

DIVERSITY AND MECHANISMS OF INSECTICIDAL ACTION OF ENTOMOPATHOGENIC NEMATODE-DERIVED TOXINS: A REVIEW

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ABSTRACT

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are important biological control agents that exploit mutualistic associations with the bacteria *Xenorhabdus* and *Photorhabdus* to rapidly kill insect hosts. These bacterial symbionts produce a diverse array of proteinaceous toxins and non-protein secondary metabolites that contribute to host killing, immune suppression, tissue degradation and cadaver preservation. This review summarizes the diversity of EPN-derived insecticidal compounds and their molecular and cellular mechanisms of action. Major toxin groups, including toxin complexes (Tcs), Makes-Caterpillars-Floppy (Mcf) toxins, Pir binary toxins and *Photorhabdus* virulence cassettes (PVCs), together with lipopeptides, polyketides and other secondary metabolites, are discussed. Their insecticidal effects involve pore formation, disruption of the midgut epithelium and cytoskeleton, apoptosis, haemocyte dysfunction, suppression of eicosanoid-mediated immunity and interference with essential cellular processes. Evidence from laboratory and field studies demonstrates activity against insects belonging to several orders, including Lepidoptera, Coleoptera and Diptera, while differences in toxin profiles and host specificity among bacterial species and strains influence their efficacy. Recent advances in genome sequencing, biosynthetic gene-cluster mining, heterologous expression and synthetic biology have expanded opportunities for discovering and developing novel EPN-derived bioinsecticides. However, challenges related to toxin purification, stabilization, formulation, host specificity, non-target effects and field performance remain important barriers to commercialization. Overall, EPN-derived toxins represent a chemically and functionally diverse resource with considerable potential for developing sustainable and innovative insect pest management strategies.

KEYWORDS: *Entomopathogenic nematodes; Insecticidal toxins; Photorhabdus; Xenorhabdus*

1. INTRODUCTION

Modern agriculture faces mounting pressure to meet the food demands of a global population exceeding 7.5 billion people (Oberemok *et al.*, 2018). Insect pests are responsible for an average 40% loss in global crop yields, amounting to an estimated \$290 billion annual impact on the global economy (FAO, 2021), with arthropods alone destroying approximately 18-26% of annual crops (Parra-Arroyo *et al.*, 2021). Pest management has relied heavily on synthetic chemical pesticides, but their injudicious and repeated application has driven widespread insecticide resistance and pest resurgence, alongside serious health and environmental consequences (Irsad *et al.*, 2023; Tomer *et al.*, 2024). This has accelerated a global shift toward safer, eco-friendly pest control strategies (Sharma *et al.*, 2019).

Biopesticides - mass-produced biological agents including bacteria, fungi, viruses, and nematodes, along with their secondary metabolites - offer an alternative to synthetic chemicals through unique modes of action and strong resistance-management potential (Rajput *et al.*, 2020). A key component of biopesticide efficacy is the production of microbial metabolites such as toxins, enzymes, and antibiotics that act directly on target pests (Irsad *et al.*, 2023). While *Bacillus thuringiensis* remains the benchmark microbial insecticidal system, research has expanded substantially into entomopathogen-based products, which typically target specific pests, spare beneficial insects, avoid air and water pollution, and decompose quickly, limiting long-term environmental persistence (Rajput *et al.*, 2020; Irsad *et al.*, 2023).

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* are obligate soil-borne insect parasites capable of suppressing pest populations within a short period. Their life cycle includes a specialized, non-feeding infective juvenile (IJ) stage, the only stage capable of actively seeking insect hosts in soil (Yuksel *et al.*, 2025). EPNs exist in mutualistic association with Gram-negative bacteria - *Steinernema* with *Xenorhabdus*, and *Heterorhabditis* with *Photorhabdus* (Stock and Hazir, 2025). IJs penetrate hosts through natural openings or the cuticle, release their symbiotic bacteria into the haemolymph, and the resulting bacterial proliferation produces an extensive arsenal of toxins, enzymes, and antimicrobial agents that induce septicemia and host death within 24-48 hours (Irsad *et al.*, 2023; Yuksel *et al.*, 2025).

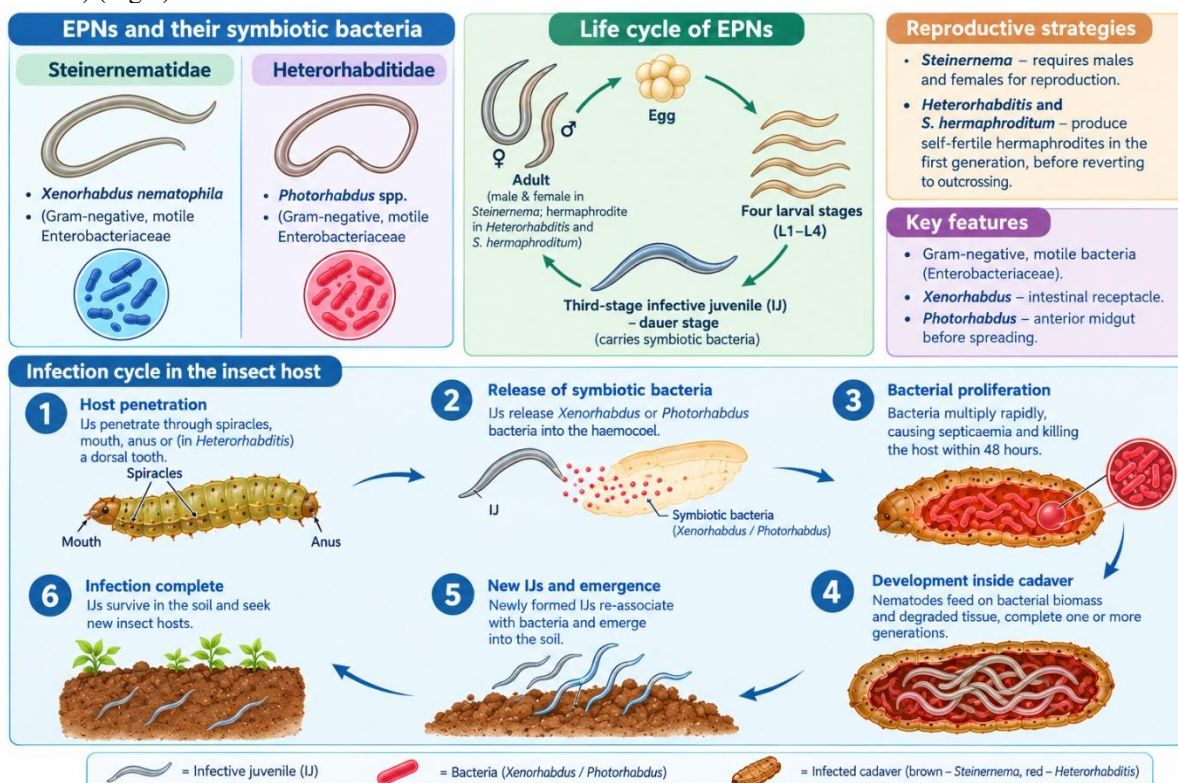
While whole EPNs are effective biocontrol agents, their field application is often limited by environmental sensitivity - susceptibility to desiccation and UV radiation - and challenges around shelf-life and mass production (Rajput *et al.*, 2020; Roy and Mukhopadhyay, 2025). This has driven interest in isolating and characterizing the toxins themselves as standalone biopesticide candidates. Several key toxin families have been identified within *Xenorhabdus* and *Photorhabdus*: pore-forming Toxin Complexes (Tcs), apoptosis-inducing Makes-Caterpillars-Floppy (Mcf) toxins, and orally toxic Pir binary toxins (Stock and Hazir, 2025). These natural products not only enhance biological control efficacy but also hold significant biotechnological potential for sustainable agriculture (Liao *et al.*, 2025). This review synthesizes current knowledge on the diversity of EPN-derived toxins and the molecular and cellular mechanisms underlying their insecticidal action.

2. Entomopathogenic Nematodes and Their Symbiotic Association

Only two of the nearly 40 nematode families associated with insects - Steinernematidae and Heterorhabditidae - are commonly used in biological control (Gaugler and Kaya, 1990). Heterorhabditidae comprises a single genus, *Heterorhabditis* Poinar, 1976, with 16 species, while Steinernematidae comprises the genus *Steinernema* Travassos, 1927 (around 95 species) and the monotypic *Neosteinerema* Nguyen and Smart, 1994 (Hunt *et al.*; Koppenhofer *et al.*). The number of recognized species has grown substantially, from 22 EPN species in 1995 to 108 in 2015 (Hunt *et al.*, 2016); as of 2025, approximately 114 species of *Steinernema* and 21 species of *Heterorhabditis* have been identified (Puza *et al.*, 2025). These families are utilized as biological control agents for a wide range of insects, serving as sustainable alternatives to chemical pesticides (Nurashikin-Khairuddin *et al.*, 2022).

EPNs, particularly species of Steinernematidae and Heterorhabditidae, are efficient biocontrol agents due to their ability to kill a wide range of insect pests rapidly through symbiosis with *Xenorhabdus* and *Photorhabdus* bacteria (Weeks and Baker). According to Poinar (1979), these nematodes are obligate insect parasites connected to bacterial symbionts (Boemare *et al.*, 1993; Forst *et al.*, 1997). Both genera are Gram-negative, motile members of the Enterobacteriaceae, housed within the modified intestines of the infective juvenile; *Xenorhabdus nematophila* colonizes the intestinal receptacle, while *Photorhabdus* species colonize the anterior midgut before spreading (Silva *et al.*, 2020).

EPNs undergo a life cycle of egg, four larval stages, and adult, with the third-stage infective juvenile (IJ) functioning as the soil-dwelling dauer stage carrying endosymbiotic bacteria (Adams *et al.*). Reproductive strategies differ between genera: *Steinernema* requires males and females, whereas *Heterorhabditis* and *S. hermaphroditum* produce self-fertile hermaphrodites in the first generation before reverting to outcrossing. Infection begins when IJs penetrate the insect through spiracles, mouth, anus, or (in *Heterorhabditis*) a dorsal tooth, releasing symbiotic bacteria into the haemocoel. These bacteria proliferate rapidly, causing septicaemia and killing the host within 48 hours (Kavya *et al.*). Inside the cadaver, nematodes feed on bacterial biomass and degraded tissue, complete one or more generations, and newly formed IJs re-associate with bacteria before emerging into the soil. The brown colour of an insect cadaver indicates Steinernematid infection, while red indicates Heterorhabditid infection, reflecting pigments released by the respective symbiotic bacteria (Bird and Akhurst) (Fig 1).



3. Diversity and Nature of EPN-Derived Toxins

Photorhabdus and *Xenorhabdus* bacteria produce bioactive compounds broadly grouped into proteinaceous toxins, peptide-based metabolites, and non-protein secondary metabolites, each playing distinct roles in insect killing, immunosuppression, and cadaver preservation.

3.1 Proteinaceous toxins

These are high-molecular-weight, virulence-gene-encoded toxins, including Toxin Complexes (Tcs), binary toxins such as PirAB, single-polypeptide toxins such as Makes-Caterpillars-Floppy (Mcf), and *Photorhabdus* Virulence Cassettes (PVCs). These proteins often cause cytotoxicity - destroying insect tissues, disrupting cell membranes or the cytoskeleton, causing septicaemia, and leading to rapid host death (Wellington *et al.*).

The Tc complex is a superfamily of pore-forming, high-molecular-weight insecticidal complexes first identified in the supernatant of two sequenced *Photorhabdus luminescens* strains, TT01 and W14, both containing a large variety of Tc genes (Duchaud *et al.*, 2003; Waterfield *et al.*, 2000; Bowen *et al.*, 1998). Using chromatography, toxin complexes were separated into four clusters - Tca, Tcb, Tcc, and Tcd (Bowen *et al.*, 1998). Tca and Tcd are orally active, while Tcc and Tcb are toxic upon direct injection into the insect haemocoel (Waterfield *et al.*, 2005). Tcs destroy midgut epithelial cells similarly to *Bacillus thuringiensis* delta-endotoxin, acting on the actin cytoskeleton via the ADP-ribosyltransferases TccC3 and TccC5 (Rodou *et al.*, 2010; Aktories *et al.*, 2014).

The Mcf gene was discovered when *Manduca sexta* larvae were infected with *Escherichia coli* carrying fragments of a 324 kDa *P. luminescens* protein, inducing caterpillar mortality (Waterfield *et al.*, 2003; Daborn *et al.*, 2002). Infected caterpillars lose body turgor, becoming 'floppy,' within 12 hours, with death around 24 hours after intoxication as Mcf1 protein rapidly kills insect haemocytes and destroys the midgut epithelium (Daborn *et al.*, 2002). An N-terminal region resembling the BH3 domain of a pro-apoptotic protein causes apoptosis when cells are intoxicated with Mcf1 (Dowling *et al.*, 2007).

Photorhabdus Virulence Cassettes are homologous to a prophage-like locus in the pADAP plasmid of *Serratia entomophila* (Yang *et al.*, 2006). Each cassette encodes 15-20 proteins resembling R-type pyocins (bacteriocins); the protein products lack direct antibacterial activity but destroy insect haemocytes, and can induce mortality in *G. mellonella* and *M. sexta* (Silva *et al.*, 2020). PVCs employ a self-contained nanosyringe delivery mechanism that breaches host cell membrane barriers independently of the bacterium, disrupting the insect host cytoskeleton (Vlisidou *et al.*, 2019).

3.2 Non-protein secondary metabolites

These small bioactive molecules include lipopeptide toxins, lipids, polyketides, and non-ribosomal peptides. For example, a low-molecular-weight lipopeptide toxin ('Xlt') from *Xenorhabdus innexi* shows larvicidal activity against mosquitoes.

3.3 Comparative overview of key insecticidal metabolites

Table 1 summarizes representative EPN-derived toxins, their chemical classification, insect targets, and proposed mechanisms of action, illustrating the substantial chemical and functional diversity within this toxin repertoire.

Species	Toxin	Chemical class	Target insect	Mechanism	Reference
<i>P. luminescens</i> subsp. <i>laumondii</i> TT01	Mcf	Polypeptide toxin	Midgut, Lepidoptera larvae (e.g. <i>Manduca sexta</i>)	Inhibits amino acid/phenolamine biosynthesis, causing paralysis	Rodou <i>et al.</i> , 2010
<i>P. luminescens</i> subsp. <i>akhurstii</i> W14	Tc	Polypeptide	Midgut epithelium, Coleoptera/Lepidoptera larvae	ADP-ribosylates actin, causing cell rounding and lysis	Hinchliffe <i>et al.</i> , 2010
<i>Xenorhabdus</i> spp.	Fabclavines	Hybrid peptide-polyketide	Broad-spectrum	Broad-spectrum bioactivity	Wenski <i>et al.</i> , 2020
<i>X. nematophila</i>	Xenorhabdycin	Proteinaceous bacteriocin	Competing bacteria in cadaver	Phage-tail-like structure used to kill competitor bacteria	Thaler <i>et al.</i> , 1995
<i>P. luminescens</i>	Photox	Polyketide	<i>Galleria mellonella</i>	ADP-ribosylates actin, causing cell death and paralysis	Visschedyk <i>et al.</i> , 2010
<i>X. nematophila</i>	Pilin subunit (17 kDa)	Polypeptide	<i>Helicoverpa armigera</i>	Haemocyte cytotoxicity; oral larvicidal activity	Kumari <i>et al.</i> , 2015
<i>P. akhurstii</i>	Galtox	Polypeptide toxin	<i>Galleria mellonella</i>	Disrupts osmotic balance of the cell	Ahuja <i>et al.</i> , 2021
<i>P. luminescens</i> strain TT01	Pir toxins	Binary protein toxin	<i>G. mellonella</i> , <i>Manduca sexta</i>	Binary toxin action	Ahantarig <i>et al.</i> , 2009

<i>P. asymbiotica</i> ATCC 43949	PVCs	Protein complex	<i>G. mellonella</i>	Apoptosis of insect phagocytes	Yang <i>et al.</i> , 2006
<i>X. nematophila</i> strain CB6	Xcn1	Proteinaceous bacteriocin	<i>Tetranychus urticae</i>	Inhibits moulting and gut microbiome function	Wei <i>et al.</i> , 2024
<i>X. nematophilus</i>	Toxin complex 1	Multi-component protein toxin	<i>Heliothis zea</i> , <i>Heliothis virescens</i>	Disrupts insect gut membranes	Sheets <i>et al.</i> , 2011
<i>X. innexi</i> strain HGB1681	Xlt	Protein toxin	<i>G. mellonella</i> , <i>M. sexta</i>	Inhibits insect virulence and reproduction	Kim <i>et al.</i> , 2017

4. Molecular and Cellular Mechanisms of Insecticidal Action

4.1 Cellular and molecular mechanisms of toxicity

Toxin complexes, Mcf toxins, Pir toxins, Photorhabdus virulence cassettes, and various secondary metabolites mediate cellular toxicity through diverse molecular mechanisms, including membrane disruption, pore formation, apoptosis induction, cytoskeletal disruption, and intracellular targeting of epithelial cells and haemocytes (Stock and Hazir, 2025). Tc toxins function as pore-forming proteins that lyse midgut epithelial cells, similar to *B. thuringiensis* delta-endotoxins, while ADP-ribosyltransferases such as TccC3 and TccC5 depolymerize actin filaments, compromising cytoskeletal integrity (Rodou *et al.*, 2010; Aktories *et al.*, 2014). Mcf toxins trigger apoptosis in haemocytes and midgut epithelium by rearranging the actin cytoskeleton, causing tissue damage and loss of turgor pressure. Pir toxins and PVCs target phagocytic haemocytes, inducing programmed cell death and suppressing phagocytosis through binary toxin action (Silva *et al.*, 2020) (Table 1).

4.2 Disruption of insect immunity, gut epithelium, and metabolism

Prostaglandins and other eicosanoids are crucial mediators of innate immune responses, whose biosynthesis is mediated by phospholipase A2 (PLA2); inhibition of PLA2, and thereby eicosanoid biosynthesis, is lethal to insects, making it a promising target for biological control (Stanley *et al.*, 2012). *Xenorhabdus*-produced toxin complexes induce immunosuppression in insects by inhibiting eicosanoid synthesis, and *X. nematophila* produces around eight suppressor metabolites of insect immunity (Silva *et al.*, 2020). *X. nematophila* and *Photorhabdus temperata* produce secondary metabolites such as benzylideneacetone, oxindole, and cis-cyclo-proline-tyrosine that block PLA2 activity, preventing arachidonic acid release essential for prostaglandin and leukotriene production, thereby disrupting cellular immune responses including haemocyte function and nodule formation (Kim *et al.*, 2005).

4.3 Comparative potency among *Xenorhabdus* and *Photorhabdus* species

Virulence and toxin potency vary significantly among species and strains due to differences in metabolite diversity, toxin gene clusters, and genomic architecture, influencing insecticidal efficacy and biopesticide development potential (Abd-Elgawad and Barna, 2022). For example, *P. luminescens* exhibits superior suppression of *Plutella xylostella* compared with some *Xenorhabdus* strains (Abdel-Razek, 2003). *X. nematophila* strains have shown insecticidal activity against representative pests across three insect orders - *Pieris brassicae* (Lepidoptera), *Aedes aegypti* (Diptera), and *Phaedon cochleariae* (Coleoptera) (Sergeant *et al.*, 2006). Notably, *Drosophila melanogaster* adults showed resistance to *X. innexi*, which was also ineffective against *Manduca sexta* larvae, while *X. nematophila* proved effective against both species (Sicard *et al.*, 2003; Kim *et al.*, 2017), highlighting host-specificity as a key variable in toxin efficacy.

4.4 Synergistic effects with bacterial metabolites and nematode secretions

Mixtures of cell-free cultures of EPN bacterial symbionts with *Bacillus thuringiensis* or *B. cereus* spores have produced synergistic toxic activity against *Phaedon brassicae*, *Plutella xylostella*, and *Spodoptera exigua* (Silva *et al.*, 2020). Combinations of *X. nematophila* or *P. temperata* with *B. thuringiensis kurstaki* and *B. thuringiensis aizawai* spores have yielded synergistic mortality in *P. xylostella* and *S. exigua* (Eom *et al.*, 2014). *Xenorhabdus* bacteria can also show synergistic activity against serious, intractable pests such as the beet armyworm when combined with other biocontrol agents (Eom *et al.*, 2014; Seongchae and Yonggyun, 2006).

5. Evidence of Insecticidal Activity Under Laboratory and Field Conditions

5.1 Cross-order activity: Lepidoptera, Coleoptera, and Diptera

Infection of *Galleria mellonella* by *Steinernema feltiae* produced rapid insecticidal activity, immune suppression, and host death within 24-48 hours; metabolomic analysis revealed strong activation of the tryptophan pathway, with 3-hydroxyanthranilic acid identified as a key contributor to EPN-mediated insect killing, achieving corrected mortality of 62.5% (Zhang *et al.*, 2022). Indigenous *Heterorhabditis bacteriophora* showed potent virulence against key Lepidoptera pests (*Helicoverpa armigera*, *Spodoptera litura*, *Agrotis segetum*), achieving high larval mortality within 120 hours, with LC50 values of 60.14 IJs/larva (third-instar *H. armigera*) and 57.90 IJs/larva (fourth-instar), and mortality increasing with larval age (Thakur *et al.*, 2022).

Both *Aedes* species larvae were orally susceptible to symbiotic bacteria: *Ae. albopictus* mortality reached 98% following exposure to *P. luminescens subsp. akhurstii* at 96 hours, and *A. aegypti* mortality reached 99% following exposure to *X. stockiae*, compared with 0-7% in controls (Park *et al.*, 2016). Pir toxins and other *Xenorhabdus*/*Photorhabdus* products act synergistically with *Bacillus thuringiensis* Cry4Ba or Bti to boost mortality in *Aedes* and *Culex*, underlining EPN-derived toxins as complementary tools for managing mosquito

insecticide resistance (Park *et al.*, 2015). Specific toxin complexes such as Tca, isolated from *P. luminescens*, have demonstrated significant oral and injectable toxicity against agricultural pests including the Colorado potato beetle (*Leptinotarsa decemlineata*) and sweetpotato whitefly (*Bemisia tabaci*) (Fatimah *et al.*, 2025).

Beyond Tc toxins, *Xenorhabdus* toxin complex proteins xptA2, xptB1, and xptC1 form tetrameric structures that attach to insect outer membranes and create pores, resulting in high insecticidal activity against lepidopteran pests including *Pieris brassicae*, *P. rapae*, and *Heliothis virescens* (Tomar *et al.*, 2022). The cooperative action of these proteins, particularly the central role of xptA1, enhances gut binding and can be up to 300 times more toxic to lepidopteran larvae compared to standalone potency (Johnson *et al.*, 2024).

5.2 Field-scale case studies

In Florida citrus groves, soil applications of *S. riobrave* against *Diaprepes abbreviatus* root weevil larvae achieved over 90% mortality at rates of 1.2×10^{10} IJs/ha, particularly at 27 degrees C in sandy soils, with irrigation delivery enhancing toxin penetration and reducing larval survival by 90% (Lacey and Georgis, 2012). Beetle grubs, especially Japanese beetle (*Popillia japonica*), are a prime target for *S. glaseri* and *H. bacteriophora*, which have proven effective when incorporated into turfgrass and maize cropping systems, illustrating broad-spectrum EPN performance against both leaf-feeding and root-dwelling pests (Raypuriya *et al.*, 2025).

6. Genomic and Biotechnological Advances

6.1 Genomic mining for toxin genes and biosynthetic pathways

Advances in genome sequencing have transformed *Xenorhabdus* and *Photorhabdus* into 'genomic goldmines' for identifying novel insecticidal agents (Hinchliffe *et al.*, 2013). Complete genome sequencing of species such as *X. nematophila* and *X. bovienii* has revealed extensive divergence in physiological and metabolic mechanisms despite similar lifestyles, indicating that evolutionary changes have followed different routes to achieve entomopathogenicity (Chaston *et al.*, 2011). These bacteria utilize specialized biosynthetic gene clusters (BGCs) to produce non-ribosomal peptides and polyketides (Meesil *et al.*, 2025), and bioinformatics tools such as antiSMASH are critical for predicting chemical diversity that traditional culture-based methods often miss - for example, antiSMASH predictions helped identify the Xlt cluster in *X. innexi*, encoding mosquitocidal toxin machinery (Kim *et al.*, 2017).

6.2 Expression in heterologous hosts and biotechnological applications

Escherichia coli is widely used for expressing individual genes, such as the tccC1 gene from *X. nematophila*, yielding recombinant toxins with high insecticidal activity (Lee *et al.*, 2004). *Bacillus subtilis* serves as an alternative host owing to strong protein secretion and reduced inclusion-body formation compared with *E. coli* (Li *et al.*, 2021). Research has also demonstrated that TcdA proteins can be expressed in transgenic *Arabidopsis thaliana* at levels sufficient to inhibit growth of pests such as the southern corn rootworm (Hinchliffe *et al.*, 2010). CRISPR-based strain engineering for enhanced toxin yield and synthetic-biology approaches for bioinsecticide design represent promising directions for future biotechnological development.

7. Challenges and Future Prospects

Despite substantial toxin diversity and well-characterized mechanisms of action, translating EPN-derived toxins into commercial biopesticides faces practical challenges: purification and stabilization of toxin compounds remain resource-intensive, toxin potency and host-specificity vary considerably between species and strains, and the ecological and non-target effects of isolated toxins (as opposed to whole living nematodes) remain comparatively understudied. Continued genomic mining, heterologous expression optimization, and a clearer mechanistic understanding of host-specificity determinants will be essential to realizing the full biopesticide potential of this toxin repertoire.

8. CONCLUSION

Entomopathogenic nematode-derived toxins represent a chemically and functionally diverse group of insecticidal agents, spanning pore-forming toxin complexes, apoptosis-inducing Mcf toxins, binary Pir toxins, and a range of non-protein secondary metabolites. Their mechanisms of action - spanning membrane disruption, cytoskeletal interference, immune suppression, and gut epithelial damage - provide multiple, often non-overlapping avenues of insecticidal activity, offering both direct biopesticide potential and valuable templates for biotechnological innovation in sustainable pest management.

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