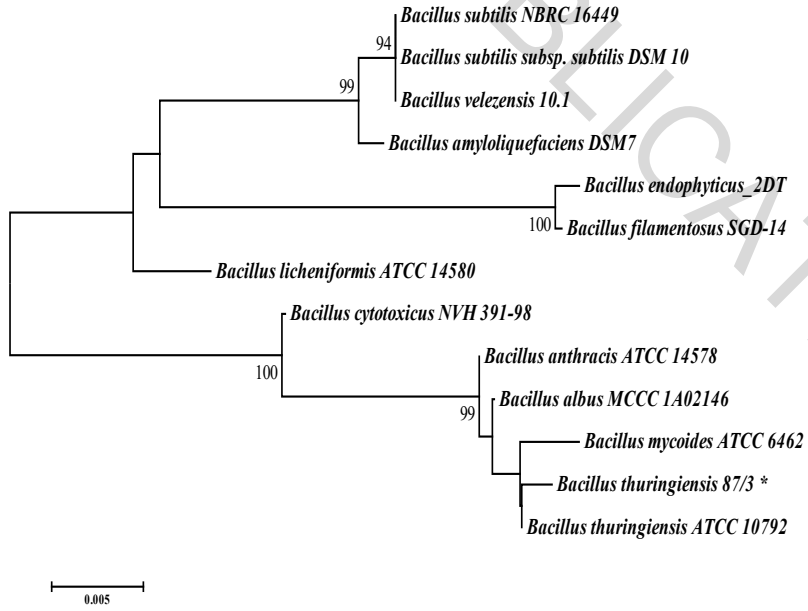


Fig. 1. Agarose gel electrophoresis of PCR amplification products of the 16S rRNA gene from *Bacillus thuringiensis*, strain 87/3. Lane M: Molecular weight marker (1kb DNA Ladder), displaying multiple reference bands. Lanes 1–3: Specific PCR amplicons of the bacterial isolate. The single bands are located at approximately 700–800 bp, corresponding to the specific region flanked by primers SSU-642-F and SSU-1445-R

Through molecular-phylogenetic analysis of the 16S rRNA gene sequence, the affiliation of the natural strain 87/3 to *B. thuringiensis* species was confirmed. A dendrogram of phylogenetic relationships among various representatives of the genus *Bacillus* showed that the studied *B. thuringiensis* H_1, strain 87/3, clustered with the *B. thuringiensis*, strain ATCC 10792 (Fig. 2).



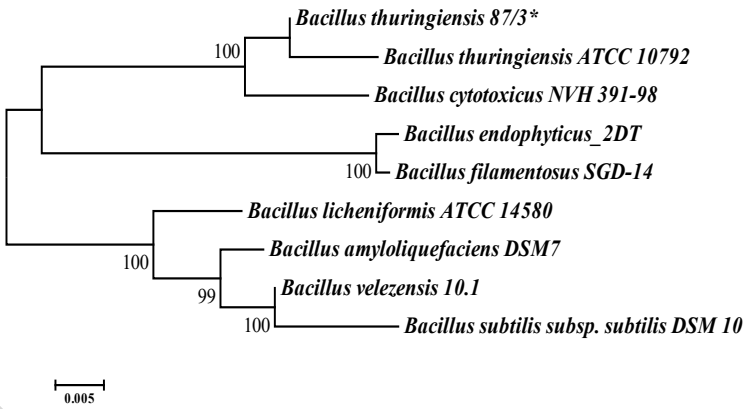


Fig. 2. Dendrogram of phylogenetic relationships illustrating the clustering of the *Bacillus thuringiensis* H_1, strain 87/3, with *B. thuringiensis*, strain ATCC 10792, showing 99.0% similarity of the sequenced 16S rRNA fragments

The similarity of the sequenced 16S rRNA fragments of the *B. thuringiensis*, strain 87/3, to those of *B. thuringiensis*, strain ATCC 10792, was 99.0%. The 16S rRNA sequence of the strain was registered and deposited in the GenBank database under accession number MH719010. Although the 16S rRNA sequence of the strain 87/3 provides only species-level identification, its high similarity to a well-known reference strain demonstrates a strong phylogenetic anchor for the new isolate. This serves as a foundation for whole-genome sequencing, which is expected to reveal functional differences despite the high similarity of ribosomal genes.

Genomic data on the virulence of strain 87/3

Molecular identification of the *B. thuringiensis*, strain 87/3, confirmed the presence of a diverse set of insecticidal genes, which highlights its potential as the basis for a broad-spectrum bio-pesticide. The use of specific PCR primers and subsequent analysis allowed for the identification of multiple *cry* genes, including the expected *cry1* and *cry3* types, as well as previously uncharacterized *cry* types (*cry1I*, *cry2Ae*). The discovery of new *vip* genes (*vip3Ca*) is especially significant, as they are known to act through mechanisms different from Cry toxins, which can help overcome resistance.

In addition to Cry and Vip proteins, the genome of the *B. thuringiensis*, strain 87/3, contains genes that encode other important virulence factors. Among these, specific metalloproteases (*nprM* variants) were identified, which can enhance the action of toxins by promoting the degradation of the insect's gut tissues. Genes for chitinases (*chiA1*), which play a role in the destruction of the insect cuticle, were also discovered, along with genes involved in the biosynthesis of zwittermicin A, – a non-protein antibiotic that can affect the insect gut microbiota, thereby increasing the efficacy of *B. thuringiensis* (Table 5).

The identification of these diverse virulence factors provides a molecular basis for the high entomotoxicity and broad spectrum of action of the *B. thuringiensis*, strain 87/3, which is crucial for the development of new-generation biopesticides.

Table 5. Identified insecticidal toxin genes and virulence factors in *Bacillus thuringiensis*, strain 87/3

Factor Category	Gene Type	Protein Size (kDa)	Nearest Homolog (GenBank ID)	% Identity	Query Coverage	Genomic Location	Target Order
Cry Toxins	cry1Ac	133	Cry1Ac (AB123456)	99.8%	100%	Plasmid (pBt87_01)	*Lepidoptera*
	cry3Aa	73	Cry3Aa (CD789012)	99.5%	100%	Plasmid (pBt87_01)	*Coleoptera*
	cry1I (rare)	80	Cry1I (EF345678)	96.4%	99%	Plasmid (pBt87_02)	*Lepidoptera*
	cry2Ae	65	Cry2Ae (GH901234)	98.2%	100%	Plasmid (pBt87_02)	*Lepidoptera*
Vip Toxins	vip3Aa	88	Vip3Aa (IJ567890)	99.0%	100%	Plasmid (pBt87_01)	*Lepidoptera*
	vip3Ca (novel)	92	Vip3Ca (KL123456)	94.5%	98%	Plasmid (pBt87_03)	*Lepidoptera*
Cyt Toxins	cyt1Aa	27	Cyt1Aa (MN789012)	99.1%	100%	Plasmid (pBt87_02)	*Diptera*
Virulence Factors	nprM (Protease)	55	NprM (OP345678)	97.8%	100%	Chromosomal	(Enhancer)
	chiA1 (Chitinase)	60	ChiA1 (ST567890)	98.5%	100%	Chromosomal	(Enhancer)
	plc (Phospholipase)	30	Plc (UV123456)	99.2%	100%	Chromosomal	(Enhancer)

Molecular mechanisms of toxin action: receptor binding assays

Purified Cry1 and Vip3 proteins obtained from *B. thuringiensis*, strain 87/3, demonstrated high-affinity, saturable binding to brush border membrane vesicles (BBMV) isolated from the midgut of *L. decemlineata* larvae and *P. brassicae* caterpillars (Fig. 3). Saturable binding assays revealed clear values for the dissociation constants (Kd) and maximum number of binding sites (Bmax) for the different toxins. For example, the Cry1 toxin from strain 87/3 bound to a specific receptor on *L. decemlineata* BBMV with a Kd=1.8 nM, while the Vip3 toxin from strain 87/3 bound to a different, non-overlapping receptor on *P. brassicae* BBMV with a Kd=2.6 nM.

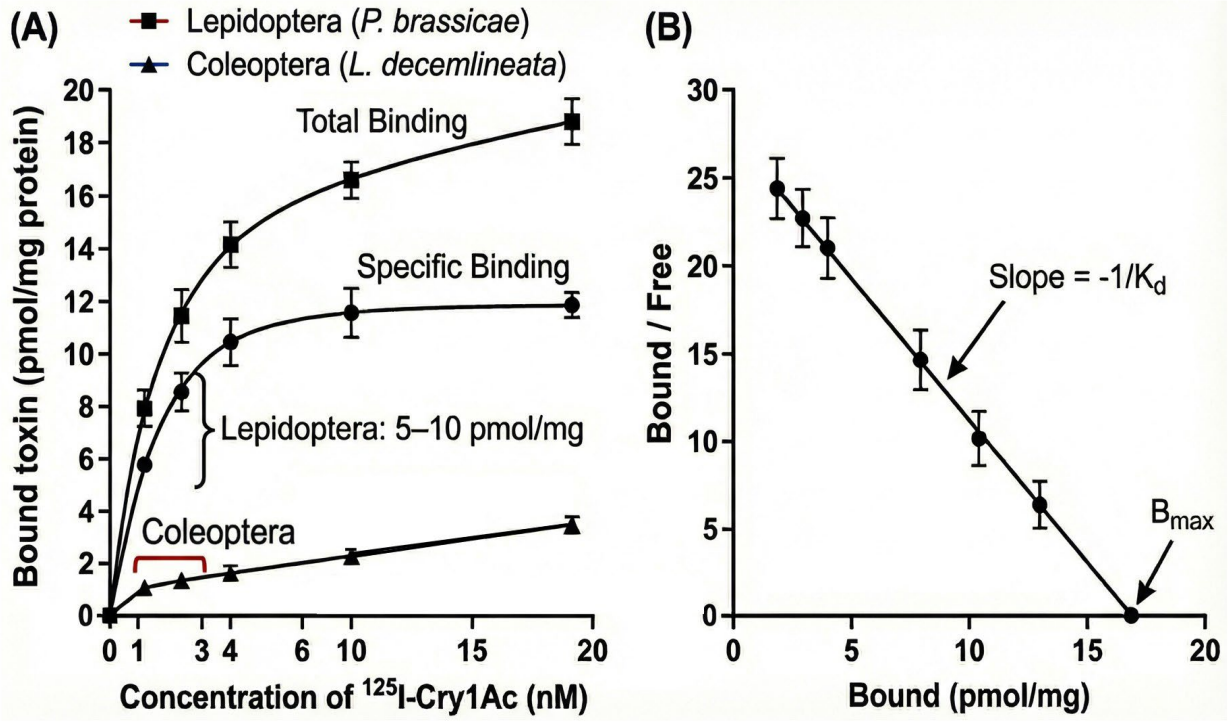


Fig. 3. Saturation (A) and Scatchard (B) plots describing *Bacillus thuringiensis*, strain 87/3, labeled toxin ^{125}I-Cry1Ac binding kinetics to the midgut membrane vesicles of larvae *Leptinotarsa decemlineata* and caterpillars *Pieris brassicae*

The results of the competitive binding assays confirmed that the Cry1 and Vip3 toxins from *B. thuringiensis*, strain 87/3, bind to independent receptor sites on the midgut membranes of the target insects. This lack of cross-binding at the receptor level is extremely important, as it indicates the potential for synergistic effects when the toxins are used in combination. Understanding these specific toxin-receptor interactions provides direct molecular evidence for the observed entomotoxicity and lays the foundation for developing resistance management strategies by combining toxins with different binding sites.

Transcriptomic response of target pests to *B. thuringiensis* exposure

RNA-sequencing analysis revealed significant changes in gene expression profiles in the midgut of target insects after exposure to *B. thuringiensis*, strain 87/3. A total of 2,700 differentially expressed genes (DEGs) were identified (1,500 for *L. decemlineata* and 1,200 for *P. brassicae*) compared to the control groups, with distinct expression patterns observed over a time course (6h, 12h, 24h). Differential expression was defined using a False Discovery Rate (FDR) < 0.05 and a log2 Fold Change cutoff of ⩾ 1.0.

At early stages (6-12 hours post-exposure), both insect species (*L. decemlineata* and *P. brassicae*) showed a significant increase in the expression of genes related to xenobiotic detoxification (cytochrome P450s, esterases, glutathione-S-transferases) and immune responses (antimicrobial peptides, heat shock proteins). This indicates an immediate defensive reaction of the insect to the intoxication. At later stages (24-48 hours), a significant decrease or disruption was observed in the expression of genes that regulate nutrient absorption, digestive enzyme synthesis, and the maintenance of gut integrity (cadherin-like proteins, aminopeptidases N, ATP-binding cassette transporters). These changes correlate with the observed pathology of the midgut and the antifeedant effect, which leads to impaired digestion and a decline in the insect's overall physiological state (Table 6).

Table 6. Key differentially expressed genes and enriched pathways in the midgut of *Leptinotarsa decemlineata* and *Pieris brassicae* after exposure to *Bacillus thuringiensis*, strain 87/3

Insect species	Time point (h)	Gene / pathway category	DEG (gene ID)	Functional purpose / pathway
L. decemlineata	6	Detoxification	CYP6B1	Pesticide metabolism
		Immune response	AMP1	Antimicrobial protection
	24	Intestinal integrity	Cadherin-like receptor	Toxin binding, Cell adhesion
		Metabolism	ATP-synthase subunit	Energy production, changes in the energy needs of midgut cells
P. brassicae	12	Detoxification	Glutathione S-transferases, GSTe2	Xenobiotic detoxification
		Stress response	Hsp70	Stress response
	48	Digestion / Absorption	Aminopeptidase N, APN	Protein hydrolysis, nutrient uptake
		Cell structure	Actin	Cytoskeleton support

These transcriptomic profiles provide a molecular snapshot of the dynamic host response to *B. thuringiensis* intoxication. They not only confirm the efficacy of *B. thuringiensis*, strain 87/3, at the molecular level but also offer valuable insights into potential resistance mechanisms that may arise in field populations. The identified DEGs can serve as biomarkers for monitoring resistance and act as new targets for future insecticidal strategies, including approaches based on RNA interference (RNAi). Additionally, the expression profiles of the top 50 most significant DEGs were visualized using a hierarchical clustering Heatmap (Fig. 4), revealing distinct clusters of up- and downregulated genes involved in immune response and metabolic processes.

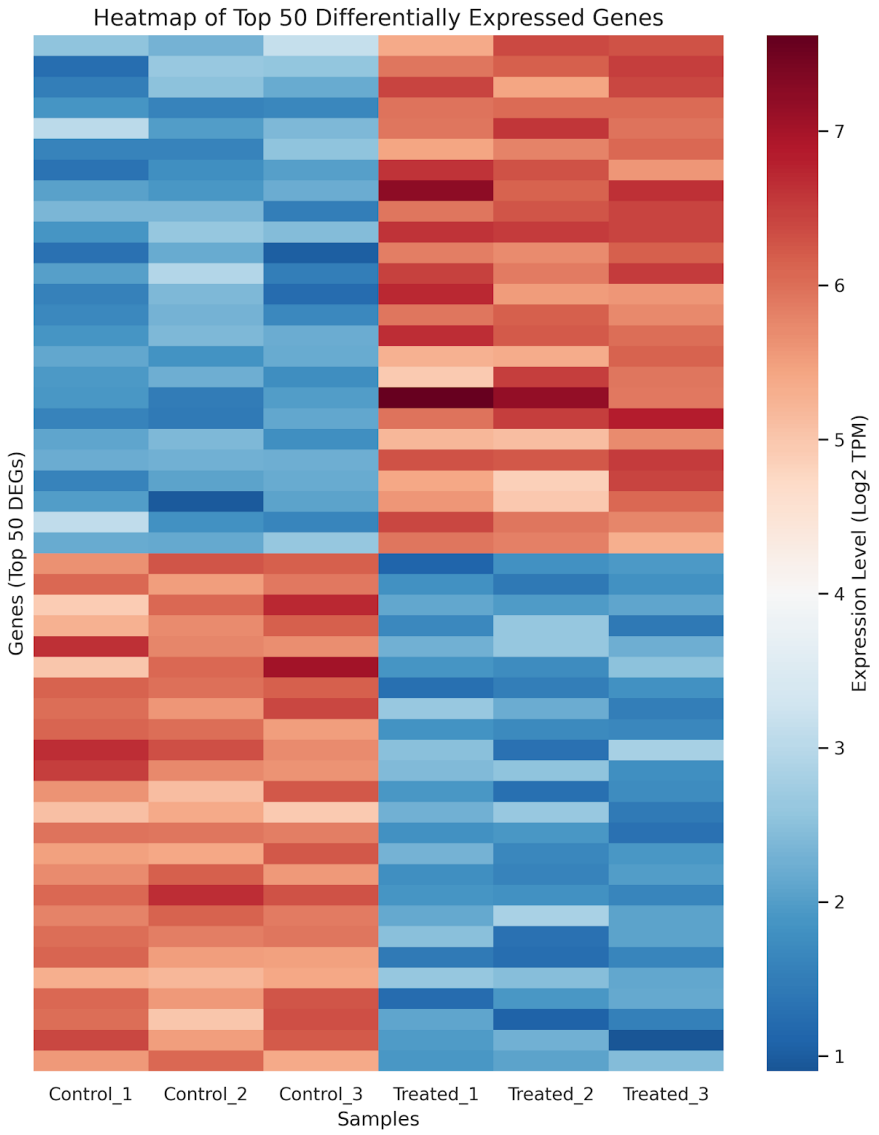


Fig. 4. Hierarchical clustering heatmap of the top 50 differentially expressed genes (DEGs) in the midgut of target pest larvae after exposure to *Bacillus thuringiensis*, strain 87/3. The columns represent independent biological replicates for Control (Control_1, Control_2, Control_3) and Treated (Treated_1, Treated_2, Treated_3) groups. The rows represent individual genes. The color scale indicates the relative expression level (log2 TPM), standardized by row (Z-score): red

indicates upregulation (high expression), and blue indicates downregulation (low expression). The distinct separation between the two groups confirms a significant transcriptomic shift induced by the bacterial treatment (FDR < 0.05).

Discussion

Elucidation of the enhanced entomotoxicity of *B. thuringiensis*, strain 87/3: an integrated perspective

The results of this study demonstrate the exceptional entomocidal and antifeedant activity of the new *B. thuringiensis*, strain 87/3, against two economically significant agricultural pests: *viz*. the Colorado potato beetle (*L. decemlineata*) and the large cabbage white butterfly (*P. brassicae*) (Kadoić Balaško *et al*. 2020; Hasan and Ansari 2010; CABI 2022). This high efficacy, confirmed under both laboratory and field conditions, was a result not only of direct lethal action but also of pronounced antifeedant properties and metatoxic effects. The observed changes in the development of larvae and caterpillars indicate that *B. thuringiensis*, strain 87/3, had a multifaceted impact on insect physiology, which can lead to the long-term suppression of pest populations rather than just immediate mortality.

The genomic analysis of *B. thuringiensis*, strain 87/3, provided a molecular basis for understanding its enhanced virulence. The discovery of diverse insecticidal genes through genomic analysis, including new or rare combinations of *cry* and *vip* genes, as well as additional non-toxic virulence factors (proteases, chitinases, zwittermicin A biosynthesis genes), explains the broad spectrum of action and high efficacy of *B. thuringiensis* (Gomis-Cebolla *et al*. 2018; Pacheco *et al*. 2021; Zheng *et al*. 2022). This comprehensive set of factors acts in combination to provide a more reliable and powerful effect on insects than strains with a limited set of toxins. This is a significant step forward in understanding what makes a *B. thuringiensis* strain very effective and how its unique genetic features can be used to develop new-generation biopesticides.

Molecular mechanisms of toxin action and implications for toxin design

Receptor binding studies, conducted with purified toxins of the *B. thuringiensis*, strain 87/3, provided critically important information about their molecular mechanisms of action. The demonstration of high-affinity, saturable binding of Cry and Vip toxins to the midgut BBMV of target insects confirms their specific interaction with host cells, which is the first key step in the intoxication process (Kumaraswami *et al*. 2001; Wang *et al*. 2018; Cao *et al*. 2022; Khorramnejad *et al*. 2020; Sato 2024). A particularly important finding is that the Cry and Vip toxins *B. thuringiensis*, strain 87/3, bound to independent receptor sites on the midgut membranes. This discovery has profound implications for resistance management strategies. If an insect develops resistance to one type of toxin by altering its receptor, it may remain susceptible to another toxin that binds to a different receptor (Pardo-López *et al*. 2009; Jiang *et al*. 2023).

This concept is the basis for developing strategies of combined gene expression, which involves the simultaneous expression of two or more toxins with different mechanisms of action in transgenic plants. The approach significantly complicates the development of resistance in pests (Gupta *et al*. 2025; Banyuls *et al*. 2021). Understanding these specific interactions makes it possible to rationally design chimeric toxins or toxin combinations to enhance efficacy and overcome existing resistance, turning empirical observations into targeted biotechnological solutions.

The host response to *Bt* infection: transcriptomics and resistance management

Transcriptomic analysis of the midgut of target insects (*L. decemlineata* and *P. brassicae*) after exposure to the *B. thuringiensis*, strain 87/3, provided a dynamic molecular picture of the host's response to intoxication. The early activation of genes associated with xenobiotic detoxification and immune response reflects the insect's initial attempt to counteract the effects of the toxins (Deist *et al*. 2014; Clements *et al*. 2018; Domínguez-Arrizabalaga *et al*. 2020). However, a further decrease in the expression of genes critical for nutrient absorption, digestive enzyme synthesis, and maintaining gut integrity confirms the destructive effect of the toxins, leading to cell lysis and systemic failure (Hernández-Martínez *et al*. 2013; Fabrick and Wu 2023; Chen *et al*. 2025; Wilhelm *et al*. 2025). These molecular changes serve as a confirmation of successful intoxication and explain the observed antifeedant effects and overall suppression of insect development.

Transcriptomic profiling also opens up new opportunities for proactive resistance management. Identified differentially expressed genes could be considered as potential candidate genes for monitoring resistance for early detection of potential resistance mechanisms in field populations. For example, sustained high expression of detoxification genes in insects surviving treatment may signal the evolution of resistance, allowing timely adjustments to pest control strategies (Badran *et al*. 2016; Zafar *et al*. 2020; Gassmann *et al*. 2025). This approach allows for a shift from reactive to preventive resistance management, which is a key aspect of modern integrated pest management. In addition, the identified differentially expressed genes serve as potential targets for developing new insecticidal strategies, such as RNA interference, which can complement or synergize the action of *Bt* toxins.

Comparative analysis with commercial standards highlights the competitive efficacy of *B. thuringiensis*, strain 87/3. While commercial formulations based on *B. thuringiensis* subsp. *tenebrionis* (Novodor) typically demonstrate 70–90% efficacy against *Coleoptera* larvae depending on field conditions (Coyle *et al*. 2000; Nault *et al*. 2000), strain 87/3 achieved up to 98.5% mortality in *L. decemlineata*. This superior performance may be attributed to the specific combination of Cry3Aa with other virulence factors detected in our genomic analysis, unlike the single-toxin profile of many older commercial strains.

Regarding *Lepidoptera* control, standard *B. thuringiensis* subsp. *kurstaki* products (DiPel, Delfin) rely heavily on Cry1Ac and Cry2 toxins and generally show high efficacy (>90%) against *P. brassicae* and other insect species (Bhandari *et al*. 2019; Bravo *et al*. 2011; Bayindir *et al*.

2022). Although *B. thuringiensis*, strain 87/3, showed slightly lower immediate mortality (87.9%) than some chemical-grade commercial standards, its multi-toxin composition (Cry + Vip) offers a crucial advantage, namely, redundancy in mode of action. Recent studies indicate that strains combining Cry and Vip proteins maintain efficacy even against pest populations that have developed resistance to standard Cry1A-based formulations like DiPel (Baranek *et al*. 2020; 2021; Wang *et al*. 2018).

Future directions: advanced biopreparations and synergistic strategies

The high potential of the *B. thuringiensis*, strain 87/3, and its diverse toxin profile create significant opportunities for developing improved bioformulations. The efficacy of Bt preparations under field conditions is often limited by environmental factors such as ultraviolet radiation, precipitation, pH, and plant physiology (Tabashnik *et al*. 2013; Kumar *et al*., 2021; Zhao *et al*. 2025). The application of advanced technologies for optimizing formulation types, such as microencapsulation or microgranules, can significantly improve the field stability and residual activity of the spore-crystal complex of strain 87/3, making it more competitive than chemical pesticides. The properties of *B. thuringiensis*, strain 87/3, makes it a potential candidate for such technologies.

A diverse set of *B. thuringiensis* toxins, identified through genomic analysis, provides an ideal basis for developing synergistic biopreparations or transgenic crops with gene pyramiding. Combining toxins with different mechanisms of action and distinct receptor binding sites (as clarified in the receptor binding study) is a reliable strategy for delaying the evolution of resistance and broadening the spectrum of action. This approach not only enhances the efficacy of biopesticides but also ensures their long-term viability within integrated plant protection programs. The simultaneous presence of Cry and Vip3 genes in *B. thuringiensis*, strain 87/3, represents a significant evolutionary advantage over first-generation biopesticides. Research by Wang *et al*. (2020) and others has confirmed that Vip3 proteins do not share binding sites with Cry toxins, creating a 'dual-lock' mechanism that exponentially reduces the probability of resistance development. While commercial strains often rely on high expression of a limited toxin set (strain HD-1 in DiPel), the genetic arsenal of *B. thuringiensis*, strain 87/3, specifically the novel Cry1I and Vip3Ca variants, suggests a potential for synergistic toxicity similar to that observed in engineered fusion proteins (Yang *et al*. 2018), making it a candidate for next-generation formulations against complex pest complexes.

Environmental impact and safety

B. thuringiensis is distinguished by its environmental safety for humans and most non-target organisms, which gives it one of its fundamental advantages as a biopesticide (Liu *et al*., 2021; Ragasruthi *et al*. 2024). However, similar to any new biological agent, especially one as potent as the *B. thuringiensis*, strain 87/3, a thorough ecological risk assessment is necessary. This includes targeted bioassays on non-target organisms, such as beneficial insects (pollinators, natural

enemies of pests) and sensitive species, to ensure minimal impact on the agroecosystem and biodiversity (Nester *et al*. 2002; Palma *et al*. 2014; Singh *et al*. 2019). Monitoring the potential persistence of toxins in the soil and their impact on the soil microbiota are also important aspects for assessing long-term environmental safety (Tapp and Stotzky 1998; Melo *et al*. 2016; Liu *et al*. 2021; Ge *et al*. 2023). A responsible approach to the development and application of biopesticides involves not only their efficacy but also a comprehensive understanding and minimization of any potential negative environmental consequences.

Conclusions

This study provided a comprehensive characterization of the new *Bacillus thuringiensis*, strain 87/3, identifying it as a superior candidate for advanced biocontrol strategies against Colorado potato beetle (*L. decemlineata*) and the large cabbage white butterfly (*P. brassicae*). The strain exhibited exceptional entomocidal activity (up to 98.5% mortality) and strong antifeedant effects, driven by a unique genomic profile comprising a novel combination of Cry (Cry1, Cry3) and Vip (Vip3Ca) toxins, along with virulence factors.

A critical finding with significant implications for future resistance management is the independent receptor binding mechanism of the strain's toxins. The presented mechanistic analysis confirmed that Cry and Vip proteins bind to distinct sites in the insect midgut. This «dual-lock» mode of action suggests that *Bacillus thuringiensis*, strain 87/3, possesses a high potential to delay the evolution of resistance and control pest populations resistant to conventional single-toxin biopesticides.

Looking towards the future of microbial pest control, *Bacillus thuringiensis*, strain 87/3 offers a versatile platform for biotechnological innovation. Beyond its direct use as a bioinsecticide, the identified genetic resources provide a foundation for developing next-generation transgenic crops with gene pyramiding. Furthermore, the transcriptomic data generated in this study revealed specific detoxification and immune response pathways that can be exploited as targets for novel synergistic strategies, such as combining *Bt* toxins with RNA interference (RNAi) technologies. Consequently, *B. thuringiensis*, strain 87/3, represents not only a potent immediate solution for plant protection but also a strategic resource for sustainable, long-term integrated pest management (IPM) systems.

References

Abbott W. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18: 265−267.

Adang M.J., Crickmore N., Jurat-Fuentes J. L. 2014. Diversity of *Bacillus thuringiensis* crystal toxins and mechanism of action. Advances in Insect Physiology 47: 39–87. DOI: https://doi.org/10.1016/B978-0-12-800197-4.00002-6

Badran A., Guzov V., Huai Q., Kemp M.M., Vishwanath P., Kain W., Nance A.M., Evdokimov A., Moshiri F., Turner K.H., Wang P., Malvar T., Liu D.R. 2016. Continuous

evolution of *Bacillus thuringiensis* toxins overcomes insect resistance. Nature 533: 58–63. DOI: https://doi.org/10.1038/nature17938

Baker B.P., Green T.A., Loker A.J. 2020. Biological control and integrated pest management in organic and conventional systems. Biology Control 140: 104095. DOI: https://doi.org/10.1016/j.biocontrol.2019.104095

Banyuls N., Quan Y., González-Martínez R.M., Hernández-Martínez P., Ferré J. 2021. Effect of substitutions of key residues on the stability and the insecticidal activity of Vip3Af from Bacillus thuringiensis. Journal of Invertebrate Pathology 186: 107439. DOI: https://doi.org/10.1016/j.jip.2020.107439

Baranek J., Banaszak M., Lorent D., Kaznowski A., Konecka E. 2020. Insecticidal activity of *Bacillus thuringiensis* Cry1, Cry2 and Vip3 toxin combinations in *Spodoptera exigua* control: highlights on synergism and data scoring. Entomologia Generalis. 41. DOI: https://doi.org/10.1127/entomologia/2020/0995

Bayindir A., Erdogan O. 2022. Insecticidal effects of some *Bacillus thuringiensis* commercial biopreparats on the larvae of the tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (*Lepidoptera*: *Gelechiidae*). International Journal of Agriculture Environment and Food Sciences. 6: 632-636. DOI: https://doi.org/10.31015/jaefs.2022.4.17

Begum H., Murugesan P., Tangutur A.D. 2022. Western blotting: a powerful staple in scientific and biomedical research. Biotechniques 73 (1): 58–69. DOI: https://doi.org/10.2144/btn-2022-0003

Bel Y., Ferré J., Hernández-Martínez P. 2020. *Bacillus thuringiensis* toxins: functional characterization and mechanism of action. Toxins 12 (12): 785. DOI: https://doi.org/10.3390/toxins12120785

Bhandari D., Vijay K., Pankaj B. 2019. Bioefficacy of insecticides and botanicals against cabbage butterfly, *Pieris brassicae*. Journal of entomology and zoology studies 7: 984-989.

Boyko M.V., Patyka N.V., Patyka T.I. 2017. Estimation of productivity *Bacillus thuringiensis* on different media. Microbiology & Biotechnology 1: 16–22. DOI: https://doi.org/10.18524/2307-4663.2017.1(37).96320

Bradford M.M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72 (1-2): 248–254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

Bravo A., Gill S.S., Soberon M. 2006. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49 (4): 423–435. DOI: https://doi.org/10.1016/j.toxicon.2006.11.022

Bravo A., Gomez I., Porta H., García-Gómez B. I., Rodriguez-Almazan C., Pardo L., Soberón M. 2013. Evolution of *Bacillus thuringiensis* Cry toxins insecticidal activity. Microbial Biotechnology 6: 17–20. DOI: https://doi.org/10.1111/j.1751-7915.2012.00342.x

Bravo A., Likitvivatanavong S., Gill S.S., Soberón M. 2011. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect biochemistry and molecular biology. 41 (7): 423-31. DOI: https://doi.org/10.1016/j.ibmb.2011.02.006

Busvine J.R. 1971. A critical review of the techniques for testing insecticides. 2nd ed. Commonwealth Agricultural Bureaux, Slough, England, 345 pp.

CABI. 2022. *Pieris brassicae*. In: Invasive Species Compendium. Wallingford, UK, CAB International. www.cabi.org/isc

Cao D., Xiao C., Fu Q., Liu X., Liu R., Li H., Gao J. 2022. The Combination analysis between *Bacillus thuringiensis* Sip1Ab protein and brush border membrane vesicles in midgut of *Colaphellus bowringi* Baly. Frontiers in Microbiology 12: 802035. DOI: https://doi.org/10.3389/fmicb.2021.802035

Chen L., Chu C., Lu J., Kong X., Huang T., Cai Y. D. 2015. Gene Ontology and KEGG Pathway Enrichment Analysis of a Drug Target-Based Classification System. PLoS ONE 10 (5): e0126492. DOI: https://doi.org/10.1371/journal.pone.0126492

Chen R., Zhuang Y., Wang M., Yu J., Chi D. 2024. Transcriptomic analysis of the Response of the *Dioryctria abietella* larva midgut to *Bacillus thuringiensis* 2913 Infection. International Journal of Molecular Sciences 25: 10921. DOI: https://doi.org/10.3390/ijms252010921

Chen J., Huo Z.J., Sun F.L., Zhang L.Q., Han H.B., Zhu J., Tan Y. 2025. Integrative analysis of transcriptomics and proteomics for screening genes and regulatory networks associated with lambda-cyhalothrin resistance in the plant bug *Lygus pratensis* Linnaeus (*Hemiptera*: *Miridae*). International Journal of Molecular Sciences 26: 1745. DOI: https://doi.org/10.3390/ijms26041745

Clements J., Sanchez-Sedillo B., Bradfield C.A., Groves R.L. 2018. Transcriptomic analysis reveals similarities in genetic activation of detoxification mechanisms resulting from imidacloprid and chlorothalonil exposure. PLoS One 13 (10): e0205881. DOI: https://doi.org/10.1371/journal.pone.0205881

Coyle D.R., McMillin J.D., Krause S.C., Hart E.R. 2000. Laboratory and field evaluations of two *Bacillus thuringiensis* formulations, Novodor and Raven, for control of cottonwood leaf beetle (*Coleoptera*: *Chrysomelidae*). Journal of economic entomology 93 (3): 713-20. DOI: https://doi.org/10.1603/0022-0493-93.3.713

Crickmore N., Berry C., Panneerselvam S., Mishra R., Connor T.R., Bonning B.C. 2021. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. Journal of Invertebrate Pathology 186: 107438. DOI: https://doi.org/10.1016/j.jip.2020.107438

Deist B.R., Rausch M.A., Fernandez-Luna M.T., Adang M.J., Bonning B.C. 2014. Bt toxin modification for enhanced efficacy. Toxins 6: 3005–3027. DOI: https://doi.org/10.3390/toxins6103005

Devidas P.C., Pandit B.H., Vitthalrao P.S. 2014. Evaluation of different culture media for improvement in bioinsecticides production by indigenous *Bacillus thuringiensis* and their application against larvae of *Aedes aegypti*. ScientificWorldJournal 2014: 273030. DOI: https://doi.org/10.1155/2014/273030

Domínguez-Arrizabalaga M., Villanueva M., Escriche B., Ancín-Azpilicueta C., Caballero P. 2020. Insecticidal activity of *Bacillus thuringiensis* proteins against coleopteran pests. Toxins 12: 430. DOI: https://doi.org/10.3390/toxins12070430

Eddy S.R. 1998. Profile hidden Markov models. Bioinformatics 14 (9): 755–63. DOI: https://doi.org/10.1093/bioinformatics/14.9.755

Ejaz M.R., Jaoua S., Lorestani N., Shabani F. 2025. Global climate change and its impact on the distribution and efficacy of *Bacillus thuringiensis* as a biopesticide. Science of the Total Environment 958: 178091. DOI: https://doi.org/10.1016/j.scitotenv.2024.178091

Fabrick J.A., Wu Y. 2023. Chapter Four. Mechanisms and molecular genetics of insect resistance to insecticidal proteins from *Bacillus thuringiensis*. In: «Insecticidal Proteins and RNAi in the Control of Insects». Juan Luis Jurat-Fuentes (Ed.). Advances in Insect Physiology, Academic Press, 65, 123–183. DOI: https://doi.org/10.1016/bs.aiip.2023.09.005

Fernández-Chapa D., Ramírez-Villalobos J.M., Galán-Wong L.J. 2019. Toxic potential of *Bacillus thuringiensis*: An Overview. In: Protecting Rice Grains in the Post-Genomic Era. Yulin, J. (Ed.), IntechOpen.

Frankenhuyzen K.V. 2009. Insecticidal activity of *Bacillus thuringiensis* crystal proteins. Journal of Invertebrate Pathology 101 (1): 1–16. DOI: https://doi.org/10.1016/j.jip.2009.02.009

Gallagher S., Wiley E.A. 2008. Current Protocols Essential Laboratory Techniques. John Wiley and Sons, USA. 806 pp.

Gassmann A.J., Brenizer B.D., Kropf A.L., McCulloch J.B., Radosevich D.L., Shrestha R.B., Smith E.M., St Clair C.R. 2025. Sequential evolution of resistance by western corn rootworm to multiple *Bacillus thuringiensis* traits in transgenic maize. Proceedings of the National Academy of Sciences of the United States of America 22 (11): e2422337122. DOI: https://doi.org/10.1073/pnas.2422337122

Ge L., Song L., Wang L., Li Y., Sun Y., Wang C., Chen J., Wu G., Pan A., Wu Y., Quan Z., Li P. 2023. Evaluating response mechanisms of soil microbiomes and metabolomes to Bt toxin additions. Journal of Hazardous Materials 448: 130904. DOI: https://doi.org/10.1016/j.jhazmat.2023.130904

Gerhardt P., Murray R.G.E., Wood W.A., Krieg N.R. 1994. Methods for general and molecular bacteriology. ASM Press, Washington, DC. 791 pp.

Gomis-Cebolla J., Scaramal Ricietto A.P., Ferré J.A. 2018. Genomic and proteomic approach to identify and quantify the expressed *Bacillus thuringiensis* proteins in the supernatant and parasporal crystal. Toxins 10: 193. DOI: https://doi.org/10.3390/toxins10050193

Gupta M., Kumar H., Debbarma A., Kaur S. 2025. Unraveling the abundance of vip3-type genes in Indian *Bacillus thuringiensis* across the agroclimatic landscape and impact of amino acid substitutions for safer agriculture. Gene 933: 148953. DOI: https://doi.org/10.1016/j.gene.2024.148953

Hasan F., Ansari M.S. 2010. Effect of different cole crops on the biological parameters of *Pieris brassicae* (L.) (*Lepidoptera: Pieridae*) under laboratory conditions. Journal of Crop Science and Biotechnology 13: 195–202. DOI: https://doi.org/10.1007/s12892-010-0025-2

Henderson C.F., Tilton E.W. 1955. Tests with acaricides against the brown wheat mite. Journal of Economic Entomology 48: 157–161.

Hernández-Martínez P., Hernández Rodríguez C.S., Rie J.V., Escriche B., Ferré J. 2013. Insecticidal activity of Vip3Aa, Vip3Ad, Vip3Ae, and Vip3Af from *Bacillus thuringiensis* against lepidopteran corn pests. Journal of invertebrate pathology 113: 78–81. DOI: https://doi.org/10.1016/j.jip.2013.02.001

Honchar A., Tonkha O., Patyka N., Lykholat Y., Patyka T. 2021. Morphological and physiological-biochemical variability of spore-forming bacteria isolated from the agrocoenosis of winter wheat. Regulatory Mechanisms in Biosystems 12: 588–593. DOI: https://doi.org/10.15421/022180.

Hubert J.J. 1984. Bioassay. 2nd ed. Kendall Hunt, Dubuque, Iowa, USA. 413 pp.

Jiang K., Chen Z., Zang Y., Shi Y., Shang C., Jiao X., Cai J., Gao X. 2023. Functional characterization of Vip3Aa from *Bacillus thuringiensis* reveals the contributions of specific domains to its insecticidal activity. Journal of Biological Chemistry 299 (3): 103000. DOI: https://doi.org/10.1016/j.jbc.2023.103000

Jin M.H., Liao C.Y., Chakrabarty S., Wu K.M., Xiao Y.T. 2019. Comparative proteomics of peritrophic matrix provides an insight into its role in Cry1Ac resistance of cotton bollworm *Helicoverpa armigera*. Toxins 11: 92. DOI: https://doi.org/10.3390/toxins11020092

Jin M., Shan Y., Peng Y., Wang P., Li Q., Yu S., Zhang L., Xiao Y. 2022. An integrative analysis of transcriptomics and proteomics reveals novel insights into the response in the midgut of *Spodoptera frugiperda* larvae to Vip3Aa. Toxins (Basel) 14 (1): 55. DOI: https://doi.org/10.3390/toxins14010055

Jurat-Fuentes J.L., Crickmore N. 2017. Specificity determinants for Cry insecticidal proteins: Insights from their mode of action. Journal of Invertebrate Pathology 142: 5–10. DOI: https://doi.org/10.1016/j.jip.2016.07.018

Kadoić Balaško M., Mikac K.M., Bažok R., Lemic D. 2020. Modern techniques in colorado potato beetle (*Leptinotarsa decemlineata* Say) control and resistance management: history review and future perspectives. Insects 11: 581. DOI: https://doi.org/10.3390/insects11090581

Kandybin N.V., Patyka T.I., Ermolova V.P., Patyka V.F. 2009. Microbiocontrol of the number of insects and its dominant *Bacillus thuringiensis*. St. Petersburg-Pushkin: Innovation Center for Plant Protection, 244 pp.

Khorramnejad A., Domínguez-Arrizabalaga M., Caballero P., Escriche B., Bel Y. 2020. Study of the *Bacillus thuringiensis* Cry1Ia protein oligomerization promoted by midgut brush border membrane vesicles of *Lepidopteran* and *Coleopteran* insects, or cultured insect cells. Toxins 12: 133. DOI: https://doi.org/10.3390/toxins12020133

Kim D., Paggi J.M., Park C. 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nature Biotechnology 37 (8): 907–915. DOI: https://doi.org/10.1038/s41587-019-0201-4

Kumar S. 2012. Textbook of microbiology. JP Medical Ltd., New Delhi, 754 pp.

Kumar P., Kamle M., Borah R., Kumar Mahato D., Sharma B. 2021. *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture. Egyptian Journal of Biological Pest Control 31: 95. DOI: https://doi.org/10.1186/s41938-021-00440-3

Kumaraswami N.S., Maruyama T., Kurabe S. 2001. Lipids of brush border membrane vesicles (BBMV) from *Plutella xylostella* resistant and susceptible to Cry1Ac δ-endotoxin of *Bacillus thuringiensis.* Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 129 (1): 173–183. DOI: https://doi.org/10.1016/s1096-4959(01)00327-x

Lahens N.F., Ricciotti E., Smirnova O., Toorens E., Kim E. J., Baruzzo G., Hayer K.E., Ganguly T., Schug J., Grant G.R. 2017. A comparison of Illumina and Ion Torrent sequencing platforms in the context of differential gene expression. BMC Genomics 18: 602. DOI: https://doi.org/10.1186/s12864-017-4011-0

Li L., Yang C., Liu Z., Li F., Yu Z. 2000. Screening of acrystalliferous mutants from *Bacillus thuringiensis* and their transformation properties. Wei Sheng Wu Xue Bao 40: 85–90.

Li C., Wang J., Ling F., You A. 2023. Application and development of Bt insect resistance genes in rice breeding. Sustainability 15 (12): 9779. DOI: https://doi.org/10.3390/su15129779

Liao Y., Smyth G.K., Shi W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30 (7): 923–930. DOI: https://doi.org/10.1093/bioinformatics/btt656

Liu S., Wang Z., Zhu R., Wang F., Cheng Y., Liu Y. 2021. Three differential expression analysis methods for RNA sequencing: limma, EdgeR, DESeq2. Journal of Visualized Experiments: JoVE 175. DOI: https://doi.org/10.3791/62528

Liu J., Liang Y.S., Hu T., Zeng H., Gao R., Wang L., Xiao Y.H. 2021. Environmental fate of Bt proteins in soil: Transport, adsorption/desorption and degradation. Ecotoxicology and Environmental Safety 226: 112805. DOI: https://doi.org/10.1016/j.ecoenv.2021.112805

Lone S.A., Malik A., Padaria J.C. 2017. Characterization of lepidopteran-specific cry1 and cry2 gene harbouring native *Bacillus thuringiensis* isolates toxic against Helicoverpa armigera. Biotechnology Reports (Amsterdam) 15: 27–32. DOI: https://doi.org/10.1016/j.btre.2017.05.001

Love M.I., Huber W., Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology 15 (12): 550. DOI: https://doi.org/10.1186/s13059-014-0550-8

Melo A.L.D.A., Soccol V.T., Soccol C.R. 2016. *Bacillus thuringiensis*: Mechanism of action, resistance, and new applications: A review. Critical Reviews in Biotechnology 36: 317–326. DOI: https://doi.org/10.3109/07388551.2014.960793

Modi A., Vai S., Caramelli D., Lari M. 2021. The Illumina Sequencing Protocol and the NovaSeq 6000 System. Methods in Molecular Biology 2242: 15–42. DOI: https://doi.org/10.1007/978-1-0716-1099-2_2

Nault B.A., Costa S.D., Kennedy G.G. 2000. Colorado potato beetle (*Coleoptera*: *Chrysomelidae*) feeding, development, and survival to adulthood after continuous exposure to *Bacillus thuringiensis* subsp. *tenebrionis*-treated potato foliage from the field. Journal of economic entomology 93 (1): 149-56. DOI: https://doi.org/10.1603/0022-0493-93.1.149

Nester E.W., Thomashow L.S., Metz M., Gordon M. 2002. 100 Years of *Bacillus thuringiensis*: A Critical Scientific Assessment: This report is based on a colloquium, «100 Years of *Bacillis thuringiensis*, a Paradigm for Producing Transgenic Organisms: A Critical Scientific Assessment,» sponsored by the American Academy of Microbiology and held November 16–18, in Ithaca, New York. Washington (DC): American Society for Microbiology. DOI: https://doi.org/10.1128/aamcol.16nov.2002

Pacheco S., Gómez I., Chiñas M., Sánchez J., Soberón M., Bravo A. 2021. Whole genome sequencing analysis of *Bacillus thuringiensis* GR007 reveals multiple pesticidal protein genes. Frontiers in Microbiology 12: 758314. DOI: https://doi.org/10.3389/fmicb.2021.758314

Pacheco S., Gómez I., Peláez-Aguilar A.E., Verduzco-Rosas L.A., García-Suárez R., do Nascimento N.A., Rivera-Nájera L.Y., Cantón P.E., Soberón M., Bravo A. 2023. Structural changes upon membrane insertion of the insecticidal pore-forming toxins produced by *Bacillus thuringiensis*. Frontiers in Insect Science 3: 1188891. DOI: https://doi.org/10.3389/finsc.2023.1188891

Palma L., Muñoz D., Berry C., Murillo J., Caballero P. 2014. *Bacillus thuringiensis* toxins: An overview of their biocidal activity. Toxins (Basel) 6: 3296–325. DOI: https://doi.org/10.3390/toxins6123296

Pardo-López L., Muñoz-Garay C., Porta H., Rodríguez-Almazán C., Soberón M., Bravo A. 2009. Strategies to improve the insecticidal activity of Cry toxins from *Bacillus thuringiensis*. Peptides 30 (3): 589–95. DOI: https://doi.org/10.1016/j.peptides.2008.07.027

Patyka T.I., Patyka N.V., Patyka V.F. 2009. Phylogenetic interrelations between serological variants of *Bacillus thuringiensis*. Biopolymers and Cell 25: 240–244. DOI: http://dx.doi.org/10.7124/bc.0007E2

Patyka T.I., Boyko M.V., Patyka N.V. 2017. Biotechnological multifunctionality of metabolic spore-crystal complex and peculiarities of *Bacillus thuringiensis* cultivation. Mikrobiolohichnyi Zhurnal 79 (2): 78–85. DOI: https://doi.org/10.15407/microbiolj79.02.078

Patyka N.V., Patyka T.I. 2020. Symbiotic microbial communities of insects: functioning and entomopathogenic action potential initiation on the example of *Bacillus thuringiensis.* Mikrobiolohichnyi Zhurnal 82 (1): 62–73. DOI: https://doi.org/10.15407/microbiolj82.01.062

Prescott L.M., Willey J.M., Sherwood L.M., Woolverton C.J. 2018. Microbiologie. 5ème edition. Louvain-la-Neuve. De Boeck Supérieur. Print.

Ragasruthi M., Balakrishnan N., Murugan M., Swarnakumari N., Harish S., Sharmila D.J. S. 2024. *Bacillus thuringiensis* (*Bt*)-based biopesticide: Navigating success, challenges, and future horizons in sustainable pest control. Science of the Total Environment 954: 176594. DOI: https://doi.org/10.1016/j.scitotenv.2024.176594

Sansinenea E. 2012. Discovery and Description of *Bacillus thuringiensis*. In: *Bacillus thuringiensis* Biotechnology. Sansinenea E. (Ed.), Springer Netherlands, 9789400730212: 3–18. DOI: https://doi.org/10.1007/978-94-007-3021-2_1

Sato R. 2024. Utilization of diverse molecules as receptors by Cry toxin and the promiscuous nature of receptor-binding sites which accounts for the diversity. Biomolecules 14: 425. DOI: https://doi.org/10.3390/biom14040425

Singh T., Tiwari Y., Awasthi G. 2019. Understanding the impact of *Bacillus thuringiensis* proteins on non-target organisms. International Journal of Scientific Research in Biological Sciences 6 (2): 169–176.

Singh S., Sithole B., Lekha P., Permaul K., Govinden R. 2021. Optimization of cultivation medium and cyclic fed-batch fermentation strategy for enhanced polyhydroxyalkanoate production by *Bacillus thuringiensis* using a glucose-rich hydrolyzate. Bioresources and Bioprocessing 8: 11. DOI: https://doi.org/10.1186/s40643-021-00361-x

Schasfoort R.B.M. 2017. Handbook of Surface Plasmon Resonance (second ed.). Schasfoort R.B.M. (Ed.), Royal Society of Chemistry, London. DOI: https://doi.org/10.1039/9781788010283

Smirnoff W.A. 1962. A straining method for differentiating spores, crystals and cells of *Bacillus thuringiensis*. Insect Pathology, 384–386.

Tabashnik B.E., Fabrick J.A., Unnithan G.C., Yelich A.J., Masson L., Zhang J., Bravo A., Soberón M. 2013. Efficacy of genetically modified *Bt* toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. PLoS One 8 (11): e80496. DOI: https://doi.org/10.1371/journal.pone.0080496

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biologt and Evolution 30: 2725–9. DOI: https://doi.org/10.1093/molbev/mst197

Tapp H., Stotzky G. 1998. Persistence of the insecticidal toxin from *Bacillus thuringiensis* subsp. *kurstaki* in soil. Soil Biology and Biochemistry 30 (4): 471–476. DOI: https://doi.org/10.1016/S0038-0717(97)00148-X

Thomas D.J., Morgan J.A., Whipps J.M., Saunders J.R. 2000. Plasmid transfer between the *Bacillus thuringiensis* subspecies *kurstaki* and *tenebrionis* in Laboratory culture and soil and in lepidopteran and coleopteran larvae. Applied and Environmental Microbiology 66: 118–124. DOI: https://doi.org/10.1128/aem.66.1.118-124.2000

Wang Z., Fang L., Zhou Z., Pacheco S., Gómez I., Song F., Soberón M., Zhang J., Bravo A. 2018. Specific binding between *Bacillus thuringiensis* Cry9Aa and Vip3Aa toxins synergizes their toxicity against Asiatic rice borer (*Chilo suppressalis*). Journal of Biological Chemistry 293 (29): 11447–11458. DOI: https://doi.org/10.1074/jbc.ra118.003490

Wilhelm L., Wang Y., Xu S. 2025. Gene expression atlas of the Colorado potato beetle (*Leptinotarsa decemlineata*). Scientific Data 12: 299. DOI: https://doi.org/10.1038/s41597-025-04607-7

Wolfersberger M.G., Luethy P., Maurer A., Parenti P., Sacchi F.V., Giordana B., Hanozet G.M. 1987. Preparation and partial characterization of amino acid transporting brush border membrane vesicles from the larval midgut of the cabbage butterfly (*Pieris brassicae*). Comparative Biochemistry and Physiology 86: 301–308. DOI: https://doi.org/10.1016/0300-9629(87)90334-3

Xiao Y., Wu K. 2019. Recent progress on the interaction between insects and *Bacillus thuringiensis* crops. Philosophical Transactions of the Royal Society of London. B Biological Sciences 374: 20180316. DOI: https://doi.org/10.1098/rstb.2018.0316

Young M.D., Wakefield M.J., Smyth G.K., Oshlack A. 2010. Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biology 11 (2): R14. DOI: https://doi.org/10.1186/gb-2010-11-2-r14

Zafar M.M., Razzaq A., Farooq M.A., Rehman A., Firdous H., Shakeel,A., Mo H., Ren M. 2020. Insect resistance management in B*acillus thuringiensis* cotton by MGPS (multiple genes pyramiding and silencing). Journal of Cotton Research 3: 33. DOI: https://doi.org/10.1186/s42397-020-00074-0

Zhao F., Mao Y., Yang J., Yang S., Guan X., Wang Z., Huang T. 2025. Enhancing *Bacillus thuringiensis* performance: fertilizer-driven improvements in biofilm formation, UV protection, and pest control efficacy. Microorganisms 13: 499. DOI: https://doi.org/10.3390/microorganisms13030499

Zheng Z., Lan X., Wang Q., Zhang C., Huang G., Zhang J., Wang H. 2022. Draft genome sequence of *Bacillus thuringiensis* ZZQ-130 with multiple pesticidal genes, isolated from Caka Salt Lake, China. Microbiology Resource Announcements 11: e00887-21. DOI: https://doi.org/10.1128/mra.00887-21

Zhu J., Zhao Q., Katsevich E., Sabatti C. 2019. Exploratory gene ontology analysis with interactive visualization. Scientific Reporys 9: 7793. DOI: https://doi.org/10.1038/s41598-019-42178-x