Characterization of plant growth promoting and antagonistic bacteria associated with the rhizosphere and phyllosphere of Platanus mexicana and Persea shiedeana trees natural hosts of ambrosia beetle.

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Abstract

It is well known that bacteria belonging to the microbiota of plants, contribute to the better development of their hosts by different mechanisms, such as, growth promotion, nutrient facilitation, stimulation of plant defenses, antagonizing pathogens or pests, and some of them are also, some microorganisms show enzymatic activities with biotechnological application in the agricultural and industrial sector. In the present study we identified and characterized fourteen bacterial strains isolated from the rhizosphere and phyllosphere of *Platanus mexicana* and *Persea schiedeana* trees; the aim of this research was to evaluate bacterial biological activities over plant growth promotion on *Arabidopsis thaliana seedlings* and antagonistic activity against the phytopathogenic fungus *Fusarium sp.*, besides studying their lytic ability when confronted with cellulose, pectin, or chitin as carbon sources. These strains were classified into the genera *Curtobacterium, Plantibacter, Bacillus, Brevibacterium, Carnobacterium, Staphylococcus, Erwinia, Serratia, Exiguobacterium* and *Yersinia*. Each individual strain exhibited revealed at least one of the characteristics evaluated. *Yersinia* sp. strain PsH3-014(14D) and *Bacillus* sp. strain Hay2-01H(7) stand out from the other strains due to their ability to promote plant growth in *A. thaliana* seedlings as well as their antagonistic activity against of *Fusarium sp.* In addition, PsH3-014(14D) also degrades pectin and chitin, while Hay2-01H (7) degrades cellulose and pectin. In contrast, *Carnobacterium gallinarum* strain Chi2-3Ri was detrimental for the development of *Arabidopsis* seedlings but it can degrade cellulose. *Erwinia* sp. strain Hay2-1H was the only strain capable of degrading all three biopolymers tested (cellulose, pectin, and chitin). Further research could be directed towards the isolation and characterization of key enzymes produced by the these strains, as well as further exploration of other metabolic capacities.

Introduction

Plants live in association with complex and diverse microbial communities whose cooperative and competitive interactions shape their own composition, abundance and persistence on plant tissues (Hassani et al. 2018). Among these microorganisms, bacteria are numerically the most abundant and diverse, not only from a phylogenetic perspective, but also from a metabolic perspective (Kopecky et al. 2019). Bacteria can promote plant health and growth through several mechanisms: for example, by providing nutrients, through nitrogen fixation (Carvalho et al. 2014) or nitrogen oxidation, solubilization of phosphate (Sharma et al. 2013), production of siderophores for iron sequestration. Bacteria can also produce “plant hormones”, signalling molecules that induce systemic resistance in plants (Viswanath et al. 2016). In addition, some bacteria outcompete, antagonise or inhibit plant pathogens (Khalil et al. 2021; Veliz et al. 2017), and can produce enzymes that convert complex and inaccessible substrates into available ones (Agostoni et al. 2017; Merlin et al. 2014).

Bacteria inhabit the rhizosphere and other plant organs such as leaves (Vacher et al. 2016), stems or fruits (Compant et al. 2019). Bacteria inhabiting the phyllosphere (leaf surface and inward into the leaf tissues) often belong to the phyla Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Bulgarelli et al. 2013). Some members of the bacterial community members are methylotrophic, nitrogen-fixing

while others strongly influence the resistance of the plant host to phytopathogens, either through microbe-microbe interactions or by stimulating the plant defense mechanisms (Vacher et al. 2016). Bacteria inhabiting the rhizosphere, called rhizobacteria, promote healthy plant development in several ways, such as by improving soil texture, pH and humidity, nutrient availability, and secreting extracellular molecules such as hormones or secondary metabolites, antibiotics, and various signalling compounds that not only modify the plant response but also affect neighbouring microorganisms (Hartmann et al. 2019). They also produce exoenzymes that initiate the degradation of biopolymers facilitating the release of monomers that are useful for both plants and neighbouring microbiota (Backer et al. 2018).

Plant growth promoting rhizobacteria (PGPR) are particularly capable of promoting plant growth (Chandran et al. 2021), making them of particular interest to agriculture. Some PGPR produce phytohormones such as auxin, cytokinin, and gibberellin that modulate endogenous hormone levels in the plant (Compant et al. 2019); others, enhance the plant host resistance to pathogen infection either through commensal-pathogen interactions (Saggese et al. 2022; Wang et al. 2022) or through modulating plant defense (Khanna et al. 2019; Viswanath et al. 2016). The production of different anthocyanins has been recognised as a plant response to various abiotic stresses such as drought, high salinity, low temperature, and excess light (Kovinich et al. 2015) or in response to biotic stresses, capable to triggering induced systemic resistance (Gruau et al. 2015; Mathys et al. 2012) against phytopathogenic fungi and bacteria.

Biocontrol agents formulated with plant growth promoting bacteria are equipped to perform other functions besides being phytopathogens or antagonists. They also provide about plant growth promotion, soil biofertilization, abiotic stress or drought mitigation (Slama et al. 2019). *Fusarium solani* is one of the the phytopathogens causing disease in many agriculturally important crops; its biocontrol has been achieved using bacteria of the genus *Bacillus, Acinetobacter, Streptomyces, Pseudomonas* as antagonists (Alam et al. 2021; Hu et al. 2014; Khalil et al. 2021; Slama et al. 2019). Some of the bacterial mechanisms involved in the antagonism are antibiosis, competition, induced systemic resistance of the host plant, siderophore production, and even hydrolytic enzyme production (Backer et al. 2018). Microorganisms capable of producing chitinolytic enzymes, such as chitinases, play an important role as biocontrol agents and pathogen antagonists (Veliz et al. 2017).

In the present study, we isolated, identified and characterized bacteria from the rhizosphere and phyllosphere of *Platanus mexicana* and *Persea schiedeana* trees, natural hosts of ambrosia beetles, and evaluated their biological activities on *Arabidopsis thaliana* seedlings and their antagonistic activity against the phytopathogenic fungus *Fusarium sp.*, INECOL_BM-06, a phytopathogenic fungus associated with the ambrosia beetle *Xylosandrus morigerus* (Carreras-Villaseñor et al. 2022), as well as enzymatic activity characteristics. Our results show that bacteria, PsH3-014(14D) isolated from *P. shiedeana* and Hay2-01H(7) from *P. mexicana*, promote plant growth in Arabidopsis seedlings and antagonistic activity against the phytopathogenic fungus *Fusarium sp*. Our results suggest the biological potential of host-associated microorganisms with potential biological activities in the biological control of diseases and pests, as a natural defense mechanism of the host plant against attack by a phytopathogenic agent.

Considering the broad spectrum of bacterial activities, found in the plant microbiome, strains with potential biotechnological application in agriculture or industry related to the transformation of agro-industrial residues or the transformation of complex carbohydrates are being sought.

Materials and methods

2.1 Isolation of bacterial strains

Root, bark and leaf samples of *Platanus mexicana* and *Persea schiedeana* trees, were collected in the “Foggy Forest Sanctuary” (19°30'47.5"N; 96°56'11.5"W) of the “Francisco Javier Clavijero” Protected Natural Area of the Institute of Ecology, A.C. (INECOL) in the city of Xalapa, México. These samples were handled and processed with sterile materials and then processed in the laboratory under axenic conditions. Five grams of each sample type; root, bark or leaf, were processed independently. Each tissue sample, was first rinsed with alcohol (96%) for one minute, followed by two subsequent one-minute rinses with sterile water; then ground and resuspended in 5 mL of a sodium phosphate solution (0.9%). Two subsequent serial dilutions (1:10) were prepared and bacterial colonies were separated from the last dilution by the cross-streak method on Luria-Bertani (LB) solid media, bark solid media (prepared with LB media (50%) plus bark extract (50%)) and V8 agar plates. Bacteria with characteristic colony morphology, were selected for purification and characterisation. Purity was confirmed by microscopic observation of the colony morphology using a Leica S8 APO stereoscopic microscope and determination of cell morphology by Gram staining using a Leica DM750 light microscope.

2.2 Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0) seeds were used for the present study. _Arabidopsis_ seeds were surface disinfected with 700 µL of 96% (v/v) ethanol for 7 min, followed by a bleach wash at 20% (v/v) for 7 min. Finally, six washes were performed with sterile distilled water and stored at 4°C for 48 hours. _Arabidopsis_ seeds were germinated and grown on 0.2x Murashige and Skoog (MS) agar medium and incubated in a plant growth chamber (Percival AR36L model) with a photoperiod of 16 h light and 8 h dark, a light intensity of 100 µmol photons m^{-2} s^{-1} and a temperature of 22 ºC.

2.3 DNA extraction, 16S rRNA gene amplification and phylogenetic identification

Biomass from each isolated and pure bacterial strain was collected on a 1.5 mL Eppendorf centrifuge tube, either from 1 mL of liquid culture or from a single colony from solid culture; then it was centrifuged at 3000 rpm for 5 min and rinsed twice with an isotonic NaCl solution (0.9%), removing the liquid and retaining the rinsed cell pellet. DNA extraction was performed as follows 160 µL of solution I (Tris base 10 mM, NaCl 60 mM, sucrose 5%, EDTA 10 mM) was added to the pellet, mixed well and the suspended cells were lysed by freezing (-80°C) and heating them (~ 90°C). Then 200 µL of solution II (Tris base 300 mM, sodium dodecyl sulphate 1.25%, sucrose 5%, EDTA 10 mM) was added and the mixture was incubated for 10 min at 65°C; then 60 µL of solution III (potassium acetate 3M) was added, vortexed and centrifuged (15,000 rpm for 10 min); finally 200 µL of supernatant was transferred to a clean 1.5 mL Eppendorf tube. The supernatant was then mixed with 420 µL of isopropanol and incubated at room temperature for 5 min, followed by centrifugation at 15,000 rpm for 10 min; the supernatant is removed to retain the resulting DNA pellet, which is then rinsed twice, with cold alcohol (70%) to resuspend with sterile double-distilled water for quantification and integrity and purity analyses for further processing. The 16SrRNA gene of each strain was amplified by 30 cycles under standard PCR conditions and reaction mix, using 27F (3´-AGAGTTTGATCCTGGCTCAG-5´) and 1492R (3´-TACCTTGTTACGACTT-5´) primers and 48°C for 30 s as annealing temperature. The PCR products were sequenced by the LABSERGEN capillary sequencing service unit, CINVESTAV, Irapuato, México.

For the phylogenetic identification, sequences were first revised using DECIPHER (v2.0) to discard chimeric sequences (Wright et al. 2012) and were further analysed using the Molecular Evolutionary Genetics Analysis Version 11 (MEGA11) (Tamura et al. 2021) and the free trial of Geneious, bioinformatics software for sequence data analysis (Kearse et al. 2012). To construct the phylogenetic trees, the closest homologous sequences used were downloaded from the National Center for Biotechnology Information using the Basic Local Alignment Search Tool (BLAST; https://blast.ncbi.nlm.nih.gov/Blast.cgi), restricted to type strain material; plus, sequences found in the SILVA rRNA database project (Quast et al. 2013). The classification was further confirmed by using the Ribosomal Database Project (RDP) tools (Cole et al. 2014). Evolutionary history was inferred using maximum likelihood Method and the Tamura-Nei model (Tamura & Nei 1993). The 16S rDNA sequences obtained were deposited in GenBank (accession numbers: OL449779-OL449799).

2.4 Arabidopsis-bacteria *in vitro* co-inoculation system

Bacteria isolated from *P. mexicana* and *P. schiedeana* were evaluated *in vitro* for their ability to promote plant growth using *Arabidopsis* seedlings (Col-0) ecotype (Ortiz-Castro et

al. 2013). Five days after germination, ten wild-type Arabidopsis (Col-0) seedlings were transferred to 0.2x MS agar medium, co-inoculated by streaking on agar medium and grown for a further period of 8 days by placing the petri dishes in the plant growth chamber (Percival AR36L) under above conditions. *Pseudomonas aeruginosa* PAO1 wild-type and *P. aeruginosa LasI-*, a quorum sensing (QS) mutant affected in the production of the QS signal, were used as negative and positive, respectively (Ortiz-Castro et al. 2011). All experiments were repeated at least three times.

2.5 Biomass and anthocyanin content

After the 8 days of co-cultivation, representative images were photographed and the fresh weight of the shoots was recorded by using an analytical balance (Ohaus, Explorer) with a 0.0001 precision valued. Anthocyanin content was determined according to Tzec-Interian et al. (2020). All experiments were repeated at least twice times.

2.6 Antagonistic activity of the bacterial strains against the phytopathogenic fungus Fusarium sp.

In order to determine whether the bacterial strains had an antagonistic effect against the growth of the pathogenic strain INECOL_BM-06 of *Fusarium* sp. provided by the Phytopathology Laboratory of the Institute of Ecology, A.C. (INECOL) (Carreras-Villaseñor et al. 2022); both organisms were co-cultured in Petri plates of potato dextrose agar (PDA) media, three Petri plates per bacterial strain plus three Petri plates only with *Fusarium sp.* only as an absolute growth control, which we consider to be the “negative control”. 10 µL of a conidial suspension ($1x10^5$ conidia/mL) was placed in the centre of the Petri dish, and 3 drops of 10

µL of a liquid bacterial culture (OD_{600} = 0.1) were placed in 3 of the 4 quadrants (at 2 mm from the edge of the Petri dish); the 4th quadrant received 10 µL of liquid LB medium to act as a negative reference for diffusible compouds. Petri dishes were incubated at 28°C for 9 days. The percentage inhibition (I) was calculated using the following formula: I=[(R-r)/R] x 100 where R is the radius of growth of *Fusarium sp.* in the negative control Petri plates and r is the radius of growth of *Fusarium sp.* in the direction of the bacterial strain evaluated. ANOVA ($\alpha \leq 0.05$) statistical analyses were performed with media means and standard deviations of the data.

2.7 Enzymatic activities of the bacterial strains

To qualitatively assess the ability of each bacterium to degrade either cellulose, pectin, or chitin; 10 µL of each bacterial liquid culture (OD_{600} = 0.1) was placed in the centre of the well containing differential culture media and were incubated at 28°C for 48 h to subsequently assess (with the appropriate detection reagent) whether the biopolymer tested was transformed during the growth of the strain. 24 microwell plates containing 1.6 mL of a differential medium (either with cellulose, pectin, or chitin). The experimental design included three replicates (one well corresponding to one replicate) of: each strain, the negative control (sterile media, not inoculated) and the positive control (sterile media, inoculated with an active strain capable of degrading the evaluated polymer). Each differential medium was prepared, and the degradation was revealed as follows:

2.7.1 Cellulolytic activity

Cellulolytic activity was evaluated with cellulose agar, each liter of which, was prepared by adding 5 mL of stock saline solution (K_2HPO_4 [5 g/L], $MgSO_4$ [2.5 g/L], NaCl [2.5 g/L], $Fe_2(SO_4)_3$ [0.5 g/L]), 1 mL of stock micronutrient solution (K_2MoO_4 [0.05 g/L], $MnSO_4$ [0.05 g/L], Na_3BO_3 [0.05 g/L], $CoNO_3$ [0.05 g/L], Cu_2SO_4 [0.05 g/L]), 1 g of NH_4NO_3 and the pH was adjusted to 6.5 before adding carboxymethylcellulose (10 g mixed with 100 mL of distilled water using a blender) and 15 g agar. Finally, it was subjected to moist heat sterilisation, and poured into 24 microwell plates. After the appropriate incubation period for bacterial growth, cellulose degradation was detected by flooding each microwell with 300 µL of Congo Red [1%], incubating at room temperature for 15 minutes, then removing the excess of Congo Red and then rinsing with 300 µL of NaCl [1M], allowing a further 15 min to elapse before recording the result. The

presence of a clear zone around surrounding the bacterial growth area, indicated degradation of the cellulose; if the media turned red without a clear halo, this indicated a negative result.

2.7.2 Pectinolytic activity

The degradation of pectin involves the activity of different enzymes operating at different pH. We evaluated it at pH 5 and pH 7, using pectin agar. Each liter of pectin agar contained 30 g of agar plus a 1:1 mixture of solution A, and solution B adjusted to pH 7 and pH 5 respectively. Both solutions A and B were prepared and stored at 5°C. Each liter of solution A contained 2 g of yeast extract and 10 g of apple (or orange) pectin. One liter of solution B contained 2 g of $(NH_4)_2SO_4$, 4 g of KH_2PO_4, 6 g of Na_2HPO_4 and 1 mL stock solution BC ($FeSO_4 \cdot 7H_2O$ [0.1 g/L], $CaCl_2$ [1 g/L], H_3BO_3 [0.01 g/L], $MnSO_4$ [0.07 g/L], $CuSO_4$ [0.05 g/L], MoO_3 [0.01 g/L]). To develop the presence or absence of pectin degradation, 300 µL of Lugol's iodine was added to each well to completely cover the media surface; after 5 minutes, the excess of the iodine was removed, and the absence of brown pigmentation surrounding the area of bacterial growth indicated pectin degradation, a completely brown surface no degradation.

2.7.3 Chitinolytic activity

Chitin degradation was assessed by detecting a pH colour change in a liquid modified basal medium supplemented with pretreated chitin and bromocresol purple ($C_{21}H_{16}Br_2O_5S$) as a pH indicator (which is yellow at 5.2 and purple at above 6.8). Per liter of “chitin media” containing 0.3 g of $MgSO_4 \cdot 7H_2O$, 3.0g of $(NH_4)_2SO_4$, 2.0 g of KH_2PO_4, 1 g of monohydrated citric acid, 4.5 g of pretreated colloidal chitin, 0.15 g of bromocresol purple; then the pH was adjusted to 2.8 with sodium acetate buffer and finally the media were subjected to moist heat sterilization. The reaction was considered positive if the media changed colour from yellow to purple after an appropriate incubation period to allow bacterial growth; if the bacteria grew but the media didn’t change colour, the reaction was considered as negative. The pre-treatment of chitin consisted in soaking 10 g of commercial colloidal chitin in 100 mL of phosphoric acid [85%], and leaving it for 24 h at 4 ^{o}C (with sporadic shaking); after this time it was washed with plenty of distilled water and filtered until it reached pH 7, then it was sterilized by moist heat, and stored at 4 C

2.8 Data analysis

For all experiments, the overall data were statistically analysed using SPSS 10 program (SPSS, Chicago, IL, USA). Univariate analyses with Tukey´s post hoc test were used to test for differences in shoot biomass and anthocyanin content. Different letters were used to indicate means that differed significantly ($P < 0.05$).

Results

3.1 Characterization and molecular identification of bacterial strains

A total of 20 bacterial strains were obtained from the root, bark and leaves of *Persea schiedeana* and 32 bacterial strains from *Platanus mexicana*. We evaluated the *in vitro* effects of these 52 strains using the Arabidopsis-bacteria system to identify bacteria with potential plant growth-promoting activity in *Arabidopsis* seedlings. We identified six strains from *Persea schiedeana* Chi 0-1C (32D), PsC1-004 (35i), PsH3-014 (14D), Chi 3–5(23), Chi 2-3Ri, and Chi 4-1Ri(68i) with plant growth promoting activity, and eight strains from *Platanus mexicana* the strains PmC1-001, Hay 2-11C (35i), Hay 2-01H (7), Hay 2-1H, PmH2-008, Hay 2-5R, PmRi2-006 and PmRi3-012 showing plant growth promotion in Arabidopsis seedlings. Analysis of partial sequences of their 16S rRNA gene showed that nine of the fourteen selected bacteria, belong to the phylum Firmicutes, three are Proteobacteria, and only two are Actinobacteria. Their closest homologous sequences were included in the construction of the phylogenetic tree illustrating their molecular identification (Fig. 1).

According to the phylogenetic analysis, five different genera were identified among the *Persea schiedeana* isolates corresponding to *Brevibacterium frigoritolerans*, *Curtobacterium pusillum*, *Yersinia* sp., *Serratia grimesii* and *Carnobacterium gallinarum.* The isolates from *Platanus mexicana* corresponded to *Staphylococcus saprophyticus*, *Plantibacter* sp., *Bacillus* sp., *Erwinia* sp. and *Exiguobacterium* sp. (Table 1).

3.2 Plant growth-promoting activity of bacteria associated with P. shiedeana and P. mexicana trees.

We evaluated the *in vitro* effects of bacteria associated with *P. shiedeana* and *P. mexicana* by using an Arabidopsis-bacteria co-culture system to elucidate the potential plant growth promoting activity in Arabidopsis seedlings. *Pseudomonas aeruginosa* PAO1 (wild type) and *LasI-* (a mutant affected in the production of the quorum sensing signal 3-oxo-dodecanoyl-homoserine lactone) were used as negative and positive controls for plant growth promotion (Figs. 2 and 3).

All bacterial strains, except for the Chi2-3Ri, had a plant growth-promoting effect in the Arabidopsis seedling co-culture, as evidence by an increase in the lateral root number and biomass as fresh shoot weight (Fig. 2B and C), similar to the effects shown by Arabidopsis seedlings co-cultured with *P. aeruginosa LasI-* compared to uninoculated Arabidopsis seedlings (Figs. 2 and 3).

The co-culture effects of bacteria on primary root growth show differential responses. Bacterial strains associated with *P. schiedeana* (Chi0-1C(32D), PsC1-004(35i), PsH3-014(14D) and Chi4-1Ri(68i)) and PmRi3-012 from *P. mexicana* show a beneficial effect on primary root growth similar to the effects in Arabidopsis seedlings co-inoculated with *P. aeruginosa LasI-*. In addition, Chi2-3Ri isolated from *P. schiedeana* and PmC1-001, Hay2-11C(35i), Hay2-01H(7), Hay2-1(35i), PmH2-008, Hay2-5R, PmRi2-006 isolated from *P. mexicana* show an inhibitory effect on primary root length similar to the effects shown in Arabidopsis seedlings co-inoculated with *P. aeruginosa* PAO1 (Figs. 2A and 3).

Plant stress caused by co-inoculation with bacterial strains was minimal, only Arabidopsis seedlings co-inoculated with Chi2-3Ri induced the accumulation of anthocyanins four times more than those inoculated with *Pseudomonas aeruginosa* PAO1, a bacterial strain used as a phytopathogenic strain, a minimal amount of anthocyanins was stimulated by the growth of Chi 3–5(23), Hay 2-1H and PmH2-008 in contact with the seedlings (Fig. 2D).

3.3 Antagonistic effects of bacterial strains against the phytopathogenic fungus Fusarium sp. strain INECOL_BM-06.

To evaluate the antagonistic activity of bacterial strains isolated from *P. schiedeana* and *P. mexicana* against the phytopathogenic fungus *Fusarium* sp., we evaluated the inhibitory radial growth of Fusarium sp. strain INECOL_BM-06 a phytopathogenic fungus (Carreras-Villaseñor et al. 2022). We found that the strains PsH3-014(14D) and Hay2-01H(7) caused a significant growth inhibition of *Fusarium sp.* strain INECOL_BM-06, corresponding to 37.2% and 45.5% respectively (Fig. 4D, J), compared to the control growth of *Fusarium* sp. strain (Fig. 4A). However, the mechanism for such inhibition, seems to be different, considering the shape of the fungal growth during the *in vitro* co-culture; a speculative suggestion is that diffusible elements were produced by Hay2-01H(7), while volatiles could have been produced by PsH3-014(14D), but further tests need to be elaborated to test these possibilities or find new ones.

3.4 Enzymatic activities of bacterial strains

Bacteria are an important source of enzymatic biological activities with various biotechnological applications. To identify the enzymatic activities of the strains isolated from *Platanus mexicana* and *Persea schiedeana* trees. We evaluated the enzymatic activity of degraded cellulose, chitin and pectin. Of the strains isolated from *P. mexicana* and *P. schiedeana*, only nine bacteria (*Brevibacterium frigoritolerans* Chi 0-1C(32D), *Serratia grimesii* Chi 3–5(23), *Carnobacterium gallinarum* Chi 2-3Ri, *Plantibacter* sp. Hay 2-11C(35i), *Bacillus* sp. Hay 2-01H(7), *Erwinia* sp. Hay 2-1H, *Bacillus* sp. PmH2-008, *Bacillus* sp. Hay 2-5R, *Exiguobacterium* sp. PmRi3-012) were able to degrade cellulose, whereas seven bacteria (Yersinia sp. PsH3-014(14D); *Staphylococcus saprophyticus* PmC1-001; *Plantibacter* sp. Hay2-11C(35i); *Bacillus* sp. Hay2-01H(7); *Erwinia* sp. Hay2-1H; *Bacillus* sp. Hay2-5R; *Exiguobacterium* sp. PmRi3-012) showed pectinolytic activities at pH 5 and pH7; and only one *Carnobacterium gallinarum* Chi4-1Ri(68i) at pH7 (Table 1). In addition, only two strains, *Yersenia* sp. PsH3-014(14D) and *Erwinia* sp. Hay 2-1H, were able to degrade chitin. *Yersinia* sp. PsH3-014(14D) was able to degrade chitin and pectin (at pH 5 and pH 7). Only *Erwinia* sp. Hay 2-1H has cellulolytic, pectinolytic and chitinolytic activities. As the assays were qualitative, further experiments would be sufficient to quantify the substrate degrading efficiency of each strain, and further protein research would be relevant to determine the nature of the enzymes produced by each strain.

Discussion

Given the biotechnological potential that bacteria isolated from different habitats around the world have shown, it is imperative to study the microbiota that tropical montane cloud forests in Mexico, as they are extremely diverse ecosystems but threatened by deforestation and land-use change (Báez-Vallejo et al. 2020). For this reason, several research groups have focused on the isolation of bacteria associated with forest species (Cazorla & Mercado-Blanco 2016), with different applications in mind, such as the biocontrol of phytopathogens (Kong et al. 2020; Reverchon et al. 2020). In the present study, the biotechnological potential of fourteen bacteria isolated from the rhizosphere and phyllosphere of two tree species from the tropical montane cloud forests (*Persea schiedeana* and *Platanus mexicana*) was evaluated, some of which showed enzymatic activities of cellulases, pectinases and chitinases, as well as biocontrol capacity against *Fusarium sp.* and promotion of *A. thaliana* seedling growth.

The biological activity exhibited by each of the isolated bacteria was congruent with previously identified characteristics according to the genus to which they belonged; the strains Hay2-11C(35i), PmRi3-012, and PmC1-001, PmRi2-006, identified as *Plantibacter* sp., *Exiguobacterium* sp. and *Staphylococcus saprophyticus*, respectively, demonstrated the ability to promote the growth of *Arabidopsis* seedlings, but were unable to inhibit the growth of *Fusarium* sp. These properties are consistent with what is described in the literature, for example, Mayer et al. 2019 found that *Plantibacter flavus* strain M259 promoted *Arabidopsis* shoot and root growth, as well as root growth in lettuce and stimulated the shoot growth of bok choy seedlings. The genus *Exiguobacterium* has an important adaptability to different extreme environments, and some strains have been studied for their ability to promote plant growth through various direct and indirect mechanisms (Pandey 2020). Santos and Rigobelo (2021) detailed that the strain IJ8 of *Staphylococcus saprophyticus* isolated from sugarcane was able to produce 45.3 µg mL^{-1} of the phytohormone IAA, it was able to fix nitrogen and solubilize phosphate, as well as exhibiting cellulolytic activity.

Bacterial strains belonging to the genera *Carnobacterium gallinarum* Chi2-3Ri and Chi 4-1Ri(68i), *Curtobacterium pusillum* PsC1-004(35i) and *Brevibacterium frigoritolerans* (Chi 0-1C(32D) were also identified. Bacteria from these genera have been reported for their ability to promote the growth of maize, pepper, and agriculturally important crops due to their ability to produce indole-3-acetic acid (IAA) and solubilize phosphorus (Egamberdieva et al. 2017; Ulfa et al. 2018; Tzec-Interián et al. 2020). They can also inhibit the growth of phytopathogens such as *Fusarium verticillioides*, *Fusarium* sp. and *Colletotrichum* sp. through the production of compounds such as phenazines (Niu et al. 2017; Chandran et al. 2021).

Three strains isolated from leaves and roots of *Platanus mexicana* (Hay 2-01H(7), PmH2-008 and Hay 2-5R), were classified as *Bacillus* sp. and showed cellulolytic and pectinolytic activities. *Bacillus* is one of the most widely used genera in the field and at the industrial level due to its ability to produce lytic enzymes, which can be exploited for the biodegradation of complex substrates rich in cellulose and pectin (Kavuthodi & Sebastian 2018; Shankar et al. 2021). Due to this quality, it is also widely used as a

biocontrol agent against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Fusarium oxysporum* (Khalil et al. 2021). Regarding the effect of *Bacillus* sp. Hay 2-01H(7), and *Bacillus* sp. Hay 2-5R on *A. thaliana*, both had a growth-promoting effect, mainly stimulating lateral root formation, causing an increase in the shoot biomass of 67.7% and 30.5%, respectively. *Bacillus* sp. PmH2-008 had little promoting effect with only a 7% increase in shoot biomass, however it stimulated the plant defence system causing a slight increase in anthocyanin production in the leaves (Fig. 3).

It is also important to highlight the enzymatic capacity found for the strains identified as *Carnobacterium gallinarum* Chi2-3Ri and Chi 4-1Ri(68i) and *Exiguobacterium* sp. PmRi3-012. Strain Chi2-3Ri was positive for cellulolytic activity and strain Chi 4-1Ri(68i) was positive for pectinolytic activity only at pH 7. Genomic analysis of *Carnobacterium* strains revealed the presence of glycoside hydrolase domains (Iskandar et al. 2017), which could correspond to different enzymatic activities such as those evaluated in this study. Strain PmRi3-012 degrades cellulose and pectin (at pH 5 and pH 7), which is consistent with the results of other studies reporting other *Exiguobacterium* strains with demonstrated ability to transform complex organic and inorganic polymers (Berlemont & Martiny 2016; Pandey 2020).

In this study it was possible to identify bacterial genera (*Yersinia* and *Erwinia*) that are reported in the literature as entomopathogenic and even able to cause diseases in plants (Ruiu 2015; Cho et al. 2019); however, according to the tests carried out, we were able to evaluate their enzyme producing potential, antagonistic capacity, and growth stimulation in seeds. *Yersinia* sp. PsH3-014(14D), isolated from the leaves of *Persea schiedeana* was able to degrade chitin and pectin from a Petri dish, promoted the growth of *A. thaliana* seedlings as evidenced by an increase in shoot biomass as well as an increased number of lateral roots compared to the sterile controls, and also showed an *in vitro* antagonistic effect against the *Fusarium* sp. strain INECOL_BM-06, inhibiting 37.21% of the radial growth of the fungus. We did not find any other reports referring to antifungal or growth promoting activities in *Yersinia* species, which is an interesting finding of our study. In the literature, different *Yersinia* species (*Y. entomophaga* and *Y. enterocolitica*) are reported as producers of insecticidal toxin complexes and enzymes (chitinases, pectate lyases, polygalacturonate lyase), that can be used for the biocontrol of coleopteran, lepidopteran, and orthopteran species (Busby et al. 2012; Hugouvieux-Cotte-Pattat et al. 2014; Samanta 2019; Paulson et al. 2021).

The strain Hay2-1H was identified as *Erwinia* sp. and shows the ability to degrade cellulose and pectin substrates. Previous work has shown that *Erwinia* species produce cellulases, chitinases and pectinases, for example *E. chrysanthemi* and *E. carotovora* (Samanta 2019). Interestingly, our results with Hay2-1H, showed growth promotion in *A. thaliana*, reflected by an increase in shoot biomass, accompanied by an increase in anthocyanin content in leaves and lateral root formation compared to the sterile control in the in vitro assays; as well as a slight antagonistic effect against *Fusarium* sp. strain INECOL_BM-06, causing 10% inhibition of fungal growth.

Finally, strain Chi 3–5(23), classified as *Serratia grimesii*, a proteobacteria able to promote the growth of *A. thaliana* seedlings, noticeable as an increment in the lateral root formation, as well as in the green shoot biomass 18.4%; the same strain, had little effect on the growth of *Fusarium* sp. strain INECOL_BM-06, causing a reduction in its growth of 11.3%. These proteobacteria also degraded cellulose. Further characterization of Chi 3–5(23) could reveal more of its properties; such as those of *Serratia grimesii* strain BXF1 (isolated from the pine nematode that causes pine wilt disease), a strain with possesses fungal and bacterial antagonistic activities, as well as plant growth regulation and modulation of nematode development (Nascimento et al. 2018).

All the strains characterized in the present study have application potential either in agriculture, or in industry, as they can be incorporated as bioinoculants to stimulate plant growth or reduce the impact of the phytopathogenic fungus *Fusarium sp.* Furthermore, they can be cultured in bioreactors to produce enzymes efficient in the transformation of different materials with cellulose, pectin, or chitin composition, such as in the textile industry, processing of cellulosic or chitin-rich residues, among others. Further research could be directed towards the characterization of such enzymes, as well as further exploration of other metabolic capacities of the strains mentioned.

Conclusion

Fourteen bacterial strains were isolated from the rhizosphere, the philosopher, and the bark of *Platanus mexicana* and *Persea schiedeana*, identified as *Curtobacterium, Plantibacter, Bacillus, Brevibacterium, Carnobacterium, Staphylococcus, Erwinia, Serratia, Exiguobacterium* and *Yersinia* genera. All have potential applications in either in agriculture or industry for their enzymatic capacity, biocontrol, and plant growth promotion. Highlighting the importance of isolating bacteria from the mountain cloud forest with biotechnological applications. Several strains possessed different capacities; strains of *Yersinia* sp. and *Bacillus* sp. stand out for their ability to promote the growth of in *A. thaliana* seedlings as well as to inhibit the growth of *Fusarium*; and to degrade at least one of biopolymers tested. On the other hand, *Erwinia* sp. was the only strain capable of degrading cellulose, pectin, and chitin. Future research could include the characterization of the enzymes produced, as well as further exploration of other metabolic capacities of the strains mentioned.

Declarations

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Author contributions

R.O.-C., A.A. and R.F.-C. obtained the funding, provided the resources, and conceived the project, B.A.A.-G. and G.E.C.-R. performed the experiments and analyzed the data, O.F.-R. and R.O.-C. supervised the research and drafted the original manuscript and figures. D.H.-M. instructed B.A.A.-G. on the analysis to evaluate the degradation activities. D.H.-M., A.A., R.F.-C. and R.O.-C. revised and edited the paper.

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Compliance with ethical requirements

This article does not contain any studies with human or animal subjects.

Declaration of competing interest

The authors have declared no conflict of interest.

References

1. Agostoni M, Hangasky JA, Marletta MA (2017) Physiological and molecular understanding of bacterial polysaccharide monooxygenases. Microbiol. Mol. Biol. Rev. 81(3):e00015-17.
2. Alam MK, Rahman MM, Jamal AHM, Islam MR (2021) Screening of antagonistic potential bacteria from rhizosphere soil against phytopathogenic fungi related to selected vegetable crops. J. Adv. Biotechnol. Exp. Ther. 4:35–43.
3. Backer R, Rokem JS, Ilangumaran G, Lamont J, Praslickova D, Ricci E, Subramanian S, Smith DL (2018) Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Front. Plant Sci. 9:1473.
4. Báez-Vallejo N, Camarena-Pozos DA, Monribot-Villanueva JL, Ramírez-Vázquez M, Carrión-Villarnovo GL, Guerrero-Analco JA, Partida-Martínez LP, Reverchon F (2020) Forest tree associated bacteria for potential biological control of *Fusarium solani* and of *Fusarium kuroshium*, causal agent of Fusarium dieback. Microbiological Research 235:126440.
5. Berlemont R, Martiny AC (2016) Glycoside Hydrolases across Environmental Microbial Communities. PLOS Comput. Biol. 12:e1005300.
6. Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and Functions of the Bacterial Microbiota of Plants. Annu. Rev. Plant Biol. 64:807–838.

7. Busby JN, Landsberg MJ, Simpson RM, Jones SA, Hankamer B, Hurst MRH, Lott JS (2012) Structural analysis of Chi1 Chitinase from Yen-Tc: The multisubunit insecticidal ABC toxin complex of *Yersinia entomophaga*. J. Mol. Biol. 415:359–371.
8. Carreras-Villaseñor N, Rodríguez-Haas JB, Martínez-Rodríguez LA, Pérez-Lira AJ, Ibarra-Laclette E, Villafán E, Castillo-Díaz AP, Ibarra-Juárez LA, Carrillo-Hernández ED, Sánchez-Rangel D (2022) Characterization of two *Fusarium solani* species complex isolates from the ambrosia beetle *Xylosandrus morigerus*. J. Fungi 8:231.
9. Carvalho TLG, Balsemão-Pires E, Saraiva RM, Ferreira PCG, Hemerly AS (2014) Nitrogen signalling in plant interactions with associative and endophytic diazotrophic bacteria. J. Exp. Bot. 65:5631–42.
10. Cazorla FM, Mercado-Blanco J (2016) Biological control of tree and woody plant diseases: an impossible task? BioControl 61:233–242.
11. Chandran H, Meena M, Swapnil P (2021) Plant Growth-Promoting Rhizobacteria as a Green Alternative for Sustainable Agriculture. Sustainability 13(19):10986.
12. Cho H, Park JY, Kim YK, Sohn S-H, Park DS, Kwon Y-S, Kim C-W, Back C-G, (2019) Whole-genome sequence of *Erwinia persicina* b64, which causes pink soft rot in onions. Microbiol. Resour. Announc. 8(1):e01302-18.
13. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, Brown CT, Porras-Alfaro A, Kuske CR, Tiedje JM (2014) Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 42:D633-D642.
14. Compant S, Samad A, Faist H, Sessitsch A (2019) A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J. Adv. Res. 19: 29–37.
15. Egamberdieva D, Wirth SJ, Alqarawi AA, Abd_Allah EF, Hashem A (2017) Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Front. Microbiol. 8:2104.
16. Gruau C, Trotel-Aziz P, Villaume S, Rabenoelina F, Clément C, Baillieul F, Aziz A (2015) *Pseudomonas fluorescens* PTA-CT2 Triggers Local and Systemic Immune Response Against *Botrytis cinerea* in Grapevine. Mol Plant-Microbe Interact 28:1117–1129.
17. Hartmann A, Fischer D, Kinzel L, Chowdhury SP, Hofmann A, Baldani JI, Rothballer M (2019) Assessment of the structural and functional diversities of plant microbiota: Achievements and challenges – A review. J. Adv. Res. 19:3–13.
18. Hassani MA, Durán P, Hacquard S (2018). Microbial interactions within the plant holobiont. Microbiome 6:58.
19. Hu W, Gao Q, Hamada MS, Dawood DH, Zheng J, Chen Y, Ma Z (2014) Potential of *Pseudomonas chlororaphis* subsp. *aurantiaca* Strain Pcho10 as a Biocontrol Agent Against *Fusarium graminearum*. Phytopathology 104:1289–97.
20. Hugouvieux-Cotte-Pattat N, Condemine G, Shevchik VE (2014) Bacterial pectate lyases, structural and functional diversity. Environ. Microbiol. Rep. 6:427–440.

21. Iskandar CF, Borges F, Taminiau B, Daube G, Zagorec M, Remenant B, Leisner JJ, Hansen MA, Sorensen SJ, Mangavel C, Cailliez-Grimal C, Revol-Junelles AM (2017) Comparative Genomic Analysis Reveals Ecological Differentiation in the Genus *Carnobacterium*. Front. Microbiol. 8:357.
22. Kavuthodi B, Sebastian D (2018) Review on bacterial production of alkaline pectinase with special emphasis on *Bacillus* species. Biosci. Biotechnol. Res. Commun. 11:18–30.
23. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647–1649.
24. Khalil MMR, Fierro-Coronado RA, Peñuelas-Rubio O, Villa-Lerma AG, Plascencia-Jatomea R, Félix-Gastélum R (2021) Rhizospheric bacteria as potential biocontrol agents against Fusarium wilt and crown and root rot diseases in tomato. Saudi J. Biol. Sci. 28:7460–71.
25. Khanna K, Jamwal VL, Sharma A, Gandhi SG, Ohri P, Bhardwaj R, Al-Huqail AA, Siddiqui MH, Marraiki N, Ahmad P (2019) Evaluation of the role of rhizobacteria in controlling root-knot nematode infection in *Lycopersicon esculentum* plants by modulation in the secondary metabolite profiles. AoB Plants 11(6):plz069.
26. Kong WL, Li PS, Wu XQ, Wu TY, Sun XR (2020) Forest tree associated bacterial diffusible and volatile organic compounds against various phytopathogenic fungi. Microorganisms 8(4):590.
27. Kopecky J, Samkova Z, Sarikhani E, Kyselková M, Omelka M, Kristufek V, Divis J, Grundmann GG, Moënne-Loccoz Y, Sagova-Mareckova M (2019) Bacterial, archaeal and micro-eukaryotic communities characterize a disease-suppressive or conducive soil and a cultivar resistant or susceptible to common scab. Sci. Rep. 9:14883.
28. Kovinich N, Kayanja G, Chanoca A, Otegui MS, Grotewold E (2015) Abiotic stresses induce different localizations of anthocyanins in *Arabidopsis*. Plant Signal. Behav. 10:e1027850.
29. Mathys J, De Cremer K, Timmermans P, Van Kerkhove S, Lievens B, Vanhaecke M, Cammue BPA, De Coninck B (2012) Genome-Wide Characterization of ISR Induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 Against *Botrytis cinerea* Infection. Front. Plant Sci. 3:108.
30. Mayer E, de Quadros PD, Fulthorpe R, Cann I (2019) *Plantibacter flavus*, *Curtobacterium herbarum*, *Paenibacillus taichungensis*, and *Rhizobium selenitireducens* Endophytes Provide Host-Specific Growth Promotion of *Arabidopsis thaliana*, Basil, Lettuce, and Bok Choy Plants. Appl. Environ. Microbiol. 85:e00383-19.
31. Merlin C, Besaury L, Niepceron M, Mchergui C, Riah W, Bureau F, Gattin I, Bodilis J (2014) Real-time PCR for quantification in soil of glycoside hydrolase family 6 cellulase genes. Lett. Appl. Microbiol. 59:284–291.
32. Nascimento F, Vicente C, Cock P, Tavares M, Rossi M, Hasegawa K, Mota M (2018) From plants to nematodes: *Serratia grimesii* BXF1 genome reveals an adaptation to the modulation of multi-species interactions. Microb. Genomics 4(7):178.

33. Niu B, Paulson JN, Zheng X, Kolter R (2017) Simplified and representative bacterial community of maize roots. Proc. Natl. Acad. Sci. 114:E2450-E2459.
34. Ortiz-Castro R, Campos-García J, López-Bucio J (2013) Rapid identification of plant-growth-promoting rhizobacteria system with Arabidopsis. Molecular Microbial Ecology of the Rhizosphere. Frans J. De Bruijn (Ed). Vol I. Wiley-Blackwell, New Jersey, pp.345–353.
35. Ortiz-Castro R, Díaz-Pérez C, Martínez-Trujillo M, del Rio RE, Campos-García J, López-Bucio J (2011) Transkingdom signaling based on bacterial cyclodipeptides with auxin activity in plants. Proc. Natl. Acad. Sci. USA 108(17):7253–7258.
36. Pandey N (2020) Exiguobacterium. In: Amaresan, N., Senthil Kumar, M., Annapurna, K., Kumar, K., Sankaranarayanan, A., Editors. Benef. Microbes Agro-Ecology, Academic Press; pp. 169–183.
37. Paulson AR, O'Callaghan M, Zhang X-X, Rainey PB, Hurst MRH (2021) *In vivo* transcriptome analysis provides insights into host-dependent expression of virulence factors by *Yersinia entomophaga* MH96, during infection of *Galleria mellonella*. G3 Genes Genomes Genetics 11:jkaa024.
38. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41(D1): D590-D596.
39. Reverchon F, Escudero-Osorio YS, Morteo-Zavaleta J, Guevara-Avendaño E, Ramírez-Vázquez M (2020) Inhibición de *Fusarium solani* por bacterias de la filósfera y rizósfera de árboles del bosque mesófilo de montaña. Biotecnología y Sustentabilidad 5(1):3–18.
40. Ruiu L (2015) Insect Pathogenic Bacteria in Integrated Pest Management. Insects 6:352–367.
41. Saggese A, De Luca Y, Baccigalupi L, Ricca E (2022) An antimicrobial peptide specifically active against Listeria monocytogenes is secreted by *Bacillus pumilus* SF214. BMC Microbiol 22:3.
42. Samanta S (2019) Microbial pectinases: a review on molecular and biotechnological perspectives. J. Microbiol. Biotechnol. FOOD Sci. 9:248–66.
43. Santos RMd, Rigobelo EC (2021) Growth-Promoting Potential of Rhizobacteria Isolated From Sugarcane. Front. Sustain. Food Syst. 5:596269.
44. Shankar T, Sankaralingam S, Balachandran C, Chinnathambi A, Nasif O, Ali Alharbi S, Park S, Baskar K (2021) Purification and characterization of carboxymethylcellulase from *Bacillus pumilus* EWBCM1 isolated from earthworm gut (*Eudrilus eugeniae*). J. King Saud Univ. Sci. 33:101261.
45. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587.
46. Slama HB, Cherif-Silini H, Chenari Bouket A, Qader M, Silini A, Yahiaoui B, Alenezi FN, Luptakova L, Triki MA, Vallat A, Oszako T, Rateb ME, Belbahri L (2019) Screening for Fusarium Antagonistic Bacteria From Contrasting Niches Designated the Endophyte *Bacillus halotolerans* as Plant Warden Against *Fusarium*. Front. Microbiol. 9:3236.
47. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10(3):512–526.

48. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol. 38(7):3022–3027.
49. Tzec-Interián JA, Desgarennes D, Carrión G, Monribot-Villanueva JL, Guerrero-Analco JA, Ferrera-Rodríguez O, Santos-Rodríguez DL, Liahut-Guin N, Caballero-Reyes GE, Ortiz-Castro R (2020) Characterization of plant growth-promoting bacteria associated with avocado trees (*Persea americana* Miller) and their potential use in the biocontrol of *Scirtothrips perseae* (avocado thrips). PLoS One 15(4): e0231215.
50. Ulfa R, Suryanto D, Munir E (2018) An ability of endophytes from blackboard tree (Alstonia scholaris) in increasing chili performance. J. Pure Appl. Microbiol. 12:815–825.
51. Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE (2016) The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. Annu. Rev. Ecol. Evol. Syst. 47:1–24.
52. Veliz EA, Martínez-Hidalgo P, Hirsch AM (2017) Chitinase-producing bacteria and their role in biocontrol. AIMS Microbiol. 3:689–705.
53. Viswanath G, Sekar J, Prabavathy VR (2016) Acyl homoserine lactone-producing rhizobacteria elicit systemic resistance in plants. Microbial-mediated induced systemic resistance in plants. Devendra K. Choudhary, Ajit Varma (Editors) Springer. pp. 135–146.
54. Wang NR, Wiesmann CL, Melnyk RA, Hossain SS, Chi M-H, Martens K, Craven K, Haney CH (2022) Commensal *Pseudomonas fluorescens* Strains Protect *Arabidopsis* from Closely Related *Pseudomonas* Pathogens in a Colonization-Dependent Manner. MBio 13:e02892-21.
55. Wright ES, Yilmaz LS, Noguera DR (2012) DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. Applied and Environmental Microbiology. 78(3):717–725.

Tables

Table 1 is available in the Supplementary Files section.

Figures

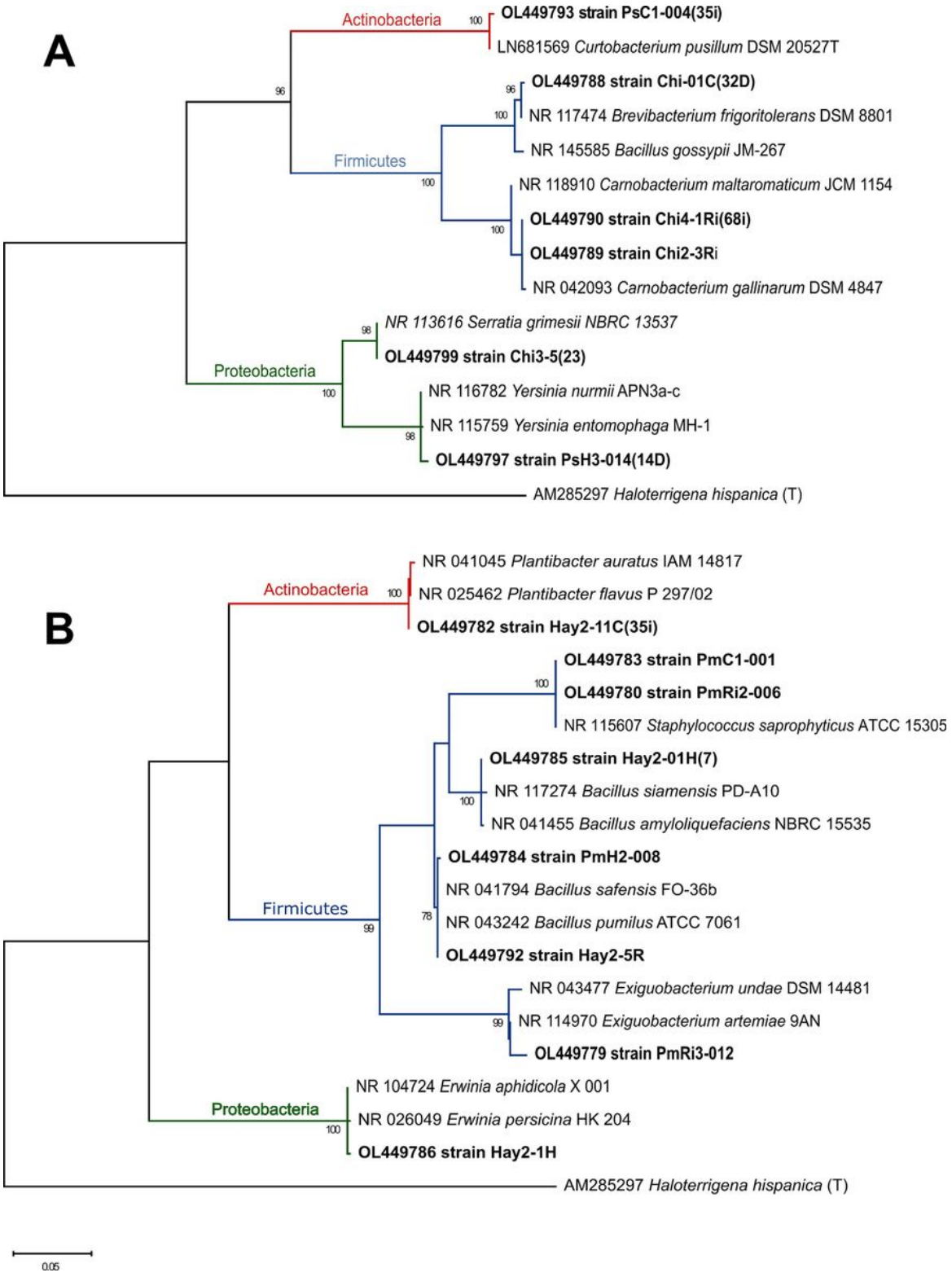

Figure 1

Phylogenetic tree of the 16S rRNA sequence of bacteria isolated from *Persea schiedeana* and *Platanus mexicana* trees. A) *Persea schiedeana, B) Platanus mexicana. Haloterrigena hispanica* was used as the outer group.

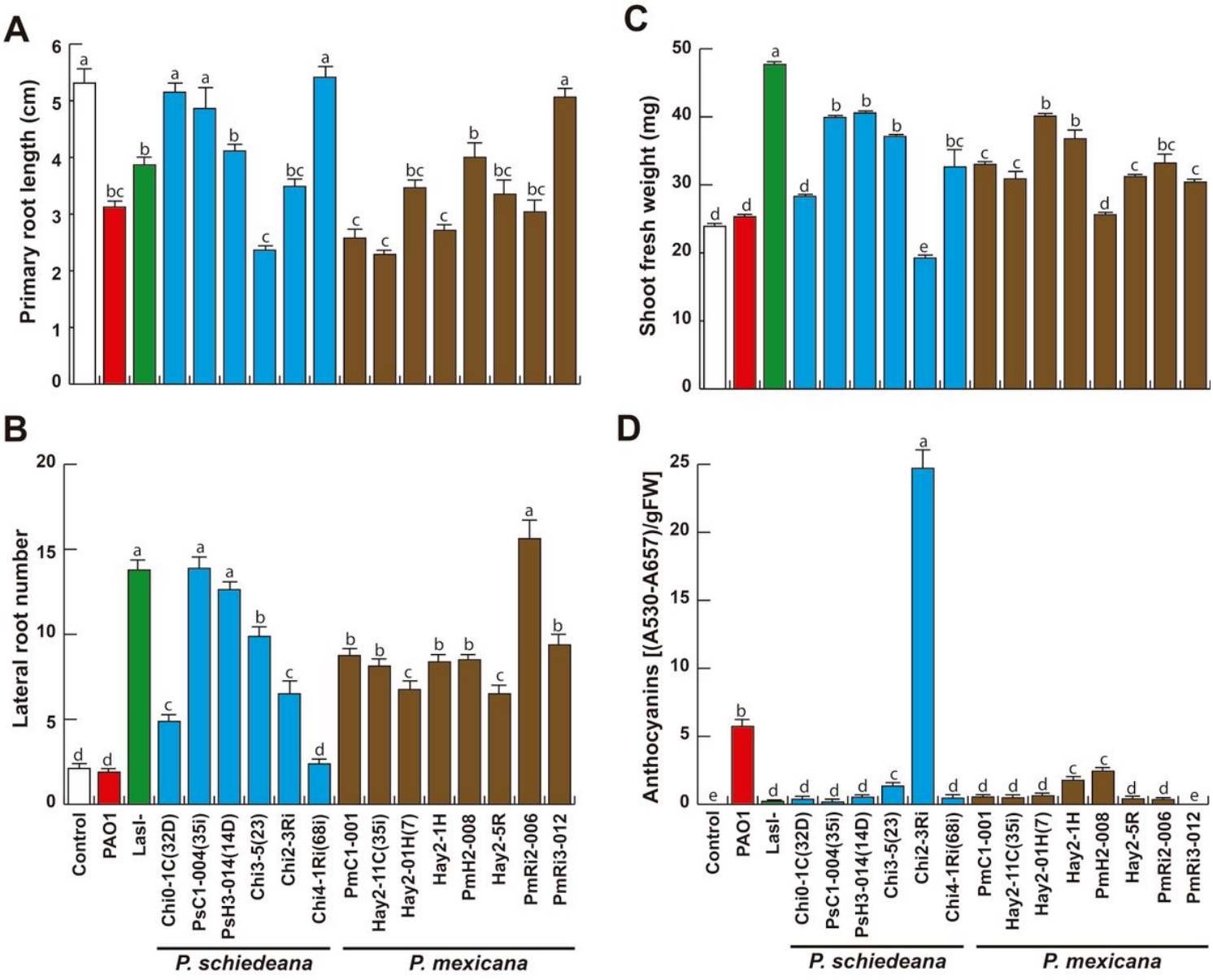

Figure 2

Effects of bacterial strains isolated from *P. schiedeana*and *P. mexicana* on the *in vitro* Arabidopsis-bacteria co-culture system. Arabidopsis seedlings from 5 d after germination were co-cultured with bacterial strains and grown for 8 d. A) Primary root length, B) Lateral root numbers, C) Shoot fresh weight, D) Anthocyanins. Data represent the mean ± standard deviation (n=24). Different letters indicate the statistical difference at $p<0.05$.

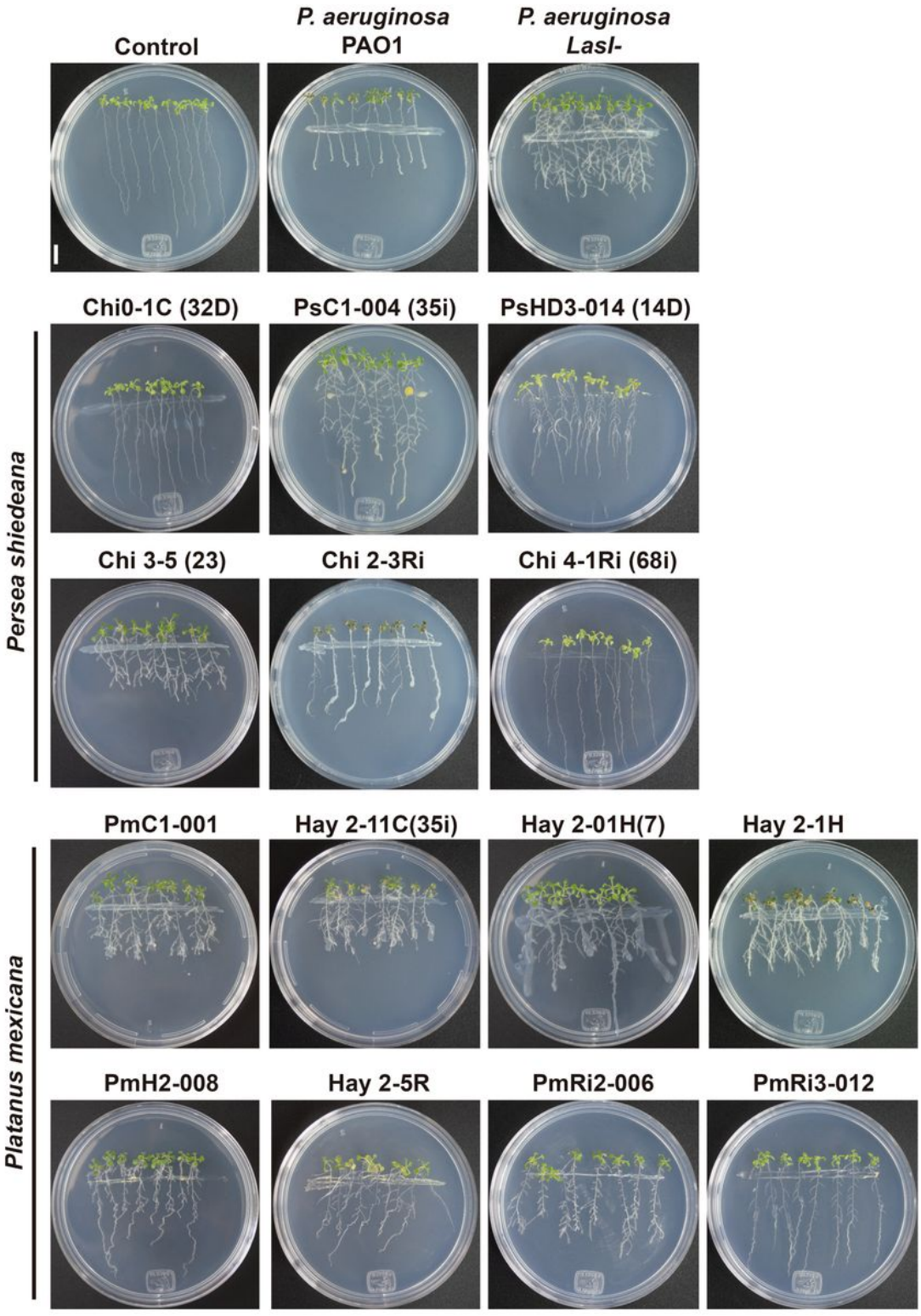

Figure 3

Arabidopsis growth responses to bacterial strains in an *in vitro* co-culture system with bacterial strains. Arabidopsis seedlings were grown on 0.2x MS agar medium and then were co-cultured with the bacterial strains isolated from *Persea schiedeana* and *Platanus mexicana*or uninoculated (control treatment). *Pseudomonas aeruginosa* PAO1 (wild-type) and *P. aeruginosa LasI-* (a quorum sensing mutant affected

in the LasI synthase) were used as pathogenic and plant growth-promoting strains, respectively. Representative images of Arabidopsis growth responses to bacterial strains. Scale bar = 1cm.

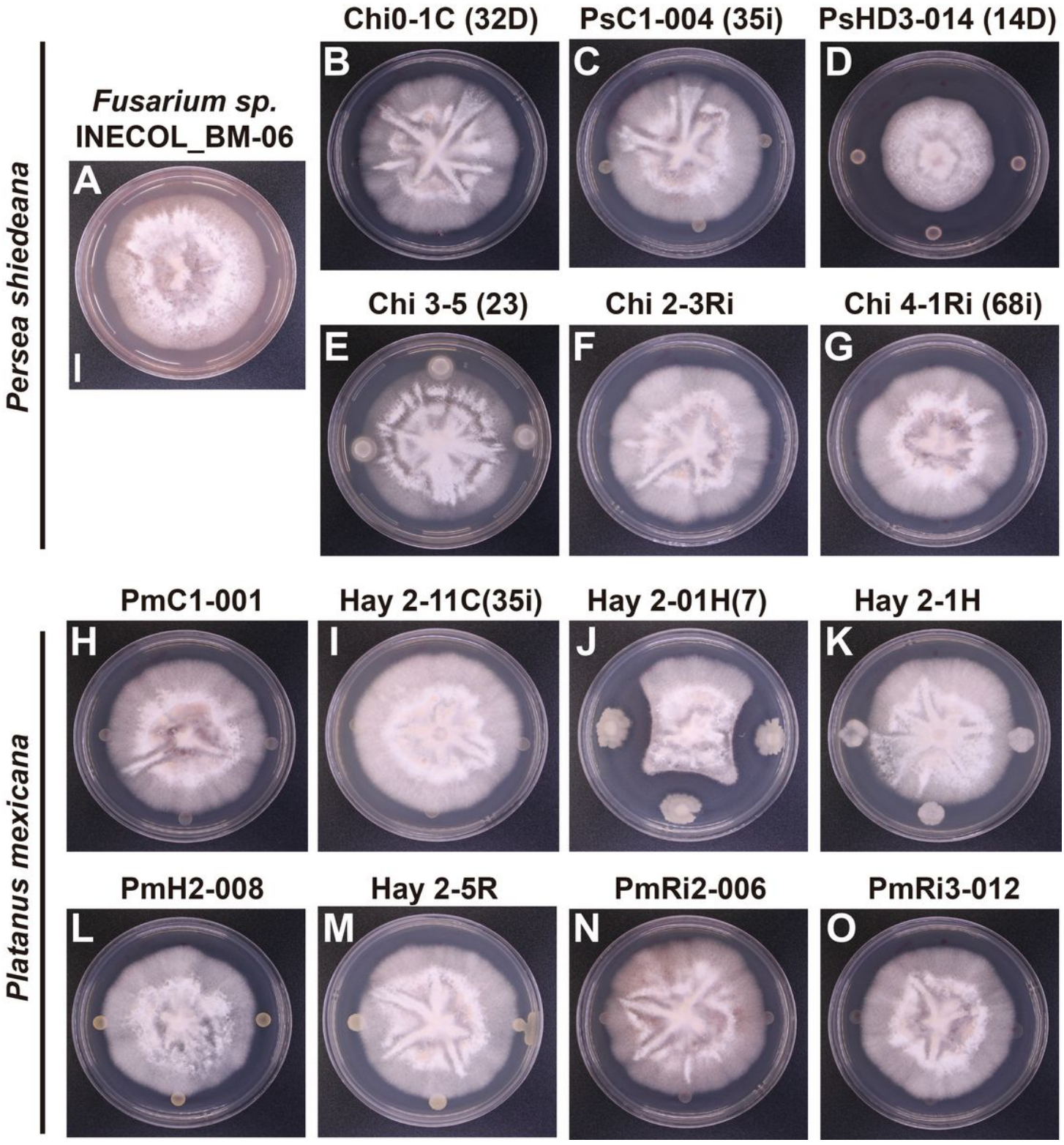

Figure 4

Co-culture of bacterial strains and *Fusarium sp.* strain INECOL_BM-06. A) *Fusarium sp.* strain INECOL_BM-06. Strains isolated from *Persea shiedeana*: B) Chi 0-1C (32D), C) PsC1-004 (35i), D) PsH3-

014(14D), E) Chi 3-5(23), F) Chi 2-3Ri, G) Chi 4-1Ri (68i). Strains isolated from *Platanus mexicana*: H) PmC1-001, I) Hay 2-11C (35i), J) Hay 2-01H (7), K) Hay 2-1H, L) PmH2-008, M) Hay 2-5R, N) PmRi2-006, O) PmRi3-012. Scale bar = 1cm.

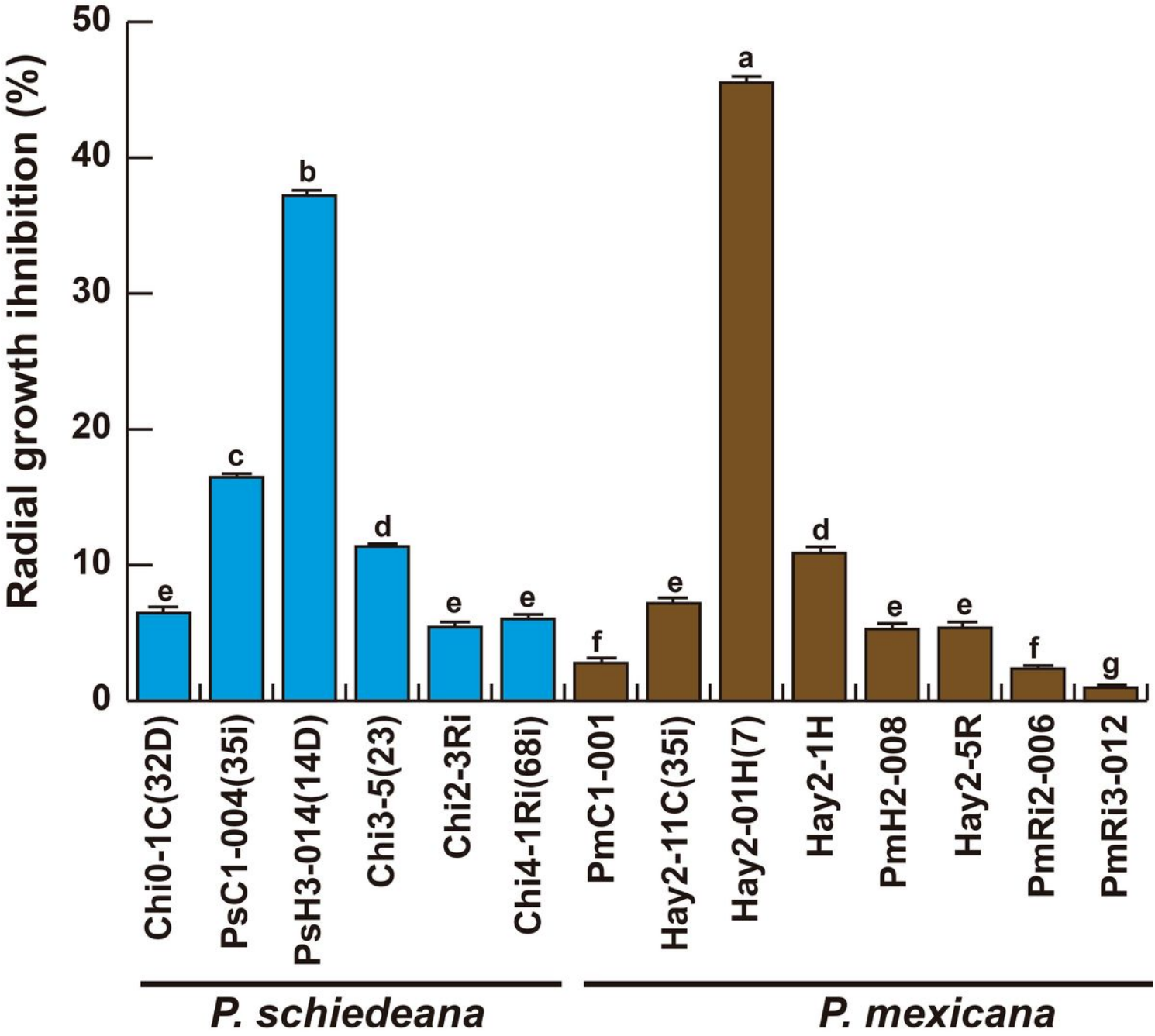

Figure 5

Effects of bacterial strains isolated from *P. shiedeana* and *P. mexicana* on the growth inhibition of *Fusarium* sp. strain INECOL_BM-06. Percentage of growth inhibition of *Fusarium sp.* strain INECOL_BM-06. caused by the antagonistic activities of the bacterial strains. The data represent the mean ± standard deviation (n=3). The experiment was repeated twice times with similar results.

Supplementary Files

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- Table1.jpg