Review

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Bacillus thuringiensis as a biofertilizer in crops and their implications in the control of phytopathogens and insect pests

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Abstract

BACKGROUND: Bacillus thuringiensis (Bt) is a spore-forming bacterium that produces insecticidal proteins and other virulence factors and is considered one of the most successful bioinsecticides available to control pests in agriculture. Currently, some Bt strains have been reported as endophyte or rhizospheric bacteria.

RESULTS: Little is known about the implications of plant-Bt interaction in crop protection. Here, we review if Bt can establish as an endophyte/rhizobacterium and evaluate if Bt as an endophyte/rhizobacterium can simultaneously act against different phytopathogens (fungi, bacteria, insects and viruses) plus promote plant growth.

CONCLUSION: Although Bt produce an arsenal of proteins with toxic effects against insect, the current knowledge suggests that Bt can be considered as a promising new plant growth promotion bacterium (PGPB). The implications of the proposed review will broaden our understanding of Bt as a versatile entomopathogen that may be able to exhibit differential behavior depending on context.

Keywords: insect pest control; insect resistance management; crop protection

1 INTRODUCTION

Bacillus thuringiensis (Bt) is an aerobic and entomopathogenic bacterium belonging to the *Bacillus cereus* group. Bt-related studies mainly focus on its insecticidal activity due to its entomopathogenic properties.[1–3] However, the natural ecology of Bt is poorly understood. Bt is ubiquitous in the soil but it is unclear whether it exists in the bulk soil in an active form or whether this is merely a 'sump' where spores are deposited for possible future consumption or distribution. The possible activity of Bt in the rhizosphere is also poorly studied with some indications that associations with roots may have a role in soil colonization.[4–6] Meanwhile, some studies have indicated that Bt may exist within plant tissues as a rhizospheric/endophytic bacterium, with implications for crop protection, as a bioprotectant and biofertilizer.[7–9]

Endophytic bacteria exist inside the plant tissues and this gives them an ability to contact with the plant's cells continually and to influence directly the plant host's metabolism.[10–12] Several studies have reported that rhizospheric/endophytic Bt isolates can stimulate both plant growth[13–30] and resistance against pathogens and pests.[16,31–48] Endophytic locations may also be advantageous since the toxicity of the Bt strains is affected by ultraviolet (UV) light (toxin inactivation) and flushing away of spores by precipitation (toxin washing).[49–51] As a result, to reduce the number of the chemical pesticide applications and improve plant production, it is of great interest to search for endophytic Bt isolates, which inhabit the internal or associated plant tissues, are less influenced by environmental factors and potentially more integrated with plant metabolism and which produce insecticidal proteins, in addition to virulence factors against phytopathogens.[52–54]

Here, we overview whether Bt as an endophyte/rhizospheric bacterium can act simultaneously against insect pests and/or phytopathogens (fungi, bacteria or virus). Moreover, we evaluate the role of Bt as a biofertilizer and bioprotectant in inoculated plants. This approach to the ecology of Bt could represent a potential alternative of Bt to be used as a bioinoculant, instead of as spray, to improve the resistance to abiotic and biotic stresses.

2 TRANSLOCATION OF *BACILLUS THURINGIENSIS* INTO PLANT TISSUES AND INTERACTION WITH OTHER PLANT GROWTH PROMOTING BACTERIA

Plants in the environment live in association with diverse, taxonomically structured communities of microorganisms. The plant microbiota can be understood as a multitude of microorganisms

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(virus-like particles, bacteria, fungi, and oomycetes) that grow associated with plants roots.[55] It has been reported that the most common bacteria present in the plant microbiome are bacteria from the genera *Pseudomonas*, *Bacillus*, *Burkholderia*, *Stenotrophomonas*, *Micrococcus*, *Pantoea* and *Microbacterium*.[10,55] Therefore, it has been suggested that the endophyte microbiome may be a subpopulation of the rhizosphere inhabiting bacteria.[10]

2.1 Presence of *Bacillus thuringiensis* in plant tissue samples and vertical transmission

Bt has been isolated from different plant tissues (root exudates, leaves samples, stems, etc)[14,23,24,28,30,38,43] and rhizosphere soil samples.[25,26,56] Specifically, Bt has been isolated from different agroeconomic crops (Fig. 1). Bt has been found to be distributed in tissues throughout the plant (roots, stem, leaves, etc.)[57,58] where the abundance of Bt cells in the rhizosphere and roots was higher than in the rest of the plant tissues (stem and leaves, etc.).[57,59] Specifically, the colonization by Bt of the plant tissues correlate with the Bt strain phylogeny in *Arabidopsis thaliana*.[24] These results suggest that the soil can act as a reservoir and the roots can act as a gate for Bt to be translocated to the plant tissues in Bt phylogeny dependent manner, perhaps as a means to increase the likelihood of infecting invertebrate hosts.[24] In addition, García-Suárez *et al*.[40] reported the presence of Bt in the seeds of *Arabidopsis thaliana* Bt colonized plants. Thus, it has been suggested that the Bt showed vertical transmission in Bt colonized plants.

2.2 Interaction of *Bacillus thuringiensis* with other plant growth promoting bacteria

Microbial interaction is established between a group of microorganisms that interact with each other to establish and maintain the relationship, which can be positive (mutualism, protocooperation and commensalism) or negative (competition, parasitism, predation and amensalism).[60] Regarding the reported interaction among Bt with other plant growth promoting bacterium (PGPB) (*Burkholderia phytofirmans*, *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and *Azospirillum brasilense*), include playing roles in the colonization efficiency, plant growth, plant nodulation (Fig. 1).[24,26,30,59,61–63] The reports published to date[22,23,30,61] showed a wide range of plant responses to the co-inoculation of Bt plus PGPB. Vidal-Quist *et al*.[24] reported that the co-inoculation with *Burkholderia phytofirmans* or *Pseudomonas fluorescens* in *Arabidopsis thaliana* showed no effect on Bt colonization levels. Rojas-Solís *et al*.[58] evaluated five different strains of *Pseudomonas fluorescens* plus Bt in *Zea mays* (corn), where the combinations of *Pseudomonas fluorescens UM16* + Bt UM96 had beneficial interactions with the plant (total fresh weight, hypocotyl length and root length), while separately the *Pseudomonas fluorescens* and Bt strains showed broad potential for colonizing the rhizosphere and promoting tomato plant growth. Mishra *et al*.[59] indicated that Bt-KR1 when co-inoculated with *Rhizobium leguminosarum-PR1* increased the nodule number, shoot weight, root weight, and total biomass, over rhizobia inoculation alone in *Pisum sativum* (pea) and *Lens culinaris* (lentils). De Almeida *et al*.[30] reported that Bt RZ2MS9 when co-inoculated with *Azospirillum brasilense* showed no effect on the dry weight of maize roots and shoots.

3 TOXICITY OF *BACILLUS THURINGIENSIS* ISOLATES WITH ENDOPHYTE/RHIZOSPHERIC BEHAVIOR AGAINST INVERTEBRATE PESTS

Most of the information on the insecticidal activity of Bt has been obtained applying the Bt products or its invertebrate-active proteins (belonging to a range of structural classes[64]) externally[1–3] or expressing the toxin genes in genetic modified crops (GMCs). However, the toxicity of Bt acting as an endophyte/rhizospheric

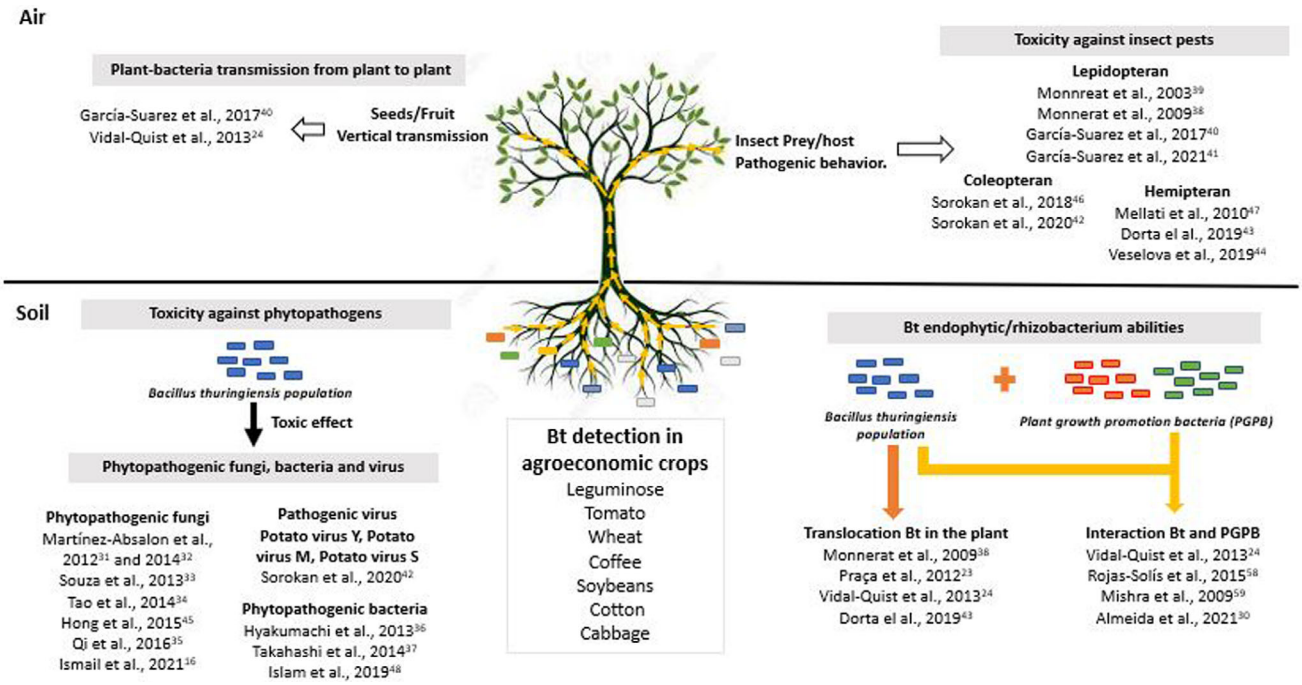

Figure 1. Roles of Bt as an endophyte/rhizospheric bacterium and their implications in the control of different kinds of phytopathogens. See References section for the whole citation of the reports indicated in the figure.

bacterium is not well characterized. The toxicity results reported to date of Bt associated with plants, corroborate that Bt can be toxic to different kinds of phytopathogens (fungi, bacteria, viruses and oomycetes) and predators (insects, nematodes)[15,16,18,31–38,40,41,43–48,65] (Fig. 1). Activity against the different targets (insect, bacteria, fungi and oomycetes) will be discussed in the following sections.

3.1 Activity against insect pests in plants colonized with Bt strains in laboratory conditions

A range of Bt strains [var. *kurstaki* (Btk), var. *israelensis* (Bti), var. *thuringiensis* (Btt), var. *azawai* (Bta) and recombinant Bt strains] have been used to colonize different plants (wheat, potatoes, beans, cotton, cabbage and orange tree) prior to tests of insecticidal activity against Lepidoptera (*Tricoplusia ni*, *Plutella xylostella* and *Spodoptera frugiperda*), Coleoptera (*Leptinotarsa decemlineata*) and Hemiptera (*Aphis gossypii*, *Schizaphis graminum* and *Diaphorina citri*).[4,38,40,43,44,46,47] The mortality of the respective pests in the plants colonized with Bt were compared to the non-treated (NT) plants and the results are summarized in Table 1. The different Bt inoculated crops (cabbage, cotton, wheat, potatoes, peanut, orange tree) showed an increase in the toxicity against insect pests. Interestingly, the increase in the toxicity compared to the NT plants have been reported in all the crops (Table 1). The toxicity differences among the Bt isolates in brassica, cotton, potatoes, wheat and orange tree may be due to the fact that Bt colonizes the plant in a phylogeny dependent manner.[24] Further analysis is needed to determine if the variability in the reported toxicity data is due to the action of Bt toxins, the activation of plant defense [systemic acquired resistance (SAR) and induced systemic response (ISR)] or whether the increased plant toxicity is not a general effect of the endophythism but rather, it could be Bt strain–plant dependent process.

As regards the effect in the insects fed with plants colonized with Bt isolates, Veselova *et al.*[44] report a reduction in the fecundity of *Schizaphis graminum* (spring green aphid, a major pest that feeds mainly on Poaceae plants like wheat, corn, oats, etc.) in 7-day-old wheat seedlings for the Bt isolates B-6066 and B-5689. Although da Costa *et al.*[64] reported no mortality of *Spodoptera frugiperda* fed in cotton plants regardless of the form of inoculation, in 11-day old cotton Bt colonized plantlets for four Bt isolates tested (S1450, S1905, S2122 and S2124). The Bt strain S2122 that showed the highest adhesion of the spore/crystal complex to the seed coats was selected for *in vitro* toxicity assays using leaves collected from 18-, 23- and 30-day old Bt colonized plantlets. The Bt strain S2122 was not toxic at the spore concentration 10^6 CFU mg^{-1} and 10^8 CFU mg^{-1} but *Spodoptera frugiperda* larvae showed a weight reduction in plants grown from seeds treated with the Bt isolate S2122.

3.2 Toxicity against insect pests of Bt strains isolated from plants naturally or artificially colonized

Few reports are published that provide toxicity of isolated Bt strains from the plants naturally colonized[39,41] or artificially inoculated[5,40] with Bt isolates. To date, three studies[5,39,41] have reported toxicity of Bt isolated from colonized (natural[39] and artificial[5,41] bacterial colonization) plants of cotton, lavender, poinsettia and *Arabidopsis thaliana*. Monnerat *et al.*[39] and García-Suárez *et al.*[41] performed toxicity assays after the Bt strains were isolated from plants. Specifically, a set of different techniques of feeding assays (leaf disk, surface contamination and drop-feeding methods) were conducted against *Anticarsia gemmatalis*, *Spodoptera frugiperda*, *Manduca sexta* and *Aedes aegypti* respectively (Table 2). The toxicity data of the respective Bt isolates after being isolated from the plant tissues indicate that the respective Bt strains were toxic. Specifically, the Bt isolates LBIT-1250L and LBIT-1251P were 2.5 and 4.1 times more active than the comparator standard strains (Bti and Btk) (Table 2). Monnerat *et al.*[39] and García-Suárez *et al.*[41] do not indicate the mortality of the respective pests in the Bt-inoculated plants (and comparison of dose rates may be difficult when plant tissue containing endophytes is used). Therefore, it cannot be determined if Bt maintain their toxicity as endophyte/rhizobacterium or free-living bacterium.

Lin *et al.*[5] inoculated 1-week-old *Arabidopsis thaliana* plants with Bt 407 Cry^- and transferred them to sterile media for a period of 48 h. These steps were repeated for 40 transfers. Over the course of the experiment, two evolved Bt lineages, E and F, showed an increase in the insecticidal activity [a significant several fold decrease in median lethal dose (LD_{50})] compared to the ancestor Bt 407 Cry^- [assessed via injection into the hemolymph of *Galleria mellonella* larvae (Table 2) in *in vivo* assays] and an increase in the hemolytic zones (assessed via hemolytic index in plate assays), compared with the ancestor. As soon of the source of the toxicity in the two evolved Bt lineages, the ancestor (Bt 407 Cry^-) produce pore-forming cytotoxins hemolysin BL (HBL), non-hemolytic enterotoxin (NHE), and cytotoxin K (CytK) regulated by different regulatory systems (PlcR, ResDE, Fnr and CcpA).[5] These regulatory systems are associated with motility, metabolism, biofilm formation and swarmer cell (the latest two trait were enhanced in the Bt lineages E and F). Therefore, the production of HBL, NHE and CytK may be the cause of the toxicity and could improve the fitness of E and F Bt lineages.

3.3 Protective effects of Bt against phytopathogens (fungi, bacteria, viruses and oomycetes)

In addition to the reported toxicity against insect pests, some Bt isolates showed protective effects against a wide range of phytopathogens (fungi, bacteria, viruses and oomycetes) (Fig. 1). The protective effects of these Bt isolates have been demonstrated *in vitro*[15,16,18,31,33,35,48,65] and in Bt colonized plants.[32,34,36,42] With regards the toxicity spectrum of these Bt isolates, they have been reported to be toxic against pathogenic fungi (*Aspergillus niger*, *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum graminicola*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Pythium ultimum*, *Verticillium dahliae*, *Verticillium longisporum*, *Urocystis agropyri*), bacteria (*Xanthomonas citri* subsp. *Citri* and *Ralstonia solanacearum*), potato viruses [potato virus Y (PVY), potato virus M (PVM), and potato virus S (PVS)] and oomycetes (*Phytophthora infestans*).[15,16,18,31-37,42,45,48,57,65-67]

Briefly, Bt isolates tested *in vitro* in toxicity assays, demonstrated that the bacteria from natural/artificially colonized plants, grown as free-living bacteria (culture media) showed activity against the respective phytopathogens assayed.[15,16,18,31–33,35,48,65] These phenomena have been reported previously[1,3] and contribute to the range of Bt pathogenicity. Regarding the *in vivo* toxicity assays a reduction in plant symptoms of infection or the number of infected plants challenged with the phytopathogenic fungi (*Botrytis cinerea* and *U. agropyri*) after been inoculated with Bt has been shown for Bt colonized plants (wheat and barrel medic plant).[32,34] Martínez-Absalón *et al.*[32] reported that the barrel medic plant (*Medicago truncalia*) inoculated first with Bt UM96 and infected afterwards with *Botrytis cinerea* showed a reduction in the disease symptoms (chlorosis, presence of grey mold, root

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browning and necrosis). Also, the protective effect was observed in plants first inoculated with Bt UM96 strains and infected with *Botrytis cinerea* at the same time. Tao *et al.*[34] reported that 12 different varieties of wheat (with different susceptibility to *U. agropyri*) inoculated with Bt strains 58-2-1 and 37-1, showed different toxicity profiles against *U. agropyri*. The strain 58-2-1 showed activity against *U. agropyri* in nine wheat varieties (highly resistant to *U. agropyri*: Yunhan-618, Bainongaikang-58, Zhengmai-9023, 04-zhong-36 and Yanzhan-4110; moderate resistant to *U. agropyri*: Jinboshi-1 and Pumai-9; highly susceptible to *U. agropyri*: Yumai-012 and Yunong-416) and no activity in three (Kaimai-20, Yunong-202 and Yubao-1) wheat varieties. As soon as the Bt strain 37-1 showed activity in seven varieties (highly resistant to *U. agropyri*: Bainongaikang-58, Zhengmai-9023, 04-zhong-36 and Yanzhan-4110; moderate resistant to *U. agropyri*: Pumai-9; highly susceptible to *U. agropyri*: Yumai-012 and Yunong-416) and no toxicity on the remaining five wheat varieties (Yunhan-618, Kaimai-20, Jinboshi-1, Yunong-202 and Yubao-1).

4 PLANT GROWTH PROMOTION AND APPLICATIONS IN PHYTOREMEDIATION OF *BACILLUS THURINGIENSIS* STRAINS

Bacteria within the taxonomic class Bacilli include well-known bacteria with endophyte/rhizopheric activity (*Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus pumilus*) available in commercial biofertilizers.[9] The endophyte/rhizopheric Bacilli bacteria can act as PGPB, stimulating the acquisition of resources and modulation of plant growth and development.[10,11] As a member of this class, Bt can also stimulate plant growth and health. Bt strains may exhibit plant growth promotion traits that are common to other well-known PGPB of the class Bacilli.[9] Plant growth promotion traits described

Table 1. Toxicity of orange tree (*Citrus sinensis* var. *osbeck*), peanut (*Phaseolus vulgaris* var. *cacahuate 72*), cabbage (*Brassica campestris* var. *chinensis* and *Brassica campestris* hybrid Matsukaze Sakata), potatoes (*Solanum tuberosum* var. early rose breeds), wheat (*Triticum aestivum* var. *salavat yulaevk*) and cotton (*Gossypium* sp. and *Gossypium* var. *delta-opal*) colonized with *Bacillus thuringiensis* (Bt) strains to insect pests

Endophyte-containing crops/infection time	Bt strain [serotype] (gene content)	Mortality (% ± SE)	References
		***Diaphorina citri* (Treatment 1/ Treatment 2) (5 DAI)†**	
Orange three (*Citrus sinensis* var. *osbeck)*	**S1302 [ND]** (*cry1Ab*, *cry3A)*	90.0 ± 5.96 a/68.0 ± 3.27 d	43
3-month-old plants	**S1450 [*kurstaki*]** (*cry1Ab*, *cry1Ac*, *cry1B*, *cry1Aa*, *cry2Aa*)	77.0 ± 6.67 ab/70.0 ± 2.11 d	
	S1989 [*israelensis*] (*cry4B*, *cry10*, *cry11*, *or cyt1A*)	82.0 ± 6.96 ab/42.0 ± 2.49 e	
	Recombinant strains		
	S2211 [ND] (*cry1Aa*)	50.0 ± 8.94 ab/——	
	S2209 [ND] (*cry1Ac*)	44.0 ± 9.91 b/——	
	S2396 [ND] (*cry1B*)	26.0 ± 5.81 bc/——	
	S2212 [ND] (*cry2Aa*)	51.3 ± 9.35 ab/36.0 ± 2.67 ef	
	S2036 [ND] (*cry4A*)	36.0 ± 5.82 b/36.0 ± 2.67 ef	
	S2037 [ND] (*cry4B*)	62.0 ± 7.06 ab/——	
	S2492 [ND] (*cry10*)	65.0 ± 5.83 ab/66.0 ± 1.63 d	
	S2038 [ND] (*cry11*)	60.0 ± 5.94 ab/66.0 ± 1.63 ef	
	S2035 [ND] (*cyt1A*)	62.0 ± 8.00 ab/54.0 ± 3.40 ef	
	S2210 [ND] (*cry1Ab*) (NC)	33.0 ± 8.70 bc/——	
	H_2O (NT)	14.4 ± 2.06 c/30.0 ± 2.11 f	
		***Schizaphis graminum* (7 DAI)**	
Wheat (*Phaseolus vulgaris* var. *cacahuate 72*)	**B-6066 [ND]** (ND)	36.3 ± 3.5	44
7-day-old plants	**B-5689 [ND]** (ND)	33.1 ± 5.2	
	H_2O (NT)	12.2 ± 1.9	
		***Leptinotarsa decemlineata* (3 DAI)**	
Potatoes (*Solanum tuberosum* var. early rose)	**B-5689 [*thuringiensis*]** (ND)	33.3 ± 3.1	46
25-day-old plants	**B-55351 [*kurstaki*]** (ND)	60.0 ± 10.6	
	H_2O (NT)	6.7 ± 0.5	
		***Tricoplusia ni* (7 DAI)‡**	
Penaut (*Phaseolus vulgaris* var. *cacahuate* 72)	**HD73 [*kurstaki*]** (*cry1Ac*) + *gfp*	48 ± 3.0	40
14-day-old plants	**H_2O** (NT)	23 ± 4.0	
		***Aphis gossypii* (5 DAI)†**	
Cotton (*Gossypium* sp.) young leaves	**29 [ND]** (ND)	76.0 ± 4.0 a	47
	40 [ND] (ND)	60.0 ± 2.6 b	
	616 [*aizawai*] (ND)	63.3 ± 2.9 b	
	1168 [ND] (ND)	73.3 ± 2.9 a	
	1576 [*aizawai*] (ND)	56.6 ± 3.7 b	

Table 1. Continued

Endophyte-containing crops/infection time	Bt strain [serotype] (gene content)	Mortality (% ± SE)	References
	H_2O (NT)	0.0 ± 0.0	
		***Pieris brassicae* (3 DAI)**	
Pak choi *Brassica campestris* var. *chinensis* 5-week-old plants	**2810-S-6 [ND]** (ND)	35 ± NA	[84]
	H_2O (NT)	No mortality observed	
		***Plutella xylostella*—*Spodoptera frugiperda* (7 DAI)**	
Cabbage and cotton *Brassica* (hybrid Matsukaze Sakata) 28-day-old plants cotton (*Gossypium* var. *delta-opal*) 28-day-old plants	**HD1 [*kurstaki*]** (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry2A*) + *gfp* (single inoculated plants)	10 ± NA—20 ± NA	[38]
	HD1 [*kurstaki*] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry2A*) + *gfp* (weekly inoculated plants)	10 ± NA—25 ± NA	
	H_2O (NT)	No mortality observed	

Note: DAI, days after inoculation. The insect toxicity assays were performed for at least 3, 5 and 7 DAI, respectively. The plants to be used in the assay have been growth to their specific development time (7-, 14-, 25-, 28-day-old plants,[38,40,44,46] 5-week-old plants,[84] 3-month-old plants[43] and young leaves[47]). All the plants had been inoculated with their respective Bt isolates prior to performing the toxicity assays. NA, the standard error was not determined in the bioassays with *Plutella xylostella*, *Spodoptera frugiperda* and *Pieris brassicae*; NC, negative control, recombinant strain S2210 harboring the gene *cry1Ab*: the Cry1Ab protein is not active against *Diaphorina citri*; ND, the serotype or gene content of Bt strains have not been determined; NT, non-treated plants, water used as a negative control; SE, standard error. The bold typeface indicate the Bt isolate used in the respective assays.
† Data (mean ± SE) followed by the same letter in each treatment did not differ statistically. See Melatti *et al.*[47] (Student–Newman Keuls test $P < 0.05$) and Dorta *et al.*[43] [generalized linear model (GLM) with a quasi-binomial distribution plus *post hoc* Tukey–Kramer test; $P < 0.05$].
‡ The SE has been interpolated from the graph published in García-Suárez *et al.*[40]

Table 2. Toxicity of *Bacillus thuringiensis* (Bt) isolates from different plant sources (naturally colonized or artificially colonized) and then re-isolated from their respective plant tissues

Plant source of the isolated Bt strain	Insect pest	Bt strains [serotype] (gene content)	Mortality		Reference
			Dose–response assays		
			LC_{50} (ng mL^{-1})	FL_{95}	
Lavender (*Lavandula angustifolia*)	*Aedes aegypti*	LBIT-1250 L [ND] (Cry4-type duplex, Cry11-type, and Cyt1-type)†	6.8	6.0–8.0	[41]
		Bti [*israelensis*]	17.6	13.0–24.2	
			LC_{50} (ng cm^{-2})	FL_{95}	
Poinsettia (*Euphorbia pulcherrima*)	*Manduca sexta*	LBIT-1251P [ND] (Cry1-type)†	1.4	1.2–1.7	
		HD1 [*kurstaki*]	5.8	4.5–7.4	
			LC_{50} (CFU/larvae)‡		
Thale cress (*Arabidopsis thaliana*)	*Galleria mellonella*	Bt 407 Cry^-	~6000 ± 1000		[5]
		Bt 407 Cry^- lineage E	~1500 ± 300		
		Bt 407 Cry^- lineage F	~1000 ± 200		
			Mortality (%) of 150 µL final culture		
Cotton (*Gossypium* sp.)	*Spodoptera frugiperda* and *Anticarsia gemmatalis*	S1974 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		[39]
		S1979 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		
		S1983 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		
		S1985 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		
		S1986 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		
		S1987 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		
		S1989 [ND] (*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*)	100		

Note: ND, the serotype or gene content of Bt strains have not been determined.
† The gene content of the respective Bt isolates, was determined by protein profile (protein band size). Since the gene content has not been confirmed with molecular techniques [polymerase chain reaction (PCR) or whole genome sequencing (WGS)], indications of protein production should be considered as preliminary data.
‡ The median lethal concentration (LC_{50}) and the standard error for *Galleria mellonella* has been interpolated from the graph published in Lin *et al.*[5]

for Bt include: synthesis of phytohormones such as IAA (indole acetic acid)[13–18,21–28,57] and ACC-deaminase,[13,17–19,21,24,26,27] biological nitrogen (N_2) fixation,[13–15] ammonia (NH_3) production,[13,16] phosphate solubilization,[14–20,57] production of sideophores[17–19,21,57] and volatile organic compounds.[29,30]

Related with the activity of Bt as a PGPB, the plants colonized with endophytic Bt improve their tolerance to abiotic stresses like heavy metal and chemical bioremediation. Improvement of plant tolerance to soil contamination (heavy metal and chemical contamination) has been found to correlate with IAA and ACC-deaminase production by the endophytic Bt strains.[13,21,27] The ACC-deaminase activity of endophytic/rhizospheric bacteria regulates the biosynthesis of ethylene in inoculated plant roots, generating longer roots and greater root density.[68,69] Babu *et al.*[21] and Sharma and Saharan[13] also reported a significant increase in the root and shoot length in *Vigna radiata* (mung bean) and *Alnus firma* (Japanese green alder) when colonized with Bt isolates, respectively. High concentrations of ethylene in the roots are common in plants under stress conditions, causing various physiological changes (including tissue abscission, short root length and senescence).[68,69] The bacterial enzyme ACC deaminase acts by degrading the plant ACC, the direct precursor of ethylene (generating α-ketobutyrate and NH_3) and preventing ethylene accumulation and, therefore, helping the plant to reduce the abiotic stress, promoting its growth and survival.[70] PGPB can assimilate tryptophan, an essential precursor of IAA synthesis, then produce IAA to induce the transcription of auxin response factors, promoting plant growth and amino acids like tryptophan are exuded into the rhizosphere along with other compounds (such as sugars and organic acids).[69] Batista *et al.*[28] reported that the endophytic Bt strain RZ2MS9 harbors the complete set of genes required in two of the four main pathways for IAA production [indole-3-pyruvate (IPA) and tryptamine (TPM) pathways]. The IAA content (time range: 3–30 h, IAA concentration range: 0.06–0.20 μg mL^{-1} with the IAA production peak at 21 h) is cell density dependent when Bt RZ2M9 are in LB medium supplemented with 1 g L^{-1} of l-tryptophan (Trp), having a constant production in the log phase and a production peak in the stationary phase. At this concentration of Trp Bt RZ2M9 produces almost five times more IAA during the stationary phase than in the control medium (LB without Trp). Finally, the application of the Bt strain RZ2MS9 to *Solanum lycopersicum* Micro-Tom (MT, tomato) increased the shoot dry weight by 24%; modified MT root architecture increasing average lateral root length by 26%; inhibited the axial root growth and changed root histology (elongation of the root cortical cells with intensified mitotic activity).

5 PLANT DEFENSE RESPONSE TO THE INOCULATION WITH *BACILLUS THURINGIENSIS* ISOLATES

The plant defense response describes a range of adaptations evolved in plants to reduce damage and improve their survival and reproduction efficiency. The general model indicates that the SAR is a 'whole-plant' resistance response that occurs following an earlier localized exposure to an abiotic/biotic stress. Meanwhile the ISR is a mechanism of plants that is activated by bacterial colonization.[71–73] The ISR resembles the SAR pathway but acts through different signaling pathways. Induction of SAR is through salicylic acid (SA) and ISR requires jasmonic acid (JA) and ethylene (Et) signaling pathways.[71–73] Some reports suggest that there is no uniform plant response, instead there seem to be different responses depending on the eliciting microbial strains, involving JA/Et signaling as well as SA signaling pathways.[74–77]

In the case of Bt colonized plants, the interaction between plant tissue and Bt triggers the plant defense responses (SAR[44,78] and ISR[36,37]). Plants colonized with Bt after being exposed to phytopathogenic bacteria, fungi or aphids showed as part of their physiological response an increase in the production of hydrogen peroxide (H_2O_2) and the activity of the following enzymes: gluconase, chitinase, ascorbate peroxidase, polyphenol oxidase and phenylalanine ammonia lyase[36,44,78–80] (Fig. 2). The signaling pathways (SAR and ISR) activated in the Bt colonized plants after being infected with a phytopathogen, are not consistent among the different reports published to date (Fig. 2). Hyakumachi *et al.*[36] and Takahashi *et al.*[37] showed that in *Solanum lycopersicum* (tomato) colonized with Bt[37] or inoculated with cell free extract (filtered supernatant),[36] respectively, and exposed to the bacterial wilt of tomato, *Ralstonia solanacearum*, induced ISR in the leaf, stem and main root tissues, but not in the lateral root tissue. In addition, the plants colonized/inoculated with Bt showed an up-regulation of several SA-responsive defense-related genes (PR-1 (P6),[36,37,74,78] PR-2, PR-1b1(p14), P4, PR-4, PR-P69E, PR-P69G[37]) and down-regulation of the JA-responsive defense-related genes [proteinase inhibitors II (PI-II) and CEVI57 (PI-CEVI57)[37]]. Burkhanova *et al.*[78] and Veselova *et al.*[44] studied *Triticum aestivum* (wheat) colonized/inoculated with two different Bt strains (B-5689 and B-6066) and exposed to the phytopathogenic fungus *Septoria nodorum* or the aphid *Schizaphis graminum* and reported the up-regulation of the SA-responsive defense-related genes (PR-1 and NADPH-oxidase), JA-responsive defense-related genes (PR-6 gene) but no difference in regulation of the PR-9 gene (SA and JA-dependent signaling cascade). Finally, Sommer *et al.*[80] described that in *Arabidopsis thaliana* inoculated with Bti and not exposed to any phytopathogen, the plant defense response activated was a different signaling pathway than the SAR or JA signaling pathway responses. More research will be needed to determine if the same/different plant infected with the same/different strains of Bt might activate the SA or JA signaling pathways.

6 EFFECTIVENESS OF THE APPLICATION OF *BACILLUS THURINGIENSIS* OR ITS METABOLITES IN FIELD CONDITIONS

Regarding the efficiency of the use of Bt (Bt isolates or Bt + PGPB) or it's metabolites in field conditions some reports have been published to date.[16,42,59,81–84] Sorokan *et al.*[42] evaluated the efficiency of potatoes colonized with Bt in the control of *Leptinotarsa decemlineata* and potato viruses (PVY, PVM, and PVS) in two different growth seasons. With regards the control of *Leptinotarsa decemlineata* in field conditions, a reduction in fecundity (number of eggs per plant) was statistically significant in two [Bt B-5351 (4.6 ± 2.2) and Bt B-6066 (~7.0 ± ~2.0)] of the three Bt-treated potatoes compared to the water-treated plants (14.0 ± 4.5). In addition, all three strains produced a reduction in the number of insects in the early and final larval instars. Particularly, plants treated with Bt B-6066 and B-5351 showed the lowest values for the early instar larvae, meanwhile for the final instar larvae the Bt B-5689-treated plant showed a reduction in the number of larvae of 50% compared to the 33% reduction in the potatoes treated with strains B-5351 and B-6066. When infection by potato viruses

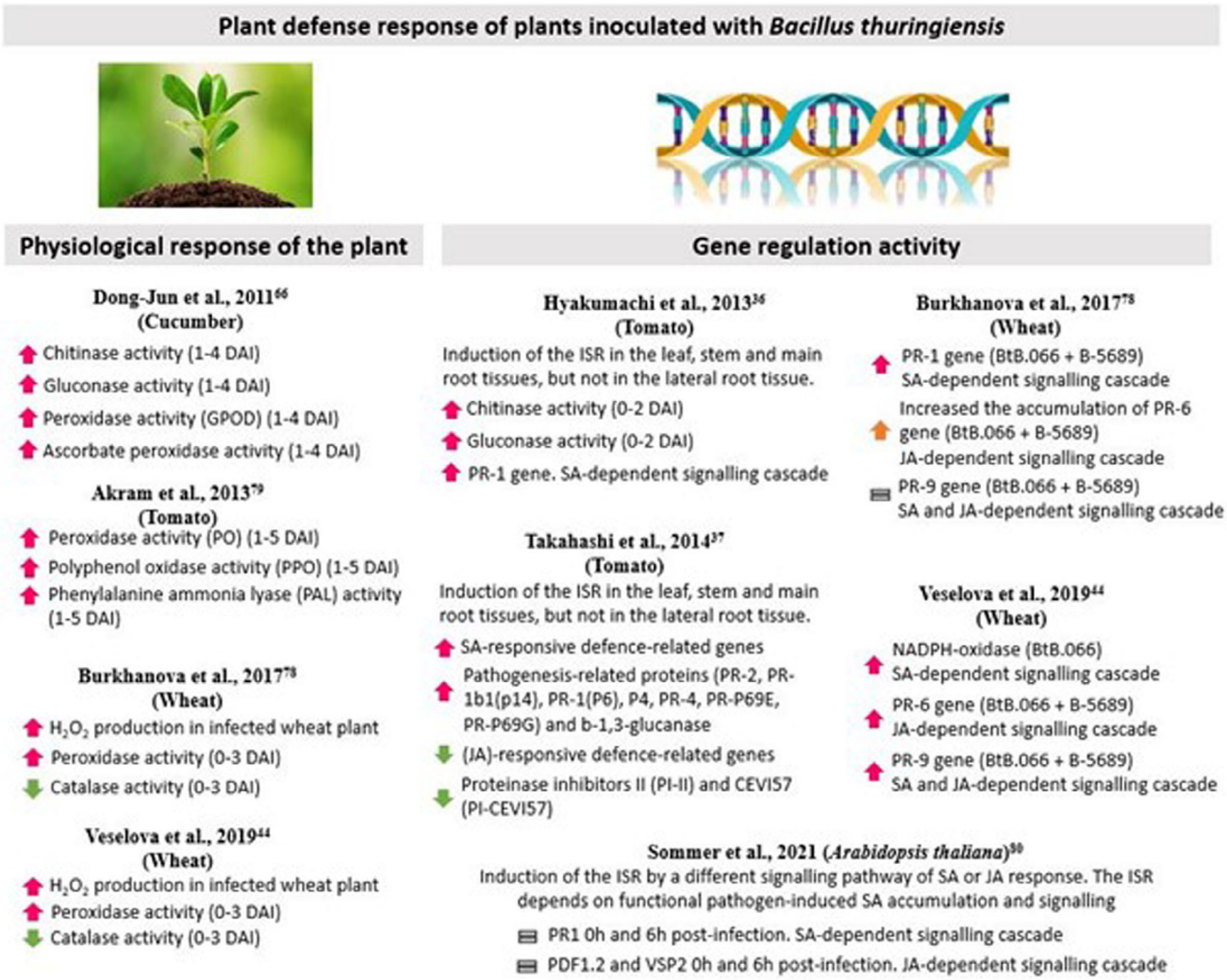

Figure 2. Plant defense response of plants inoculated with endophytic Bt strains. Pink upright arrows indicate gene up-regulation, orange upright arrows signify slight gene up-regulation while the green down arrows indicate gene down-regulation. See References section for the whole citation of the reports indicated in the figure.

was assessed, a significant reduction in the incidence (infected plants per plot) was observed for PVS, PVM and PVY in the two growth seasons. For PVS, PVM and PVY the Bt isolate B-6066 showed the greatest reduction in incidence for all the potato viruses (single or double inoculated) with between 0 and 15% infected plants compared to the 40–70% for water-treated control potato plants.

Regards the efficacy of Bt as PGPB, there are only a few published studies on the use of Bt or its combination with other PGPB (*Burkholderia ambifaria*)[84] or commercial biofertilizer microbial agents (*Azospirillum brasilense*)[59] in field conditions. Bandopadhyay *et al*.[83] reported a significant increase in seed germination, shoot height, root length, leaf diameter, vigor index, fruit weight, seed weight, total fresh weight and dry weight of *Abelmoschus esculentus* (okra) colonized with Bt. Also, the *Abelmoschus esculentus* colonized with Bt showed increases of 68% in protein content in leaves, 70% catalase activity, 52% peroxidase activity, 66% soluble sugar content, 34% protein content and more than 75% phosphorus content compared to untreated plants. Ferrarezi *et al*.[61] reported the use in field conditions of Bt isolate RZMS9 with *Azospirillum brasilense* in maize fields, the treatment of Bt RZ2MS9 + *Azospirillum brasilense* in maize plants significantly increased plant height by 2.8% and 2.6% and stalk diameter by 9% and 6.9%, while the inoculation of *Azospirillum brasilense* and Bt RZ2MS9 individually did not differ from the control. Also in field conditions, the inoculation with Bt had no effect either on the composition of the maize-associated bacterial community (Gammaproteobacteria, Betaproteobacteria, Actinobacteria, Alphaproteobacteria, Cytophagia, and Bacilli) or on the total bacterial biomass. However, significant differences in the richness and in the community structure have been detected in the different plant niches analyzed.

As an alternative to inoculating the whole PGPB to the plant, Ismail *et al*.[16] compared the effect of applying plant hormones exogenously [IAA, benzyl adenine (BA)] and metabolites of Bt PB2 in *Phaseolus vulgaris* (beans). The metabolites of Bt PB2 were obtained from the supernatant (incubated 6 days at 28 °C) with ethyl acetate (1:1 *v/v* 10 h at 4 °C). The solvent layer (containing metabolites) was separated and evaporated to get the crude metabolites. A concentration of 100 ppm was applied to the plant leaves from top to bottom with a spray atomizer, the treatments were applied to 15-, 30- and 50-day old seedlings. Results showed

that the bacterial metabolites of Bt PB2 surpassed the exogenously applied hormones in increasing the plant biomass, photosynthetic pigments, carbohydrate and protein contents, antioxidant enzyme activity, endogenous hormones, and yield traits.

7 OPEN QUESTIONS ABOUT THE USE OF *BACILLUS THURINGIENSIS* AS PLANT GROWTH PROMOTION BACTERIA

Most of the experimental evidence, prove that Bt can act as entomopathogen when it is applied as spray to the leaves surface.[1–3] But the activity of Bt as a PGPB had been out of focus during the last 20 years, since the first isolation of endophytic Bt stains from cotton.[39] Therefore, there are research gaps on the soil microbial ecology of Bt and plant–Bt interactions to be addressed. With regards the soil microbial ecology of Bt, the soil phase of Bt had been considered as a 'sump of spores'.[1–3] Nevertheless, the reported Bt strain with endophytic/rhizospheric activity during the Bt soil phase give rise to research questions: (i) how frequently are these strains distributed in nature?, (ii) how does Bt recognize the different environments and modifies their behavior?, (iii) how does Bt interact with other members of the soil microbiome (PGPB) and plant growth promotion fungi (PGPF)? In the case of the plant–Bt interaction Vidal-Quist *et al*.[24] reported a correlation between strain phylogeny and colonization levels in *Arabidopsis thaliana*. However, little is known if this correlation can be done in other plant species. Furthermore, it is not known which genetic determinants are involved in the plant–Bt interaction and how to make the interaction specific. Further experimentation is needed to answer these open questions and expand our knowledge of Bt as a highly versatile entomopathogen able to adapt to different environments.

8 CONCLUSIONS

Bt synthesizes an extraordinary diversity of insecticidal proteins and has demonstrated its potential and safety as a biocontrol agent over more than five decades. Over this time Bt has been used in field conditions as sprays or, more recently, generating Genetic Modified Organism (GMO) that encode Bt pesticidal proteins.

Current research suggests that Bt can also be considered as a promising new PGPB that is able to promote plant growth and act against phytopathogens in addition to insect pests. The consideration of Bt as a PGPB will broaden our understanding as a versatile entomopathogen by exhibiting differential behaviors depending on context. Moreover, the current research fit with the European Green Deal priority, specifically with the 'Farm to Fork' strategy by the use a well-known and harmless entomopathogenic bacteria to reach out sustainable food production.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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