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# Biotechnological advances in *Bacillus thuringiensis* and its toxins: Recent updates

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**Abstract** *Bacillus thuringiensis* (*Bt*) and its products are commonly used to control insect pests. The main issue with these bacteria is their limited field stability. These constraints fueled interest in using several molecular, biological, and biotechnological techniques to develop new recombinant *Bt* toxins with a broader insect spectrum, improved environmental stability and more efficient delivery of toxins to pest insects control. However, the potential environmental impact of using recombinant *Bt* strains and genetically engineered *Bt* crops includes gene flows into wild species and their unintended consequences on parasitoids and predators. The development of new

hybrid/mutated *Bt* insecticidal toxins, with enhanced insecticidal activity and/or a broader spectrum of target insects, will continue to be a useful strategy for controlling resistant insect pests and delaying resistance evolution. Furthermore, the use of other genes encoding non-*Bt* proteins with insecticidal properties and different modes of action, such as protease inhibitors, lectins, cholesterol oxidases and chitinases, isolated from various sources will be critical in providing new weapons for the fight against insect damage. This review thoroughly describes recent advances and the most recent updates in the new potential applications of *Bt*, making it a remarkable new cell factory that can be employed to control pests.

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## 1 Introduction

*Bacillus thuringiensis* (*Bt*) is the most widely used microbial biopesticide in agriculture, forestry and mosquito control (Ortiz and Sansinenea 2021a). *Bt* was first discovered in Japan in 1901 by Shigetane Ishiwata; however, it was not until 1915 that it could be fully identified by the German bacteriologist Ernst Berliner, who named this bacterium *Bacillus thuringiensis* (Ortiz and Sansinenea 2021b). Insecticides based on *Bt* have been used for decades to control a

wide range of insect pests, primarily lepidopteran (caterpillars), Dipteran (mosquitoes and black flies), and Coleopteran (beetle larvae) (Sanchis 2011). *Bt* is characterized by the production, during sporulation, of endotoxin proteins, known as Cry proteins, that accumulate and form a crystal inclusion body. Insects must consume/ingest these Cry proteins in order to feel their effect until the insect dies. Following ingestion, the alkaline conditions within the insect midgut cause the solubilization of the crystals, converting them into toxic core fragments (Sansinenea 2019). These toxic proteins bind to receptors (glycoproteins or glycolipids) located on insect midgut epithelial cells (Bravo et al. 2011).

After binding, the toxin changes its conformation, allowing it to insert into the cell membrane and form a cation-selective channel (Bravo et al. 2013). When enough of these channels are formed, several cations enter to the cell. This causes an osmotic imbalance within the cell, resulting in the loss of the midgut epithelium integrity. This allows alkaline gut juices and bacteria to pass through the midgut basement membrane, killing the insect. When used as sprays, these toxins are ineffective in preventing insects from attacking the plant's roots or internal parts of the plant (Sanahuja et al. 2011). These limitations sparked interest in developing new genetically modified plants and bacteria to express Cry and other *Bt*-insecticidal genes in order to provide a more efficient toxin delivery system to control these insects (Azizoglu and Karabörklü 2021).

Continuous advances in biotechnological techniques such as genetic engineering with the assistance of computational biology have resulted in further development and discoveries about *Bt*. In this context, various research groups worldwide were very interested in finding novel Cry toxins with new range of inhibitory activities and high level of toxicity as an alternative against insect pest that have developed greater resistance levels (Hou et al. 2019; Crickmore et al. 2021; Lazarte et al. 2021). As a result, continuous strains improvement, using current genome data, development of genetically modified (GM) microorganisms, are becoming unavoidable toolkits for achieving heterologous expression of non-native genes and improvement native producers to develop genetically improved strains (Liu et al. 2017; Azizoglu et al. 2020). Today's new generation methods, such as simulation and dynamics studies,

*Bt* genomics, quantitative structure–activity relationships, molecular docking and structure/function prediction methods, are described. These methods are essentially required for the numerous preparations and preprocessing steps that are involved.

The current review focuses on an update on recent advances in the new potential applications of *Bt* and its toxins, analyzing them from different perspectives, in an effort to generate critical discussion that can help in understanding their potential benefits and impacts.

## 2 New characteristics and properties of *Bacillus thuringiensis*

*Bt* is commonly and widely used as a host-specific and safe biopesticide and also has been extensively exploited as a gene source for the development and production of GM-*Bt* crops (Salehi Jouzani et al. 2017; Pinos et al. 2021; Pohare et al. 2021; Tetreau et al. 2021). In light of the scientific benefits of these biotechnological approaches, several other novel characteristics for *Bt* have recently been explored. Toxicity against nematodes, mites, ticks and mollusks and anticancer activities, antagonistic effects against bacteria and fungi that cause disease in plants and animals, biofertilizer and plant growth-promoting (PGP) activities, biodegradation and bioremediation of different pollutants, such as heavy metals, pesticides and petroleum derivatives, metal nanoparticles biosynthesis (e.g. silver and gold) and also biosynthesis of different value-added biopolymers, such as polyhydroxyalkanoate, are among these new environmental and industrial features (Salehi Jouzani et al. 2017).

### 2.1 Biocontrol activities against nematodes, bacteria, fungi, spiders and mollusca

The capability of some *Bt* strains to control plant and animal pathogenic nematodes, bacteria, fungi, spiders and snails has been efficiently demonstrated (Table 1). Some *Bt* strains have shown high nematocidal activity against various phytopathogenic nematodes, including the root-knot nematode *Meloidogyne incognita* (Khalil and Abd El-Naby 2018; Choi et al. 2020; Ramalakshmi et al. 2020; Leong et al. 2021; Verduzco-Rosas et al. 2021), the cereal cyst nematode *Heterodera avenae* (Ahmed et al. 2018), the soybean

**Table 1** Biocontrol activities of *Bt* strains against plant and animal pathogenic nematodes, bacteria, fungi, spiders and snails

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
Biocontrol of plant pathogens (Antagonist)	KYC	Control of <i>R. solani</i> , <i>F. oxysporum</i> , <i>F. graminearum</i>	The strain promoted the growth of tomato plants by reducing root gall numbers and fungal effects under in vivo conditions	Choi et al. (2020)
	TbL-22 and TbL-26	Antagonistic activity against citrus canker disease caused by <i>X. citri subsp. citri</i> ( <i>Xcc</i> )	TbL-22 showed high antagonistic activity against streptomycin resistant <i>Xcc</i> strains with inhibition zones of about 20 mm, and also reduced canker incidence on infected leaves by 64.05%	Islam et al. (2019)
	BCN10	Antifungal activities against five common postharvest spoilage fungi on loquats, <i>F. oxysporum</i> , <i>Botryosphaeria</i> sp., <i>T. atroviride</i> , <i>C. gloeosporioides</i> , and <i>P. expansum</i>	The strain was able to suppress the mycelial growth of all targeted pathogens according to inhibition ratio in the double petri-dish assay as well as disease incidence and disease diameter on loquat fruits	He et al. (2020)
	ABTS-1857	Suppressing gray mold fungus ( <i>B. cinerea</i> ) in tomato based on induced systemic resistance	The gray mold disease was significantly suppressed when rhizospheres were drenched with the inoculum. The strain increased the expression levels of defense-related genes (PR-1(P6) and P4) in tomato seedlings, suggest that the strain has the potential to suppress gray mold disease through systemic activation of the plant defense system	Yoshida et al. (2019)
	Different <i>Bt</i> isolates from tomato roots	Antagonistic activity against the two phytopathogenic fungi <i>V. dahliae</i> and <i>V. longisporum</i> via production of different antifungal metabolites	High antifungal activities against <i>Verrucillium</i> species in vitro	Hollensteiner et al. (2017)
	IMC8	Biological control of powdery mildew ( <i>E. pulchra</i> ) on flowering dogwood ( <i>C. florida</i> ) through antibiosis by production of antifungal and antibacterial compounds	It could effectively control <i>E. pulchra</i> in growth chamber, greenhouse and shade house environments compared to the control	Rotich et al. (2020)
	Without name	Production of different antifungal compounds, including indole 1, tryptamine 2, N-acetyl tryptamine 3, phenethylamine 4, phenethyl acetamide 5, and 3-methylN-(2'-phenylethyl) butyramide 6	High antifungal activities against plant pathogenic fungi, including <i>F. oxysporum</i> , <i>Alternaria</i> sp., and <i>M. roreri</i> (the causal agent of cacao disease)	Vaca et al. (2020)
	Without name	Antagonistic effect on bacterial wilt of tomato, caused by <i>R. solanacearum</i> (Rs)	It significantly suppressed the incidence of bacterial wilt (67%) and promoted plant growth. Higher plant heights (72.70–111.00 cm), stem girth (1.63–1.90 cm) and number of leaves (103.33–125.33) were recorded for plants inoculated with <i>Bt</i>	Akintokun et al. (2019)
	LTS-209 and VITSJ-01	Antagonistic effect on Fusarium wilt of cucumber caused by <i>F. oxysporum f.sp cucumerinum</i>	They could suppress Fusarium wilt of cucumber in lab and greenhouse experiments	Akintokun et al. (2020)
	BMB171	Production of antimicrobial lipopeptide thumolysin	Broad spectrum of antibacterial and anti-nematode activities	Zheng et al. (2018)

Table 1 (continued)

Type of new applications	The studied <i>Br</i> strains	Specific application and mode of action	Efficiency	Reference
	SY33.3	Antagonistic effects against plant pathogenic fungi, <i>F. oxysporum f.sp. niveum</i> , <i>V. dahliae</i> and <i>A. niger</i> , due to chitinase production	Strong antagonistic effects against the studied plant pathogenic fungi in dual culture experiments	Azizoglu et al. (2021)
	Dipel®	Antagonistic effects against Asian soybean rust ( <i>P. pachyrhizii</i> ), Downy mildew ( <i>P. manshurica</i> ) and powdery mildew ( <i>M. diffusa</i> ) as most important soybean diseases, by inducing $\beta$ -1,3 glucanase enzyme and phytoalexin glyceollin production	The results of in vitro, in vivo and field assays, showed that <i>Br</i> Dipel® can reduce soybean foliar diseases by production of antimicrobial compounds	Müller et al. (2019)
	Without name	Induction of tomato systemic resistance to the necrotrophic fusarium wilt caused by the fungus <i>F. oxysporum f. sp. lycopersici</i> by reducing level of H <sub>2</sub> O <sub>2</sub> and increasing transcription of the antioxidant enzymes genes such as superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST)	Priming tomato resistance against fusarium wilt	Zibanezhadian et al. (2022)
	Berliner 1915	Antifungal activity surfactin against maize fungal phytopathogen <i>F. graminearum</i> Schwabe by producing surfactin	The growth of <i>F. graminearum</i> was successfully inhibited by surfactin at 80% concentration	Muddasir et al. (2021)
Nematicidal activities against plant and animal pathogenic nematodes	BC and BD	Nematicidal activity against the root knot nematode, <i>M. incognita</i>	The BC and BD strains showed highest nematicidal activity against J2 of <i>M. incognita</i> with LC <sub>50</sub> values of 0.12 and 0.23 $\mu\text{g mL}^{-1}$ of protein	Ramakshmi et al. (2020)
	LBIT-107		Spore-crystal suspensions decreased up to 90% the number of galls of <i>M. incognita</i>	Verduzco-Rosas et al. (2021)
	KYC		The bacterial culture filtrates significantly reduced the hatching rate of the nematode egg and caused J2 mortality	Choi et al. (2020)
	GC91	Nematicidal activity against citrus nematode, <i>T. semi-penetrans</i>	60.7% reduction of nematode population	El-Saedy et al. (2019)
	BRC-QX12	Nematicidal activity against the pine wood nematode ( <i>B. xylophilus</i> )	LC <sub>50</sub> of 32.13 $\mu\text{g mL}^{-1}$	Huang et al. (2018)
	Cry toxins		App6Aa2 (LC <sub>50</sub> =49.71 $\mu\text{g mL}^{-1}$ ), Cry13Aa1 (LC <sub>50</sub> =53.17 $\mu\text{g mL}^{-1}$ ), Cry12Aa1 (LC <sub>50</sub> =58.88 $\mu\text{g mL}^{-1}$ )	Guo et al. (2022)
	Cry14Ab expressed in transgenic soybean	Nematicidal activity against the soybean cyst nematode <i>H. glycines</i>	Cry14Ab-expressing soybean reduced <i>H. glycines</i> reproduction at both the mid-season (43%) and end-of-season (60%) assessment times	Kahn et al. (2021)

**Table 1** (continued)

Type of new applications	The studied <i>Br</i> strains	Specific application and mode of action	Efficiency	Reference
	MH032-003	Nematicidal activity against the cereal cyst nematode <i>H. avenae</i>	The isolate significantly reduced nematode infection of wheat roots when juveniles were used as inoculum after 10 days post inoculation	Ahmed et al. (2018)
	GP526	Nematicidal and ovicidal activity against the zoonotic nematode <i>A. caninum</i>	The strain showed a nematicidal effect with an $LT_{50}$ of 35.8 h, $LC_{50}$ of $60 \mu\text{g mL}^{-1}$ and an ovicidal effect with an $LC_{50}$ of $94.9 \mu\text{g/ml}$	Dunstand-Guzmán et al. (2020)
		Ovicidal and cestocidal effects on the canine and human parasite <i>D. caninum</i>	The lethal concentration of toxins on eggs was $600 \mu\text{g mL}^{-1}$	Peña et al. (2013)
		Anthelmintic effect against the gill fish trematode <i>C. formosanus</i>	The $LC_{50}$ with solubilized protein was $83.8 \mu\text{g mL}^{-1}$	Mendoza-Estrada et al. (2016)
Biocontrol of spiders	<i>Br kurstaki</i>	Activity against the spider mite <i>E. orientalis</i>	$10 \text{ mg}$ crude <i>Btk</i> toxin completely eliminated the mites from the infested plants within 12 days of treatment	Veloortalpill Narayanan et al. (2018)
	<i>Br</i> 26.4 and <i>Br</i> 13.4	Activity against the spider mite <i>E. orientalis</i>	<i>Br</i> 26.4 ( $LC_{50} = 1.533 \text{ mg mL}^{-1}$ ) and <i>Br</i> 13.4 ( $LC_{50} = 1.385 \text{ mg mL}^{-1}$ ) showed the highest mortalities to adults and larvae	Alahyane et al. (2019)
	GP532	Acaricidal activity against <i>P. cuniculi</i> mites (psoroptic mange) in rabbits	GP532 application resulted in a decreased infestation rate in rabbits by 76.80% at day 30	Dunstand-Guzmán et al. (2017)
Molluscicidal activity	Qalyubia	Molluscicidal activity against <i>B. alexandrina</i>	The strain showed mortality rate from 20% to 100% at five treatment concentrations of 100–500 $\text{mg mL}^{-1}$ . The $LC_{50}$ and $LC_{90}$ values for <i>Qalyubia</i> isolate were 133.27 $\text{mg mL}^{-1}$ and 270.32 $\text{mg mL}^{-1}$ , respectively	Abd El-Ghany et al. (2017)

cyst nematode *H. glycines* (Kahn et al. 2021), the citrus nematode *Tylenchulus semipenetrans* (El-Saedy et al. 2019), and the pine wood nematode *Bursaphelenchus xylophilus* (Huang et al. 2018; Guo et al. 2022). Additionally, it has demonstrated nematocidal activities against zoonotic and animal pathogenic nematodes, including the *Ancylostoma caninum* (Dunstand-Guzmán et al. 2020), the cestode *Dipylidium caninum* and the gill fish trematode *Centrocestus formosanus* (Peña et al. 2013; Mendoza-Estrada et al. 2016). Furthermore, many *Bt* strains have strong antagonistic activities against various plant pathogenic bacteria and fungi. *Bt* strains have been shown to effectively control the bacterial wilt agent, *Ralstonia solanacearum* (Akintokun, et al. 2019) and *Xanthomonas citri* subsp. *citri* (Islam et al. 2019).

*Bt* strains have also demonstrated antagonistic activities against a wide range of plant pathogenic fungi. Among these are the common postharvest spoilage fungi *Fusarium oxysporum* (Vaca et al. 2020), *Botryosphaeria* sp. (causing canker in many crops, ornamental plants, landscape and forest trees and shrubs), *Colletotrichum gloeosporioides* (the agent of postharvest disease of many tropical fruits), and *Penicillium expansum* which causes blue mold in apples and produces mycotoxin patulin (He et al. 2020), gray mold fungus *Botrytis cinerea* (Yoshida et al. 2019), *Verticillium dahliae* and *V. longisporum* (Hollensteiner et al. 2017; Azizoglu et al. 2021), powdery mildew *Erysiphe pulchra* on flowering dogwood (Rotich et al. 2020), *Alternaria* sp, and *Moniliophthora roreri*, the causal agent of cacao disease (Vaca et al. 2020), *F. oxysporum f.sp. cucumerinum* (Akintokun, et al. 2020), *F. oxysporum f.sp. niveum*, *Aspergillus niger* (Azizoglu et al. 2021), Asian soybean rust, (*Phakopsora pachyrhizi*), soybean downy mildew (*Peronospora manshurica*) and powdery mildew (*Microsphaera diffusa*) (Müller et al. 2019), *F. oxysporum f. sp. lycopersici* (Zibanezhadian et al. 2022) and *F. graminearum* (Muddasir et al. 2021).

According to the studies, these antagonistic effects are achieved through the production of various antifungal and antibacterial metabolites, such as surfactin, tryptamine, N-acetyl tryptamine, phenethylamine, phenethyl acetamide, thumolycin, chitinase,  $\beta$ -1,3 glucanase, phytoalexin glyceollin, or by lowering the level of  $H_2O_2$  by increasing transcription of the antioxidant enzymes genes such as superoxide dismutase

(SOD), catalase (CAT), and glutathione S-transferase (GST), and inducing systemic resistance (Table 1).

Previous reports have also shown that *Bt* strains possess acaricidal effects on the soft tick *Argas persicus* (fowl tick or poultry tick), the hard tick *Hyalomma dromedarii* which is a blood feeding ectoparasite infesting camels (Hassanain et al. 1997), the three host black-legged or deer tick, *Ixodes scapularis* (Zhioua et al. 1999), *Acarus siro* (an acarid mite in stored products), *Tyrophagus putrescentiae* (a ubiquitous mite in soil, stored products and house dusts infesting food and causing allergies in people), *Dermatophagoides farinae* (an allergenic mite), *Lepidoglyphus destructor*, producing allergens (Erban et al. 2009), *Varroa destructor*, the most devastating external parasite of honey bees (Alquisira-Ramírez et al. 2014), *Tetranychus macfarlanei* (Neethu et al. 2016), and *T. putrescentiae* (Ahmed et al. 2016).

Recently, some studies have exhibited the acaricidal activity of *Bt* strains against other spiders, including the oriental red spider mite, *Eutetranychus orientalis*, which is a major pest of citrus in many countries (Velooralappil Narayanan et al. 2018; Alahyane et al. 2019) and *Psoroptes cuniculi*, the common ear mite of rabbits (Dunstand-Guzmán et al. 2017) with an  $LC_{50}$  of  $1-10 \text{ mg mL}^{-1}$ . In addition, several *Bt* strains exhibited molluscicidal activity against *Biomphalaria alexandrina* snails, with the  $LC_{50}$  and  $LC_{90}$  values of  $133.27 \text{ mg mL}^{-1}$  and  $270.32 \text{ mg mL}^{-1}$ , respectively (Abd El-Ghany and Abd El-Ghany 2017) (Table 1).

## 2.2 Enhancing PGPR activities and abiotic stress tolerance by *Bt* strains

Plant growth-promoting rhizobacteria (PGPR) are bacteria that have beneficial effects on plant growth. It has been widely documented that some *Bt* strains have the ability to colonize plant roots and promote plant growth development through the production of various phytohormones and beneficial compounds (Salehi Jouzani et al. 2017; Azizoglu 2019). Previously, several researchers have validated PGPR activity of *Bt* strains on different plants such as soybean, field pea and lentils (Mishra et al. 2009). Many *Bt* strains in addition to biopesticide activity, produce several metabolites, such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, indole-3-acetic acid (IAA), proline and phosphate

solubilizing enzymes which enhance plant growth under different abiotic stress conditions including salinity and drought by enhancing antioxidant activities (superoxide dismutase, catalase and ascorbate peroxidase) and reducing plant oxidative damage to lipids (Armada et al. 2015a; 2015b, Ortiz et al. 2015). In addition, some *Bt* strains enhance phosphate solubilization, biostimulation (IAA synthesis) and biocontrol activities through the production of cyanhydric acid, siderophores and 2, 3-butanediol (Cherif-Silini et al. 2016).

Over the past five years, new studies have confirmed previous findings on the PGPR activities of *Bt* as well as evidenced new PGPR activities of *Bt* on some other plants, including tomato (Batista et al. 2021), maize (de Almeida et al. 2021), *Abelmoschus esculentus* (Bandopadhyay 2020), pepper (Jo et al. 2020), radish (Li et al. 2019) and wheat (Delfim et al. 2018, 2020, 2021) through different mechanisms. Furthermore, several *Bt* strains have improved drought and salinity tolerance in various crops by modulating antioxidant metabolism (Kahtani et al. 2021), dehalogenase production (Oyewusi et al. 2021) and synthesis of a bacteriocin, thuricin17 (Subramanian et al. 2021) (Table 2).

### 2.3 Detoxification, biosorption or bioremediation of pollutants and heavy metals by *Bt* strains

The industrial development of human societies around the world resulted in the large-scale production and diffusion of petroleum derivatives, pesticides, herbicides and heavy metals, which are today recognized as the most important risk factors for human health and the environment. When these substances enter the environment, they quickly accumulate in the food chain, posing a threat the higher trophic levels. As a result, detoxification or bioremediation of these hazardous substances using green technologies is critical (Salehi Jouzani et al. 2017).

Over the past five years, different studies have demonstrated how certain *Bt* strains can detoxify or biodegrade a number of these pollutants (Table 3). Among them are the insecticides pyrethroid cypermethrin (Biolli et al. 2021), chlorpyrifos (Ambreen and Yasmin 2021; Al-Zubaidi et al. 2021), triazophos and dimethoate (Ambreen et al. 2020), bendiocarb (Muñoz-Martínez et al. 2021), 3, 5, 6-Trichloro-2-pyridinol (Ambreen and Yasmin 2021) and

quinalphos (Gangireddygarri et al. 2017). The mechanism of pesticides detoxification and biodegradation of *Bt* strains involves the production of different enzymes, such as organophosphorus phosphatases (OPPs) and extracellular acidic and alkaline phosphatases (Ambreen et al. 2020).

Another potential use of *Bt* strains is the detoxification and biodegradation of industrial wastes, which has been successfully demonstrated for a variety of wastes, such as methylene blue dye (Wu et al. 2022), manganese peroxidase (MnP), lignin peroxidase (LiP), and NADH-DCIP reductase, nitrogen compounds (Xu et al. 2021), bisphenol A (Li et al. 2018), benzo (a) pyrene degradation (as one of polycyclic aromatic hydrocarbons (PAHs)) (Lu et al. 2019), anthracene (Tarafdar et al. 2017), phenol (Fu et al. 2022; Ereqat et al. 2018), phenanthrene as a polycyclic aromatic hydrocarbon (Tarafdar et al. 2018) and low-density polyethylene (Ray 2019). Besides that, some *Bt* strains can detoxify various drugs and pharmaceuticals, including ibuprofen (Marchlewicz et al. 2017), erythromycin (Zhou et al. 2018) and naproxen via tetrahydrofolate-dependent O-demethylase activity, as well as salicylic acid via catechol cleavage (Górny et al. 2019a; Dzionek et al. 2020). Another important verified feature of several *Bt* strains is heavy metals bioprecipitation and bioremediation. Bioprecipitation of different metals, through the production of different enzymes, such as organophosphorus phosphatases (OPPs), arsenate reductase, and extracellular acidic or alkaline phosphatases, includes nickel, manganese (Ambreen et al. 2020; Jeevaraj et al. 2022), chromium (Suresh et al. 2021), cadmium (Han et al. 2018; Ambreen et al. 2020; Shah et al. 2020), Pb (Li et al. 2019), europium (Pan et al. 2017), arsenic (Altowayti et al. 2019; Banerjee et al. 2022), mercury (Asare et al. 2018; Saranya et al. 2019) (Table 3). The main mechanisms used in these strains are accumulation, degradation or mineralization of toxic heavy metals.

### 2.4 Anti-cancer and hygiene properties of *Bt* strains

In recent decades, unfortunately, a significant increase in the global prevalence of cancer as one of the most important causes of death has been occurred. The limitations of suitable treatment methods have led to intense interest and curiosity of scientists in search of new and effective treatments.



**Table 2** PGPR activities and enhancing abiotic stress tolerance by *Bt* strains

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
Biofertilizer (PGPR)	RZ2MS9	Auxin production	The shoot dry weight and lateral root length of tomato increased by 24 and 26%, respectively	Batista et al. (2021)
	RZ2MS9	Indoles (IAA) production, colonizing maize endophytically and altering production of volatile organic compounds	It enhanced maize's roots and shoots dry weight around 50% and 80%, respectively at lab and greenhouse levels	de Almeida et al. (2021)
	A5-BRSC	Phosphate solubilizing and phytohormone producing activity, as well as stimulating the growth of potted plants	<i>A. excrucians</i> plants exhibited 68% more protein content in leaves, 70% more catalase and 52% more peroxidase activity, 66% increase in the soluble sugar content, 34% more protein content and more than 75% phosphorus content in pods under field conditions	Bandopadhyay (2020)
	KNU-07	IAA production, siderophore production, phosphate solubilization, and urease activities	The inoculated pepper plants exhibited significant increases in root length (30.7%), shoot length (19.7%), and total dry biomass (30.7%) at lab level	Jo et al. (2020)
Abiotic stress tolerance	HC-2 and BC	Reducing the water-soluble Cd (34–56%) and Pb (31–54%) concentrations and increasing the pH and NH <sub>4</sub> <sup>+</sup> concentration	They increased the dry weight of radish roots (18.4–22.8%) and leaves (37.8–39.9%) and decreased Cd (28–94%) and Pb (22–63%) content in the radish roots under field conditions	Li et al. (2019)
	AG-82	Phosphate solubilizing	Some organic P (Po) and inorganic P (Pi) fractions were achieved and improved P uptake by wheat crops	Delfim et al. (2018, (2020), (2021)
	MH161336	Bt and silicon modulate antioxidant metabolism and improve the physiological traits to confer salt tolerance in lettuce	Leaves number, head weight (g), total yield, relative water content and chlorophyll a, b in stressed lettuce plants were considerably enhanced	Kahtani et al. (2021)
	H2	Enhancing salinity-stability by production of dehalogenase	The production of dehalogenase results in degradation of haloacids, haloacetates, and chloropyrifos under extreme salinity (35% NaCl)	Oyewusi et al. (2021)
	NEB17	Promotion of the growth more effectively under salt stress conditions by producing a bacteriocin, thuricin17 (Th17)	Th17 as a biostimulant or the live bacterial inoculum could be used for crop production under salt conditions	Subramanian et al. (2021)

**Table 3** Detoxification or bioremediation of hazardous materials by *Bt* strains

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
Pesticides detoxification and biodegradation	Berliner	Biodegradation of the pyrethroid cypermethrin (Cyp)	It could result in 83.5% biodegradation of Cyp with the production of 12.0 mg·L <sup>-1</sup> 3-phenoxybenzoic acid after 5 days at lab level. Experiments in soil showed 16.7 and 36.6% degradation of Cyp under biotic (native microbiome) and abiotic conditions	Bioroli et al. (2021)
	MB497	Degrading Chlorpyrifos, Triazophos and Dimethoate by production of organophosphorus phosphatases (OPPs), extracellular acidic and alkaline phosphatases	81–94.6% degradation of pesticides by alkaline OPPs, while 61–70.5% by acidic OPPs	Ambreen et al. (2020)
	Without name	Biodegradation of the insecticide Bendiocarb	The strain immobilized in a biofilm reactor, using fragments of volcanic rock (tezonite) degraded more than 90% of the insecticide with high removal rates	Muñoz-Martínez et al. (2021)
	MB497	Biodegradation of Chlorpyrifos and 3, 5, 6-Trichloro-2-pyridinol	99% degradation of the spiked CPF (200 mg L <sup>-1</sup> ) after 9 days and 90.57% degradation of TCP (28 mg L <sup>-1</sup> ), after 72 h in M-9 broth, soil slurry and soil microcosm	Ambreen and Yasmin (2021)
Detoxification and biodegradation of industrial waste	<i>Bti</i>	Biodegradation of chlorpyrifos pesticide using silver bio-nanoparticles produced by <i>Bt</i>	Silver bio-nanoparticles from <i>Bti</i> extracts lead to biodegradation of chlorpyrifos completely without forming harmful products	Al-Zubaidi et al. (2021)
	OPI	Biodegradation of quinalphos	The strain grew on quinalphos with a generation time of 28.38 min or 0.473 h in logarithmic phase. Maximum degradation of quinalphos was observed with an inoculum of 1.0 OD, an optimum pH (6.5–7.5), and an optimum temperature of 35–37°C	Gangireddygarri et al. (2017)
	F5	Decolorization and biodegradation of methylene blue dye (MB) by production of laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP), and NADH-DCIP reductase	Decolorization rate reached 95% after 12 h and the phytotoxicity of MB degraded metabolites was significantly lower than that of the parent compound	Wu et al. (2022)
	WXXN-23	Biological nitrogen removal from wastewater by a novel heterotrophic nitrification and aerobic denitrification (HNAD) pathway	The strain was effective at wastewater treatment, with TN, NH <sub>4</sub> <sup>+</sup> -N, NO <sub>3</sub> <sup>-</sup> -N and NO <sub>2</sub> <sup>-</sup> -N removal efficiencies of 82.12, 86.74, 90.74 and 100%, respectively	Xu et al. (2021)
	GIMCC1.817	Bisphenol A (BPA) degradation	The degradation efficiency of 1 μM of BPA by 1 g L <sup>-1</sup> of <i>Bt</i> was up to 85% after 24 h	Li et al. (2018)
GIMCC1.817	Benzo (a) pyrene degradation (as one of polycyclic aromatic hydrocarbons (PAHs)) by cytochrome P450 hydroxylase	Biosorption and degradation of benzo (a) pyrene efficiencies were approximately 90 and 80%, respectively	Lu et al. (2019)	
AT. ISM. 1	Biodegradation of anthracene	The degradation efficiency of the strain has been estimated to be around 91% (for 40 mg L <sup>-1</sup> of anthracene concentration) after 2 weeks of incubation at 33–36°C and initial pH of 6.8–7	Tarafdar et al. (2017)	

Table 3 (continued)

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
	A1	Phenol degradation	The removal efficiency of phenol (increased from 1.56% to 13.78%) by the <i>A. calamus</i> plant and <i>Bt</i> A1 consortium was higher than that of the independent <i>A. calamus</i> system	Fu et al. (2022)
	J20	Phenol degradation	Phenol degradation efficiency of the free and immobilized cells of J20 was about 88.6 and 100% versus of 700 mg L <sup>-1</sup> of phenol in 120 h	Ereqat et al. (2018)
	AT.ISM.11	Adsorption-synergic biodegradation of a polycyclic aromatic hydrocarbon (phenanthrene, Phe)	The strain and application of buckypaper as a bio-carrier enhanced Phe biodegradation of 93.81%	Tarafdar et al. (2018)
	Without name	Biodegradation of low-density polyethylene (LDPE)	Co-culture of the <i>Bt</i> and <i>L. sphaericus</i> strains could significantly degrade LDPE	Ray (2019)
Drug detoxification	B1 (2015b)	Naproxen biodegradation by production of tetrahydrofolate-dependent O-demethylase activity and salicylic acid via catechol cleavage	The strain could significantly degrade naproxen and could be applied in water and wastewater treatment systems that have been contaminated with non-steroidal anti-inflammatory drugs	Górny et al. (2019b)
	B1 (2015b)	Biodegradation of ibuprofen	The maximum specific ibuprofen removal rate and the value of the half-saturation constant were $q_{max} = 0.24 \pm 0.02$ mg mL <sup>-1</sup> h <sup>-1</sup> and $K_s = 2.12 \pm 0.56$ mg L <sup>-1</sup> , respectively	Marchlewicz, et al. (2017)
	GIMCC1.817	Biodegradation of erythromycin	The strain could effectively remove 77–86% and degrade 53% of 1 $\mu$ M erythromycin within 24 h	Zhou et al. (2018)
	B1 (2015b)	Degradation of naproxen by immobilization of <i>Bt</i> B1 (2015b) on loofah sponge	The immobilized cells degraded naproxen (1 mg L <sup>-1</sup> ) faster in the presence of autochthonous microflora than in a monoculture trickling filter	Dzionek et al. (2020)
Bio-precipitation and bioremediation of heavy metals	GL-1	Phosphate solubilizing	The strain could use Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> , FePO <sub>4</sub> , and AlPO <sub>4</sub> as the sole P resource and significantly increase water-soluble phosphate concentrations. Pot experiment found that inoculation decreased soil Pb phyto-availability and consequently inhibited its uptake by lettuce	Liu et al. (2021)
	MB497	Bio-precipitation of selected metals (Ni, Mn, Cr and Cd) by production of organophosphorus phosphatases (OPPs), extracellular acidic and alkaline phosphatases	Both acidic and alkaline OPPs were capable of bio-precipitation of selected metals (Ni, Mn, Cr and Cd) up to 86–100%	Ambreen et al. (2020)
	HZM7	Biosorption and extraction of europium	The bacteria had high adsorption capacity of Eu <sup>3+</sup> (achieves as high as 160 mg g <sup>-1</sup> ) and the adsorbed Eu <sup>3+</sup> could be desorbed by HCl or EDTA solution	Pan et al. (2017)
	WS3	The adsorptive removal of As (III) using biomass of arsenic resistant <i>Bt</i> strain	The maximal As (III) loading capacity was determined as 10.94 mg g <sup>-1</sup>	Altowayti et al. (2019)

Table 3 (continued)

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
	IAGS 199	Alleviating cadmium-induced phytotoxicity in <i>C. annuum</i>	Application of <i>Bt</i> putrescine established a synergistic role in the mitigation of Cd-induced stress through modulating physicochemical features of <i>C. annuum</i> plants	Shah et al. (2020)
	X30	Reducing heavy metal accumulation	The strain reduced the edible tissue Cd and Pb uptake through decreasing Cd and Pb availability in the soil and increasing Cd or Pb translocation from the roots to the leaves of the radish	Han et al. (2018)
	KPWPI	Bioremediation of arsenic by production of arsenate reductase	The strain contained <i>arsC</i> , <i>arsB</i> and <i>arsR</i> genes involved in arsenate tolerance. The inducible <i>arsC</i> gene was cloned, expressed and the purified ArsC protein showed profound enzyme activity with the KM and Kcat values as 25 $\mu\text{M}$ and 0.00119 $\text{s}^{-1}$ , respectively	Banerjee et al. (2022)
	CASKS3	Biosorption of mercury	The strain showed adsorption ability of 38–62 at 800 $\text{mg}^{-1}$ to 200 ppm	Saranya et al. (2019)
	V45	Bioremediation of hexavalent chromium-contaminated wastewater	The minimum tolerance of chromium was found up to 1000 $\mu\text{g mL}^{-1}$ . V45 could tolerate Cr (VI) (520 $\mu\text{g mL}^{-1}$ ). Similarly, it was able to tolerate other metals such as $\text{Hg}^{2+}$ (40 $\mu\text{g mL}^{-1}$ ), $\text{Cu}^{2+}$ (30 $\mu\text{g mL}^{-1}$ ), $\text{Ni}^{2+}$ (60 $\mu\text{g mL}^{-1}$ ), $\text{Zn}^{2+}$ (40 $\mu\text{g mL}^{-1}$ ), and $\text{Pb}^{2+}$ (30 $\mu\text{g mL}^{-1}$ ). It could decrease Cr(VI) at a primary concentration of 50 $\mu\text{g mL}^{-1}$ up to 86.42%	Suresh et al. (2021)
	HC-2	Cd and Pb accumulation of radish in a heavy metal-contaminated farmland under field conditions	The strain and biochar significantly increased the dry weight of radish roots (18.4–22.8%) and leaves (37.8–39.9%) and decreased Cd (28–94%) and Pb (22–63%) content in the radish roots compared with the control	Li et al. (2019)
	C20	Multi metal tolerance	C20 was tolerant to heavy metals (cadmium, nickel and lead) and survived in heavy metal mixture fortified medium with high recovery rate and increased colony forming units	Jeevaraj et al. (2022)
	MC28	Bio sorbent for mercury in groundwater from some selected gold mining communities	At optimal conditions of the strain, contact time of six days and 35 °C, 99.94–99.98% of mercury in groundwater samples were removed	Asare et al. (2018)

Cry proteins without insecticidal activity are known as parasporins (PSs). Cry31A (parasporin-1 (PS1)), Cry41A (parasporin-3 (PS3)), Cry45A (parasporin-4 (PS4)), Cry46A (parasporin-2 (PS2)), Cry63A (parasporin-6 (PS6)) and Cry64A (parasporin-5 (PS5)) are the most known PSs. Parasporins after digestion by proteases show effective cytotoxic activity against human cancer cells, but interestingly they do not affect normal cells. Up to now, 19 different parasporins grouped into six subclasses (PS1, PS2, PS3, PS4, PS5, and PS6) based on their amino acid sequence homology have been identified (<https://www.fitc.pref.fukuoka.jp/parasporin/index.htm> (accessed June 30, 2022)).

The antitumor activities of the parasporins have been reported against human cervical cancer cells (HeLa), SiHa, murine lymphoma L5178YR cell line, human leukemia T cells (MOLT-4), CEM-SS, human uterus endometrium adenocarcinoma cell lines Hec-1A and KLE, myeloid leukemia cells (HL60) and liver (hepatocyte) cancer cell (HepG2), human epithelial colorectal adenocarcinoma cell line (CACO-2), endometrial adenocarcinoma (Sawano), adherent human colon cancer cells (HT-29), HCT-250, HCT 116 and SW620, human prostate cancer cell line (PC-3), human histiocytic lymphoma (U-937), human breast cancer cell lines (MCF-7 and MDA-MB231) and Jurkat cells (Salehi Jouzani et al. 2017).

Recent discoveries over the last five years have increased hopes for practical applications of *Bt* strains in cancer treatment. Moazamian et al. (2018) demonstrated that *Bt kurstaki* and *sotto* had cytotoxic activities against human colon and blood cancer cells via parasporin synthesis. Aberkane et al. (2020) confirmed the antitumor activity of the native Algerian *Bt* strain BDzG against laryngeal and alveolar cancers by production of parasporins. Other scientists have shown anti-cancer activities of *Bt* strains against breast cancer cells (Borin et al. 2021; Melo and Kitada 2020), as well as against human leukemic cells (Oktay et al. 2018; Beena et al. 2019) (Table 4). Recently, it has been confirmed that RNases secreted by some *Bt* strains have antiviral activity against Human Influenza Virus A/Aichi/2/68 (H3N2). In experiments with mice infected with H3N2, preparations provided reliable protection for infected animals comparable to that of the reference drug Tamiflu (Andreeva et al. 2020).

## 2.5 *Bt* as source for production of nano-and biomaterials

Metal nanoparticles with advanced physicochemical properties have found widespread applications in a variety of industrial sectors during the last few decades. Different plants, algae, fungi, bacteria and other biological-based products have been employed to biosynthesize different metal nanoparticles. Biosynthesis of silver (Khaleghi et al. 2019), TiO<sub>2</sub> (Jalali et al. 2020) and gold (Patil et al. 2018) nanoparticles by *Bt* strains has been recently described. Production of other biomaterials, such as vitamin B complex by hydrolyzing hair waste (Hassan et al. 2020), bioplastic (Polyhydroxyalkanoate (PHA)) (Singh et al. 2021), PHB-HV copolymer (Ponnusamy et al. 2019), chitoooligosaccharides (Santos-Moriano et al. 2018), melanin (Cao et al. 2018) and  $\alpha$ -amylase enzyme (Al-Khafaji et al. 2021) using various *Bt* have all been thoroughly investigated. Additionally, *Bt* has been also utilized to design a biofilter containing *Bt*-inoculated beads to remove trimethylamine (TMA). This compound is a toxic and odorous pollutant which is generally produced in different manufacturing processes (Santawee et al. 2019). Moreover, inoculation of a *Bt* strain in *E. cordifolius* plant-microbial fuel cell could enhance the electric power output from such microbial fuel cell (Treesubuntorn et al. 2019; Treesubuntorn and Thiravetyan 2021) (Table 5).

## 3 The success of *Bacillus thuringiensis* in the biological control

The mode of action of *Bt* and its narrow spectrum toxicity against a number of insect pests have made it an effective and successful bioinsecticide. Aside this significant benefit, *Bt* is a natural insecticide that is harmless for non-target organisms and leaves no polluting residues behind. This bacteria sporulates at the same time it produces the crystal proteins, making it simple to formulate the bioinsecticide as a spore-crystal complex, which is less expensive to achieve than preparations containing the crystal alone. (Radhakrishnan et al. 2017). *Bt* products must be safe, remain effective and have a long shelf life. To achieve this goal Cry proteins and spores are formulated with some protecting agents. These protectants should be as environmentally safe as possible

**Table 4** Anti-cancer and anti-viral activities of *Bt* strains

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
Anti-cancer	<i>Bt kurstaki</i> and <i>Bt sotto</i>	Cytolytic activities against toward human colon and blood cancer cells via parasporin production	High cytolytic activities against cancer cell lines	Moazamian et al. (2018)
	KAU 41	Inducing apoptosis in cancer cells through intrinsic pathway	The strain produces non-hemolytic homogenous crystals showing specific cytotoxicity towards cancer cells. HeLa cells were highly susceptible to this peptide with IC50 1 $\mu\text{g mL}^{-1}$ and showed characteristics of apoptosis	Chubicka et al. (2018)
	Native Algerian <i>Bt</i> strain BDzGs	Activity against laryngeal and alveolar cancers by production of parasporins	High cytotoxic activity against human laryngeal carcinoma, was observed with an IC50 equal to 2.33 $\mu\text{g mL}^{-1}$ , while moderate cytotoxicity against adenocarcinomic human alveolar basal epithelial (A549) cells has been shown with IC50 equal to 18.54 $\mu\text{g mL}^{-1}$	Aberkane et al. (2020)
	RSK CAS4	Anticancer activity of extracellular polysaccharides	Extracellular polysaccharide inhibited the growth of cancer cells in a dose-dependent manner and maximum anticancer activity was found to be 76% against liver cancer at 1000 $\mu\text{g mL}^{-1}$	Ramamoorthy et al. (2018)
	A13	Inducing cytotoxic effect by late apoptosis against breast cancer cells by producing parasporin A13-2	The protein A13-2 showed the highest cytotoxic activity against breast cancer cell line MCF-7 (13% cell viability at 6 $\mu\text{g mL}^{-1}$ )	Borin et al. (2021)
	KAU 59	Inducing cell cycle arrest and apoptotic cell death in human leukemic cells	The parasporin isolated from the strain KAU 59 causes S phase cell cycle arrest and apoptotic cell death in human lymphocytic leukemia cell line Jurkat	Beena et al. (2019)
	A14d2	Producing a parasporin with cytotoxic activity against MCF-7 breast cancer cells	The A14d2 strain parasporin was efficient with an LD <sub>50</sub> of 14.83 $\mu\text{g mL}^{-1}$ and a protein concentration of 520 $\mu\text{g mL}^{-1}$	Melo and Kitada (2020)
	<i>Bt-Ba14</i>	Cytotoxicity against human cancer cell lines	Parasporal protein from <i>Bt-Ba14</i> exhibited the highest cytotoxicity against all three cancer cells, HeLa, PC-3 and A549	Oktaş et al. (2018)
Anti-viral	Gi-443, Gi-466, and Cb-527	Antimicrobial activity of RNases contained enzymes produced by the <i>Bt</i> strain against Human Influenza Virus A/Aichi/2/68 (H3N2)	In experiments on mice infected with 10 LD50 influenza virus strain A/Aichi/2/68 (H3N2), the effective variants of preparations based on culture fluid of <i>Bt</i> strains were selected for preventive administration that provided reliable protection of infected animals, close to that of the reference drug Tamiflu	Andreeva et al. (2020)

**Table 5** *Bt* as source for production of nano-and bio-materials

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
Nanoparticles biosynthesis	GL22	Biosynthesis of silver nanoparticles	The spherical biosynthesized silver nanoparticles with a 42 nm average size have good antibacterial properties at low concentrations (MIC=6.25–12.5 µg mL <sup>-1</sup> ). The highest rate of bacterial biofilm degeneration (> 90%) was observed at 6 µg mL <sup>-1</sup> concentration	Khaleghi et al. (2019)
	<i>Bt kurstaki</i> KD-2	Biosynthesis of different polymorphs of TiO <sub>2</sub> nanoparticles	TiO <sub>2</sub> nanoparticles produced by the strain could significantly protect <i>Bt</i> formulations from Ultra Violet radiations	Jalali et al. (2020)
	<i>Bt</i> SV2 (JN315886)	Biosynthesis of gold nanoparticles (GNPs)	The strain could effectively produce GNPs. GNPs could enhance protection of <i>Bt</i> formulation from sunlight irradiation, and also enhanced activity against <i>Ae. aegypti</i> (23.10%) and <i>An. subpictus</i> (27.24%)	Patil et al. (2018)
Other industrial applications	MT1	Valorization by hydrolyzing hair waste using keratinase enzymes produced by <i>Bt</i> and exploitation of the keratinous hydrolysate to produce vitamin B complex	Donkey hairs were treated with metallokeratinase, emancipating eight essential and eight more non-essential amino acids, which were subsequently utilized by <i>Saccharomyces cerevisiae</i> strain ATCC 64,712, to produce vitamin B-complex	Hassan et al. (2020)
	DF7 (Accession no. KC020161)	Bioplastic (Polyhydroxyalkanoate (PHA)) production	After fermentation process optimization, the cell biomass of 20.99 g L <sup>-1</sup> , PHA concentration of 14.28 g L <sup>-1</sup> , PHA yield of 68.03%, and PHA productivity of 0.219 g L <sup>-1</sup> h <sup>-1</sup> was achieved	Singh et al. (2021)
	CP010577.1	Increasing of electricity production from <i>E. cordifolius</i> -microbial fuel cell by inoculating <i>Bt</i>	<i>Bt</i> effectively enhanced the maximum power density, producing about 20–35 mW m <sup>-2</sup> of maximum power density, which maintained up to 120 days	Treesubstorn and Thiravetyan (2021), Treesubstorn et al. (2019)
	<i>Bt</i> aizawai	Efficient conversion of chitosan into chitooligosaccharides by a chitosanolytic activity from <i>Bt</i>	A 10 g L <sup>-1</sup> solution of chitosan with 600–800 kDa enzyme derived from <i>Bt</i> was fully converted into oligosaccharides in 55 h	Santos-Moriano et al. (2018)
	E101	Production of PHB-HV copolymer	Both qualitative (FTIR and NMR) and quantitative (GC-MS) characterizations revealed that the polymer produced by <i>Bt</i> E101 contains poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer	Ponnusamy et al. (2019)

**Table 5** (continued)

Type of new applications	The studied <i>Bt</i> strains	Specific application and mode of action	Efficiency	Reference
	L-7601	High production of melanin	High production of melanin	Cao et al. (2018)
	Not mentioned	Inoculation of coir pith–glucose syrup beads as a packing material in trimethylamine (fishy odor) biofilter	The pilot-scale biofilter containing <i>Bt</i> -inoculated coir pith–glucose syrup beads could remove 72.52 mg m <sup>-3</sup> of gaseous trimethylamine (100%) within 1 h	Santawee et al. (2019)
	KS4	α-amylase enzyme production	10% corn medium with 35 °C incubation temperature resulted in the highest amylase production by the strain (16.39 unit mL <sup>-1</sup> )	Al-Khafaji et al. (2021)
	A102	PHA (SCL-SCL) copolymer production	The strain grown in optimized RC medium yielded PHA of about 3.25 g L <sup>-1</sup>	Suguna et al. (2020)

while still being safe for usage by avoiding chemical compounds.

Since *Bt* products entered the market they have faced some challenges and difficulties. First, *Bt* spray cannot be distributed uniformly to all parts of the plant and far less inside the plant tissues. Furthermore, when is applied as spray formulation on plant crops *Bt* is easily degraded by UV light and can be removed by water runoff. As a result, multiple pesticide treatments need be administered to crops in order to provide the necessary insect protection. It is also important to emphasize that early instar larvae are affected by *Bt* since older larvae are more tolerant. For the aforementioned reasons, *Bt*-based formulation persists for only a few days on the leaf surface, having thus a limited efficacy (Gao et al. 2018). These problems have limited the market for *Bt* biopesticides, and thus several solutions have been developed to address these issues (Jallouli et al. 2020).

As mentioned one of the key issues is that formulations based on *Bt* are easily degraded by UV. This problem was initially resolved by adding to the spore-crystal preparations chemical screens. However, the utilization of these chemical screens has certain detrimental environmental consequences. Therefore, another option to protect these formulations from UV damage was to encapsulate the Cry proteins into the bacterium *P. fluorescens* since crystal proteins are even more vulnerable to degradation than the spores. However, this encapsulation does not ensure that the bacteria can persist in soil or water for as long as *Bt* spores (Sanahuja et al. 2011). Another strategy involves the use of melanin, a fully natural photoprotective agent, that is easily biodegradable in nature and capable of absorbing radiations, and that has been utilized to protect *Bt* against UV damage (Sansinenea and Ortiz 2015; Sansinenea et al. 2015).

Another important concern with *Bt* biopesticides used as sprays is their restricted field application, as spray applications are mainly used to treat the aerial parts of the plant (essentially the leaves) rather than the entire crop. This problem has been solved by employing GM-*Bt* crops which are been genetically modified by introducing a *Bt* gene, allowing the plant to expresses a Cry toxin in all the parts of the plant. The use of GM-*Bt* crops was a very controversial issue, with much debate around the introduction of this type of due to the possible risk to human health from ingesting these types of foods (Krogh et al.



2020). Several studies have been conducted to assess the risks associated with environmental safety and human health, most concluding that the use of GM-*Bt* crops is safe and associated with health cost savings (Kouser et al. 2019). Over the past years, despite all the controversies, GM-*Bt* crops have gained prominence and have been widely used. However, because of the high cost for generating GM-*Bt* crops, environmentally-friendly and inexpensive sprayable formulations still have a great potential for agricultural use (Singh et al. 2019).

Currently, *Bt*-based formulations, including Dipel®, Javelin®, Thuricide®, and Bactospeine® are used and marketed to control several insect pests of different order such as, lepidopteran. The *Bt* subsp, *israelensis*, which is commercially available under the trade names of VectoBac®, Teknar®, and Bac-timos®, is also particularly important because it is largely used to control dipteran insects which are vectors of tropical diseases like malaria, dengue fever, Nile virus, Zika virus, and Chikungunya virus (Derua et al. 2018). Commercially and currently used *Bt* based-biopesticides developed are listed in Table 6.

Despite the problems that *Bt*-based biopesticides have had to solve, they are the most commonly used biocontrol agents in the world (Kumar et al. 2021) and its formulations have been applied, over the years, to multiple crops to protect them, with remarkable success, against a high number of insect pests (do Nascimento et al. 2022).

#### 4 Advances in biotechnology of *Bacillus thuringiensis*

Natural *Bt* strains have been employed in the first generation of *Bt*-based bioinsecticides. However, when more than one main insect pest is targeted per crop, these *Bt* products often have a too narrow range of activity and their persistence in the field is also deemed to be too poor, due to solar radiation and environmental degradation of their insecticidal toxins by soil microorganisms (Karabörklü et al. 2018). Furthermore, when applied as sprays, these biological products are ineffective in preventing insects from attacking the plant's roots or internal parts (Sanahuja et al. 2011). These limitations sparked interest in developing new recombinant *Bt* insecticides with a broader insect spectrum, improved environmental

stability, and more efficient toxin delivery to control agricultural pests (Karabörklü et al. 2018). Wild strains are given novel insecticidal capabilities, utilizing a variety of molecular, biological, and biotechnological strategies, which helped to tackle the problem of narrow insect pest specificity (Azizoglu and Karabörklü 2021). Research first focused on producing novel *Bt* strains, either through conjugation or recombination, with the aim of expanding the set of toxins they produce and creating new toxin combinations.

##### 4.1 Expression of *Bt* cry genes in recombinant heterologous microbial hosts or non-sporulating *Bt*

Various strategies have been adopted to protect *Bt* insecticidal properties from environmental degradation and maintain their insecticidal activity for as long as feasible after being released into the environment (Sanchis 2012). To increase the persistence of Cry toxins in the field, researchers developed recombinant *P. fluorescens* cells (Mycogen Corp.'s Cell-cap® encapsulation process) or GM-asporogenic *Bt* strains in which the mother cell lysis is completely blocked without affecting insecticidal crystal protein production (Lereclus et al. 1995; Sanchis et al. 1999; Quan et al. 2020). In both cases, the crystal proteins are protected against deactivation by UV light and environmental degradation by encapsulating them in *P. fluorescens* or asporogenic *Bt* ghost cells (Quan et al. 2020). This also has the advantage, in the case of non-sporulating *Bt* strains, of releasing the GM-microorganisms into non-viable environment, thus minimizing the environmental impact caused by the dispersion of large quantities of viable spores. Some researchers have also documented a synergistic effect of chitinases, involved in peritrophic membrane degradation, and Cry proteins (Karabörklü et al. 2018; Martínez-Zavala et al. 2020).

As an illustration, a *Bt* subsp, *israelensis* (*Bti*) strain was engineered to produce inclusions of ChiA74Δsp, an endochitinase of *Bt* lacking its secretion signal peptide. This recombinant strain formed parasporal bodies made up of Cry4Aa, Cry4Ba, Cry11Aa, Cyt1Aa, and ChiA74sp inclusions and was two times more toxic than the parental *Bti* against *A. aegypti* (Juárez-Hernández et al. 2015). The ChiA74sp was also expressed in a *Bt* HD73 strain that naturally contains a Cry1Ac; as a result, the Cry1Ac

**Table 6** Commercial *Bt*-based insecticides currently in use

Microorganism	Action	Brand name	Producer company
<i>B. thuringiensis</i> var, <i>aizawai</i>	Insecticide	XenTari	Valent BioSciences
		Agree	Certis
		Turex	
<i>B. thuringiensis</i> var, <i>israelensis</i>	Insecticide	Solbit	Green Biotech, Korea
		Bactimos	Valent BioSciences
		Teknar	
		VectoBac	
		VectoMax	
		Aquabac	Becker Microbial
		Bacticide	Biotech Int'l
<i>B. thuringiensis</i> var, <i>kurstaki</i>	Insecticide	BTI granules	Clarke Mos. Cont
		Dipel	Valent BioSciences
		Foray	
		Cordalene	Agrichem
		Lipel Sp	Som Phytopharma
		Lipel	Agri Life
		Biolep	Biotech international
		BMP 123	Becker microbial
		Baturad	Agrindustrial S.A
		Belthirul	Probelte S.A
		Deliver	Certis
		Delfin	
		Condor	
		Crymax	
		Javelin WG	
		Lepinox WG	
		Turex	
Turicide			
Safer BTK	Woodstream Canada		
Rapax	Ecogen/Intrachem		
Lepinox plus			
<i>B. thuringiensis</i> var, <i>tenebrionis</i>	Insecticide	Novodor	Valent BioSciences

was more toxic to *S. frugiperda*, one of the major insect pest that is less susceptible to Cry1A proteins (González-Ponce et al. 2017).

#### 4.2 Construction of mutagenized toxins or protein chimaeras

Mutagenesis of Cry toxins is originally employed to investigate the role of certain Cry toxin domains/regions in the mechanism of toxicity, with a few of those changes leading to Cry improved toxin molecules (Deist et al. 2014). This knowledge is used to edit *cry* genes through site-directed mutagenesis of

certain amino acids, resulting in novel toxins with a broader target spectrum or increased toxicity to specific pests (Gatehouse 2008; Vílchez 2020). Other specific manipulations in *Bt*-based products included the introduction of a chymotrypsin cleavage site into the Cry3A toxin, which resulted in improved Cry3A protoxin activation and increased activity against the western corn rootworm (*Diabrotica virgifera virgifera*), a very important maize pest, that was mainly insensitive to the parental non modified Cry3A toxin (Walters et al. 2008). Similarly, a designed Cry1A-bMod toxin lacking Cry1Ab helix-1 has been found to be efficacious against a resistant population of a

bollworm (*Pectinophora gossypiella*), whose resistance is linked to a cadherin receptor gene deletion. By bypassing the absence of cadherin receptor in *P. gossypiella*, the Cry1AbMod toxin was able to kill insects that developed resistance to the toxin Cry1Ab as a result of receptor modification (Muñoz-Garay et al. 2009). Finally, it was shown that this deletion enhanced the oligomerization of these toxins in solution in the absence of cadherin, and that Cry1AMod toxins could also counteract resistance in various other insect species where resistance was not associated with cadherin mutations. It was also demonstrated that the Cry1AbMod provided little or no advantage against some resistant strains of other pests with altered cadherin genes (Tabashnik et al. 2011), implying that improved oligomerization was the key element that was responsible for the higher potency of the modified toxin.

For the past two decades, scientists have attempted to create hybrid (or chimaeric) Cry proteins with varied degrees of toxicity and binding specificity and/or affinity to their target insect pests. Many hybrid toxins have been created by fusing or substituting Cry toxin domains (mostly Cry toxin domains II and III) from two or more different Cry proteins (Pardo-López et al. 2009). Ecogen created and inserted the Cry1Ac/1F chimeric toxin into the *Bt* strain EG7826, which is more toxic to the fall armyworm (*S. frugiperda*) than the parental Cry1Ac and Cry1F toxins. This strain was approved as the active ingredient in Lepinox™, a biological pesticide. Similarly, a chimeric Cry1C/Ab toxin consisting of a modified Cry1C toxin with first 731 amino acids of Cry1C (domains I and II) and last 432 amino acids (domain III) of Cry1Ab exhibited improved efficacy against Egyptian cotton leaf worm (*S. littoralis*) and lesser beet armyworm (*S. exigua*) pests (Sanchis et al. 1999). Walters and colleagues have also developed a hybrid Cry1Ab/Cry3A toxin that is toxic to the western corn rootworm, which is unaffected by either parent toxin (Walters et al. 2010). Similar outcomes were obtained by fusing the 600 amino acid residues at the N-terminus of Vip3Ac1 and the 189 amino acid residues at the C-terminus of Vip3Aa1, creating a new chimeric Vip3 protein, called Vip3AcAa, with a wider insecticidal spectrum for lepidopteran insect pests (Chen et al. 2017a). More recently, two new chimeric Cry1A.2 and Cry1B.2 proteins containing elements from three different Cry1 proteins, by exchanging domains.

Cry1A.2 and Cry1B.2 differ in receptor utilization from one another and have also distinct receptor usage patterns than other commercially available *Bt* proteins such as Cry1Ac, Cry1A.105, Cry1F.842, Cry2Ab2, and Vip3A, that were also described (Chen et al. 2021).

#### 4.3 The use of *Bt* Cry toxins in genetically modified crops

Bacteria that produce *Bt* toxins are non-systemic insecticides, meaning they have no impact on insects that do not come into direct contact with the insecticidal spore and crystals preparations (which includes sap sucking and piercing insects, fruit borers and tunneling and root-feeding pests etc.). Most of these constraints have now be addressed while minimizing non-target fauna exposure by genetically modifying important agricultural plants to express Cry and other *Bt*-insecticidal genes throughout the growing season.

Corn producing Cry1Ab and cotton producing Cry1Ac were the first genetically modified (GM) *Bt* crops to produce only one *Bt* toxin. The principal strategy for delaying insect resistance development for these first generation *Bt* crops that only expressed one Cry toxin in each plant, was to plant refuges of non-*Bt* crops near *Bt* crops in the field. Resistance management can be aided by combining multiple *Bt* genes, active against the same set of target pests but recognizing distinct midgut receptors (gene pyramiding). Commercial Bollgard II cotton containing two *Bt* toxins, Cry1Ac and Cry2Ab, which target the same lepidopteran pests in two different ways was developed (Liu et al. 2022). The issue of the Cry toxins' narrow insect pest specificity, which prevents the toxins present in a given transgenic plant from effectively controlling all of the major pests that attack a crop, was addressed by expressing multiple *Bt* toxins active against different target insect pests in the same GM-*Bt* crop; Monsanto's YieldGard Plus maize expressing in addition to a lepidopteran active *cry1Ab1*, a synthetic variant of the *cry3Bb1* gene providing resistance against coleopteran corn borer pest exemplifies this gene stacking strategy. Similarly, GM-*Bt* crops modified to express lepidopteran-active Vip3 proteins, which have no homology in sequence or binding locations with Cry proteins (Adamczyk et al. 2008; Syed et al. 2020), alongside other lepidopteran-active Cry proteins were the following generation of GM-*Bt*

plants that were developed to manage resistant pests and delay resistance evolution (Carrière et al. 2015). For example, when the chimeric *vip3AcAa* gene and the *cry1Ac* gene were introduced into cotton, the resulting transgenic cotton line displayed increased toxicity against pests that were resistant or mildly tolerant to Cry1A proteins (Chen et al 2017b). In addition, when the *cry1Ac*, *cry2Ab*, and *vip3Aa* genes were combined in the same plant, like in Bollgard® III GM-*Bt*-cotton, the toxicity against some target pests was also significantly increased. Combining *Bt cry* gene expression with other non-*Bt* insecticidal techniques, such as RNA interference (RNAi), aimed at silencing expression of selected genes implicated in insect immune-related pathways, has also recently been examined as a new possible and alternative strategy for crop protection against insect pests (Liu et al. 2020; Paddock et al. 2021).

Ni and colleagues created a transgenic cotton pyramid that combined a *Bt* toxin with double stranded RNA (dsRNA) designed to interfere with the metabolism of *H. armigera* juvenile hormone. The scientists found that the synergistic action of *Bt* toxins and RNAi(s) could delay or prevent the emergence of resistance in cotton pests (Ni et al. 2017). The SmartStax® PRO maize, which expresses a double-stranded hairpin RNA (dsRNA) targeting the *DvSnf7* gene (*Diabrotica virgifera* (Dv)+sucrose-non-fermenting (*snf*) locus) a putative homolog of yeast *Snf7*, and an ortholog of the *Drosophila vps32/Snf7* protein involved in intracellular protein trafficking, is another example of this technology. *Snf7* is a class E vacuolar sorting proteins conserved in many organisms including humans (*hSnf7*), mouse (*mSnf7*), fruit fly *Drosophila* (*Shrub*), *Diabrotica virgifera* (*DvSnf7*), responsible for internalizing, transporting, sorting, and degradation of transmembrane proteins (Bolognesi et al. 2012). Knockdown of this *Snf7* ortholog in western corn rootworm (WCR) increases the accumulation of damaged organelles, misfolded proteins, and toxic compounds, negatively affecting cell physiology and homeostasis. This disruption inhibits larval growth and development, leading to WCR death (Reinders et al. 2022). In another study, Baum et al. (2007) characterized 125 genes whose silencing resulted in considerable WCR mortality. Fourteen of these genes caused mortality in 50% of WCR at doses less than 5.2 ng dsRNA cm<sup>-2</sup> of the artificial diet (Paddock et al. 2021). These genes

include putative *V-ATPase* A and D subunits, *ESCRT I Vps28*, *III Vps2* and *III Snf* orthologs, a  $\beta$ -subunit of a *COPI* capomer, ribosomal proteins, a proteasome ortholog,  $\alpha$ -actin, tubulin, and an *RNA polymerase II* ortholog (Baum et al. 2007; Paddock et al. 2021).

This product expresses this dsRNA together with three corn rootworm-active *Bt* proteins (Cry3Bb1 and Cry34Ab1/Cry35Ab1), as well as three lepidopteran-active *Bt* proteins (Cry1A.105, Cry2Ab2, and Cry1F) (Head et al. 2017). The toxicity of *Bt* against *Bombyx mori* (Lepidoptera: Bombycidae) and its reproduction in insect cadavers were both significantly increased by RNA interference-mediated knockdown of a *B. mori* hemocyte-specific cathepsin (*Cat L*)-like cysteine protease gene, possibly due to its role in the degradation of engulfed bacteria in haemocyte lysosomes (Yang et al. 2022). Therefore, this gene is also a potential target for future pest control methods that aim to improve the insecticidal potency of *Bt*-based biopesticides.

## 5 Ecological impact of *Bacillus thuringiensis* toxins

### 5.1 Possible impacts of recombinant *Bt* toxins and GM-*Bt* crops on natural enemies

Although *Bt* is a unique pathogen used for insect pest control worldwide (Azizoglu et al. 2016; Azizoglu et al. 2019), and is considered as safe for non-target organisms due to its high host-specificity, there is growing evidence of unintended effects of its use to beneficial insects (Azizoglu et al. 2020; Belousova et al. 2021). Potential risks of recombinant *Bt* strains and GM-*Bt* crops to the environment include their impact on non-target beneficial species such as parasitoids and predators (Azizoglu et al. 2020). However, scientists have conducted intensive research to address these concerns. One of the main goals is to increase its host specificity using biotechnology while seeking to lower spraying requirements and increasing its long-term persistence, to achieve better efficacy and lower insect resistance (Castagnola and Jurat-Fuentes 2012; Karabörklü et al. 2018; Azizoglu and Karabörklü 2021).

## 5.2 Parasitoids

Parasitoids are essential agents of insect pest management due to their ability to seek for and find their hosts. When parasitizing insect pests, they may be exposed to *Bt* toxins and, affected by the *Bt* toxins in addition to their intended target pests, either directly or indirectly (Belousova et al. 2021). Therefore, assessing the possible risks of recombinant *Bt* toxins and GM-*Bt* crops on parasitoids is critical, and some recent research has focused on this topic. Investigations were conducted on the effects of a *Pseudomonas-cry9Aa* strain, which harbors the *cry9Aa* from the *Bt* ssp. *galleriae*, on the larval parasitoid, *Exorista larvarum* (Diptera: Tachinidae). When the host larvae, *Galleria mellonella* (Lepidoptera: Pyralidae) was treated with *Pseudomonas-cry9Aa*, *E. larvarum* did not show any decrease in the egg laying, parasitism rate, pupal weight or the number of offspring (Marchetti et al. 2009). In contrast, despite the fact that the Cry3A toxin has no negative effect on the potato aphid pest *Macrosiphum euphorbiae* (Hemiptera: Aphididae), GM-*Bt*-potatoes were reported to significantly impact the survival, weight gain and fecundity of the wasp *Aphidius nigripes* (Hymenoptera: Aphididae), a parasitoid of the aphid potato pest (Ashouri 2004; Raspor and Cingel 2021).

In another study, Xu et al. (2019) reported that GM-*Bt* maize had no effect on the attractiveness of the egg parasitoid *Trichogramma ostrinae* (Hymenoptera: Trichogrammatidae) compared with non-*Bt* isogenic plants. GM-*Bt* corn (Cry1Ab) has also been reported to have no detrimental effects on the aphid parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Aphididae), in terms of foraging and egg laying efficiency, and no evidence has been found that these herbivore parasitoids can distinguish between GM-*Bt* corn and non-*Bt* corn. Although numerous studies have shown that GM-*Bt* crops have no negative effects on the herbivore parasitoids, GM-*Bt* crops might indirectly influence natural enemies due to changes in tritrophic interactions (plant-herbivore-parasitoids/predator) in various food chains (Han et al. 2016; De Bortoli et al. 2017).

## 5.3 Predators

The predators may be indirectly affected as they eat insects that may have ingested *Bt* toxins. It has been

reported that the GM-*Bt* UV173A strain (Cry3Aa7) showed high insecticidal activity against Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) whereas the parental strain *Bt* UV17 (Cry1Ba) was highly toxic to the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). However, no effect on non-target insects belonging to different insect orders (Diptera, Hymenoptera, Coleoptera, Lepidoptera, Hemiptera, Homoptera, Thysanoptera, Orthoptera, and Neuroptera) (Wang et al. 2008). Similarly, it has been reported that *Chrysoperla carnea* (Neuroptera; Chrysopidae) that fed on *Helicoverpa armigera* (Lepidoptera; Noctuidae) larvae that were exposed to the GM-*Bt* EG11070 (Cry1Ac), GM-*Bt* EG7077 (Cry1Ab) and, GM-*Bt* EG7699 (Cry2Ab) strains have not been adversely affected. Furthermore, when this predator was directly exposed to the Cry1A toxin, no detrimental effects were observed at the toxin binding sites in the midgut epithelium of this predator (Rodrigo-Simón et al. 2006). Another research confirmed that feeding larvae of this predator on aphids reared on GM-*Bt* corn did not affect the pupation or adult emergence rates of *C. carnea* (Moussa et al. 2018).

Various studies on tritrophic test systems that include a variety of prey and host species, as Cry-toxin carriers, have been conducted to examine the effects of GM-*Bt* crops on parasitoids and predators. These included prey or host species that were: (1) susceptible to the Cry toxins (lepidopteran and coleopteran pests that were targets of GM-*Bt* crops), (2) non-sensitive to the Cry toxins due to their taxonomic affiliation (aphids, leafhoppers, mites, thrips, etc.), and (3) target herbivores that have evolved resistance to the Cry toxins. One challenge with tritrophic studies is that they can produce inaccurate results when susceptible herbivores that are sublethally affected by the Cry toxins are used as preys, as this might have negative consequences on the predators due to the reduced quality of the prey rather than the toxin itself (Naranjo 2009; Romeis et al. 2019). In summary, while GM-*Bt* and recombinant toxins have been shown in several experiments to have no negative effects on predators, the mode of action of these toxins can vary depending on the toxin, its concentration, and the predator species. However; there may be a risk changing prey preference by the predators as consumption of Cry toxins by the prey may lead to low-quality food for predators (Azizoglu et al. 2020).

## 6 Conclusions and recommendations

Novel biological control tactics aimed at targeting specific insect immunity proteins or interfering with insect development-related genes combined with *Bt cry* gene expression are likely to form the foundation for the next generation of insect-resistant transgenic plants. Downregulation of insect pest gene expression using RNA interference (RNAi) is already a promising strategy for effective insect control (Zhang et al. 2017; Darlington et al. 2022). More research is already being done to find novel and essential gene targets that can elicit a strong RNAi response. However, caution should be taken to ensure that these new target genes also do not belong to non-target beneficial insects and adversely affect the populations of beneficial insects. The RNAi technique is expected to be able to manage a larger range of insects, particularly sap-sucking insects, which GM-*Bt* crops have failed to control. However, it is important to keep in mind that to properly implement this technology, both the amount of dsRNA taken by the insect, as well as the systemic dissemination of the dsRNA throughout all of the target insects' cells, must be high enough to achieve significant silencing of the selected target gene. Furthermore, the efficacy of the RNAi response via ingestion of dsRNA when feeding on transgenic plants producing hairpin RNA differs between species due to dsRNA degradation by insect nucleases. Therefore, many insect species, especially significant lepidopteran pests, remain resistant in their reaction to RNA interference efficiency. As a result, this method may not be suitable for a wide range of pests, as no single protocol is appropriate for all species, and should instead be applied on a case-by-case basis.

Plant genome editing using CRISPR/Cas9-based approaches could also possibly be employed as a supplement to further boost pest resistance caused by transgenes that already produce *Bt* toxins. Although this technique has primarily been used to combat virus, fungal, and bacterial plant diseases (Borrelli et al. 2018), some studies have already suggested that altering plant volatile blends, which insects recognize as clues for host selection and oviposition site detection, through genome editing, has also the potential to be used in insect pest management (Beale et al. 2006). Lu and colleagues recently demonstrated that knocking down a gene that catalyzes the conversion of tryptamine to serotonin, an important chemical

that insects rely on for development, immunity, and behavior, reduced the growth of the rice brown plant hopper (*Nilaparvata lugens*), one of the most serious pests in rice production (Lu et al. 2018). In conclusion, combining traditional host plant resistance with the use of *Bt* toxins and non-*Bt* insecticidal proteins, together with plant genome editing and gene silencing technologies, can provide the tools needed to slow the evolution of resistance in the field and ensure the success of future biocontrol programs. In addition to being widely used as a pesticide and as a gene source for genetic engineering, *Bt* has been shown to have potential applications in many other fields and/or industries. There have been several studies in the past few years that indicate that *Bt* can be used to control pathogenic fungi and bacteria in both plants and animals and as a biological control agent of plant nematodes and mites. It can also be used to stimulate plant development, and even to clean up different pollutants or biosynthesis of different nanoparticles. Another application of *Bt* is the production of cytotoxic parasporins, which target cancer cells. These proteins need to be studied further to understand their mechanism of action and the specific cancer cell receptors they target so that researchers can work towards developing useful products for human health.

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### Declarations

**Conflict of interest** No potential conflict of interest was reported by the authors.

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