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Characterization of Plant Growth–Promoting Traits of Bacteria Isolated from Larval Guts of Diamondback Moth *Plutella xylostella* (Lepidoptera: Plutellidae)

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Abstract Eight bacterial isolates from the larval guts of Diamondback moths (*Plutella xylostella*) were tested for their plant growth–promoting (PGP) traits and effects on early plant growth. All of the strains tested positive for nitrogen fixation and indole 3-acetic acid (IAA) and salicylic acid production but negative for hydrogen cyanide and pectinase production. In addition, five of the isolates exhibited significant levels of tricalcium phosphate and zinc oxide solubilization; six isolates were able to oxidize sulfur in growth media; and four isolates tested positive for chitinase and β-1,3-glucanase activities. Based on their IAA production, six strains including four that were 1-aminocyclopropane-1-carboxylate (ACC) deaminase positive and two that were ACC deaminase negative were tested for PGP activity on the early growth of canola and tomato seeds under gnotobiotic conditions. *Acinetobacter* sp. PSGB04 significantly increased root length (41%), seedling vigor, and dry biomass (30%) of the canola test plants, whereas *Pseudomonas* sp. PRGB06 inhibited the mycelial growth of *Botrytis cinerea*, *Colletotrichum coccodes*, *C. gleospoiroides*, *Rhizoctonia solani*, and *Sclerotia sclerotiorum* under in vitro conditions. A significant increase, greater than that of the control, was also noted for growth parameters of the tomato test plants when the seeds were treated with PRGB06. Therefore, the results of the present study suggest that bacteria associated with insect larval guts possess PGP traits and positively influence plant growth. Therefore, insect gut bacteria as effective PGP agents represent an unexplored niche and may broaden the spectrum of beneficial bacteria available for crop production.

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Introduction

Insects (Class Insecta) are a major group of arthropods and are the most diverse group of animals on the earth; there exist > 1 million more described insect species than all other animal groups combined [5, 34]. This enormous diversity stems from their ability to survive under different ecologic conditions and metabolize several food sources. In addition, insects harbor a large number of microbes in their gut and thus serve as an arsenal of microbial diversity [5]. In turn, the tremendous adaptability of insects can also be attributed to the versatile roles played by their microbial partners. For instance, the presence of diazotrophic bacteria in insect gut helps with nitrogen uptake [1]; the production of indole derivatives and siderophores by symbiotic insect gut bacteria has been documented to exhibit antagonistic activity against pathogenic bacteria and fungi [5, 11, 19, 27]; and the production of extracellular hydrolytic enzyme chitinase helps maintain the physical property of the peritrophic membrane, which is imperative for nutrition diffusion [5].

In previous reports, the different genera of bacteria associated with insect guts were closely related to bacteria from the rhizosphere, phyllosphere, and soil [11, 16], indicating the existence of a tritrophic interaction between phytophagous insects, crop plants, and their microbial associates [16], the beneficial effects of which may include the production of phytohormones, associative nitrogen fixation, enzyme activity, improved mineral uptake, and suppression of pathogenic and deleterious organisms.

Therefore, these bacteria have been referred to as plant growth–promoting bacteria (PGPB) [3, 7, 23, 28].

Most reported PGPB are rhizosphere, phyllosphere, and soil isolates, except for a few that come from milk and cow dung [23, 33]. The microbial population (4×10^5 to 2×10^{11} /mL gut suspension) in insect guts is greater than that in the phyllosphere (3×10^5 /cm^2) [14], representing a well-explored niche for beneficial bacterial isolation. It is assumed that many insect species derive their microbiota from the surrounding environment, e.g., the phylloplane of food plants [5, 16]. Presumably, nutrient cycling rates in the gut environment are much higher and the potential disturbances more dramatic than those in soil and plants. Hence, insect-gut bacterial strains are more metabolically versatile than isolates occurring elsewhere. Insects also have well-developed mouth parts, which are used to grind and shred organic substances, making insects more accessible for microbial colonization [5]. Therefore, the isolation and characterization of microbes from such specialized dynamic environments represent a step forward in the development of bioinoculants that can be used to increase crop production. Recently, the present investigators reported on the isolation and characterization of bacterial strains from a lepidopteran insect, the Diamondback moth (DBM; *Plutella xylostella*). The contribution of gut bacteria to the nutrition of the host insect, based on influencing quantitative food-use efficiency through the production of chitinase enzyme, was also investigated. In addition, the effect of the siderophores produced by the gut bacterial strains against entomopathogenic fungi was reported as a mechanism of antagonism [11]. Accordingly, because the bacterial traits imparting a beneficial effect on their host insect may also have a constructive influence on crop growth [5, 7], the present study tested the plant growth–promoting (PGP) traits of DBM gut isolates. In addition, the ability of the selected bacterial strains to augment plant growth under gnotobiotic conditions was evaluated using canola and tomato as test plant species.

Materials and Methods

Bacterial and Fungal Strains

The hemolytic property of bacterial strains, initially isolated from the gut of DBM grown in Chinese cabbage under laboratory conditions, was determined according to Benson [2]. Five *Acinetobacter* sp. (PSGB03, PSGB04, PSGB05, PRGB15, and PRGB16), one *Pseudomonas* sp. (PRGB06), and two *Serratia* sp. (PRGB11 and PSGB13), all showing nonhemolytic behavior, were chosen for this study. All of these bacterial strains were previously isolated from fourth-instar DBM larvae using nutrient, Luria Bertani broth, and MacConkey agar media (Difco Laboratories, Detroit, MI) and identified based on their physical, biochemical, and molecular characteristics [11]. The 16S rRNA sequences of the respective bacteria were deposited at GenBank (http://www.ncbi.nlm.nih.gov/). Pure cultures of the bacterial strains were maintained in 50% glycerol at −80°C. The phytopathogenic fungi (Table 2) were obtained from the Korean Agricultural Culture Collection (KACC; Suwon, Republic of Korea) and maintained on potato dextrose agar (PDA) plates.

Mineral Solubilization

The ability of the bacteria to solubize mineral phosphate (P) and zinc (Zn) was tested on Pikovskaya's medium [29], which was supplemented with 0.5% TCP (Ca_3 $(PO_4)_2$ (0.5 g/100 mL media containing 998.45 µg 'P'/mL) or 0.12% ZnO. P solubilization in liquid media was quantified according to the method of Murphy and Riley [21]. Zn-solubilization efficiency (%) was calculated based on the zone diameter/colony diameter × 100 [25]. Sulfur-oxidizing potential was tested in a mineral salts thiosulfate medium, and the sulfate concentration in the spent medium was quantified turbidometrically at 340 nm [18].

Nitrogen Fixation, 1-aminocyclopropane-1-carboxylate deaminase Activity, and Phytohormone Production (indole-3-acetic and salicylic acids)

Acetylene reduction activity (ARA) of the bacterial strains was performed using the method of Hardy et al. [9]. The presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity in the bacteria was quantified by measuring the amount of α-ketobutyrate produced as a result of the enzymatic cleavage of ACC [28]. The quantification of indole-3-acetic acid (IAA) and salicylic acid (SA) was performed according to Gordon and Weber [6] and Meyer and Abdallah [20], respectively.

Production of Siderophore, Hydrolytic Enzymes, and Antifungal Metabolites (Hydrocyanic Acid and Ammonia) and in vitro Antagonism

A qualitative assay of siderophore and chitinase production was conducted in CAS (chrome azural S) agar and peptone agar media amended with 1.5% (v/v) colloidal chitin as carbon source, respectively [22, 32]; β-1,3-glucanase activity was quantified according to Nelson [24]; and pectinase production was tested in M9 medium [3]. To qualitatively determine hydrocyanic acid (HCN) production, the bacterial strains were streaked on King's B agar

medium amended with 4.4 g glycine/L. Filter paper, Whatman no. 1, was cut into uniform strips, 8 cm long and 0.5 cm wide; saturated with an alkaline picrate solution (0.5 % picric acid + Na_2CO_3; pH 13); and placed inside the lid of a Petri dish. The plates were then sealed air-tight with parafilm and incubated at 30°C for 48 hours. Thereafter, a color change in the sodium picrate present in the filter paper from yellow to reddish brown was considered to be an indication of HCN production [15]. Ammonia-producing ability of the bacterial isolates was tested in peptone broth [35], and their antifungal activity was tested against phytopathogenic fungi on PDA plates according to the dual-culture technique [7], in which a suspension of 10^6 conidia/mL of the fungal pathogen to be tested was spread on PDA, with a 6-mm diameter well in the centre, into which 6 µL (10^8 colony-forming units/mL) of the bacterial cells were inoculated. The plates were then incubated at 30°C until fungal mycelia covered the agar surface of the control plate without bacterial cells; the plate was then examined for a zone of inhibition. Wherever necessary, the protein content of the cells was determined using the standard Lowry method [17].

Gnotobiotic Growth Pouch Assay

Four ACC deaminase-positive (*Acinetobacter* sp. PSGB03, PSGB04, PSGB05, and PRGB15) and two ACC deaminase-negative (*Pseudomonas* sp. PRGB06 and *Serratia* sp. PRGB11) strains were further selected to test their PGP potential using canola and tomato as test plant species. Both plant species are sensitive to ethylene level at early growth stages. Seed treatment and gnotobiotic growth pouch assay were performed according to Penrose and Glick [28]. The primary root length of canola and tomato seeds was measured at days 5 and 10 after sowing, and seedling vigor [13] and dry biomass (oven dried at 70°C to a constant weight) were recorded at day 15 after sowing.

Statistical Analysis

Data were statistically analyzed by analysis of variance using the general linear model developed by the SAS Institute (version 9.1; Cary, NC), and means were compared using the least significant difference (LSD) method; $P \leq 0.05$ was considered significant.

Nucleotide Sequence Accession Numbers

The National Center for Biotechnology Information GenBank accession numbers for the sequences of bacterial strains PSGB03, PSGB04, PSGB05, PRGB15, PRGB16, PRGB06, PRGB11, and PSGB13 are EF195353, EF195354, EF195355, EF195345, EF195346, EF195341, EF195344, and EF195352, respectively.

Results

Results for the various PGP traits of the selected gut bacterial strains are presented in Table 1. All of the bacterial strains were found to solubilize P or Zn in the plate-based assay, as evidenced by the formation of a clear halo around the colony. Meanwhile, in the broth-based assay, maximum P solubilization was observed with *Acinetobacter* sp. strains (ranging from 479 to 547 µg P/mL), followed by *Pseudomonas* (250 µg P/mL) and *Serratia* sp. strains (120 to 215 µg P/mL). With the exception of two *Acinetobacter* sp. strains (PRGB15 and PRGB16), all of the bacterial strains were found to accumulate sulfate in the culture media.

The determination of ARA in gut bacterial isolates indicated their ability to fix dinitrogen, which was substantiated by the amount of ethylene (2–3 nmol/h/mg protein) that evolved as a result of acetylene reduction by the bacterial strains. All of the *Acinetobacter* sp. strains (except PSGB13 and PRGB16) showed significant ACC deaminase activity, as evidenced by the production of α-ketobutyrate, yet none of the *Pseudomonas* or *Serratia* strains were found to possess ACC deaminase activity. All of the strains produced a significant amount of IAA and SA, and all of then were also positive for siderophore and ammonia production but negative for pectinase and HCN production. *Acinetobacter* sp. strains PSGB03 and PRGB15 and *Serratia* sp. strains PRGB11 and PSGB13 showed a positive reaction in the chitinase production test. Among the four strains that showed positivity for *β*-1,3-glucanase production, maximum activity was recorded for the chitinase-negative *Pseudomonas* sp. PRGB06 (8 µg glucose/min/mg protein).

Antagonistic activity of the bacterial strains was evaluated in terms of inhibition-zone diameter as an indicator of the reduction in growth of the various test phytopathogenic fungi (Table 2). Among the eight isolates, *Pseudomonas* sp. PRGB06 was found to be the most effective biocontrol agent: It had an inhibitory effect on the growth of five of the eight tested fungi. Maximum inhibition was observed with the growth of *C. gloespoiroides* (3.6 cm) and *R. solani* (3.0 cm), the causal agents of anthracnose and sheath blight disease in red peppers and rice, respectively. None of the isolates inhibited the growth of *C. acutatum*, *C. capsici*, and *F. oxysporum*, yet *Serratia* sp. PRGB11 and all of the *Acinetobacter* sp. strains had an inhibitory effect on *Phytophthora capsici*.

Table 1 Plant-growth-promoting characteristics of bacterial strains isolated from larval guts of Diamondback Moth (DBM) – *Plutellaxylostella*

Bacterial strains[†]	Manner of direct PGP ability						Manner of indirect PGP ability		
	Mineral solubilization/oxidation			Production of enzymes and phytohormones			Production of biocontrol determinants		
	Soluble P (μg/mL)	Zn SE (%)	Sulfate content (μg/mL)	ARA (nmol ethylene/*h*/ mg protein)	ACCD (nmol α-KB min/mg protein)	IAA (μg/mL)	Salicylic acid (μg/mL)	Siderophore	Chitinase	β-1,3-glucanase (μg glucose/min/ mg protein)
Acinetobacter sp. PSGB03	458 ± 15^{b}	286 ± 5^{d}	7.7 ± 1.3^{ab}	2.7 ± 0.2^{bc}	60.4 ± 0.6^{d}	7.9 ± 0.2^{e}	5.8 ± 0.5^{d}	+	+	1.3 ± 0.1^{c}
Acinetobacter sp. PSGB04	468 ± 6^{b}	300 ± 3^{c}	2.4 ± 0.9^{c}	2.0 ± 0.4^{e}	572 ± 1.2^{a}	8.3 ± 0^{dc}	4.7 ± 0.3^{e}	+	–	0.7 ± 0.1^{d}
Acinetobacter sp. PSGB05	479 ± 35^{b}	271 ± 2^{e}	6.5 ± 0.6^{b}	2.8 ± 0.1^{bc}	133 ± 1.4^{b}	8.0 ± 0.1^{e}	6.0 ± 0.3^{d}	+	–	–
Acinetobacter sp. PRGB15	540 ± 20^{a}	300 ± 4^{c}	–	2.8 ± 0.2^{ab}	86.5 ± 1.2^{c}	8.2 ± 0^{d}	3.1 ± 0.3^{f}	+	+	–
Acinetobacter sp. PRGB16	547 ± 59^{a}	314 ± 10^{b}	–	2.2 ± 0.2^{de}	–	8.2 ± 0^{dc}	7.2 ± 0.6^{c}	+	–	2.4 ± 0.2^{b}
Pseudomonas sp. PRGB06	250 ± 35^{c}	171 ± 1^{g}	10.0 ± 3.4^{a}	3.0 ± 0.4^{ab}	–	10.0 ± 0.1^{a}	6.8 ± 0.4^{c}	+	–	8.0 ± 0.4^{a}
Serratia sp. PRGB11	216 ± 5^{c}	250 ± 3^{f}	8.0 ± 1.2^{ab}	2.4 ± 0.3^{cd}	–	8.7 ± 0^{b}	8.0 ± 0.4^{b}	+	+	–
Serratia sp. PSGB13	120 ± 18^{d}	514 ± 3^{a}	2.4 ± 0.9^{c}	3.3 ± 0.4^{a}	–	8.4 ± 0.1^{c}	10.1 ± 0.7^{a}	+	+	–
LSD ($P \leq 0.05$)	43	7.0	3.3	0.4	3.5	0.2	0.7			0.4

[†] All the strains were positive for ammonia production and negative for HCN and pectinase production. SE, solubilization efficiency; ARA, acetylene reduction assay; IAA, indole-3-acetic acid; ACCD, 1-aminocyclopropane-1-carboxylate deaminase; α-KB, α-ketobutyrate; +, presence of trait; –, absence of trait. The values indicate the mean ± SE of three replications. In the same column, significant differences according to the LSD at 0.05% levels are indicated by different letters

The effect of the selected bacterial strains on the growth of canola and tomatoes under gnotobiotic conditions is listed in Table 3. The *Acinetobacter* sp. strains with ACC deaminase activity significantly increased canola root length by 35% to 41% compared with the control. The two isolates (PRGB06 and PRGB11) that did not produce ACC deaminase also increased canola root length. Meanwhile, *Pseudomonas* sp. PRGB06 increased tomato root length by 23%, which was similar to the root-length increase elicited by ACC deaminase-producing *Acinetobacter* sp. PSGB05. However, the magnitude of ACC deaminase activity did not have any significant effect on canola and tomato root lengths. Increased seedling vigor and canola (30%) and tomato (26%) dry biomass caused by seed inoculation of *Acinetobacter* sp. PSGB04 and *Pseudomonas* sp. PRGB06, respectively, reflected the effect of increased root and shoot length (data not shown).

Discussion

Mineral solubilization and increasing mineral availability are regarded as the most important traits directly associated with PGPBs. All of the strains tested in this study solubilized TCP and ZnO and oxidized S. Diverse groups of microorganisms including *Pseudomonas*, *Serratia*, and *Acinetobacter* species employ a variety of solubilization reactions, such as acidification, chelation, exchange reactions, and production of gluconic acid, to release soluble from insoluble P [7, 8, 26, 30]. It may be possible that the glucose provided as carbon source in the growth media may aid gluconic acid production and eventually lead to P solubilization [8]. In contrast, the amount of P released by *Acinetobacter* sp. strains was higher than that released by *Pseudomonas* and *Serratia* species, proving once again the fact that the members of the former groups are proficient in P solubilization [30]. Cattelan et al. [3] have already reported the P-solubilizing potential of *Acinetobacter* sp. and its effect on soybean growth in phosphorus-deficient soil.

Although literature related to the bacterial solubilization of Zn is limited, its importance to crop production is evident. The Zn-solubilizing potential of a few bacterial genera such as *Bacillus*, *Pseudomonas*, and the diazotrophic *Gluconacetobacter* has already been studied [31, 35], and a positive correlation between the potential for P and Zn solubilization has also been reported [35], as evidenced by the effect of the *Acinetobacter* genus in the present study. In addition, the trait for S oxidation by the bacterial strains would enhance the production of sulfates and makes them available for plant growth [36]. Interestingly, all of the bacterial strains in the present study also produced an iron-chelating compound siderophore.

Table 2 Antagonistic activities of DBM gut bacterium *Pseudomonas* sp. PRGB06 against phytopathogenic fungi

Phytopathogenic fungi	Host	Disease	Inhibition zone diameter (cm)
Botrytis cinerea	Tomato	Gray mold	1.8 ± 0.4^{bc}
Colletotrichum acutatum KACC 41932	Strawberry	Leaf spot	–
C. capsici KACC 40978	Red pepper	Anthracnose	–
C. coccodes KACC 40008	Red pepper	Anthracnose	1.2 ± 0.1^{c}
C. gloespoiroides KACC 40690	Red pepper	Anthracnose	3.6 ± 0.1^{a}
Fusarium oxysporum f. sp. *niveum* KACC 40902	Muskmelon	Wilt	–
Rhizoctonia solani KACC 40106	Rice	Sheath blight	3.0 ± 0.4^{a}
Sclerotia sclerotiorum	Ginseng	Wilt	2.3 ± 0.2^{b}
P. capsici KACC 40483*	Red pepper	Anthracnose	–
LSD ($P \leq 0.05$)			0.7

–, absence of antagonism

* Growth of *P. capsici* was inhibited by *Serratia* sp. PRGB11 (1.4 ± 0.3), *Acinetobacter* sp. strains PRGB15 (1.8 ± 0.6), PRGB16 (1.6 ± 0.4), PSGB03 (1.6 ± 0.5), PSGB04 (1.6 ± 0.2), and PSGB05 (1.6 ± 0.3). Values in parentheses after strain names indicate diameter of inhibition zone (cm). Values are the mean ± SE of three replications. In the same column, significant differences according to the LSD at 0.05% levels are indicated by different letters

Table 3 Effect of DBM larval gut bacterial strain seed treatment on early growth of canola and tomato under gnotobiotic conditions

Treatment	Root length (cm)		Seedling vigor		Dry biomass (mg)	
	Canola	Tomato	Canola	Tomato	Canola	Tomato
Acinetobacter sp. PSGB03	8.0 ± 0.2^{a} (39)	5.7 ± 0.3^{c} (9)	1048 ± 4^{a}	892 ± 2^{c}	136 ± 6^{bc} (17)	96 ± 0.5^{b} (22)
Acinetobacter sp. PSGB04	8.0 ± 0^{a} (41)	5.5 ± 0.3^{d} (3)	1007 ± 4^{b}	835 ± 9^{d}	151 ± 2^{a} (30)	100 ± 2.8^{a}(26)
Acinetobacter sp. PSGB05	7.7 ± 0^{ab} (35)	6.4 ± 0.1^{a} (21)	957 ± 6^{d}	937 ± 9^{b}	150 ± 1^{a} (30)	83 ± 1.8^{c} (5)
Acinetobacter sp. PRGB15	7.9 ± 0.6^{a} (38)	5.6 ± 0.2^{dc} (7)	995 ± 9^{c}	819 ± 5^{e}	128 ± 1^{c} (11)	89 ± 1.4^{c} (13)
Pseudomonas sp. PRGB06	7.2 ± 0.1^{b} (27)	6.5 ± 0.1^{a} (23)	881 ± 4^{g}	982 ± 2^{a}	136 ± 7^{abc} (17)	100 ± 1.0^{a} (26)
Serratia sp. PRGB11	8.0 ± 0.1^{a} (41)	6.1 ± 0.2^{ba} (16)	930 ± 7^{e}	934 ± 7^{b}	138 ± 3^{b} (19)	91 ± 1.0^{c} (15)
Control	5.7 ± 0.5^{c}	5.3 ± 0.6^{d}	920 ± 6^{f}	811 ± 8^{f}	116 ± 11^{d}	79 ± 1.7^{c}
LSD ($P \leq 0.05$)	0.6	0.4	5.0	7.0	9.0	2.0

The values indicate the mean ± SE of three replications. In the same column, significant differences according to LSD at 0.05% levels are indicated by different letters. Values in parentheses indicate the percentage increase over the control

Crowley et al. [4] previously reported the existence of a siderophore-mediated iron-transport system in oats and suggested that siderophores produced by rhizosphere micro-organisms can supply iron to plants that have mechanisms for using these compounds under iron-limited conditions.

Next to mineral solubilization, nitrogen fixation and the production of phytohormones and enzymes, such as ACC deaminase, are considered as direct PGP traits of benevolent bacterial strains [3, 28]. Plant-associated nitrogen-fixing bacteria are regarded as a possible alternative for inorganic nitrogen fertilizers, and diazotrophic bacterial strains have previously been discovered in association with insects that depend on gut bacterial symbionts as a major source of nitrogen [1]. The *Acinetobacter* sp. strains in this study all showed ACC deaminase enzyme activity, and the role of ACC deaminase in decreasing ethylene levels by the enzymatic hydrolysis of ACC into α-ketobutyrate and ammonia has been presented as one of the major mechanisms of PGPBs in promoting root and plant growth [3, 7, 28]. All of the bacterial isolates in this study produced varying amounts of IAA and SA in the broth-based assays, and PGPBs have been reported to influence plant growth by contributing to the host plant's endogenous pool of phytohormones, such as IAA and SA.

The production of SA, siderophores, and cell-wall lytic enzymes such as chitinase and β-1,3-glucanase is considered important to biocontrol [7, 22]. Bacterially produced SA mediates induced systemic resistance (ISR) in plants and works as an antimicrobial agent against various pathogens [12]. In addition to mediating ISR, SA also acts as a precursor for the production of siderophores, the presence of which pre-empts iron in an environment like soil, rhizosphere, phyllosphere etc., and nutritionally

challenges invading pathogens [22]. Although the antagonistic potential of non–chitinase producing *Pseudomonas* sp. PRGB06 toward entomopathogenic fungi has already been reported [11], the results of the present study proved the antagonistic activity of this bacterial strain toward more than one phytopathogenic fungus. The present results were also in accordance with those of previous studies, which reported a lack of correlation between chitinase production and antagonism against pathogenic fungi [7]. Several mechanisms have already been described for biocontrol agents to explain their antagonistic effect on pathogenic fungi in vitro and in vivo, and these mechanisms may vary in different strains of the same species [7, 21]. In this study, the chitinase-producing bacterial strains were only effective against one phytopathogenic fungi, *P. capsici*, which has little chitin in its cell wall, implying that the observed biocontrol activity of the bacterial strains may have been caused by the production of β-1,3-glucanase, a siderophore, and/or SA [22]. Therefore, the application of bacterial strains with multifaceted traits for PGP activity is more beneficial.

Bacterial inoculation has been reported to have a positive influence on various plant-growth parameters, including root and shoot length, seedling vigor, and dry biomass. ACC deaminase and SA-producing bacteria has been reported to prevent the inhibition of root elongation by decreasing the level of growth-limiting ethylene through hydrolytic cleavage (deaminase activity) of the ethylene-biosynthesis precursor ACC and inhibiting the activity of ACS (ACC synthase) [10, 12, 27]. However, the significant increase in the root length of the crop plants treated with ACC deaminase-negative *Pseudomonas* sp. PRGB06 suggests that the effects of other PGP traits such as IAA, SA, cytokinin, and gibberellic acid should also be considered in addition to just ACC deaminase activity [3, 7, 28]. The absence of any concomitant increase in root length relative to the magnitude of ACC deaminase activity in this study also confirms the previous report that an optimum of $\geq$ 20 nmol α-ketobutyrate/h/mg protein is sufficient for bacterial strains to have PGP properties [28]. Nonetheless, growth promotion of the canola and tomato test plants under gnotobiotic conditions confirmed the beneficial effects of insect (i.e., DBM) gut bacterial strains on crop growth.

The bacterial strains tested negative for deleterious traits, such as pectinase and HCN production; the latter interferes with the cytochrome P_{450} system in crop plants [15], thus rendering it an antagonistic trait [22]. In conclusion, the PGP traits of the DBM gut bacterial strains and their positive effects on the growth of the tested plant species suggested that insect guts may be a potential niche for the isolation of beneficial PGP bacteria. Furthermore, the use of such bacterial strains, with multiple beneficial traits and lack of negative traits, as a bioinoculants can simultaneously increase plant growth and mineral uptake by crop plants, eventually increasing crop yield.

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