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Review

Bacillus Thuringiensis as a Biofertilizer in Crops and Their Implications in the Control of Phyto-pathogens and Insect Pests

Joaquin Gomis-Cebolla[1*] and Colin Berry[2]

[1]Institute for Conservation and Improvement of Valencian Agrodiversity (COMAV), Universitat Politècnica de València, Camí de Vera 14, 46022 Valencia, Spain.
[2]School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK; Berry@cardiff.ac.uk; Tel.: +44-29-20874508
* Correspondence: jgomceb1@upvnet.upv.es

Abstract

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. Bt strains have been reported as endophyte or rhizospheric bacteria, but little is known about the implications of this property of Bt 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. The implications of the proposed review will broaden our understanding of Bt as a versatile entomopathogen by exhibiting 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

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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 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 bioinoculant instead as spray to improve the resistance to biotic stresses.

2. Translocation of *Bacillus thuringiensis* into plant tissues and interaction with other plant growth promoting bacteria.

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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 (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 tissues samples and vertical transmission

Bt have 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 (Figure 1). Regarding the Bt distribution in the plant tissues, it was present throughout the plant (roots, stem, leaves, etc.)[57,58] where the in the rhizosphere and roots the abundance of Bt was higher than in the rest of the plant tissues (stem and leaves, etc.)[57,59]. These results suggests 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 the aim to increase the likelihood of infecting invertebrate hosts[24]. In addition, García-Suárez et al, 2017[40] reported the presence of Bt in the seeds of *Arabidopsis thaliana* Bt colonized plants. Thus, it has been suggested that the Bt showed a 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, proto-cooperation and commensalism) or negative (competition, parasitism, predation and ammensalism)[60]. In the case of the interaction of Bt with other plant growth

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promoting bacteria (PGPB) (*Burkholderia phytofirmans*, *Pseudomonas fluorescens*, *Rhizobium leguminosarum* and *Azospirillum brasilense*), include playing roles in the colonization efficiency, plant growth, plant nodulation (Figure 1)[24,26,30,59,61–63]. The reports published up to date[22,23,30,61] showed a wide range of plant response to the co-inoculation of Bt plus PGPB bacteria. Vidal-Quist et al., 2013[24] reported that the co-inoculation with *B. phytofirmans* or *P. fluorescens* in *A. thaliana* showed no effect on Bt colonization levels. Rojas-Solís et al., 2015[58] evaluated five different strains of *P. fluorescens* plus Bt in *Zea mays* (corn), where the combinations of *P. fluorescens UM16* + Bt UM96 had beneficial interaction (total fresh weight, hypocotyl length and root length) with the plant, while separately the *P. fluorescens* and Bt strains showed broad potential for colonizing the rhizosphere and promoting tomato plant growth. Mishra et al., 2009[59] indicated that Bt -KR1 when co-inoculated with *R. 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). Almeida et al., 2021[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 GMO crops. However, the toxicity of Bt acting as a endophyte/rhizospheric 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] (Figure 1). Activity against the

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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 lab 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 colonise 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 increased were compared to the non-treated plants (NT), 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 compare to the NT plants have been reported in all the crops (Table 1). Regarding the toxicity differences among the Bt isolates in brassica, cotton, potatoes, wheat and orange tree could may be due to the fact that Bt colonizes the plant in a phylogeny dependent manner[24]. Further analysis is need it to determine if the variability in the reported toxicity data is due to the action of Bt toxins, the activation of plant defence (Systemic Acquired Resistance (SAR) and Induced Systemic Response (ISR)) or the increase plant toxicity it is not a general effect of the endophythism, it could be Bt strain-plant dependent process.

As regard the effect in the insects fed with plants colonized with Bt isolates, Veselova et al., 2019[44] report a reduction in the insect fecundity of *Schizaphis graminum* (spring green aphid, a major pest that feed mainly Poaceae plants like wheat, corn, oat, etc.) in 7-day-old wheat seedlings for the Bt isolates B-6066 and B-5689. Although da Costa et al., 2020[64] reported no mortality of *S. frugiperda* fed in cotton plants regardless of the form of inoculation, in 11-dat old cotton Bt colonized plantules for four Bt isolates

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tested (S1450, S1905, S2122 and S2124). The Bt strain showed the highest adhesion of the spore/crystal complex to the seed coats, so it was selected for toxicity *in vitro* assays of leaves collected at 18-, 23- and 30-day old Bt colonized plantules. The Bt strain was not toxic at the spore concentration 10^6 CFU/mg and 10^8 CFU/mg but *S. frugiperda* larvae showed a weight reduction in plants grown from seeds treated with 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 reports[5,39,41] reported toxicity of isolated Bt from colonized (natural[39] and artificial[5,41] bacterial colonization) plants of cotton, lavender, poinsettia and *Arabidopsis thaliana*. Monnerat et al., 2003[39] and García-Suárez et al., 2021[41] performed toxicity assays after the Bt strains were isolated form the plant. 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., 2003[39] and García-Suárez et al., 2021[41] do not indicate the mortality of the respective pests in the Bt-inoculated plants. Therefore, it cannot be determined if Bt kept their toxicity as a endophyte/rhizobacterium or free-living bacterium.

In the case of Lin et al., 2021[5] a 1-week-old *A. thaliana* plants was inoculated with Bt 407 Cry^- and transfer to steril media. Bot incubations were done for a period of 48h. These steps were repeated for 40 transfers. Because of the experimental evolution experiment, two evolved Bt lineages E and F showed an increase in the activity compared to the

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ancestor Bt 407 Cry$^-$ in *in vivo* toxicity assays Specifically, the Bt lineages E and F showed a significant several fold decrease in LD_{50} compared to the ancestor strain, via injection into the hemolymph of *Galleria mellonella* insect larvae (Table 2). Also, in *in vitro* assays the evolved Bt lineages E and F showed an increased hemolytic zone compared with the ancestor.

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) (Figure 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* [*R. solanacearum* discussed in section 7]), potato viruses (Potato virus Y (PVY), Potato virus M (PVM), and Potato virus S (PVS) [commented in section 8]) and oomycetes (*Phytophthora infestans*)[15,16,18,31–37,42,45,48,57,65].

Briefly, Bt isolated tested *in vitro* toxicity assays demonstrated that the bacteria from natural/artificial colonized plants grown as a free-living bacterium (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 Bt pathogenicity. Regarding the Bt colonized plants[32,34] a reduction in the plant symptoms or the number of infected plants against phytopathogenic fungi (*B. cinerea* and *U. agropyri*) is shown. Martínez-Absalón et al., 2014[32] reported that the barrel medic plants (*Medicago truncalia*) inoculated with Bt UM96 and *B. cinerea* showed a reduction in the disease symptoms

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(chlorosis, presence of grey mould, root browning and necrosis). Also, the protective effect was observed in plants first inoculated with Bt UM96 strains and infected with *B. cinerea*. Tao et al., 2014[34] reported that the twelve varieties of wheat inoculated with Bt strains 58-2-1 and 37-1, showed a different toxicity profile against *U. agropyri*. The strain 58-2-1 showed activity against *U. agropyri* in nine wheat varieties and no activity in three wheat varieties. As soon as the Bt strain 37-1 showed activity on the seven varieties and no toxicity on the remaining five wheat varieties.

4. Plant growth promotion and pathogenicity traits 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 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 N_2 fixation[13–15], ammonia production (NH_3)[13,16], phosphate solubilization[14–20,57], production of sideophores[17–19,21,57] and volatile organic compounds [29,30].

Also, Bt colonized plants exhibit traits that increase the plant protection against phytopathogens. Those traits are a set of enzymes that impair or reduce the development of phytopathogens (fungi, bacteria and viruses). It has been reported that Bt could produce the enzymes amylase, cellulase, proteases, pectinase, xylase[16,57], gluconase[66],

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chitinases[32,66] AiiA lactonase[67] and RNAse activity[42]. Regarding the role of the Bt toxins in the insect mortality increase in Bt colonized plants it is not well understood. The plants inoculated with different strains of Bt showed an increase in the insect mortality compare to the non-treated plants (Table 1 and 2). But little it is known if the mortality reported is caused by the Bt toxins, secondary metabolites produced by Bt, the activation of the plant defence response, etc. Further research will be need it to determine the role of the Bt toxins in the insect mortality reported in the Bt colonized plants.

5. Applications of the Plant Growth Promotion traits of *Bacillus thuringiensis* in phytoremediation.

Related with the activity of Bt as a PGPB, the plants colonised with endophytic Bt improve their resistance against abiotic stresses, 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., 2013[21] and Sharma et al., 2016[13] also reported a significant increase in the root and shoot length in *Vigna radiata* (mung bean) and *Alnus firma* (park tree) when colonised 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 ammonia) and preventing ethylene accumulation and, therefore, helping the plant to reduce the abiotic stress, promoting its growth and survival[70]. For the role of the IAA, it has been proposed, that the roots of the plant exude various compounds to the rhizosphere,

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such as sugars, organic acids and amino acids like tryptophan Glick et al.,2014[69]. PGPB can assimilate tryptophan, an essential precursor of IAA synthesis, then produce IAA to induce the transcription of auxin response factors, promoting plant growth. Batista et al., 2021[28], reported that the endophytic Bt strain RZ2MS9 harbours 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: 3h to 30h, IAA concentration range: 0.06 to 0.20 µg/ml with an IAA production peak at 21h with a concentration of 0.20 µg/ml) is cell density dependent when Bt RZ2M9 are in LB medium supplemented with 1 g/l 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 (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).

6. Plant defense response to the inoculation with *Bacillus thuringiensis* isolates.

The plant defense response describe a range of adaptations evolved in the plants to reduce the damage and improve their survival and reproduction efficiency. The general model indicate 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 signalling pathways. Induction of SAR is through salicylic acid (SA) and ISR requires jasmonic acid (JA) and ethylene (Et)

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signalling pathways[71–73]. Regarding the plant response some reports suggest that there is no uniform, instead there seem to be different responses depending on the eliciting microbial strains, involving JA/ET signalling as well as SA signalling pathways[74–77].

In the case of Bt colonized plants, the interaction between plant tissue and Bt triggers the plant defence responses (Systemic Acquired Resistance (SAR)[44,78] and Induced Systemic Response (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 H_2O_2 and the activity of the following enzymes: gluconase, chitinase, ascorbate peroxidase, polyphenol oxidase and phenylalanine ammonia lyase[36,44,78–80] (Figure 2). The signalling pathways (SAR and ISR) activated in the Bt colonized plants after been infected with a phytopathogen, are not consistent among the different reports published up to date (Figure 2). Hyakumachi et al., 2013[36] and Takahashi et al., 2014[37] showed that in *Solanum lycopersicum* (tomato) colonised with Bt (37) or inoculated with cell free extract (filtrated 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 defence-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 defence-related genes (Proteinase inhibitors II (PI-II) and CEVI57 (PI-CEVI57)[37]). Burkhanova et al., 2017[78] and Veselova et al., 2019[44] studied *Triticum aestivum* (wheat) colonised/inoculated with two different Bt strains (B-5689 and B-6066) and exposed to the phytopathogenic fungus *Septoria nodorum* or the aphid *Schizaphis graminum* reported the up-regulation of the SA-responsive defence-related genes (PR-1 and NADPH-oxidase), JA-responsive defence-related genes (PR-6 gene) but no difference in regulation of the PR-9 gene (SA and JA-dependent signalling cascade). Finally, Sommer et al., 2021[81] described that in *Arabidopsis thaliana* inoculated

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with Bti and not exposed to any phytopathogen, the plant defence response activated was a different signalling pathway than the SAR or JA signalling 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 signalling pathways.

7. Effectiveness of the application of *Bacillus thuringiensis*or it's metabolites in field conditions.

To date few reports have been published about the successful use of Bt as a in field conditions[16,42,59,82–84]. Sorokan et al., 2020[42] evaluate the efficiency of potatoes colonized with Bt in the control of *L. decemlineata* and potato viruses (Potato virus Y (PVY), Potato virus M (PVM), and Potato virus S (PVS)) in two different growth seasons. With regards the control of *L. 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 instar was observed. 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 plants with strains B-5351 and B-6066. When infection by potato viruses was assessed, a significant reduction in the incidence (infected plants/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 incidence reduction in the all the potato virus (single or double inoculated) with between 0-15% infected plants compared to the 40-70% of water-treated control potato plants.

Regarding the efficacy of Bt as PGPB, there are few published studies on the use of Bt or its combination with other PGPB (*Burkholderia ambifaria*)[84] or commercial

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biofertilizer microbial agents (*Azospirillum brasilense*)[59] in field conditions. Bandopadhyay et al., 2020[84] 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 *A. 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., 2022[59] reported the use in field conditions of Bt isolate RZMS9 with *A. brasilense* in maize fields, the treatment of Bt RZ2MS9 + *A. 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 Ab and Bt RZ2MS9 individually do 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 inoculate the whole PGPB to the plant, Ismail et al., 2021[16] compared the effect of applying exogenously plant hormones (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 28ºC) with ethyl acetate (1:1 v/v 10 h at 4ºC). 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 up to down with a spray atomizer, the treatments were done at 15-, 30- and 50-days old seedlings. As a result, 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.

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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 GMO that encode Bt pesticidal proteins. With the current knowledge Bt can also be considered as a new promise PGPB that it is able to promote the plant growth and act against phytopathogens in addition to insect pests.

However, many questions remain about the soil microbial ecology of Bt: What is the role of endophytic/rhizospheric Bt strains within the plant? How frequently are these strains distributed in nature? How does Bt interact with other members of the plant microbiome? Further experimentation is need to answer these questions and expand our knowledge of Bt as a highly versatile entomopathogen able to adapt to different environments.

Conflicts of Interest.

The authors declare no conflict of interest.

References

1. Palma L, Muñoz D, Berry C, Murillo J, Caballero P. *Bacillus thuringiensis* toxins: An overview of their biocidal activity. Vol. 6, Toxins. MDPI; 2014. p. 3296–325.
2. Chakroun M, Banyuls N, Bel Y, Escriche B, Ferré J. Bacterial Vegetative Insecticidal Proteins (Vip) from Entomopathogenic Bacteria. Microbiology and Molecular Biology Reviews. 2016 Jun;80(2):329–50.
3. Jouzani GS, Valijanian E, Sharafi R. *Bacillus thuringiensis*: a successful insecticide with new environmental features and tidings. Vol. 101, Applied Microbiology and Biotechnology. Springer Verlag; 2017. p. 2691–711.
4. Bishop AH, Rachwal PA, Vaid A. Identification of genes required by *Bacillus thuringiensis* for survival in soil by transposon-directed insertion site sequencing. Curr Microbiol. 2014 Apr;68(4):477–85.
5. Lin Y, Alstrup M, Pang JKY, Maróti G, Er-Rafik M, Tourasse N, et al. Adaptation of *Bacillus thuringiensis* to Plant Colonization Affects Differentiation and Toxicity. mSystems. 2021 Oct 12;
6. Rocha FYO, Negrisoli Júnior AS, de Matos GF, Gitahy P de M, Rossi CN, Vidal MS, et al. Endophytic *Bacillus* Bacteria Living in Sugarcane Plant Tissues and *Telchin licus licus* Larvae

(Drury) (Lepidoptera: Castniidae): The Symbiosis That May Open New Paths in the Biological Control. Front Microbiol. 2021 May 12;12.

7. Raddadi N, Cherif A, Ouzari H, Marzorati M, Brusetti L, Boudabous A, et al. *Bacillus thuringiensis* beyond insect biocontrol: plant growth promotion and biosafety of polyvalent strains. Ann Microbiol. 2007;57(4):481–94.
8. Sauka D. *Bacillus thuringiensis* and their Endophytic Capabilities. EC Microbiol. 2017;9(9):27–31.
9. Azizoglu U. *Bacillus thuringiensis* as a Biofertilizer and Biostimulator: a Mini-Review of the Little-Known Plant Growth-Promoting Properties of Bt. Vol. 76, Current Microbiology. Springer New York LLC; 2019. p. 1379–85.
10. Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. Plant growth-promoting bacterial endophytes. Vol. 183, Microbiological Research. Elsevier GmbH; 2016. p. 92–9.
11. Orozco-Mosqueda M del C, Santoyo G. Plant-microbial endophytes interactions: Scrutinizing their beneficial mechanisms from genomic explorations. Vol. 25, Current Plant Biology. Elsevier B.V.; 2021.
12. Afzal I, Shinwari ZK, Sikandar S, Shahzad S. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Vol. 221, Microbiological Research. Elsevier GmbH; 2019. p. 36–49.
13. Sharma N, Saharan B. Bacterization Effect of Culture Containing 1-aminocyclopropane-1-carboxylic Acid Deaminase Activity Implicated for Plant Development. Br Microbiol Res J. 2016 Jan 10;16(1):1–10.
14. Batista BD, Lacava PT, Ferrari A, Teixeira-Silva NS, Bonatelli ML, Tsui S, et al. Screening of tropically derived, multi-trait plant growth-promoting rhizobacteria and evaluation of corn and soybean colonization ability. Microbiol Res. 2018 Jan 1;206:33–42.
15. Fernandes TA, Oliveira MC, Batista F de C, Ribeiro VP, Gomes EA, Valicente FH, et al. Potencial Multifuncional de Isolados de *Bacillus thuringiensis* para Controle de Fungos Fitopatogenicos e Promoçao de Crescimiento Vegetal. BOLETIM DE PESQUISA E DESENVOLVIMENTO 198 [Internet]. 2019;1679–0154. Available from: www.embrapa.br/fale-conosco/sac
16. Ismail MA, Amin MA, Eid AM, Hassan SED, Mahgoub HAM, Lashin I, et al. Comparative study between exogenously applied plant growth hormones versus metabolites of microbial endophytes as plant growth-promoting for *Phaseolus vulgaris* L. Cells. 2021 May 1;10(5).
17. Raddadi N, Cherif A, Boudabous A, Daffonchio D. Screening of plant growth promoting traits of *Bacillus thuringiensis*. Ann Microbiol. 2008;58(1):47–52.
18. Cherif-Silini H, Silini A, Yahiaoui B, Ouzari I, Boudabous A. Phylogenetic and plant-growth-promoting characteristics of *Bacillus* isolated from the wheat rhizosphere. Ann Microbiol. 2016 Sep 1;66(3):1087–97.
19. Lyngwi NA, Nongkhlaw M, Kalita D, Joshi SR. Bioprospecting of plant growth promoting bacilli and related genera prevalent in soils of pristine sacred groves: Biochemical and molecular approach. PLoS One. 2016 Apr 1;11(4).
20. Sauka DH, Piccinetti CF, Vallejo DA, Onco MI, Pérez MP, Benintende GB. New entomopathogenic strain of *Bacillus thuringiensis* is able to solubilize different sources of inorganic phosphates. Applied Soil Ecology. 2021 Apr 1;160.
21. Babu AG, Kim JD, Oh BT. Enhancement of heavy metal phytoremediation by *Alnus firma* with endophytic *Bacillus thuringiensis* GDB-1. J Hazard Mater. 2013 Apr 5;250–251:477–83.
22. Mishra PK, Mishra S, Selvakumar G, Kundu S, Shankar Gupta H. Enhanced soybean (*Glycine max* L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thuringiensis*-KR1. Acta Agric Scand B Soil Plant Sci. 2009 Mar;59(2):189–96.
23. Praça LB, Gomes ACMM, Cabral Glaucia, Martins ES, Sujii ER, Monnerat RG. Endophytic Colonization by Brazilian Strains of *Bacillus thuringiensis* on Cabbage Seedlings Grown *in Vitro*. Bt Research. 2012;
24. Vidal-Quist JC, Rogers HJ, Mahenthiralingam E, Berry C. *Bacillus thuringiensis* colonises plant roots in a phylogeny-dependent manner. FEMS Microbiol Ecol. 2013 Dec;86(3):474–89.

25. Armada E, Roldán A, Azcon R. Differential Activity of Autochthonous Bacteria in Controlling Drought Stress in Native Lavandula and Salvia Plants Species Under Drought Conditions in Natural Arid Soil. Microb Ecol. 2014 Feb 1;67(2):410–20.
26. Armada E, Probanza A, Roldán A, Azcón R. Native plant growth promoting bacteria Bacillus thuringiensis and mixed or individual mycorrhizal species improved drought tolerance and oxidative metabolism in Lavandula dentata plants. J Plant Physiol. 2016 Mar 15;192:1–12.
27. Suyamud B, Thiravetyan P, Panyapinyopol B, Inthorn D. *Dracaena sanderiana* endophytic bacteria interactions: Effect of endophyte inoculation on bisphenol A removal. Ecotoxicol Environ Saf. 2018 Aug 15;157:318–26.
28. Batista BD, Dourado MN, Figueredo EF, Hortencio RO, Marques JPR, Piotto FA, et al. The auxin-producing *Bacillus thuringiensis* RZ2MS9 promotes the growth and modifies the root architecture of tomato (*Solanum lycopersicum* cv. Micro-Tom). Arch Microbiol. 2021 Sep 1;203(7):3869–82.
29. Timmusk S, Abd El-Daim IA, Copolovici L, Tanilas T, Kännaste A, Behers L, et al. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: Enhanced biomass production and reduced emissions of stress volatiles. PLoS One. 2014 May 8;9(5).
30. de Almeida JR, Bonatelli ML, Batista BD, Teixeira-Silva NS, Mondin M, dos Santos RC, et al. *Bacillus thuringiensis* RZ2MS9, a tropical plant growth-promoting rhizobacterium, colonizes maize endophytically and alters the plant's production of volatile organic compounds during co-inoculation with *Azospirillum brasilense* Ab-V5. Environ Microbiol Rep. 2021 Dec 1;13(6):812–21.
31. Martinez-Absalon SC, del C Orozco-Mosqueda M, Martinez-Pacheco MM, Farias-Rodriguez R, Govindappa M, Santoyo G. Isolation and molecular characterization of a novel strain of *Bacillus* with antifungal activity from the sorghum rhizosphere. Genet Mol Res. 2012;11(3):2665–73.
32. Martínez-Absalón S, Rojas-Solís D, Hernández-León R, Prieto-Barajas C, Orozco-Mosqueda M del C, Peña-Cabriales JJ, et al. Potential use and mode of action of the new strain *Bacillus thuringiensis* UM96 for the biological control of the grey mould phytopathogen *Botrytis cinerea*. Biocontrol Sci Technol. 2014 Dec 1;24(12):1349–62.
33. Souza A, Cruz JC, Sousa NR, Procópio ARL, Silva GF. Endophytic bacteria from banana cultivars and their antifungal activity. Genetics and Molecular Research. 2014 Oct 27;13(4):8661–70.
34. Tao A, Pang F, Huang S, Yu G, Li B, Wang T. Characterisation of endophytic *Bacillus thuringiensis* strains isolated from wheat plants as biocontrol agents against wheat flag smut. Biocontrol Sci Technol. 2014;24(8):901–24.
35. Qi J, Aiuchi D, Tani M, Asano SI, Koike M. Potential of Entomopathogenic *Bacillus thuringiensis* as Plant Growth Promoting Rhizobacteria and Biological Control Agents for Tomato *Fusarium* Wilt. Vol. 2, International Journal of Environmental & Agriculture Research (IJOEAR) ISSN. 2016.
36. Hyakumachi M, Nishimura M, Arakawa T, Asano S, Yoshida S, Tsushima S, et al. *Bacillus thuringiensis* suppresses bacterial wilt disease caused by *Ralstonia solanacearum* with systemic induction of defense-related gene expression in tomato. Microbes Environ. 2013;28(1):128–34.
37. Takahashi H, Nakaho K, Ishihara T, Ando S, Wada T, Kanayama Y, et al. Transcriptional profile of tomato roots exhibiting *Bacillus thuringiensis*-induced resistance to *Ralstonia solanacearum*. Plant Cell Rep. 2014 Jan 1;33(1):99–110.
38. Monnerat RG, Soares CM, Capdeville G, Jones G, Martins ÉS, Praça L, et al. Translocation and insecticidal activity of *Bacillus thuringiensis* living inside of plants. Microb Biotechnol. 2009 Jul;2(4):512–20.
39. Monnerat R, Cavalcanti dos Santos R, Carvalho Barros P, Cardoso Batista A, Berry C. Isolamento e caracterização de estirpes de *Bacillus thuringiensis* endofíticas de algodão. Comunicado Técnico 98 Embrapa. 2003;0102–0099.
40. García-Suárez R, Verduzco-Rosas LA, del Rincón-Castro MC, Délano-Frier JP, Ibarra JE. Translocation of *Bacillus thuringiensis* in *Phaseolus vulgaris* tissues and vertical transmission in *Arabidopsis thaliana*. J Appl Microbiol. 2017 Apr 1;122(4):1092–100.
41. García-Suárez R, Verduzco-Rosas LA, Ibarra JE. Isolation and characterization of two highly insecticidal, endophytic strains of *Bacillus thuringiensis*. FEMS Microbiol Ecol. 2021 Jul 1;97(7).

42. Sorokan A, Cherepanova E, Burkhanova G, Veselova S, Rumyantsev S, Alekseev V, et al. Endophytic *Bacillus* spp. as a Prospective Biological Tool for Control of Viral Diseases and Non-vector *Leptinotarsa decemlineata* Say. in *Solanum tuberosum* L. Front Microbiol. 2020 Oct 15;11.
43. Dorta S de O, Balbinotte J, Monnerat R, Lopes JRS, da Cunha T, Zanardi OZ, et al. Selection of *Bacillus thuringiensis* strains in citrus and their pathogenicity to *Diaphorina citri* (Hemiptera: Liviidae) nymphs. Insect Sci. 2020 Jun 1;27(3):519–30.
44. Veselova S v., Burkhanova GF, Rumyantsev SD, Blagova DK, Maksimov I v. Strains of *Bacillus* spp. Regulate Wheat Resistance to Greenbug Aphid *Schizaphis graminum* Rond. Appl Biochem Microbiol. 2019 Jan 1;55(1):41–7.
45. Hong CE, Jo SH, Moon JY, Lee JS, Kwon SY, Park JM. Isolation of novel leaf-inhabiting endophytic bacteria in *Arabidopsis thaliana* and their antagonistic effects on phytophathogens. Plant Biotechnol Rep. 2015 Nov 1;9(6):451–8.
46. Sorokan A v., Benkovskaya G v., Blagova DK, Maksimova TI, Maksimov I v. Defense Responses and Changes in Symbiotic Gut Microflora in the Colorado Potato Beetle *Leptinotarsa decemlineata* under the Effect of Endophytic Bacteria from the Genus Bacillus. J Evol Biochem Physiol. 2018 Jul;54(4):300–7.
47. Melatti V, Praça L, Martins É, Sujii E, Berry C, Monnerat RG. Selection of *Bacillus thuringiensis* Strains Toxic Against Cotton Aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). BioAssay. 2010 Dec 7;5(0).
48. Islam MN, Ali MS, Choi SJ, Hyun JW, Baek KH. Biocontrol of citrus canker disease caused by *Xanthomonas citri* subsp. *citri* using an endophytic *Bacillus thuringiensis*. Plant Pathol J (Faisalabad). 2019;35(5):486–97.
49. Myasnik M, Manasherob R, Ben-Dov E, Zaritsky A, Margalith Y, Barak Z. Comparative sensitivity to UV-B radiation of two *Bacillus thuringiensis* subspecies and other *Bacillus* sp. Curr Microbiol. 2001;43(2):140–3.
50. Cucchi A, Sanchez De Rivas C. SASP (Small, Acid-Soluble Spore Proteins) and Spore Properties in *Bacillus thuringiensis israelensis* and *Bacillus sphaericus*. Cucchi CURRENT MICROBIOLOGY. 1998;36:220–5.
51. Hadapad AB, Vijayalakshmi N, Hire RS, Dongre TK. Effect of ultraviolet radiation on spore viability and mosquitocidal activity of an indigenous ISPC-8 *Bacillus sphaericus* Neide strain. Acta Trop. 2008 Aug;107(2):113–6.
52. Ruiu L. Plant-Growth-Promoting Bacteria (PGPB) against Insects and Other Agricultural Pests. Agronomy. 2020 Jun 1;10(6).
53. di Benedetto NA, Corbo MR, Campaniello D, Cataldi MP, Bevilacqua A, Sinigaglia M, et al. The role of plant growth promoting bacteria in improving nitrogen use efficiency for sustainable crop production: A focus on wheat. AIMS Microbiol. 2017;3(3):413–34.
54. dos Santos RM, Diaz PAE, Lobo LLB, Rigobelo EC. Use of Plant Growth-Promoting Rhizobacteria in Maize and Sugarcane: Characteristics and Applications. Vol. 4, Frontiers in Sustainable Food Systems. Frontiers Media S.A.; 2020.
55. Chialva M, Lanfranco L, Bonfante P. The plant microbiota: composition, functions, and engineering. Vol. 73, Current Opinion in Biotechnology. Elsevier Ltd; 2022. p. 135–42.
56. Armada E, Leite MFA, Medina A, Azcón R, Kuramae EE. Native bacteria promote plant growth under drought stress condition without impacting the rhizomicrobiome. FEMS Microbiol Ecol. 2018 Jul 1;94(7).
57. Tshikantwa TS, Ullah MW, He F, Yang G. Current trends and potential applications of microbial interactions for human welfare. Vol. 9, Frontiers in Microbiology. Frontiers Media S.A.; 2018.
58. Rojas-Solis D, Hernandez-Pacheco CE, Santoyo G. Evaluation of *Bacillus* and *Pseudomonas* to colonize the rhizosphere and their effect on growth promotion in tomato (*Physalis ixocarpa* Brot. ex Horm.). Rev Chapingo Ser Hortic. 2016 Jan 1;22(1):45–57.
59. Mishra PK, Mishra S, Selvakumar G, Bisht JK, Kundu S, Gupta HS. Coinoculation of *Bacillus thuringeinsis*-KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea

(*Pisum sativum* L.) and lentil (*Lens culinaris* L.). World J Microbiol Biotechnol. 2009 May;25(5):753–61.

60. Khan N, Bano A, Babar MA. Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in *Cicer arietinum* L. PLoS One. 2019 Mar 1;14(3).
61. Ferrarezi JA, Carvalho-Estrada P de A, Batista BD, Aniceto RM, Tschoeke BAP, Andrade PA de M, et al. Effects of inoculation with plant growth-promoting rhizobacteria from the Brazilian Amazon on the bacterial community associated with maize in field. Applied Soil Ecology. 2022 Feb 1;170.
62. Crickmore N, Berry C, Panneerselvam S, Mishra R, Connor TR, Bonning BC. A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. J Invertebr Pathol. 2021 Nov 1;186.
63. Hollensteiner J, Wemheuer F, Harting R, Kolarzyk AM, Diaz Valerio SM, Poehlein A, et al. *Bacillus thuringiensis* and *Bacillus weihenstephanensis* inhibit the growth of phytopathogenic *Verticillium* species. Front Microbiol. 2017 Jan 18;7(JAN).
64. da Costa FSS, Praça LB, Gomes ACMM, dos Santos RC, Soares CMS, Monnerat RG. *Bacillus thuringiensis* effect on the vegetative development of cotton plants and the biocontrol of *Spodoptera frugiperda*. Agronomy. 2020 Dec 1;10(12).
65. Hernández-Pacheco CE, Orozco-Mosqueda M del C, Flores A, Valencia-Cantero E, Santoyo G. Tissue-specific diversity of bacterial endophytes in Mexican husk tomato plants (*Physalis ixocarpa* Brot. ex Horm.), and screening for their multiple plant growth-promoting activities. Curr Res Microb Sci. 2021 Dec 1;2.
66. Dong-Jun S, Nguyen DMC, Song YS, Jung WJ. Induction of Defense Response Against Rhizoctonia solani in Cucumber Plants by Endophytic Bacterium *Bacillus thuringiensis* GS1. J Microbiol Biotechnol. 2012 Jan 7;22(3):407–15.
67. Anandan K, Vittal RR. Quorum quenching activity of AiiA lactonase KMMI17 from endophytic *Bacillus thuringiensis* KMCL07 on AHL-mediated pathogenic phenotype in *Pseudomonas aeruginosa*. Microb Pathog. 2019 Jul 1;132:230–42.
68. Arshad M, Saleem M, Hussain S. Perspectives of bacterial ACC deaminase in phytoremediation. Vol. 25, Trends in Biotechnology. 2007. p. 356–62.
69. Glick BR. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res. 2014 Jan 20;169(1):30–9.
70. Santoyo G, Equihua A, Flores A, Sepulveda E, Valencia-Cantero E, Sanchez-Yañez JM, et al. Plant Growth Promotion by ACC Deaminase-Producing Bacilli Under Salt Stress Conditions. In 2019. p. 81–95.
71. Pieterse CMJ, van Wees CMS, Hoffland E, van Pelt JA, van Loon LC. Systemic Resistance in *Arabidopsis* lnduced by Biocontrol Bacteria is lndependent of Salicylic Acid Accumulation and Pat hogenesis-Related Gene Expression. Vol. 8, The Plant Cell. American Society of Plant Physiologists; 1996.
72. Pieterse CMJ, van der Does D, Zamioudis C, Leon-Reyes A, van Wees SCM. Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol. 2012;28:489–521.
73. van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic Resistance Induced by rhizosphere bacteria [Internet]. Vol. 36, Annu. Rev. Phytopathol. 1998. Available from: www.annualreviews.org
74. Kojima H, Motaher Hossain M, Kubota M, Hyakumachi M. Involvement of the Salicylic Acid Signaling Pathway in the Systemic Resistance Induced in *Arabidopsis* by Plant Growth-Promoting Fungus *Fusarium equiseti* GF19-1. Vol. 62, J. Oleo Sci. 2013.
75. Nie P, Li X, Wang S, Guo J, Zhao H, Niu D. Induced systemic resistance against *Botrytis cinerea* by *Bacillus cereus* AR156 through a JA/ET- and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in *Arabidopsis*. Front Plant Sci. 2017 Feb 28;8.
76. van de Mortel JE, de Vos RCH, Dekkers E, Pineda A, Guillod L, Bouwmeester K, et al. Metabolic and transcriptomic changes induced in arabidopsis by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiol. 2012;160(4):2173–88.

 doi:10.20944/preprints202210.0254.v1

77. Wu L, Huang Z, Li X, Ma L, Gu Q, Wu H, et al. Stomatal closure and SA-, JA/ET-signaling pathways are essential for *Bacillus amyloliquefaciens* FZB42 to restrict leaf disease caused by *Phytophthora nicotianae* in *Nicotiana benthamiana*. Front Microbiol. 2018 Apr 27;9(APR).
78. Burkhanova GF, Veselova S v., Sorokan' A v., Blagova DK, Nuzhnaya T v., Maksimov I v. Strains of *Bacillus* ssp. regulate wheat resistance to *Septoria nodorum* Berk. Appl Biochem Microbiol. 2017 May 1;53(3):346–52.
79. Akram W, Mahboob A, Javed AA. *Bacillus thuringiensis* strain 199 can induce systemic resistance in tomato against *Fusarium* wilt. Eur J Microbiol Immunol (Bp). 2013 Dec;3(4):275–80.
80. Sommer A, Wenig M, Knappe C, Kublik S, Fösel B, Corina Vlot A. Induced systemic resistance impacts the phyllosphere microbiome through plant-1 microbe-microbe interactions 2 3. bioRxiv 20210113426583. 2021
81. Khan N, Bano A, Zandi P. Effects of exogenously applied plant growth regulators in combination with pgpr on the physiology and root growth of chickpea (*Cicer arietinum*) and their role in drought tolerance. J Plant Interact. 2018 Jan 1;13(1):239–47.
82. Khan N, Bano AMD, Babar A. Impacts of plant growth promoters and plant growth regulators on rainfed agriculture. PLoS One. 2020 Apr 1;15(4).
83. Bandopadhyay S. Application of plant growth promoting *Bacillus thuringiensis* as biofertilizer on *Abelmoschus esculentus* plants under field condition. J Pure Appl Microbiol. 2020 Jun 1;14(2):1287–94.
84. Prabhakar A, Bishop AH. Effect of *Bacillus thuringiensis* naturally colonising *Brassica campestris var. chinensis* leaves on neonate larvae of *Pieris brassicae*. J Invertebr Pathol. 2009 Mar;100(3):193–4.

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TABLES

Table 1. Toxicity of orange tree (*Citrus sinensis var osbeck*), peanut (*Phaseolus vulgaris var. cacahuate 72*), cabbage (*Brassica campestris var. chinensis and B. 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 Bt strains to insect pests.

Endophyte-containing Crops/ Infection time	***Bt* strain [serotype] (gene content)**	**Mortality (% ±SE)**	**Reference**
		D. citri **(Treatment 1/Treatment 2) (5 DAI)***	
Orange three (*Citrus sinensis var osbeck)* 3-month-old plants	**S1302 [ND]** (*cry1Ab, cry3A)*	90.0 ± 5.96 a/68.0 ± 3.27 d	43
	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	
		S. graminum **(7 DAI)**	
Wheat (*Phaseolus vulgaris var. cacahuate 72*) 7-day-old plants	**B-6066 [ND]** (ND)	36.3 ± 3.5	44
	B-5689 [ND] (ND)	33.1 ± 5.2	
	H_2O (NT)	12.2 ± 1.9	
		L. decemlineata **(3 DAI)**	
Potatoes (*Solanum tuberosum* var early rose) 25-day-old plants	**B-5689 [*thuringiensis*]** (ND)	33.3 ± 3.1	46
	B-55351 [*kurstaki*] (ND)	60.0 ± 10.6	
	H_2O (NT)	6.7 ± 0.5	

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Table 1 cont. Toxicity of orange tree (*Citrus sinensis var osbeck*), peanut (*Phaseolus vulgaris var. cacahuate 72*), cabbage (*Brassica campestris var. chinensis and B. 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 Bt strains to insect pests.

Endophyte-containing Crops/ Infection time	***Bt* strains [serotype] (gene content)**	**Mortality (% ±SE)**	**Reference**
		***T. ni* (7 days DAI)†**	
Penaut (*Phaseolus vulgaris* var cacahuate 72) 14-day old plants	**HD73 [*kurstaki*]** (*cry1Ac*) + *gfp*	48 ± 3.0	40
	H_2O (NT)	23 ± 4.0	
		Aphis gossypii* (5 days 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	
	H_2O (NT)	0.0 ± 0.0	
		***Pieris brassicae* (3 days 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	
		***P. xylostella* — *S. frugiperda* (7 days DAI)**	
Cabbage and Cotton *Brassica (hybrid Matsukaze Sakata)* 28-day old plants	**HD1 [*kurstaki*]** (*cry1Aa, cry1Ab, cry1Ac, cry2A*) + *gfp* (single inoculated plants)	10 ± NA — 20 ± NA	38
Cotton (*Gossypium var Delta-Opal*) 28-day old plants	**HD1[*kurstaki*]** (*cry1Aa, cry1Ab, cry1Ac, cry2A*) + *gfp* (weekly inoculated plants)	10 ± NA — 25 ± NA	
	H_2O (NT)	No mortality observed	

SE: Standard error

ND: The serotype or gene content of *Bacillus thuringiensis* strains have not been determined.

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NT: Non treated plants, water used as a negative control.

DAI: The insect toxicity assay have been performed for at least 3, 5 and 7 days, respectively, when the plant reach 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 respectively Bt isolates prior to perform the toxicity assays.

NC: Negative control, recombinant strain S2210 harbouring the gene *cry1Ab*: the Cry1Ab protein is not active against *D. citri*.

NA: The standard error was not determined in the bioassays with *P. xylostella*, *S. frugiperda* and *Pieris brassicae*.

* Data (mean ± SE) followed by the same letter in each treatment did not differ statistically. See Melatti et al., 2010[47] (Student-Newman Keuls test $P < 0.05$) and Dorta et al., 2019[43] (GLM with a quasi-binomial distribution plus post hoc Tukey–Kramer test; $P < 0.05$) for further details of the statistical analyses performed.

† The standard error has been interpolated from the graph published in García-Suárez et al., 2017[40].

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Table 2. Toxicity of the Bt isolates from different plant sources (naturally colonized or artificially colonized) and then reisolated 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)	FL_{95}	
Lavender (*Lavandula angustifolia*)	*A. aegypti*	LBIT-1250L [ND] (Cry4-type duplex , Cry11-type, and Cyt1-type)*	6.8	6.0-8.0	
		Bti [*israelensis*]	17.6	13.0-24.2	41
			LC_{50} (ng/cm^2)	FL_{95}	
Poinsettia (*Euphorbia pulcherrima*)	*M. 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)*	*G. mellonella*	Bt 407 Cry$^-$	~6,000 ± 1,000		
		Bt 407 Cry$^-$ lineage E	~1,500 ± 300		5
		Bt 407 Cry$^-$ lineage F	~1,000 ± 200		
			Mortality (%) of 150µl final culture		
Cotton (*Gossypium sp*)	*S. frugiperda and A. gemmatalis*	S1974 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B*)	100		
		S1979 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		
		S1983 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		
		S1985 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		39
		S1986 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		
		S1987 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		
		S1989 [ND] (*cry1Aa, cry1Ab, cry1Ac, cry1B)*	100		

ND: The serotype or gene content of *Bacillus thuringiensis* 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 have not been confirmed with molecular techniques (PCR or whole genome sequencing (WGS)), it be considered as preliminary data.† The LC50 and the standard error for *G. mellonella* has been interpolated from the graph published in Lin et al., 2021.

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FIGURES

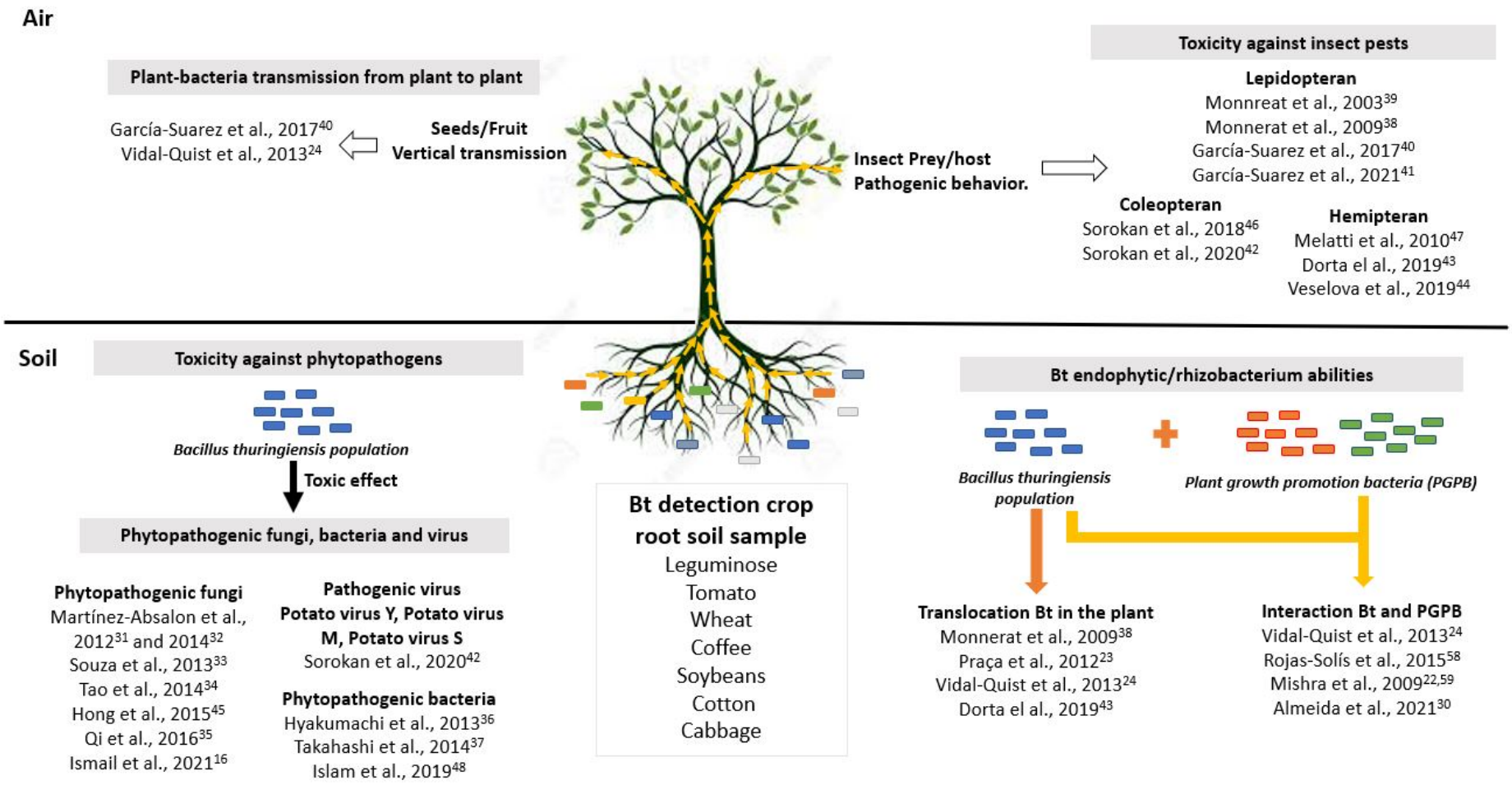

Figure 1. Role of Bt as endophyte/rhizospheric bacterium and their implications in the control of different kinds of phytopathogens.

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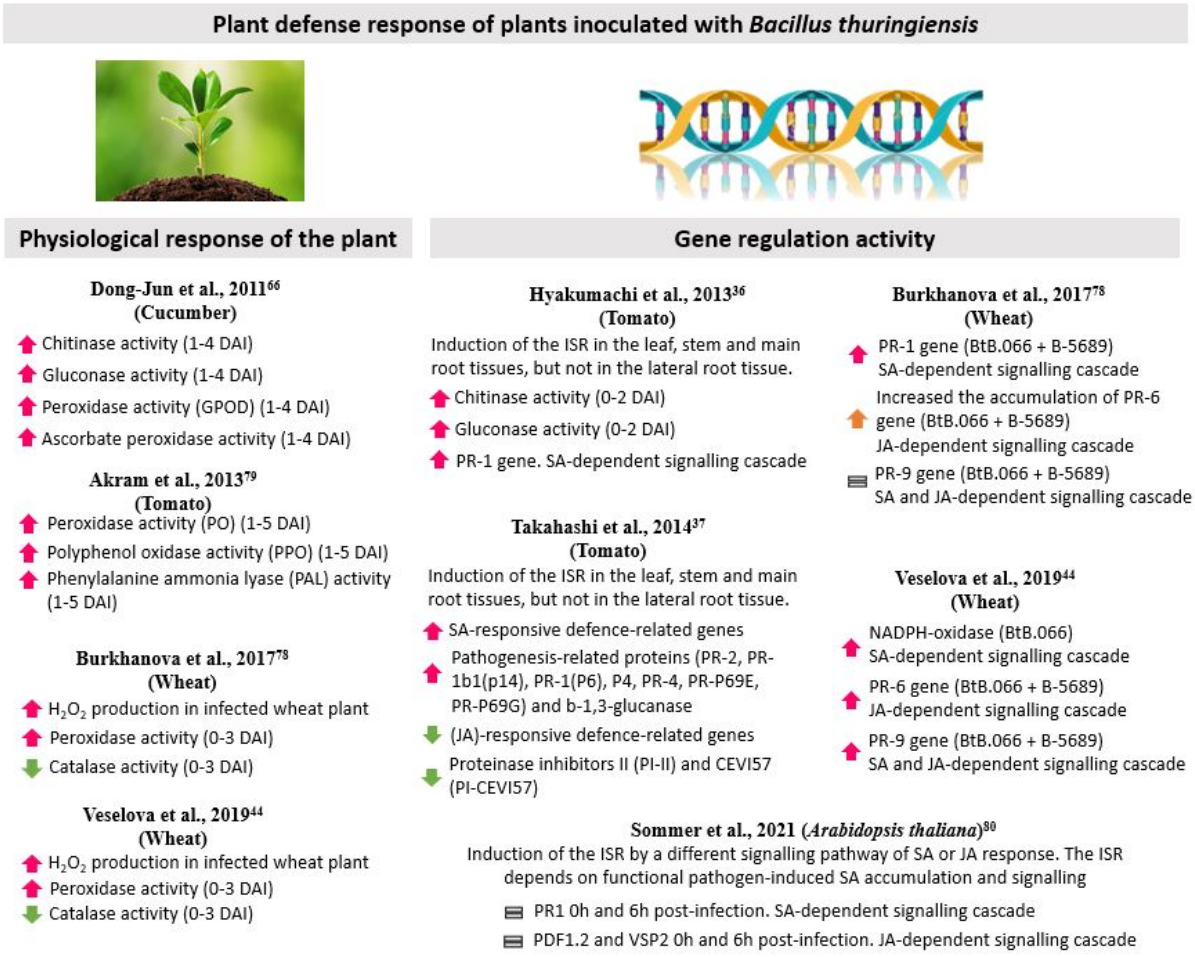

Figure 2. Plant defence response of plant inoculated with endophytic Bt strains. Pink right arrows indicate gene up-regulation, orange right arrows meaning slightly gene up-regulation while the Green down arrows indicate gene down-regulation. See References section for the whole citation of the reports indicated in the figure.