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Bacterial insecticides beyond *Bacillus thuringiensis*

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Abstract

Bacterial insecticides have emerged as a biotechnological strategy for controlling insect pests in crops, primarily due to the ineffectiveness of synthetic pesticides, which have led to resistance in insect populations due to their overuse. For many years, *Bacillus thuringiensis* has been recognized as the most promising microorganism for this purpose, while other entomopathogenic bacteria, which have demonstrated efficacy against various insect orders, have been largely overlooked. These entomopathogenic bacteria employ diverse mechanisms to control insect pests, including damage via contact or ingestion, disruption of intestinal cell integrity, interference with the insect's central nervous system, and alterations to reproductive processes. Among the insecticidal compounds produced by these bacteria are Cry homologous toxins, non-homologous toxins, biosurfactant-type compounds, macrocyclic lactones, lipopeptides, chitinases, and other metabolites that remain underexplored. This paper provides a comprehensive overview of the current state of lesser-known entomopathogenic bacteria utilized in the development of these biopesticides. It discusses the advantages and disadvantages of these biotechnological products, the mechanisms of action of entomopathogenic bacteria, genetic engineering strategies aimed at enhancing these biopesticides, and the application of these bacteria in commercial production. Additionally, the paper reviews patents related to this field and examines the role of insecticidal bacteria as inducers of systemic resistance (ISR) in plants, highlighting their potential for the development of commercial bioproducts.

Keywords Entomopathogenic bacterial, Insect, Commercial products, Patents

Introduction

The intensification of agriculture driven by population growth is becoming increasingly apparent, with projections indicating that agricultural production must increase by 70% by 2050 to meet rising food demands (Sunjka et al. 2022). However, this intensification is accompanied by significant economic losses worldwide, primarily due to plant pathogens that have developed resistance to traditional pesticides, which can result in losses of up to 42% in major crops (Glare et al. 2012; Aneja et al. 2016; Samada et al. 2020).

Currently, several alternatives exist for managing plant pathogens, including the development of resistant crop varieties and the use of synthetic agrochemicals. However, the latter approach has notable limitations. Environmental problems stemming from excessive chemical use in agroecosystems have become evident, leading to issues such as the emergence of pest resistance, the elimination of beneficial microorganisms and non-target organisms, the rapid proliferation of secondary pests, and the accumulation of pesticide residues in food, which poses potential health risks, including cancer and fetal damage (Gupta and Dikshit 2010; Singh et al. 2014; Mishra et al.

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2015; Hakim et al. 2020; Kumar et al. 2021; Perez et al. 2011; Hernandez et al. 2022).

In light of these challenges, biopesticides have been developed as biotechnological products that offer an ecological and sustainable alternative to chemical pesticides in agroecosystems (Glare et al. 2012; Prabha et al. 2017; Hakim et al. 2020). Research indicates that biopesticides can outperform synthetic pesticides when applied at optimal times and concentrations (Shah et al. 2013). Furthermore, they effectively reduce product losses without compromising quality and are considered environmentally friendly due to their biodegradable nature (Leng et al. 2011; Kumar et al. 2021).

The United States Environmental Protection Agency (EPA) categorizes biopesticides into three groups based on their active ingredients: plant-incorporated protective agents (PIPs), which are produced through transgenic methods that induce the synthesis of natural insecticidal compounds in plant tissues; biochemical biopesticides, which are derived from natural compounds produced by insects and plants; and microbial pesticides, which are based on bacteria, fungi, viruses, or microalgae (Gupta et al. 2010; Seiber et al. 2014; Nathan et al. 2014; Mishra et al. 2015; Aneja et al. 2016; Kachhawa 2017; Lengai et al. 2018; Hakim et al. 2020) (Fig. 1).

Bacterial insecticides are natural formulations derived from live bacteria or their metabolites, specifically designed to control insect pests through highly specific mechanisms while exhibiting low toxicity to non-target organisms, including beneficial insects (Thakore 2006; Mazid et al. 2011; Nathan et al. 2014; Kumar and Singh 2015; Hakim et al. 2020).

Historically, most commercial products have been developed from the bacterium *Bacillus thuringiensis* (Bt). In fact, products based on the genus *Bacillus* represent the most significant class of commercially available crop protection products (Jacobsen et al. 2004; Samada et al. 2020). However, other notable bacterial genera have not received adequate recognition. This review will focus on the exploration of alternative entomopathogenic bacteria as a foundation for formulating biopesticides. It will discuss insecticidal molecules and their modes of action, highlight some commercial products, outline genetic engineering strategies employed in their production, and present patents awarded or in progress related to these products and their role as inducers of systemic resistance (ISR) in plants as part of the state of the art.

Bacterial insecticides: advantages and disadvantages

Bacterial insecticides were first developed in the 1950s and, due to their ease of mass production, became the first microbial pesticides to be commercialized. For over 40 years, these products have been in use and currently account for 90% of biopesticides marketed globally (Nathan et al. 2014; Osman et al. 2015; Tabashnik and Carrière 2017; Villareal et al. 2017; Kumar et al. 2021). While these insecticides offer advantages such as high effectiveness and specificity, they also face challenges, including low stability, high production costs, and inconsistent results in field trials (Cooping and Menn 2000;

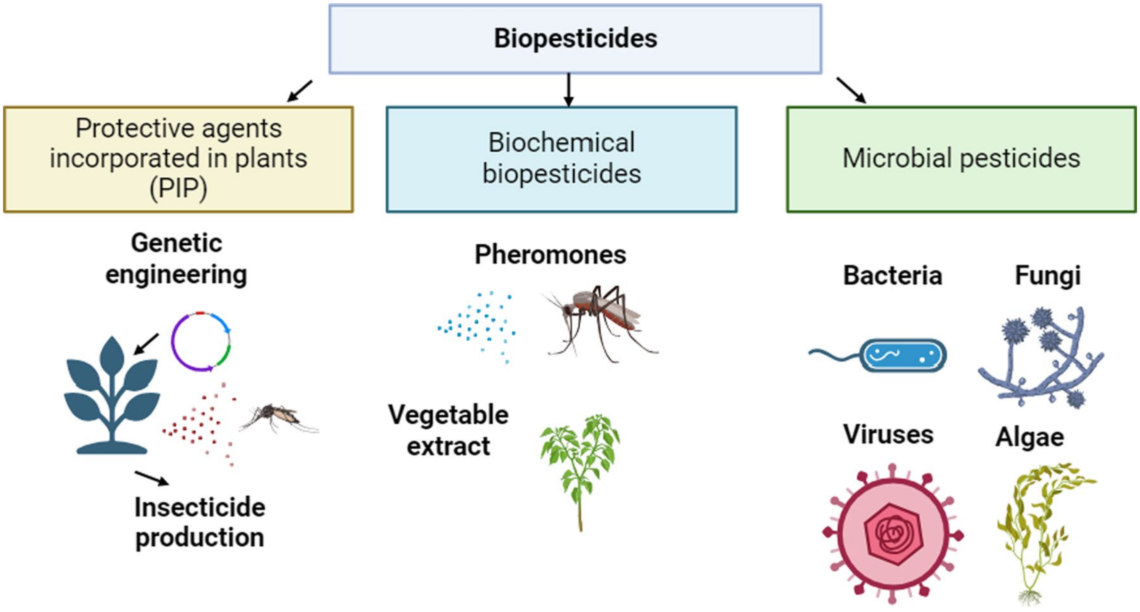


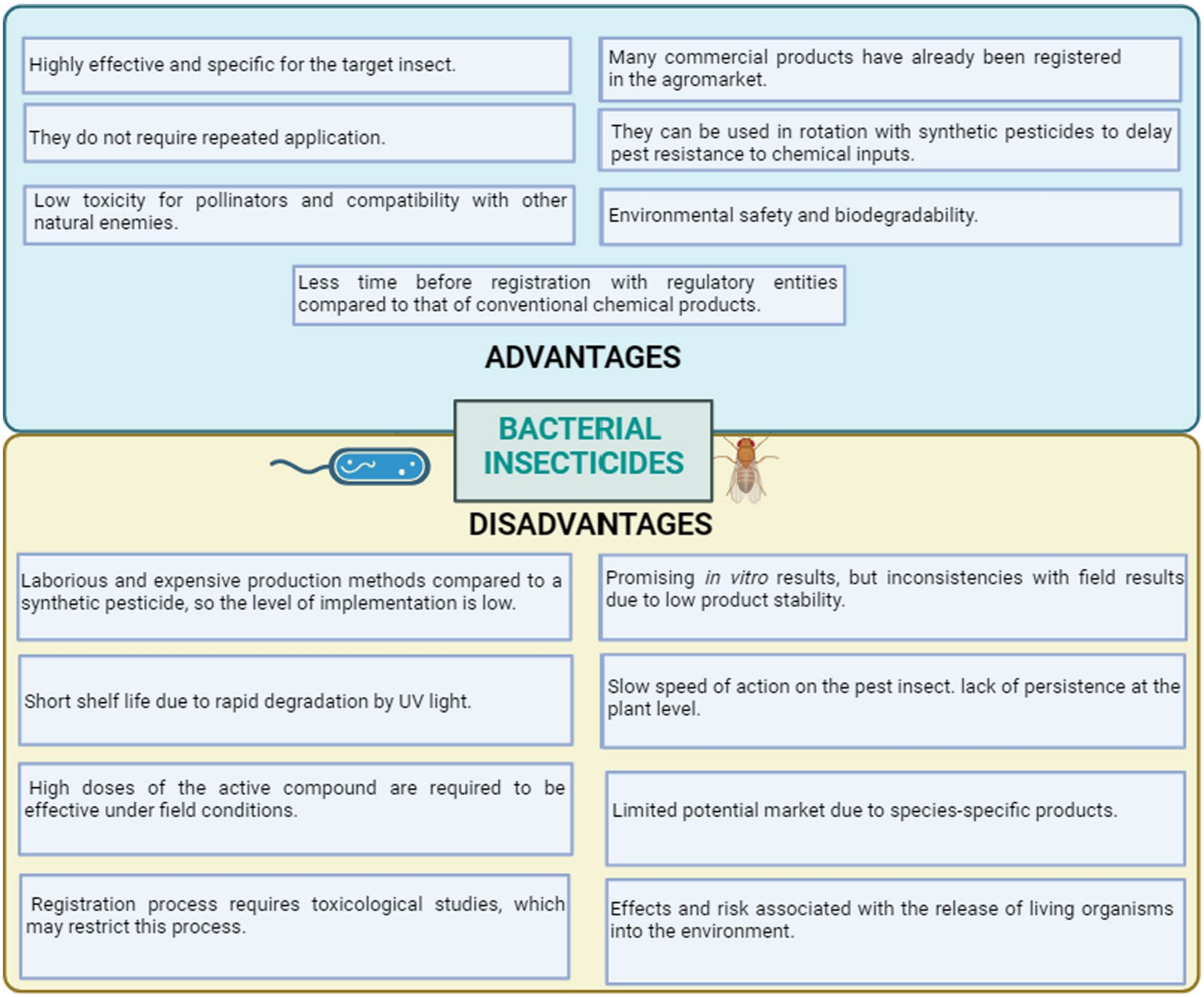
Fig. 1 Types of biopesticide according to the United States Environmental Protection Agency (EPA)

Gullino et al. 2005; Hynes and Boyetchko 2006; Gupta et al. 2010; Xu et al. 2011; Chandler et al. 2011; Leng et al. 2011; Glare et al. 2012; Gasic and Tanovic 2013; Bhattacharjee and Dey 2014; Siever et al. 2014; Ritika and Utpal 2014; Mishra et al. 2015; Mnif et al. 2015; Kumar and Singh 2015; Tijjani et al. 2016; Aneja et al. 2016; Shiberu et al. 2016; Kachhawa 2017; Lengai et al. 2018; Samada and Tambunan 2020; Hakim et al. 2020; Kumar et al. 2021) (Fig. 2).

Entomopathogenic bacteria as insecticides

By the 1980s, approximately 100 bacteria had been identified as entomopathogenic, yet only four *Bacillus thuringiensis*, *Bacillus popilliae*, *Bacillus lentimorbus*, and *Bacillus sphaericus* had been extensively studied as agents for insect control (Miller et al. 1983). Among these, *Bacillus thuringiensis* (Bt) has received the most attention, leading to the development of commercial products. Bt is a Gram-positive, spore-forming bacterium that is non-pathogenic to humans and typically found in soil and the guts of various insects, particularly Lepidoptera. Globally, there are around 175 registered biopesticides with approximately 700 active compounds available for use (Hakim et al. 2020). In the United States, over 150 commercial products are available, while the European Union market features only 60 analogous products (Kumar 2021).

Bacteria used as pesticides can be either Gram-positive or Gram-negative. Gram-positive bacteria can be further classified into endospore-forming and non-endospore-forming types, with endospore-forming bacteria being



Highly effective and specific for the target insect.

Many commercial products have already been registered in the agromarket.

They do not require repeated application.

They can be used in rotation with synthetic pesticides to delay pest resistance to chemical inputs.

Low toxicity for pollinators and compatibility with other natural enemies.

Environmental safety and biodegradability.

Less time before registration with regulatory entities compared to that of conventional chemical products.

Laborious and expensive production methods compared to a synthetic pesticide, so the level of implementation is low.

Promising *in vitro* results, but inconsistencies with field results due to low product stability.

Short shelf life due to rapid degradation by UV light.

Slow speed of action on the pest insect. lack of persistence at the plant level.

High doses of the active compound are required to be effective under field conditions.

Limited potential market due to species-specific products.

Registration process requires toxicological studies, which may restrict this process.

Effects and risk associated with the release of living organisms into the environment.

Fig. 2 Advantages and disadvantages of using bacterial insecticides

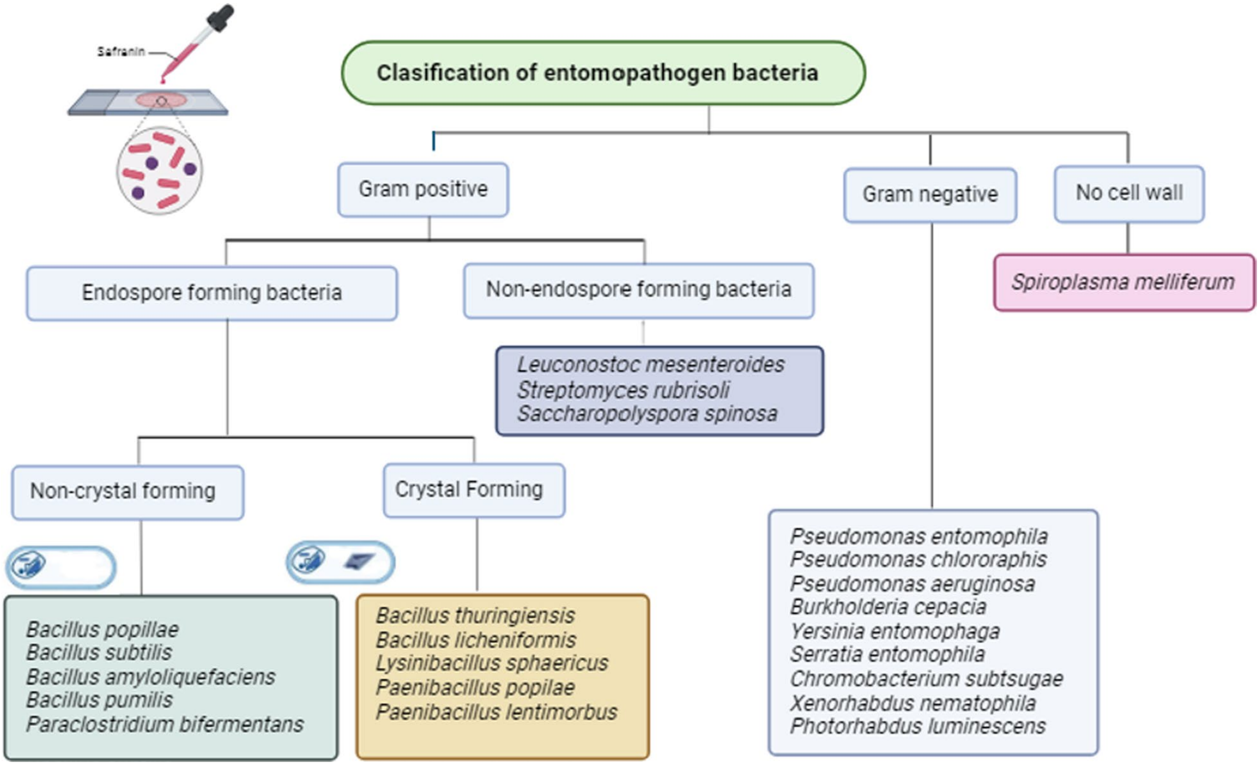


Fig. 3 Classification of entomopathogenic bacteria used as biopesticides

the most widely studied for commercial applications (Kumar et al. 2021) (Fig. 3).

Currently, a wide diversity of bacteria is recognized for their potential use in the production of bioinsecticides. Most of these bacteria belong to the families *Bacillaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Lactobacillaceae*, *Micrococcaceae*, and *Streptococcaceae* (Ruiu 2015; Kachhawa 2017; Azizoglu et al. 2020). Notable species within the *Bacillaceae* family include *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *Bacillus licheniformis*, as well as *Paenibacillus popilliae* and *Brevibacillus laterosporus* (Mnif et al. 2015; Villareal et al. 2017). Additionally, species from the *Pseudomonadaceae* family, such as *Pseudomonas entomophila* and *Pseudomonas aeruginosa*, along with those from the *Yersiniaceae* family like *Yersinia entomophaga*, are also noteworthy (Raaijmakers and Mazzola 2012; Prabha et al. 2017; Villareal et al. 2017; Kumar et al. 2021). Furthermore, insecticidal bacteria of the genera *Xenorhabdus* and *Photorhabdus* form a symbiotic relationship with entomopathogenic nematodes from the genera *Steinernema* and *Heterorhabditis*. During infection, the nematode acts as a vector, transporting the bacteria to the larva of the target insect, where the bacteria are disseminated into the hemolymph, leading to septicemia (Chattopadhyay et al. 2004) (Table 1).

Mechanisms of action of entomopathogenic bacteria

Entomopathogenic bacteria employ various mechanisms to infect, colonize, and ultimately eliminate their insect hosts. The primary strategies include inducing contact damage, producing toxins that disrupt the insect's digestive system, generating molecules that target the insect's nervous system, and interfering with reproduction and development. These mechanisms can operate independently or in synergy, enhancing their overall effectiveness (Jurat-Fuentes and Jackson 2012).

Production of contact damage

Chitinases are enzymes that degrade chitin, a structural polysaccharide found in the exoskeletons of insects and crustaceans. Chitin plays a crucial role in preventing water loss and forming a protective barrier against pathogens and environmental stressors (Veliz et al. 2017). The degradation of chitin can weaken the exoskeleton, rendering the insect unable to support its own weight and increasing its susceptibility to microbial infections. Additionally, the presence of these enzymes can disrupt the molting process, leading to improper formation of the

Table 1 Entomopathogenic bacteria used for the production of bacterial insecticides

Bacteria	Characteristics	Insecticidal molecule	Mechanism of action	Biological target	References
Bacillus pumilus	Gram-positive, aerobic, spore-forming bacillus with cream-coloured colonies with concentric ring-like appearance, ubiquitous distribution	Uncharacterised enzymes and secondary metabolites produced during sporulation	Not determined	*Aulacorthum solani; Aphis gossypii; Ceratitis capitata*	Kahia et al. (2021), Molina et al. (2010)
Bacillus amyloliquefaciens	Gram-positive, aerobic, oval spore-forming bacillus, clustered colonies with mucoid appearance and metallic grey colour, ubiquitous distribution	Biosurfactant	Contact	*Anopheles stephensi, Culex quinquefasciatus, Aedes aegypti*	Geetha et al. (2011), Saint-Pierre et al. (2023)
Bacillus licheniformis	Gram positive bacillus, aerobic, ubiquitous distribution	Rhomboid parasporal crystal, chitinases, exopolysaccharide	Contact, midgut and muscle involvement in larvae	*Anopheles stephensi, Aedes aegypti, Globitermes sulphureus*	Yan et al. (2007), Abinaya et al. (2018), Hussin et al. (2020)
Bacillus subtilis	Gram-positive, aerobic, spore-forming bacillus	Lipopeptide	Contact, histopathological changes in the intestine	*Drosophila melanogaster, Culex quinquefasciatus, Anopheles stephensi, Aedes aegypti, Prays oleae, Spodoptera littoralis, Ephestia kuehniella*	Assie et al. (2002), Das and Mukherjee (2006), Getha et al. (2010), Manonmani et al. (2011), Ghribi et al. (2011), Ghribi et al. (2012a; b)
Brevibacillus laterosporus	Gram-positive, aerobic, motile, spore-forming bacillus with production of a canoe-shaped lamellar body that attaches to one side of the spore after lysis of the sporangium providing a typical morphological feature, ubiquitous distribution	Binary toxin ISP1A and ISP2A	By ingestion causing histopathological changes in the intestine by rupture of epithelial cells	*Musca domestica,Aedes aegypti*	Riu et al. (2012, 2013)
Lysinibacillus sphaericus	Gram-positive, aerobic, spore-forming, spherical bacillus with ubiquitous distribution	Binary toxin (Bin A/Bin B), Mtx 1, Mtx2, Mtx 3, Spaericolysin, S-layer	Ingestion of spores or toxins affecting the microvilli of intestinal epithelial cells	*Anopheles sp, Mansonia* sp, *Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, Culex tritaeniorhynchis*	Ampofo (1995), El-Bendary (2006), Ruiu (2015), Mnif et al. (2015), Cooping and Menn (2000), Kumar et al. (2021)
Paenibacillus popillae	Gram-positive bacillus, aerobic or facultative anaerobic, ubiquitous distribution. Obligate pathogen	Cry homologous protein, chitinase	By ingestion of spores and by contact	*Popillia japonica, Blitopertha orientalis., Phyllophaga elenans, Phyllophaga menetriesi*	Heimpel and Angus (1960), Yokoyama et al. (2004), Ruiu (2015), Grady et al. (2016)
Paenibacillus lentimorbus	Gram-positive bacillus. Obligate pathogen	Cry protein homologue	By ingestion of spores	*Popillia japonica, Blitopertha orientalis*	Yokoyama et al. (2004), Rippere et al. (1998)
Paraclostridium bifermentans	Gram-positive, anaerobic, motile, spore-forming bacillus	Cbm71 Protein Cbm 17.1 Protein Cbm 17.2 Protein	Ingestion causing intestinal damage	*Aedes egypti, Anopheles gambiae*	Barloy et al. (1996), Qureshi et al. (2014)
Leuconostoc pseudomesenteroides	Coccus Gram-positive, microaerophilic	Not determined	Not determined	*Drosophila suzukii, D. melanogaster, Acyrthosiphon pisum*	Hiebert et al. (2020)

Table 1 (continued)

Bacteria	Characteristics	Insecticidal molecule	Mechanism of action	Biological target	References
Streptomyces rubrisoli	Gram-positive, filamentous bacteria abundant in soil	Macrocyclic lactones, avermectins (B1a and B1b) and emamectin Antimycin A, flavensomycin, macrotetralides, piericidins, prasinones and prasinones	By contact and ingestion, affecting the peripheral nervous system of the insect causing muscular paralysis	*Spodopetra littoralis, Aedes aegypti, Anopheles sp, Culex* sp.	Osman et al. (2007), Kekuda et al. (2010), Yap et al. (2022)
Saccharopolyspora spinosa	Actinobacteria, Gram-positive, aerobic, immobile, present in soil	Spinosina	By contact or ingestion causing paralysis and death of the insect	*Aedes aegypti; Spodoptera littoralis*	Basma et al. (2005), Thavara et al. (2009), Kirst 2010
Pseudomonas entomophila	Gram-negative bacillus, motile, ubiquitous distribution	Biosurfactants, lipopeptides, rhamnolipids. Insecticidal toxin	By ingestion causing intestinal damage	*Drosophila melanogaster*	Vodovar et al. (2005)
Pseudomonas chlororaphis	Gram-positive aerobic mesophilic bacillus	Fit D protein, chitinases, phospholipase C, biosurfactants, hidrogen cianide (HCN), cyclic lipopeptides (orphamide A and B)	By ingestion causing intestinal damage and haemocele	*Galleria mellonella*; *Drosophila melanogaster*	Flury et al. (2017), Anderson et al. (2018)
Pseudomonas aeruginosa	Gram-negative bacillus, motile, aerobic, ubiquitous distribution	Exotoxin, rhamnolipids, chitinases (Chi C)	By ingestion causing damage at intestinal level Contact at cuticle level	*Aedes egypti*	Lalithambika et al. (2014), Silva et al. (2015), Lalithambika et al. (2016), Bodykevich et al. (2022)
Burkholderia cepacia	Gram-negative bacillus, motile, aerobic, symbiotic	Unidentified bacterial metabolites	By ingestion. Decreases insect egg deposition	*Riptortus pedestris*	Kill et al. (2014), Cordova et al. (2013)
Chromobacterium subtsugae	Gram-negative, facultative aerobic, motile bacillus with polar flagellum	Unidentified thermostable proteins	Ingestion causing intestinal damage	*Diabrotica virgifera*, *Nezara viridula*	Martin et al. (2007a; b)
Yersinia entomophaga	Gram-negative bacillus motile, non-spore-forming	Toxic complex Yen-tc (A, B, C) and chitinases	By ingestion of metabolites involved in the degradation of the peritrophic membrane of the gut facilitating entry of toxins	*Costelytra zealandica; Acrossidius tasmaniae, Listronotus bonariensis, Plutella xylostella*	Hurst et al. (2011), Landsberg et al. (2011), Busby et al. (2012)
Serratia entomophila	Gram-negative bacillus, facultative anaerobic, non-spore forming	Plasmid pADAP, PEP proteins (Sep A, Sep B, Sep C), chitinases, lipases	By ingestion causing intestinal damage	*Costelytra zealandica, Heliothis ermigera, Spodoptera litura, Plutella xylostella*	Hurst et al. (2000), Chattopadhyay et al. (2012)
Xenorhabdus nematophila	Gram-negative, facultative aerobic, motile. nematode symbionts	Insecticidal toxin (A24tox) (Xin)	By contact and ingestión	*Phytoseiulus persimilis Neoseiulus californicus*	Cevizci et al. (2020)
Photorhabdus luminescens	Gram-negative, facultative anaerobic, bioluminescent, nematode symbionts	Insecticidal toxin (TC), toxin (Mcf1, Mcf 2). Pir proteins	By contact and ingestión	*Manduca sexta*	Blackburn et al. (1998)

new exoskeleton, which may result in deformities, inability to shed the old exoskeleton, or death during molting (Winssy et al. 2024). Bacteria that produce such enzymes include *Serratia* spp. and certain actinomycete species such as *Streptomyces* spp. (Kwak et al. 2015).

Production of gut-level damage

Many entomopathogenic bacteria produce lethal toxins, which can be proteins, peptides, or secondary metabolites that interfere with vital physiological processes in insects. Approximately 90% of bacterial insecticides are derived from endotoxin-producing bacteria that enter the insect when larvae consume plant leaves (Mishra et al. 2015; Kachhawa 2017). Once ingested, these bacteria are lysed by the alkaline environment of the gut, releasing toxins as inclusions or inactive protoxins. These protoxins are then solubilized and activated by proteases, such as trypsin (Drobniewski et al. 1989; Ruiu et al. 2018). During activation, peptides from the N-terminal and C-terminal regions are cleaved by proteases, generating the active toxin, which binds to receptors on the membranes of intestinal epithelial cells. This binding triggers a conformational change in the toxin, forming crystalline inclusions that create pores or ion channels within the receptors, leading to cell lysis and disruption of transport processes. Consequently, the insect becomes paralyzed, ceases feeding, and ultimately dies from starvation within 48 h (Jisha et al. 2013) (Fig. 4).

Some variants of insecticidal toxins exhibit species-specific activity (Nathan et al. 2014; Aneja et al. 2016; Kachhawa 2017; Jurat et al. 2012). Variations in the gene sequences of these toxins can lead to different affinities for gut receptors, resulting in distinct insecticidal activities among various bacteria (Pigott et al. 2007). The molecules studied from the aforementioned bacteria display significant chemical diversity, with some not yet fully characterized. This challenges the notion that only proteins homologous to the Cry proteins of *Bacillus thuringiensis* (Bt) possess insecticidal properties. While most mechanisms of action for these entomopathogenic bacteria involve ingestion or contact, the specifics remain unclear for other species, particularly regarding their biological targets, which include insects from the orders Lepidoptera and Coleoptera. Utilizing sporulated bacteria in product development enhances viability and stability over time (Villareal et al. 2017).

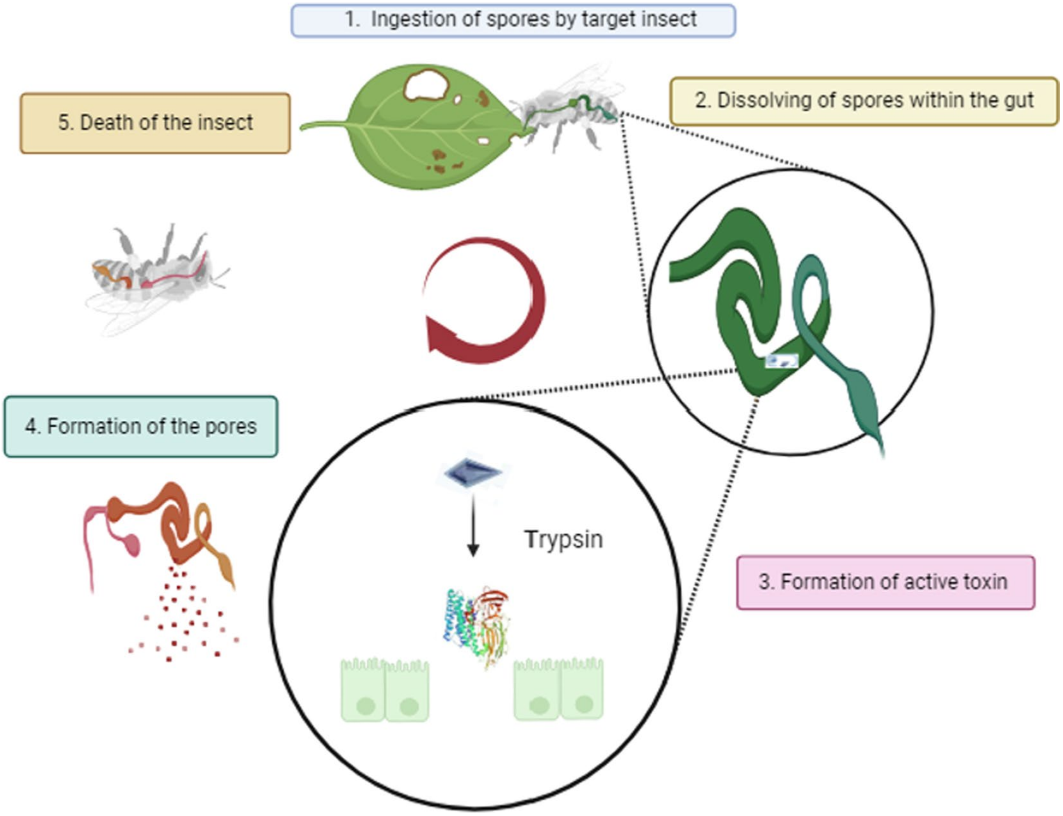


Fig. 4 Toxin production and damage to the insect gut

Examples of diseases caused by Cry-like proteins include Cbm 17.1 and Cbm 17.2, produced by *Paraclostridium biofermentans*, which are highly effective against flies and mosquitoes (Qureshi et al. 2014). Additionally, *Paenibacillus popilliae* is responsible for milky spore disease in beetles, characterized by an opaque white coloration due to the accumulation of sporulating bacteria in the hemolymph. This disease can be fatal if the infective dose of spores is sufficiently high during the early stages of larval development (Grady et al. 2016).

Another spore-producing bacterium, *Lysinibacillus sphaericus*, is a facultatively aerobic, terminally spore-forming, and motile bacterium found in soil and aquatic environments. It utilizes amino acids and organic acids as carbon sources, as it lacks genes for sugar-degrading enzymes. This bacterium can accumulate hydrocarbons and immobilize heavy metals due to its paracrystalline S-layer. Strains of *Lysinibacillus sphaericus* have shown high larvicidal efficacy, producing a toxin similar to that of Bt, which typically damages the epithelial microvilli in the insect midgut. This leads to the cessation of feeding and ultimately the insect's death (Kumar et al. 2021).

Involvement of the insect nervous system and behavior

Certain bacteria can impact the nervous systems of insects as direct pathogens, behavior-modifying symbionts, or as causative agents of specific neurological diseases (Table 1). For instance, *Streptomyces rubrisoli* and *Saccharopolyspora spinosa* produce macrocyclic lactones that affect insect nervous systems, leading to paralysis and eventual death. Examples of these molecules include the avermectins—such as emamectin, avermectin, ivermectin, and abamectin and milbemycins, including milbemectin. Both classes of lactones have been utilized as antiparasitics in animals and for insect control in various crops (Pérez-Cogollo et al. 2018).

These compounds reach nerve endings and trigger the release of gamma-aminobutyric acid (GABA), causing GABA-activated Cl^- ion channels to open. This hyperpolarizes the membrane potential of nerve cells, blocking signal transmission to the insect's peripheral nervous system and inhibiting neurotransmitter release, ultimately resulting in paralysis (Martin et al. 2002; Khan and Khan 2023). Unlike other avermectins, spinosins activate nicotinic receptors on the postsynaptic membrane through an unknown mechanism, leading to nervous system excitation, prostration, and paralysis. Some avermectins may also affect other physiological systems in target organisms. For example, ivermectin can impair muscle function and excretion in parasites, facilitating the clearance of infestations (Gonzalez et al. 2009; Lumaret et al. 2012). Marques et al. (2020) evaluated a spinosad-based formulation called Tracer® on the stingless bee foragers, *Plebeia lucii* Moure (Apidae: Meliponini), and found that bee locomotion, both walking and flying, was reduced with increasing spinosad concentrations.

Interference with insect reproduction and development

Wolbachia pipientis is a Gram-negative bacterium belonging to the order Rickettsiales that affects the reproductive systems of its hosts. This endosymbiotic bacterium predominantly resides in the gonads of various insects, with some estimates suggesting that approximately 16% of arthropods harbor *Wolbachia* spp. It can also infect spiders and mites (Kaur et al. 2021). *Wolbachia* is known to induce several reproductive processes, including feminization, parthenogenesis, male killing, and cytoplasmic incompatibility (Shropshire et al. 2020).

Feminization leads to genetic males developing phenotypically as females, though the molecular mechanisms underlying this process remain unknown. In cases of parthenogenesis, uninfected species typically exhibit arrhenotokous parthenogenesis due to their haplodiploid sex determination system. However, virgin mothers infected with *Wolbachia* produce all-female offspring from their unfertilized eggs, switching from arrhenotokous to telitokous parthenogenesis. Male killing results in a female-biased sex ratio in several arthropods by selectively eliminating males (Fukui et al. 2015). Additionally, this bacterium has potential applications in controlling nematodes and parasites, such as *Plasmodium* spp., the causative agents of malaria (Bourtzis et al. 2014).

Burkholderia cepacia is an aerobic, motile, non-fermenting, Gram-negative bacterium known for its significant metabolic versatility. It is important for colonizing soil, water, and plants and serves as a symbiont in legume root nodules (Rojas-Rojas et al. 2019). This bacterium has successfully colonized the guts of insects, leading to reduced egg deposition. In contrast, *Spiroplasma* spp., belonging to the group Mollicutes, are helicoid-shaped bacteria that lack a cell wall and are obligate symbionts in certain insects. They feed on trehalose, the predominant sugar in insect hemolymph, causing symptoms such as tremors, paralysis, and behavioral changes. In *Drosophila melanogaster*, infection with *Spiroplasma melliferum* has been associated with increased mortality of male progeny during embryonic or larval stages and a female-biased sex ratio in subsequent generations, although the underlying mechanism remains unclear (Anbutsu and Fukatsu 2011).

Genetic engineering strategies for the improvement of genes encoding insecticidal molecules

Advancements in molecular biology, genetic engineering, and protein engineering have facilitated the development of bacterial insecticides (Azizoglu et al. 2020). Initial genetic modifications of biopesticides employed recombinant DNA technology and protein engineering in *Bacillus thuringiensis*, particularly in the subspecies *Kurstaki*, to create strains with novel insecticidal proteins effective against codling moths in maize (All et al. 1994; Federici et al. 2005). As of 2012, there were no commercially available biopesticides based on genetically modified microorganisms due to regulatory constraints (Glare 2012). However, genes encoding insecticidal toxins have now been isolated, characterized, manipulated, and expressed in various organisms to create new combinations of toxins with broader activity (Azizoglu et al. 2020). Recombinant DNA technologies have been employed to enhance insecticidal efficacy by increasing protein synthesis and facilitating the creation of new endotoxin combinations (Karabörklü et al. 2018). Furthermore, genetic engineering can enhance the resilience of entomopathogens to adverse environmental conditions, potentially increasing their effectiveness in the field (Azizoglu et al. 2020).

These approaches have primarily targeted entomopathogenic bacteria from the genera *Bacillus*, *Lysinibacillus*, and *Pseudomonas* (Azizoglu et al. 2020). A notable study reported the transformation of the *cry1Ab* gene from *Bacillus thuringiensis* strain LM-466 into *B. subtilis* and *B. licheniformis* strains, evaluating their insecticidal activity against *Tuta absoluta* larvae, with an LC50 comparable to that of the original *B. thuringiensis* strain (Theoduloz et al. 2003). Other *cry* and *cyt* genes from *B. thuringiensis* have been transferred to *Lysinibacillus sphaericus* strains, resulting in tenfold increased toxicity against *Culex* spp. (Federici et al. 2003).

Research has also focused on *Serratia entomophila*, where a plasmid carrying the *sepABC* genes, responsible for inducing feeding cessation and mortality in *Costelytra zealandica* larvae, was inserted into *Escherichia coli*. The recombinant strains displayed the desired insecticidal traits (Hurst et al. 2004). An alternative approach involves the transconjugation of endotoxin-encoding genes in plasmids or cloning *Bacillus thuringiensis* genes for expression in alternative hosts such as *Pseudomonas fluorescens*, a common plant-associated bacterium, or other endophytic microorganisms (Federici et al. 2005).

Liu et al. (2010) identified the toxin production gene (*tccC*) in *Pseudomonas taiwanensis*, which was overexpressed in *E. coli*, resulting in increased mortality in *Drosophila melanogaster*. In another study, the *chiABC* gene from *Serratia marcescens* was inserted and overexpressed in *E. coli*, yielding recombinant chitinases that exhibited up to 80% insecticidal activity against *Malacosoma neustria* and *Helicoverpa armigera* (Danışmazoğlu et al. 2015). Finally, Zhang et al. (2020) conducted genome reduction of *Bacillus amyloliquefaciens* LL3 through metabolic engineering to enhance surfactin production, a lipopeptide biosurfactant with potential insecticidal properties. The original strain's *sfrA* operon, crucial for surfactin production, was optimized by removing unnecessary genomic regions, promoting rapid growth and high operon expression (Zhang et al. 2020).

Another strategy for plant protection against insects involves transferring genes encoding insecticidal proteins to crops. This approach minimizes the need for continuous insecticide application, as it facilitates the constant production of Bt proteins (Federici et al. 2005). However, studies indicate that only genes derived from *Bacillus thuringiensis* have been effectively transferred to various plant species such as cotton, tomato, and eggplant, yielding economic benefits for growers (Leng et al. 2011; Nathan et al. 2014; Siever et al. 2014; Mishra et al. 2015). In contrast, research involving other entomopathogenic bacterial species remains in its early stages.

While recombinant DNA technology offers the potential to modify insecticide modes of action by altering host specificity or enhancing efficacy, regulatory restrictions on the use of genetically modified organisms (GMOs) have made research in this area less appealing for commercial product development. Concerns about the potential for gene flow to wild species, development of insect resistance, and negative impacts on beneficial organisms further complicate the landscape (Azizoglu et al. 2020).

Bacterial insecticides as commercial products

The European Union currently leads the global market for commercial biological control products, offering incentives for the registration of low-risk biological control agents (Glare 2012). In contrast, bacterial insecticides in the United States are regulated by the Division of Biopesticides and Pollution Prevention within the Environmental Protection Agency (EPA) (Leahy et al. 2014). Despite existing regulations, there is a pressing need for enhancements to current legislation (Kumar et al. 2021) to streamline the registration process for new products, especially in developing countries. The lengthy and costly nature of current registration procedures poses significant challenges (Kumar et al. 2016a, b).

While biopesticides represent only 5% of plant protection products worldwide, their growth has accelerated in recent decades. Hakim et al. (2020) report that there are currently 175 biopesticides registered globally, encompassing 700 active substances. The biopesticide market was valued at approximately \$3 billion in 2013 and is projected to reach \$4.5 billion by 2023 (Lahlali et al. 2022; Hernández et al. 2022). Notably, products derived from *Bacillus*, *Burkholderia*, *Pseudomonas chlororaphis*,

Table 2 Commercial products made from different entomopathogenic bacteria

Trade name	Entomopathogenic bacterium	Target insect	References
Nortica 10 WP, VOTiVO FS seed treatment Bionemagon	*Bacillus firmus*	Nematodes	Arthurs and Dara (2019), Ruiu (2018)
Majestene, Venerate	*Burkholderia* spp	Chewing insects and mites	Ruiu (2018)
Tracer ™ 120, Conserve	*Saccharopolyspora spinosa*	Unspecified insects	Ruiu (2018)
Grandevo	*Chromobacterium subtsugae*	Chewing insects and mites	Ruiu (2018)
Vectolex® GC	*Lysinibacillus sphaericus*	Unspecified insects	Cooping and Menn (2000)
Cedomon®, Cerall®, Cedress®	*Pseudomonas chlororaphis*	Unspecified insects	Anderson et al. (2018)
Bioshield™	*Serratia entomophila*	Unspecified insects	Cooping and Menn (2000)

and *Serratia entomophila* are among the most effective against various orders of insects (Table 2).

The development of a commercial product is a complex, multi-step process that begins with the selection of promising microorganisms. These are initially evaluated through in vitro tests against various pathogens using techniques such as disc diffusion, agar well diffusion, and the poisoned food technique before undergoing larger-scale assessments (Jahangiriana et al. 2013). It has become evident that many microorganisms deemed promising at the in vitro level often fail to perform effectively in greenhouse formulations, rendering them unfeasible as commercial products (Hynes and Boyetchko 2006).

The active component may consist of metabolites produced by a microorganism. In such cases, various analytical techniques, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography coupled with mass spectrometry (GC–MS), are employed for characterization (Lengai et al. 2018). Following this, the fermentation stage is critical, requiring careful consideration of the type of fermentation (solid or submerged), culture medium composition, oxygen transfer, incubation temperature, collection timing, and additional treatments (Montazeri and Greaves 2002).

Next, the formulation of the active compound begins. At this stage, it is essential to optimize the combination of active ingredients, carrier materials, emulsifiers, surfactants, and other components to enhance stability and efficacy while minimizing degradation from environmental factors during field application (Lengai et al. 2018; Hynes and Boyetchko 2006). The initial step involves selecting an appropriate carrier that facilitates the controlled release of the active ingredient. Inert materials, such as petroleum distillates, starch, talc, clay, and water, serve as stabilizers to prolong the shelf life of the product. Additionally, emulsifying agents or surfactants, such as soap, may be incorporated to enhance effectiveness (Lengai et al. 2018). Adjuvants that conserve moisture and protect the active ingredient from UV radiation or desiccation must also be selected based on microbial physiology studies (Burges 1998; Boyetchko et al. 2002; Hynes and Boyetchko 2006).

Another important consideration during formulation is enhancing the controlled release of the active compound by testing various product presentations. For example, slow-release granules or spray formulations for foliar applications could improve coverage with the active agent (Glare 2012). Despite progress in these areas, unexpected challenges in the fermentation and formulation processes, as well as inadequate market management, have led to unfinished products or the withdrawal of products from the market (Hynes and Boyetchko 2006).

While advancements have been made in developing commercial products based on various entomopathogenic bacteria, *Bacillus thuringiensis* (Bt)-based products maintain a competitive edge. This advantage stems from years of research on Bt, which has elucidated its biology, mechanisms of action, and spectrum of efficacy. Such foundational knowledge has informed improvements in product manufacturing, including the selection of suitable carriers for controlled release, effective application methods, strategies for extending product shelf life, and safety assurances. These factors contribute to lower production costs and increased profitability for manufacturers. However, despite these advances, limitations remain, including rapid deactivation upon light exposure, short duration of activity, low lethality, and high sensitivity to environmental conditions (Ayilara et al. 2023). Continued research is essential to overcome these challenges and establish competitive alternatives for managing insect pests.

Patents related to bacterial insecticides

To date, patents related to the production of bacterial insecticides based on entomopathogenic bacteria other than *Bacillus thuringiensis* (Bt) are limited. This scarcity may be attributed to the insufficient large-scale production studies of these alternative bacterial inputs and the challenges associated with optimizing their formulations. The existing patents primarily focus on the production of insecticidal metabolites derived from certain species of phytopathogenic bacteria or improvements in the synthesis of these compounds through genetic engineering processes. The countries most active in this field include the United States and the European Union (Table 3).

Entomopathogenic bacteria that can induce systemic resistance (ISR) in plants

Induction of systemic resistance (ISR) is a physiological state that enhances a plant's defense capacity, elicited by plant growth-promoting rhizobacteria (PGPR). These bacteria can induce local resistance and subsequently transfer it to other parts of the plant, achieving global induction (Ilham et al. 2019). In response to pathogen or insect attacks, as well as abiotic stresses, plants can rapidly activate various cellular defense mechanisms, including increased activity of chitinases, β-1,3-glucanases, and peroxidases, along with the accumulation of phytoalexins and the formation of protective layers composed of lignin and hydroxyproline-rich glycoproteins (Archana et al. 2011; Jatoi et al. 2019). Among the genera recognized as inducers of systemic resistance are some insecticidal bacteria previously discussed in this review, underscoring their potential relevance as bioproducts.

Wei et al. (1996) conducted greenhouse studies that demonstrated the ability of the PGPR *Bacillus pumilus* INR7 to promote plant growth and induce systemic resistance against cucumber diseases. When applied as a seed treatment, this strain significantly increased protection against anthracnose caused by *Colletotrichum orbiculare*. Furthermore, a combined inoculation of *Bacillus pumilus* INR7, *Curtobacterium flaccumfaciens* MEl, and *Bacillus subtilis* GB0 effectively controlled cucumber angular leaf spot caused by *Pseudomonas syringae* pv. *lachrymans* (Raupach et al. 2000).

Li et al. (2020) evaluated rhizospheric isolates from tobacco and found that *B. pumilus* strain S2-3–3 significantly reduced disease severity in tobacco by inducing systemic resistance while also promoting growth through the production of indoleacetic acid (IAA). In bell pepper plants, disease severity decreased when roots were drenched with strain S2-3–2, leading to enhanced plant weight and chlorophyll content compared to untreated controls. Ilham et al. (2019) highlighted selected strains of *Bacillus amyloliquefaciens* (I3) and *Trichoderma harzianum* in inducing systemic resistance in *Arabidopsis thaliana*, revealing that these microorganisms can trigger defense pathways associated with salicylic acid and jasmonic acid, unlike plants treated with chemical elicitors.

Abdelkhalek et al. (2020) reported the antiviral activity of *Bacillus licheniformis* strain POT1 against alfalfa mosaic virus (AMV) in potato plants. Dual foliar applications of crop filtrate 24 h before and after inoculation with AMV proved most effective, resulting in an 86.79% reduction in viral accumulation and improvements in various growth parameters. Transcriptional analysis indicated that thirteen genes related to phenylpropanoid, chlorogenic acid, and flavonoid biosynthetic pathways were induced after treatments, with anthocyanin, a type of flavonoid, playing a crucial role in plant defense against viral infection.

Bharathi et al. (2004) found that *Bacillus subtilis* provided protection against rot and progressive death of chili bell pepper (*Capsicum annuum*) fruits caused by *Colletotrichum capsici*, and PGPR significantly enhanced seed germination and seedling vigor. Saravanakumar et al. (2007) evaluated bioformulations of PGPR (*Pseudomonas* sp. and *Bacillus subtilis*) for their efficacy against vesicular blight disease (*Exobasidium vexans*) in tea (*Camellia sinensis*) under field conditions, finding that foliar applications consistently reduced the incidence of blight disease.

Wang et al. (2015) identified and characterized a protein elicitor secreted by *Brevibacillus laterosporus* strain A60, named PeBL1. When expressed in *Escherichia coli*, this recombinant protein induced a hypersensitive response (HR) and systemic resistance in *Nicotiana benthamiana*, triggering a cascade of plant defense responses, including reactive oxygen species (ROS) production and phenolic compound deposition. In a related study, Jatoi et al. (2019) reported that the protein inducer PeBL2 (17.2 kDa) encoded by the PeBL2 gene could similarly induce HR in tobacco, generating ROS and systemic resistance against *Botrytis cinerea*.

Shabanamol et al. (2017) explored the biocontrol mechanisms of *Lysinibacillus sphaericus*, a diazotrophic endophyte of rice, against the rice sheath blight pathogen *Rhizoctonia solani*. This endophyte induced systemic resistance, achieving 100% growth inhibition of the fungal pathogen through the production of volatile organic compounds and siderophores, as well as hydrogen cyanide (HCN) and ammonia. Under greenhouse conditions, foliar and soil applications of *L. sphaericus* significantly reduced disease incidence by accumulating defense enzymes, including peroxidases and polyphenol oxidases, and enhancing phenolic compound levels. Kumar et al. (2016a, b) found that inoculation of tobacco

Table 3 Patents related to bacterial insecticides based on other entomopathogenic bacteria

Name authors	Patent code	Country	Title	Description of the invention
Heins S, Manker D, Jimenez D, Marrone P. (1999)	US6001637	United States	*Bacillus pumilus* strain for controlling corn rootworm, nematode and armyworm infestations	Patent for the use of a strain of *B. pumilus* active against insects such as *Diabrotica undecimpunctata* and *Spodoptera exigua*
Hellwege E, Andersch W, Gladbach B, Stenzel K, Springer B. (2020)	US10542757 B2	United States	Compositions comprising a biological control agent and an insecticide	Development of biological control agents based on several species of the genus *Bacillus* sp. (*B. chitinosporus*, *B. mycoides*, *B. pumilus*, *B. subtilis*), *Muscodor albus*, *M. roseu*, *Rhodococcus globerulus*, and strains of *Streptomyces galbus* and *Streptomyces* sp.
Beattie J, Crawford M, Eads J, Donovan B. (2017)	AU2017212500 B2	Australia	Compositions and methods for controlling insect pests	The invention indicates the composition and methods for obtaining insecticidal polynucleotides that are used for the control and prevention of various insect species
Kurt S, Eda R, Thorsten J. (2013)	WO2014147534	European Union	Synergistic compositions comprising a *Bacillus subtilis* strain and a pesticide	The invention indicates the formulation of a pesticide based on *Bacillus subtilis* strain FB17 or a cell-free cell extract or a metabolite obtained from the bacterium
Brahm L, Liebmann D, Wilhelm B, Gewehr M (2014)	CA2890635	Canada	Synergistic pesticidal mixtures comprising *Bacillus subtilis* MBI-600	The invention indicates pesticide production based on *B. subtilis* MBI-600 and its potential uses; the invention includes the method of application to generate plant protection
Asolkar D, Koivunen D, Marrone D, Huang H, Cordova K. (2011)	EP2539432B1	European Union	Isolated bacterial strain of the genus *Burkholderia* and pesticidal metabolites therefrom	The invention indicates the cultivation and production of insecticidal metabolites from the bacterium *Burkholderia* sp, and shows the method for isolating such metabolites from the culture medium supernatant which can be used for the control of various plant pathogens including insects
Hahne C, Jarrod L, Russell C, (2018)	AU2018237187	Australia	Combinations of *Yersinia entomophaga* and pesticides or other substances	The invention indicates the combination of the bacteria *Yersinia entomophaga* and *Yersinia nurmii*, their toxins and other metabolites of agricultural interest as insecticides, repellents, antimicrobials, biostimulants, among others
Tiangang K, Ran L, Zixin D. (2022)	WO2022262384	European Union	Method for increasing yield of spinosyns of *Saccharopolyspora spinosa*	The invention relates the use of genetic engineering to increase the production of spinosins by the bacterium *Saccharopolyspora spinosa* by overexpressing the *spnF/spn P* genes

plants with *Paenibacillus lentimorbus* B-30488 increased the accumulation of defense-related enzymes in response to cucumber mosaic virus infection.

Kim et al. (2008) reported that root colonization by *Pseudomonas chlororaphis* O6 in cucumber induced ISR against *Corynespora cassiicola*. Molecular studies identified the cucumber galactinol synthetase gene (*CsGolS1*), with transcription levels increasing upon treatment with strain O6, correlating with heightened resistance to *Botrytis cinerea* and *Erwinia carotovora*. Khalimi et al. (2011) evaluated formulations of *P. aeruginosa* to enhance growth and induce ISR in soybean plants against soybean stunting virus under greenhouse conditions. These formulations significantly increased plant growth, reducing disease incidence from 15 to 80%, while untreated plants exhibited up to 90% infection rates.

Finally, Ezziyyani et al. (2017) demonstrated that inoculating bell pepper stems with *Phytophthora capsici* and treating the roots with antagonists *Burkholderia cepacia* and *Trichoderma harzianum* together induced a defensive response. Stem infection resulted in a hypersensitive reaction, but necrosis was slowed in plants treated with both antagonists, evidenced by increased production of proteins with β-1,3-glucanase activity, a component of the hypersensitive defense system.

Conclusion

The production of commercial bacterial bioinsecticides is a complex process that requires extensive research and multiple stages of development. While a microorganism may demonstrate effectiveness in laboratory settings (in vitro), it may not perform as expected in field conditions. Challenges in scaling up production and formulating these microorganisms need to be addressed to ensure viable market management. Despite these hurdles, the production of bacterial bioinsecticides has significantly increased worldwide, particularly in developed countries. Current market offerings predominantly include mixtures of bacteria, such as various species within the genus *Bacillus* (e.g., *B. chitinosporus*, *B. mycoides*, *B. pumilus*, and *B. subtilis*). These bacteria produce toxins analogous to Cry proteins, which can cause intestinal damage and starvation in target insects. Additionally, certain actinomycetes, like *Streptomyces* species, cause contact damage, while bacteria such as *Streptomyces rubrisoli* and *Saccharopolyspora spinosa* produce macrocyclic lactones that affect the insect nervous system. Other notable microorganisms, including *Wolbachia pipientis*, *Burkholderia cepacia*, and *Spiroplasma melliferum*, can disrupt the reproductive systems of their hosts. With the advancement of genetic engineering, there has been manipulation of genes encoding insecticidal toxins across various organisms to create novel combinations that enhance efficacy, broaden the spectrum of action against additional insect species, and increase resistance to environmental factors. Moreover, efforts are underway to transfer genes encoding insecticidal proteins into plants, which could reduce the need for repeated insecticide applications in crops. In addition to their insecticidal properties, some of these bacteria can also enhance systemic resistance (ISR) in plants, thereby increasing interest in developing commercial bioproducts. However, legislative changes are necessary to streamline the product registration process, which is currently lengthy and costly in many countries. Such reforms are essential for bacterial bioinsecticides to compete with traditional chemical insecticides on price, achieve mass production, and contribute positively to soil health and environmental sustainability. Furthermore, additional research is required to provide scientific evidence that addresses regulatory concerns about genetically modified organisms, particularly regarding potential gene flow to wild species, insect resistance development, and ecological impacts. This approach will help mitigate resistance and apprehension among regulatory bodies, facilitating the responsible release of beneficial microbial products.

Abbreviations

AMV	Alfalfa mosaic virus
Bt	*Bacillus thuringiensis*
EPA	Environmental Protection Agency
GABA	Gamma-aminobutyric acid
GC–MS	Gas chromatography coupled with mass spectrometry
GMOs	Genetically modified organisms
HCN	Hidrogen cianide
HPLC	High-performance liquid chromatography
IAA	Indoleacetic acid
ISR	Inducer systemic resistance
PGPR	Plant growth-promoting rhizobacteria
PIPs	Plant-incorporated protective agents
ROS	Reactive oxygen species
TLC	Thin-layer chromatography

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Author contributions

MB contributed to the study's conception and design. MB and JC drew the diagrams and tables, performed the data collection, analyzed the data, and wrote the manuscript. All authors read and approved the final manuscript.

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References

Abdelkhalek A, Al-Askar A, Behiry S. *Bacillus licheniformis* strain POT1 mediated polyphenol biosynthetic pathways genes activation and systemic resistance in potato plants against Alfalfa mosaic virus. Sci Rep. 2020;10:16120. https://doi.org/10.1038/s41598-020-72676-2.

Abinaya M, Vaseeharan B, Divya M, Vijayakumar S, Govindarajan M, Alharbi N, et al. Structural characterization of *Bacillus licheniformis* Dahb1 exopolysaccharide—antimicrobial potential and larvicidal activity on malaria and Zika virus mosquito vectors. Environ Sci Pollut Res. 2018;25:18604–19. https://doi.org/10.1007/s11356-018-2002-6.

All JN, Stancil JD, Johnson TB, Gouger R. A Genetically-Modified *Bacillus thuringiensis* product effective for control of the fall armyworm (Lepidoptera: Noctuidae) on Corn. Fla Entomol. 1994;77(4):437–40. https://doi.org/10.2307/3495697.

Ampofo J. Use of local raw materials for the production of *Bacillus sphaericus* insecticide in Ghana. Biocontrol Sci Technol. 1995;4:417–24. https://doi.org/10.1002/jobm.200510585.

Anbutsu H, Fukatsu T. Spiroplasma as a model insect endosymbiont. Environ Microbiol Rep. 2011;3:144–53. https://doi.org/10.1111/j.1758-2229.2010.00240.x.

Anderson A, Kim Y. Biopesticides produced by plant-probiotic *Pseudomonas chlororaphis* isolates. Crop Prot. 2018;105:62–9. https://doi.org/10.1016/j.cropro.2017.11.009.

Aneja K, Khan S, Aneja A. Biopesticides an eco-friendly pest management approach in agriculture: status and prospects. KAVAKA. 2016;47:145–54.

Annapurna K, Amod Kumar L, Vithal Kumar V, Govindasamy PB, Ramadoss D. PGPR-induced systemic resistance (ISR) in plant disease management. In: Maheshwari DK, editor. Bacteria in agrobiology: disease management. Berlin: Springer; 2013. p. 405–25. https://doi.org/10.1007/978-3-642-33639-3_15.

Arthurs S, Dara S. Microbial biopesticides for invertebrate pests and their markets in the United States. J Invertebr Pathol. 2019;165:13–21. https://doi.org/10.1016/j.jip.2018.01.008.

Asolkar D, Koivunen D, Marrone D, Huang H, Cordova K. Patent EP2539432B1. European Union. 2011.

Assie L, Deleu M, Arnaud L, Paquot M, Thonart P, Gaspar Ch, et al. Insecticide activity of surfactins and iturins from a biopesticide *Bacillus subtilis* Cohn (S499 strain). Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet. 2002;67:647–55.

Ayilara M, Adeleke B, Akinola S, Fayose C, Adeyemi U, Gbadegesin L, et al. Biopesticides as a promising alternative to synthetic pesticides: a case for microbial pesticides, phytopesticides, and nanobiopesticides. Front Microbiol. 2023;14:1040901. https://doi.org/10.3389/fmicb.2023.104090.

Azizoglu U, Jouzani G, Yilmaz N, Baz E, Ozkok D. Genetically modified entomopathogenic bacteria, recent developments, benefits and impacts: a review. Sci Total Environ. 2020. https://doi.org/10.1016/j.scitotenv.2020.139169.

Barloy F, Delècluse A, Nicolas L, Lecadet M. Cloning and expression of the first anaerobic toxin gene from Clostridium bifermentans subsp malaysia, encoding a new mosquitocidal protein with homologies to *Bacillus thuringiensis* delta-endotoxins. J Bacteriol. 1996;178:3099–105. https://doi.org/10.1128/jb.178.11.3099-3105.1996.

Basma M, Metwally M, Atalla F. Metabolites derived from fermentation of *Saccharopolyspora spinosa* as insecticide for the cotton leafworm *Spodoptera littoralis* (BOISD) (Noctuidae – lepidoptera). J Agric Sci Mansoura Univ. 2005;30:1685–92. https://doi.org/10.21608/jppp.2005.238790.

Baumberger N, Baulcombe DE. Arabidopsis ARGONAUTE1 is an RNA slicer that selectively recruits rnicroRNAs and short interfering RNAs. Proc Natl Acad Sci. 2005;102:11928–33. https://doi.org/10.1073/pnas.0505461102.

Beattie J, Crawford M, Eads J, Donovan B. Patent AU 2017212500 B2. Australia. 2017.

Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R. Rhizobacteria based bioformulations for the management of fruit rot infection in chillies. Crop Prot. 2004;23:835–43. https://doi.org/10.1016/j.cropro.2004.01.007.

Bhattacharjee R, Dey U. An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. Afr J Microbiol Res. 2014;8(17):1749–62.

Blackburn M, Golubeva E, Bowen D, French R. A novel insecticidal toxin from *Photorhabdus luminescens*, toxin complex a (*Tca*), and its histopathological effects on the midgut of *Manduca sexta*. Appl Environ Microbiol. 1998;64(8):3036–41. https://doi.org/10.1128/aem.64.8.3036-3041.1998.

Bodykevich G, de Leon M, Li S, Tom K. Cloning *chiC* from insecticidal *Pseudomonas aeruginosa* PAO1. UJEMI. 2022;27:1–11.

Bourtzis K, Dobson S, Xi Z, Rasgon J, Calvitti M, Moreira L, et al. Harnessing mosquito–*Wolbachia* symbiosis for vector and disease control. Acta Trop. 2014;132(1):S150–63. https://doi.org/10.1016/J.ACTATROPICA.2013.11.004.

Boyetchko S, Rosskopf E, Caesar A, Charudattan, R. Biological weed control with pathogens: search for candidates to applications. Appl Mycol Biotechnol. 2002;2:239–74. https://doi.org/10.1016/S1874-5334(02)80013-2.

Brahm L, Liebmann D, Wilhelm B, Gewehr M. Patent CA2890635. Canada. 2014.

Burges H, editor. Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. 1998. ISBN 978-94-011-4926-6. https://doi.org/10.1007/978-94-011-4926-6.

Busby J, Landsberg M, Simpson J, Jones M, Hankamer A, Hurst M, et al. Structural analysis of *Chi1* chitinase from *Yen-Tc*: The multisubunit insecticidal ABC toxin complex of *Yersinia entomophaga*. J Mol Biol. 2012;415:359–71. https://doi.org/10.1016/j.jmb.2011.11.018.

Cevizci D, Ulug D, Cimen H, Touray M, Hazir S, Cakmak I. Mode of entry of secondary metabolites of the bacteria Xenorhabdus szentirmaii and *X. nematophila* into *Tetranychus urticae*, and their toxicity to the predatory mites *Phytoseiulus persimilis* and *Neoseiulus californicus*. J Invertebr Pathol. 2020;174:107418. https://doi.org/10.1016/j.jip.2020.107418.

Chambers J, Jelen A, Gilbert M, Jany C, Johnson T, Gawron-Burke C. Isolation and characterization of a novel insecticidal crystal protein gene from *Bacillus thuringiensis* subsp. aizawai. J Bacteriol. 1991;173(13):3966–76. https://doi.org/10.1128/jb.173.13.3966-3976.1991.

Chandler D, Bailey A, Tatchell G, Davidson G, Greaves J, Grant W. The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc Lond B Biol Sci. 2011;366:1987–98. https://doi.org/10.1098/rstb.2010.0390.

Chapman M, Hoy M. Relative toxicity of *Bacillus thuringiensis* var. tenebrionis to the two-spotted spider mite (Tetranychus urticae Koch) and its predator Metaseiulus occidentalis (Nesbitt) (Acari, Tetranychidae and Phytoseiidae). J Appl Entomol. 1991;111(1–5):147–54. https://doi.org/10.1111/j.1439-0418.1991.tb00305.x.

Chattopadhyay A, Bhatnagar N, Bhatnagar R. Bacterial insecticidal toxins. Crit Rev Microbiol. 2004;30(1):33–54. https://doi.org/10.1080/10408410490270712.

Chattopadhyay P, Chatterjee S, Gorthi S, Sen S. Exploring agricultural potentiality of *Serratia entomophila* AB2: dual property of biopesticide and biofertilizer. Br Biotechnol J. 2012;2(1):1.

Cooping L, Menn J. Biopesticides: a review of their action, applications and efficacy. Pest Manag Sci. 2000;56:651–76. https://doi.org/10.1002/1526-4998(200008)56:8<651::AID-PS201>3.0.CO;2-U.

Cordova A, Fernandez L, Koivunen M, Yang A, Flor-Weiler L, Marrone P. Isolation and characterization of Burkholderia rinojensis sp nov, a non-Burkholderia cepacia complex soil bacterium with insecticidal and miticidal activities. Appl Environ Microbiol. 2013;79:7669–78. https://doi.org/10.1128/AEM.02365-13.

Dalmay T, Hamilton A, Rudd S, Angell S, Baulcombe D. An RNA-Dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell. 2000;101:543–53. https://doi.org/10.1016/s0092-8674(00)80864-8.

Danışmazoğlu M, Demir I, Sezen K, Muratoğlu H, Nalçacioğlu R. Cloning and expression of chitinase A, B, and C (*chiA*, *chiB*, *chiC*) genes from *Serratia marcescens* originating from *Helicoverpa armigera* and determining their activities. Turk J Biol. 2015;39:78–87. https://doi.org/10.3906/biy-1404-31.

Das K, Mukherjee A. Assessment of mosquito larvicidal potency of cyclic lipopeptides produced by *Bacillus subtilis* strains. Acta Trop. 2006;97:168–73. https://doi.org/10.1080/09583159550039611.

de Maagd R. *Bacillus thuringiensis*-based products for insect pest control. In: Lugtenberg B, editor. Principles of plant-microbe interactions: microbes for sustainable agriculture. Cham: Springer; 2015. p. 185–92. https://doi.org/10.1007/978-3-319-08575-3_20.

Drobniewski F, Ellar D. Purification and properties of a 28-Kilodalton hemolytic and mosquitocidal protein toxin of Bacillus thuringiensis subsp darmstadiensis. J Bacteriol. 1989;171(6):3060–7. https://doi.org/10.1128/jb.171.6.3060-3067.1989.

El-Bendary M. *Bacillus thuringiensis* and *Bacillus sphaericus* biopesticides production. J Basic Microbiol. 2006;46(2):158–70. https://doi.org/10.1002/jobm.200510585.

Ezziyyani M, Hamdache A, Egea C, Requena M, Candela M. Production of pathogenesis-related proteins during the induction of resistance to *Phytophthora capsici* in pepper plants treated with *Burkholderia cepacia* and *Trichoderma harzianum* in combination compatible. J Mater Environ Sci. 2017;8(S):4785–95.

Federici B. Insecticidal bacteria: an overwhelming success for invertebrate pathology. J Invertebr Pathol. 2005;89(1):30–8. https://doi.org/10.1016/j.jip.2005.06.007.

Federici B, Park H, Bideshi D, Wirth M, Johnson J. Recombinant bacteria for mosquito control. J Exp Biol. 2003;206:3877–85. https://doi.org/10.1242/jeb.00643.

Flury P, Vesga P, Péchy-Tarr M, Aellen N, Dennert F, Hofer N, et al. Antimicrobial and insecticidal: cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial *Pseudomonas* strains CHA0, CMR12a, and PCL1391 contribute to insect killing. Front Microbiol. 2017;8:100. https://doi.org/10.3389/fmicb.2017.00100.

Fukui T, Kawamoto M, Shoji K, Kiuchi T, Sugano S, Shimada T, Suzuki Y, Katzuma S. The endosymbiotic bacterium wolbachia selectively kills male hosts by targeting the masculinizing gene. PLoS Pathog. 2015;11(7):e1005048. https://doi.org/10.1371/journal.ppat.1005048.

Gasic S, Tanovic B. Role of biopesticides in crop protection: present status and future prospect. Pesticidi i Fitomedicina. 2013;28:97–102. https://doi.org/10.2298/PIF1302097G.

Geetha I, Manonmani A, Paily K. Identification and characterization of a mosquito pupicidal metabolite of a Bacillus subtilis subsp. subtilis strain. Appl Microbiol Biotechnol. 2010;86:1737–44. https://doi.org/10.1007/s00253-010-2449-y.

Geetha I, Manonmani A, Prabakaran G. *Bacillus amyloliquefaciens*: a mosquitocidal bacterium from mangrove forests of Andaman & Nicobar islands, India. Acta Trop. 2011;120:155–9. https://doi.org/10.1016/j.actatropica.2011.07.006.

Ghribi D, Mnif I, Boukedi H, Kammoun R, Ellouze-Chaabouni S. Statistical optimization of low-cost medium for economical production of *Bacillus subtilis* biosurfactant, a biocontrol agent for the olive moth *Prays oleae*. Afr J Microbiol Res. 2011;5:4927–36. https://doi.org/10.5897/AJMR11.1125.

Ghribi D, Elleuch M, Abdelkefi L, Ellouze-Chaabouni S. Evaluation of larvicidal potency of *Bacillus subtilis* SPB1 biosurfactant against *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae and influence of abiotic factors on its insecticidal activity. J Stored Prod Res. 2012;48:68–72. https://doi.org/10.1016/j.jspr.2011.10.002.

Ghribi D, Abdelkefi-Mesrati L, Boukedi H, Elleuch M, Ellouze-Chaabouni S, Tounsi S. The impact of the *Bacillus subtilis* SPB1 biosurfactant on the midgut histology of *Spodoptera littoralis* (Lepidoptera: Noctuidae) and determination of its putative receptor. J Invertebr Pathol. 2012;109:183–6. https://doi.org/10.1016/j.jip.2011.10.014.

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Kohl J, et al. Have biopesticides come of age? Trends Biotechnol. 2012;30(5):250–8. https://doi.org/10.1016/j.tibtech.2012.01.003.

González A, Sahagún A, Diez M, Fernández N, Sierra V, García J. The pharmacokinetics and metabolism of ivermectin in domestic animal species. Vet J. 2009;179(1):25–37. https://doi.org/10.1016/j.tvjl.2007.07.011.

Gordon R, Haynes W, Pang C. The genus *Bacillus*. In: Agricultural Handbook. No. 427. 1973; Washington, DC: United states Department of Agriculture.

Grady E, MacDonald J, Liu L, Richman A, Chun YZ. Current knowledge and perspectives of *Paenibacillus*: a review. Microb Cell Fact. 2016;15:203. https://doi.org/10.1186/s12934-016-0603-7.

Gullino M. Environmental impact and risk analysis of bacterial and fungal biocontrol agents. Phytoparasitica. 2005;33:3–6. https://doi.org/10.1007/BF02980918.

Gupta S, Dikshit A. Biopesticides: an ecofriendly approach for pest control. J Biopestic. 2010;3:186–8.

Hahne C, Jarrod L, Russell C. Patent AU2018237187. Australia. 2018.

Hakim L, Friend U. Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. J Biol Sci. 2020;20:66–76. https://doi.org/10.3844/ojbsci.2020.66.76.

Heimpel A, Angus T. Bacterial insecticides. Bacteriol Rev. 1960;24:266–88. https://doi.org/10.1128/br.24.3.266-288.1960.

Heins S, Manker D, Jimenez D, Marrone P. Patent US6,001,637. USA. 1999.

Hellwege E, Andersch W, Gladbach B, Stenzel K, Springer B. Patent US 10,542,757 B2. 2020

Hernandez F, Miranda A, Rodríguez C, Giraldo C, Sáez A. A potential strategies in the biopesticide formulations: a bibliometric analysis. Agron. 2022;12:2665. https://doi.org/10.3390/agronomy12112665.

Hiebert N, Kessel T, Skaljac M, Spohn M, Vilcinskas A, Zin W. The Gram-positive bacterium *Leuconostoc pseudomesenteroides* shows insecticidal activity against Drosophilid and Aphid Pests. InSects. 2020;11:471. https://doi.org/10.3390/insects11080471.

Hoshino T. Violacein and related tryptophan metabolites produced by *Chromobacterium violaceum*: biosynthetic mechanism and pathway for construction of violacein core. Appl Microbiol Biotechnol. 2011;91:1463–75. https://doi.org/10.1007/s00253-011-3468-z.

Huang C. The application and prospect of planting biotechnique in the control of pests and diseases. J Anhui Agric Sci. 2001;29:320–4. https://doi.org/10.3126/ijasbt.v8i2.28326.

Hurst M, Glare T, Jackson T, Ronson C. Plasmid-located pathogenicity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photorhabdus luminescens*. J Bacteriol. 2000;182:5127–38. https://doi.org/10.1128/JB.182.18.5127-5138.2000.

Hurst M, Glare T, Jackson T. Cloning *Serratia entomophila* anti feeding genes- a putative defective prophage active against the grass grub *Costelytra zealandica*. J Bacteriol. 2004;186:5116–28. https://doi.org/10.1128/JB.186.15.5116-5128.2004.

Hurst M, Becher A, Young S, Nelson T, Glare T. *Yersinia entomophaga* sp. Nov., isolated from the New Zealand grass grub Costelytra zealandica. Int J Syst Evol Microbiol. 2011;61:844–9. https://doi.org/10.1099/ijs.0.024406-0.

Hussin N, Ab MA. Termiticidal activity of chitinase enzyme of *Bacillus licheniformis*, a symbiont isolated from the gut of *Globitermes sulphureus* worker. Biocatal Agric Biotechnol. 2020;24:101548. https://doi.org/10.1016/j.bcab.2020.101548.

Hynes R, Boyetchko R. Research initiatives in the art and science of biopesticide formulations Russell K. Soil Biol Biochem. 2006;38:845–9. https://doi.org/10.1016/j.soilbio.2005.07.003.

Ibrahim N, Hussien A, Hatem A, Aldebis H, Vargas E. Persistence of the transformed *Paenibacillus polymyxa* expressing *CRY1C* in the plant leaves and its effect on chlorophyll and carotenoid. Life Sci J. 2014;11:433–42.

Ilham B, Noureddine C, Philippe G, Mohammed E, Brahim E, Sophie A, et al. Induced systemic resistance (ISR) in *Arabidopsis thaliana* by *Bacillus amyloliquefaciens* and *Trichoderma harzianum* used as seed treatments. Agriculture. 2019;9:166. https://doi.org/10.3390/agriculture9080166.

Jacobsen B, Zidack N, Larson B. The role of Bacillus-based biological control agents in integrated pest management systems: plant diseases. Phytopathology. 2004;94:1272–5. https://doi.org/10.1094/phyto.2004.94.11.1272.

Jahangiriana H, Haron M, Mohd H, Roshanak R, Leili A, Majid R, et al. Well diffusion method for valuation of antibacterial activity of copper phenyl fatty hydroxamate synthesized from canola and palm Kernel Oils. J Nanomater Biostructures. 2013;3:1263–70.

Jatoi G, Lihua G, Xiufen Y, Gadhi M, Keerio A, Abdulle Y, et al. A novel protein elicitor *PeBL2*, from *Brevibacillus laterosporus* A60, induces systemic resistance against *Botrytis cinerea* in tobacco plant. Plant Pathol J. 2019;35(3):208–18. https://doi.org/10.5423/PPJ.OA.11.2018.0276.

Jisha V, Smitha R, Benjamin S. An overview on the crystal toxins from *Bacillus thuringiensis*. Adv Microbiol. 2013;3:462–72. https://doi.org/10.4236/aim.2013.35062.

Jurat J, Jackson T. Bacterial entomopathogens. Insect Pathol. 2012. https://doi.org/10.1016/B978-0-12-384984-7.00008-7.

Kachhawa D. Microorganisms as a biopesticides. J Entomol Zool Stud. 2017;5(3):468–73.

Kageyama D, Anbutsu H, Shimada M, Fukatsu T. *Spiroplasma* infection causes either early or late male killing in Drosophila, depending on maternal host age. Naturwissenschaften. 2007;94:333–7. https://doi.org/10.1007/s00114-006-0195-x.

Kahia M, Nguyen T, McCune F, Naasz R, Antoun H, Fournier V. Insecticidal effect of *Bacillus pumilus* PTB180 and *Bacillus subtilis* PTB185 used alone and in combination against the foxglove aphid and the melon aphid (Hemiptera: Aphididae). Can Entomol. 2021;153(6):726–40.

Karabörklü S, Azizoglu U, Azizoglu Z. Recombinant entomopathogenic agents: a review of biotechnological approaches to pest insect control. World J Microbiol Biotechnol. 2018;34:14. https://doi.org/10.1007/s11274-017-2397-0.

Kaur R, Shropshire J, Cross K, Leigh B, Mansueto A, Stewart V, et al. Living in the endosymbiotic world of *Wolbachia*: A centennial review. Cell Host Microbe. 2021;29:879–93. https://doi.org/10.1016/J.CHOM.2021.03.006.

Kekuda P, Shobha K, Onkarappa R. Potent insecticidal activity of two *Streptomyces* species isolated from the soils of the Western ghats of Agumbe, Karnataka. J Nat Pharm. 2010;1:30–2. https://doi.org/10.4103/2229-5119.73584.

Khalimi K, Suprapta D. Induction of plant resistance against Soybean stunt virus using some formulations of *Pseudomonas aeruginosa*. J ASSAAS. 2011;17:98–105.

Khan H, Khan T. Efficacy of entomopathogenic fungi against three major stored insect pests, *Rhyzopertha dominica*, *Sitophilus zeamais* and *Trogoderma granarium*. J Stored Prod Res. 2023;104:102188.

Kil Y, Seo M, Kang D, Oh S, Cho H, Youn Y, et al. Effects of Enterobacteria (Burkholderia sp.) on development of Riptortus pedestris. J Fac Agric Kyushu Univ. 2014;59:77–84. https://doi.org/10.5109/1434382.

Kim M, Cho S, Kang E, Im Y, Hwangbo H, Kim Y, et al. Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization. Mol Plant Microbe Interact. 2008;21:1643–53. https://doi.org/10.1094/mpmi-21-12-1643.

Kirst H. The spinosyn family of insecticides: Realizing the potential of natural products research. J Antibiot. 2010;63:101–11. https://doi.org/10.1038/ja.2010.5.

Kumar S, Singh A. Biopesticides: present status and the future prospects. J Fertil Pestic. 2015;6:100–29. https://doi.org/10.4172/jbfbp.1000e129.

Kumar S, Chauhan P, Agrawal L, Raj R, Srivastava A, Gupta S, et al. *Paenibacillus lentimorbus* inoculation enhances tobacco growth and extenuates the virulence of *Cucumber mosaic* virus. PLoS ONE. 2016b;11(3):e0149980. https://doi.org/10.1371/journal.pone.0149980.

Kumar J, Ramlal A, Mallick D, Mishra V. An overview of some biopesticides and their importance in plant protection for commercial acceptance. Plants. 2021;10:1185. https://doi.org/10.3390/plants10061185.

Kumar N, Verma M, Prakash J, Mishra J. Regulation of Biopesticides: Global concerns and policies in Arora, editors. Bioformulations: for sustainable agriculture, Springer: India; 2016. https://doi.org/10.1007/978-81-322-2779-3_16

Kurt S, Eda R, Thorsten J. Patent WO2014147534. European Union. 2013.

Kwak K, Han M, Nam S, Choi J, Lee Y, Kim H, Park K. Comparing the mortality of *Protaetia brevitarsis seulensis* (Coleoptera: Cetoniidae) caused by entomopathogenic bacteria and *Serratia marcescens* (Enterobacteriales: Enterobacteriaceae). Int J Ind Entomol. 2015;30(2):40–4.

Lahlali R, Ezrari S, Radouane N, Kenfaoui J, Esmaeel Q, El Hamss H, et al. A biological control of plant pathogens: a global perspective. Microorganisms. 2022;10:596. https://doi.org/10.3390/microorganisms10030596.

Lalithambika B, Vani C. *Pseudomonas aeruginosa* KUN2, extracellular toxins A potential source for the control of dengue vector. J Vector Borne Dis. 2016;53:105–11. https://doi.org/10.1007/s11356-017-9714-x.

Lalithambika B, Vani C, Tittes A. Biological control of dengue vector using *Pseudomonas fluorescens*. Re J Rec Sci. 2014;3:344–51.

Lambert B, Peferoen M. Insecticidal promise of *Bacillus thuringiensis*. Bioscience. 1992;42:1121–2. https://doi.org/10.2307/131165210.2307/1311652.

Landsberg M, Jones S, Rothnagel R, Busby J, Marshall S, Simpson R, et al. Structure of the *Yersinia entomophaga* toxin complex and implications for insecticidal activity. PNAS. 2011;108:20544–9. https://doi.org/10.1073/pnas.1111155108.

Leahy J, Mendelsohn M, Kough J, Jones R, Berckes N. Biopesticide oversight and registration at the U.S. environmental protection agency. In: Gross AD, Coats JR, Duke SO, Seiber JN, editors. Biopesticides: State of the Art and Future Opportunities. Washington: American Chemical Society; 2014. p. 3–18.

Lee A, Bulla Jr, Jun B, Purnima R. WO1993003619A1. 1991.

Leng P, Zhang Z, Pan G, Zhao M. Applications and development trends in biopesticides. Afr J Biotechnol. 2011;10:19864–73. https://doi.org/10.5897/AJBX11.009.

Lengai G, Muthomi J. Biopesticides and their role in sustainable agricultural production. J Biosci Med. 2018;6:7–41. https://doi.org/10.4236/jbm.2018.66002.

Li W, Lee S, Cho Y, Ghim S, Jung H. Mediation of induced systemic resistance by the plant growth promoting rhizobacteria *Bacillus pumilus* S2–3-2. Mol Bio Rep. 2020;47:8429–38. https://doi.org/10.1007/s11033-020-05883-9.

Liu J, Lin Y, Chang S, Zeng Y, Wang S. Molecular cloning and characterization of an insecticidal toxin from *Pseudomonas taiwanensis*. J Agric Food Chem. 2010;58:12343–9. https://doi.org/10.1021/jf103604r.

Lumaret J, Errouissi F, Floate K, Römbke J, Wardhaugh K. A review on the toxicity and non-target effects of macrocyclic lactones in terrestrial and aquatic environments. Curr Pharm Biotechnol. 2012;13:1004–60. https://doi.org/10.2174/138920112800399257.

Manonmani A, Geetha I, Bhuvaneswari S. Enhanced production of mosquitocidal cyclic lipopeptide from *Bacillus subtilis* subsp. subtilis. Ind J Med Res. 2011;134:476–82.

Mao Y, Cai W, Wang J, Hong G, Tao X, Wang L, et al. Silencing a cotton bollworm P450 monooxygenase gene by plant-mediated RNAi impairs larval tolerance of gossypol. Nat Biotechnol. 2007;25:1307–13. https://doi.org/10.1038/nbt1352.

Marques R, Lima M, Marques R, Bernardes RC. A spinosad-based formulation reduces the survival and alters the behavior of the stingless bee *Plebeia lucii*. Neotrop Entomol. 2020;49:578–85. https://doi.org/10.1007/s13744-020-00766-x.

Martin P, Gundersen-Rindal D, Blackburn M, Buyer J. Chromobacterium subtsugae sp. Nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. Int J Syst Evol Microbiol. 2007;57(5):993–9. https://doi.org/10.1099/ijs.0.64611-0.

Martin P, Hirose E, Aldrich J. Toxicity of *Chromobacterium subtsugae* to southern green stink bug (Heteroptera: Pentatomidae) and corn rootworm (Coleoptera: Chrysomelidae). J Econ Entomol. 2007;100:680–4.

Mazid S, Kalida JC, Rajkhowa RC. A review on the use of biopesticides in insect pest management. Int J Sci Adv Technol. 2011;1:169–78.

Miller L, Lingg A, Bulla L. Bacterial, viral and fungal insecticides. Science. 1983;219:715–21.

Mishra J, Tewari S, Singh S, Kumar N. In: Arora N. editors. Plant Microbes Symbiosis: Applied Facets. 2015. https://doi.org/10.1007/978-81-322-2068-8_2.

Mnif I, Ghribi D. Potential of bacterial derived biopesticides in pest management. Crop Prot. 2015;77:52–64. https://doi.org/10.1016/j.cropro.2015.07.017.

Molina C, Cana-Roca J, Osuna A, Vilchez S. Selection of a *Bacillus pumilus* strain highly active against *Ceratitis capitata* (Wiedemann) larvae. Appl Environ Microbiol. 2010;76(5):1320–7.

Montazeri M, Greaves MP. Effects of culture age, washing and storage conditions on desiccation tolerance of *Colletotrichum truncatum* conidia. Biocontrol Sci Techn. 2002;12:95–105.

Morales-Rodriguez A, Peck DC. Synergies between biological and neonicotinoid insecticides for the curative control of the white grubs *Amphimallon majale* and *Popillia japonica*. Biol Control. 2009;51:169–80. https://doi.org/10.1016/j.biocontrol.2009.06.008.

Nathan S. A review of biopesticides and their mode of action against insect pests. Environmental sustainability: role of green technologies. 2014. pp 49–63.

Osman G, Mostafa S, Sonya M. Antagonistic and insecticidal activities of some *Streptomyces* isolates. Pak J Biotechnol. 2007;4:65–71.

Osman G, El-Ghareeb D, Already R, Assaeedi A, Organji S, Abulreesh H, et al. Bioinsecticide *Bacillus thuringiensis* a comprehensive review. Egypt J Biol Pest Control. 2015; 25(1).

Pardo-López L, Soberón M, Bravo A. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. FEMS Microbiol Rev. 2013;37:3–22. https://doi.org/10.1111/j.1574-6976.2012.00341.x.

Perez A, Romero D, de Vicente A. Plant protection and growth stimulation by microorganisms: biotechnological applications of Bacilli in agriculture. Curr Opin Biotechnol. 2011;22:187–93. https://doi.org/10.1016/j.copbio.2010.12.003.

Pérez-Cogollo LC, Rodríguez-Vivas RI, Basto-Estrella GS, Reyes-Novelo E, Martínez-Morales I, Ojeda-Chi MM, et al. Toxicidad y efectos adversos de las lactonas macrocíclicas sobre los escarabajos estercoleros: una revisión. Rev Mex Biodivers. 2018;89:1293–314. https://doi.org/10.22201/ib.20078706e.2018.4.2508.

Pigott C, Ellar D. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol Mol Biol Rev. 2007;71:255–81. https://doi.org/10.1128/MMBR.00034-06.

Prabha S, Yadav A, Kumar A, Yadav A, Yadav H, Kumar S, et al. Biopesticides: an alternative and eco-friendly source for the control of pests in agricultural crops. Plant Archives. 2017;16:902–6.

Qureshi N, Chawla S, Likitvivatanavong S, Lee H, Gill S. The cry toxin operon of clostridium bifermentans subsp. Malaysia is highly toxic to aedes larval mosquitoes. Appl Environ Microbiol. 2014;80:5689–97. https://doi.org/10.1128/AEM.01139-14.

Raaijmakers J, Mazzola M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu Rev Phytopathol. 2012;50(1):403–24.

Raupach G, Taensa I, Kloepper J. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis. 2000;84:1073–5. https://doi.org/10.1094/pdis.2000.84.10.1073.

Rippere K, Tran M, Yousten A, Hilu K, Klein M. *Bacillus popilliae* and *Bacillus lentimorbus*, bacteria causing milky disease in Japanese beetles and related scarab larvae. Int J Syst Evol Microbiol. 1998;48:395–402.

Ritika B, Utpal D. An overview of fungal and bacterial biopesticides to control plant pathogens/diseases. Afr J Microbiol Res. 2014;8:1749–62.

Rojas F, López D, Meza G, Méndez A, Ibarra J, Estrada P. El controvertido complejo *Burkholderia cepacia*, un grupo de especies promotoras del crecimiento vegetal y patógenas de plantas, animales y humanos. Rev Argent Microbiol. 2019;51:84–92. https://doi.org/10.1016/J.RAM.2018.01.002.

Ruiu L. *Brevibacillus laterosporus*, a pathogen of invertebrates and a broad-spectrum antimicrobial species. InSects. 2013;4:476–92. https://doi.org/10.3390/insects4030476.

Ruiu L. Insect pathogenic bacteria in integrated pest management. InSects. 2015;6:352–67. https://doi.org/10.3390/insects6020352.

Ruiu L. Microbial biopesticides in agroecosystems. Agronomy. 2018;8:235. https://doi.org/10.3390/agronomy8110235.

Ruiu L, Floris I, Satta A, Ellar DJ. Toxicity of a *Brevibacillus laterosporus* strain lacking parasporal crystals against *Musca domestica* and *Aedes aegypti*. Biol Control. 2007;43:136–43. https://doi.org/10.1016/j.biocontrol.2007.07.002.

Ruiu L, Satta A, Floris I. Observations on house fly larvae midgut ultrastructure after *Brevibacillus laterosporus* ingestion. J Invertebr Pathol. 2012;111:211–6. https://doi.org/10.1016/j.jip.2012.08.005.

Ruiu L, Satta A, Floreis I. Emerging entomopathogenic bacteria for insect pest management. Bull Insectology. 2013;66:181–6.

Sachidanandham R, Jayaraman K. Formation of spontaneous asporogenic variants of *Bacillus thuringiensis* subsp. galleriae in continuous cultures. Appl Microbiol Biotechnol. 1993;40:504–7. https://doi.org/10.1007/BF00175739.

Saint-Pierre G, Henríquez D, Paredes L, Gaete M. Bacillus amyloliquefaciens. Rev Chilena Infectol. 2023;40:289–90. https://doi.org/10.4067/s0716-10182023000300289.

Samada L, Tambunan U. Biopesticides as promising alternatives to chemical pesticides: a review of their current and future status. J Biol Sci. 2020;20:66–76. https://doi.org/10.3844/ojbsci.2020.66.76.

Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P. *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnol J. 2011;9:283–300. https://doi.org/10.1111/j.1467-7652.2011.00595.x.

Saravanakumar D, Vijayakumar C, Kumar N, Samiyappan R. PGPR-induced defense responses in the tea plant against blister blight disease. Crop Prot. 2007;26:556–65. https://doi.org/10.1016/j.cropro.2006.05.007.

Schäfer L, Volk F, Kleespies R, Jehle J, Wennmann J. Elucidating the genomic history of commercially used Bacillus thuringiensis subsp. tenebrionis strain NB176. Front Cell Infect Microbiol. 2023;13:1129177. https://doi.org/10.3389/fcimb.2023.1129177.

Shabanamol S, Sreekumar J, Jisha M. Bioprospecting endophytic diazotrophic *Lysinibacillus sphaericus* as biocontrol agents of rice sheath blight disease. 3Biotech. 2017;7:337. https://doi.org/10.1007/s13205-017-0956-6.

Shah J, Inayatullah M, Sohail K, Shah S, Shah S, Iqbal T, et al. Efficacy of botanical extracts and a chemical pesticide against tomato fruit worm. Helicoverpa Armigera SJA. 2013;1:93–6.

Shiberu T, Getu E. Assessment of selected botanical extracts against Liriomyza species (diptera: agromyzidae) on tomato under glasshouse condition. Int J Fauna Biol Stud. 2016;1:87–90.

Shropshire J, Leigh B, Bordenstein S. Symbiont-mediated cytoplasmic incompatibility: what have we learned in 50 years? eLife. 2020;9:e61989. https://doi.org/10.7554/eLife.61989.

Sieber J, Coats J, Duke S, Gross A. Biopesticides: state of the arte and future opportunities. J Agric Food Chem. 2014;62:11613–9. https://doi.org/10.1021/jf504252n.

Silva V, Lovaglio R, Von Zuben C, Contiero J. Rhamnolipids: solution against *Aedes aegypti*? Front Microbiol. 2015;6:1–5. https://doi.org/10.3389/fmicb.2015.00088.

Singh H. Management of plant pathogen with microorganisms. Proc Indian Nant Sci. 2014;80:443–54. https://doi.org/10.16943/ptinsa/2014/v80i2/55120.

Starnes R, Liu C, Marrone P. History, use, and future of microbial insecticides. Am Entomol. 1993;39(2):83–91. https://doi.org/10.1093/ae/39.2.8310.1093/ae/39.2.83.

Suprapta D. Potential of microbial antagonists as biocontrol agents against plant fungal Pathogens. ISSAAS. 2012;2:1–8.

Tabashnik B, Carrière Y. Surge in insect resistance to transgenic crops and prospects for sustainability. Nat Biotechnol. 2017;35:926–35. https://doi.org/10.1038/nbt.3974.

Thakore Y. The biopesticide market for global agricultural use. Ind Biotechnol. 2006;2(3):194–208.

Thavara U, Tawatsin A, Asavadachanukorn P, Mulla M. Field evaluation in thailand of spinosad, a larvicide derived from *Saccharopolyspora spinosa* (actinomycetales) against *Aedes aegypti* larvae. Southeast Asian J Trop Med Public Health. 2009;40:235–42.

Theoduloz C, Vega A, Salazar M, González E, Meza-Basso L. Expression of a *Bacillus thuringiensis* delta-endotoxin *cry1Ab* gene in *Bacillus subtilis* and *Bacillus licheniformis* strains that naturally colonize the phylloplane of tomato plants (*Lycopersicon esculentum*, Mills). J Appl Microbiol. 2003;94:375–81. https://doi.org/10.1046/j.1365-2672.2003.01840.x.

Thorne L, Garduno F, Thompson T, Decker D, Zounes M, Wild M, et al. Structural similarity between the lepidoptera- and diptera-specific insecticidal endotoxin genes of Bacillus thuringiensis subsp. "kurstaki" and "israelensis." J Bacteriol. 1986;166:801–11. https://doi.org/10.1128/jb.166.3.801-811.1986.

Tiangang K, Ran L, Zixin D. Patent WO2022262384. European Union. 2022.

Tijjani A, Bashir KA, Mohammed I, Muhammad A, Gambo A, Musa H. Biopesticides for pests control: a review. J Biopest Agric. 2016;3:6–13.

Veliz E, Martínez P, Hirsch A. Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiol. 2017;3(3):689–705. https://doi.org/10.3934/microbiol.2017.3.689.

Villaverde J, Villaverde B, Sevilla-Morán P, Sandín-España C, López-Goti C, Alonso-Prados JL. Biopesticides in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Manag Sci. 2014;70:2–5. https://doi.org/10.1002/ps.3663.

Vodovar N, Vinals M, Liehl P, Basset A, Degrouard J, Spellman P, et al. Drosophila host defense after oral infection by and entomopathogenic Pseudomonas species. Proc Natl Acad Sci USA. 2005;102:11414–9. https://doi.org/10.1073/pnas.0502240102.

Vodovar N, Vallenet D, Cruveiller S, Rouy Z, Barbe V, Acosta C, et al. Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. Nat Biotechnol. 2006;24:673–9. https://doi.org/10.1038/nbt1212.

Waltz E. USDA approves next-generation GM potato. Nat Biotechnol. 2015;33:12–3. https://doi.org/10.1038/nbt0115-12.

Wang MB, Abbott DC, Waterhouse PM. A single copy of a virus-derived transgene encoding hairpin RNA gives immunity to barley yellow dwarf virus. Mol Plant Pathol. 2000;1:347–56. https://doi.org/10.1046/j.1364-3703.2000.00038.x.

Wang H, Yang X, Guo L, Zeng H, Qiu D. PeBL1, a novel protein elicitor from *Brevibacillus laterosporus* strain A60, activates defense responses and systemic resistance in *Nicotiana benthamiana*. Appl Environ Microbiol. 2015;81(8):2706–16. https://doi.org/10.1128/AEM.03586-14.

Wei G, Kloepper J, Tuzun S. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology. 1996;86:221–4.

Winssy T, Gopal N, Indiragandhi P, Balasubramani V, Anandham R. Chitinase producing gut-associated bacteria affected the survivability of the insect *Spodoptera frugiperda*. Front Biosci. 2024;16(2):15. https://doi.org/10.31083/j.fbe1602015.

Xu X, Jeffries P, Pautasso M, Jeger M. Combined use of biocontrol agents to manage plant diseases in theory and practice. Phytopathology. 2011;101(9):1024–31. https://doi.org/10.1094/PHYTO-08-10-0216.

Yan M, Roehrl M, Wang J. Discovery of cristalline inclusions in *Bacillus licheniformis* that resemble parasporal cristal of *Bacillus thuringiensis*. Can J Microbiol. 2007;53:1111–5. https://doi.org/10.1139/W07-076.

Yap Z, Azman A, Abubakar S, Low V. Streptomyces derivatives as an insecticide: current perspectives, challenges and future research needs for mosquito control. Acta Trop. 2022;229:106381. https://doi.org/10.1016/j.actatropica.2022.106381.

Yokoyama T, Tanaka M, Hasegawa M. Novel cry gene from *Paenibacillus lentimorbus* strain *Semadara inhibits* ingestion and promotes insecticidal activity in *Anomala cuprea* larvae. J Invertebr Pathol. 2004;85:25–32. https://doi.org/10.1016/j.jip.2003.12.009.

Zamore PD, Tuschl T, Sharp PA, Bartel DP. RNAi: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell. 2000;101(1):25–33. https://doi.org/10.1016/S0092-8674(00)80620-0.

Zhang F, Huo K, Song X, Quan Y, Wang S, Zhang Z, et al. Engineering of a genome-reduced strain *Bacillus amyloliquefaciens* for enhancing surfactin production. Microb Cell. 2020;9:223. https://doi.org/10.1186/s12934-020-01485-z.