Saudi Journal of Biological Sciences 28 (2021) 7460–7471

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Rhizospheric bacteria as potential biocontrol agents against *Fusarium* wilt and crown and root rot diseases in tomato

Md. Masudur Rahman Khalil [a], Rosario Alicia Fierro-Coronado [a], Ofelda Peñuelas-Rubio [a,b], Alma Guadalupe Villa-Lerma [c,d], Rigoberto Plascencia-Jatomea [c], Rubén Félix-Gastélum [e], Ignacio Eduardo Maldonado-Mendoza [a,*]

[a] *Departamento de Biotecnología Agrícola, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR)-Unidad Sinaloa, Instituto Politécnico Nacional, CP 81101 Guasave, Sinaloa, Mexico*
[b] *Departamento de Ingenierías, Instituto Tecnológico del Valle del Yaqui, Tecnológico Nacional de México, CP 85276 Bácum, Sonora, Mexico*
[c] *SYME Agroinsumos Innovadores S.A. de C.V., CP 85225 Navojoa, Sonora, Mexico*
[d] *Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, Campus Náinari, CP 85130 Ciudad Obregón, Sonora, Mexico*
[e] *Departamento de Ciencias Naturales y Exactas, Universidad Autónoma de Occidente, Unidad Regional Los Mochis, CP 81217 Los Mochis, Sinaloa, Mexico*

ARTICLE INFO

Article history:
Received 13 April 2021
Revised 13 August 2021
Accepted 15 August 2021
Available online 26 August 2021

Keywords:
Acinetobacter
Antagonist
Bacillus
Plant-growth promotion
Tomato hybrid specificity

ABSTRACT

The discovery of novel biocontrol agents requires the continuous scrutiny of native microorganisms to ensure that they will be useful on a regional scale. The goal of the present work was to discover novel antagonistic bacteria against *Fusarium oxysporum* ff. spp. *lycopersici* race 3 (*Fol* R3) and *radicis-lycopersici* (*Forl*) causing *Fusarium* wilt disease and *Fusarium* crown and root rot of tomatoes, respectively. High-throughput liquid antagonism screening of 1,875 rhizospheric bacterial strains followed by dual confrontation assays in 96-well plates was used to select bacteria exhibiting > 50% fungal growth inhibition. In a second dual confrontation assay in 10-cm Petri dishes, bacteria showing > 20% *Fol* R3 or *Forl* growth inhibition were further screened using a blood hemolysis test. After discarding β-hemolytic bacteria, a seedling antagonistic assay was performed to select five potential antagonists. A phylogenetic analysis of 16S rRNA identified one strain as *Acinetobacter calcoaceticus* (*Ac*DB3) and four strains as members of the genus *Bacillus* (*B. amyloliquefaciens Ba*MA26, *Bacillus siamensis Bsi*DA2, *B. subtilis Bs*TA16 and *B. thuringiensis Bt*MB9). Greenhouse assays demonstrated that *Bs*TA16 and *Ac*DB3 were the most promising antagonists against *Fol* R3 and *Forl*, respectively. Pathogen biocontrol and growth promotion mechanisms used by these bacteria include the production of siderophores, biofilm, proteases, endoglucanases and indole acetic acid, and phosphate solubilization. These five bacteria exerted differential responses on pathogen control depending on the tomato hybrid, and on the growth stage of tomatoes. We report for the first time the use of an *Acinetobacter calcoaceticus* isolate (*Ac*DB3) to control *Forl* in tomato under greenhouse conditions.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important agricultural crops, and can be grown both in the field and under greenhouse conditions. In 2019, Mexico was ranked ninth worldwide in terms of tomato production, with 4.27 million tons harvested annually (FAOSTAT, 2019). Sinaloa is the main contributing state, accounting for 22.21% of the total national tomato production and exports in excess of 372.5 million USD annually (SIAP-SAGARPA, 2019).

Tomato production may be severely affected by *F. oxysporum* f. sp. *lycopersici* (*Fol*) races 1, 2 and 3, which cause *Fusarium* wilt disease, and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*), which causes *Fusarium* crown and root rot of tomatoes (*FCRRT*) (Jarvis and Shoemaker, 1978). The presence of *Forl* (Ramírez-Estrada and Leyva-Mir, 1990) and *Fol* race 3 (*Fol* R3) (Valenzuela-Ureta et al., 1996) has already been reported in Sinaloa state, Mexico. *Fol* R3

* Corresponding author.
E-mail address: imaldona@ipn.mx (I.E. Maldonado-Mendoza).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.08.043
1319-562X/

causes vascular wilting in young tomato plants. Diseased plants display yellowing, foliage wilting, and discoloration of the vascular tissue to the point of dark brown coloration, stunting, and the eventual death of the entire plant (Aydi-Ben-Abdallah, et al., 2016). In contrast, *Forl* causes *FCRRT*, which affects the crown and roots of tomato plants. Field infection with *Forl* produces stunted plants, with lower leaves that turn yellow and wilt. Wilting occurs during the warmest part of the day, and then plants recover at night. *Fol* R3 and *Forl* co-infections are common in tomato plants under greenhouse and open field conditions (Debbi et al., 2018). Tomato production in open field conditions in India can be reduced up to 45% by *Fol*-induced wilting (Elanchezhiyan et al., 2018). *FCRRT*-induced losses caused by *Forl* in greenhouse tomato yields have been reported at levels of up to 60% in greenhouses in southwestern Ontario (Canada) (Salim et al., 2017), and 90% in Tunisia (Hibar et al., 2007). In Sinaloa, Mexico, *Fol* R3 and *Forl* are now major concerns due to their serious effects on tomato production. These devastating diseases can decrease tomato yields by up to 50% in open field and greenhouse conditions (Apodaca-Sánchez et al., 2002).

The use of chemical fungicides to control these diseases is of limited benefit since their level of control is not very effective, and they have negative impacts on the environment and human health (Hu et al., 2015). Biological control represents an environmentally friendly approach that uses microorganisms (i.e. bacteria and fungi) capable of inhibiting or suppressing pathogen populations (Chow et al., 2018), making it a suitable alternative for managing phytopathogenic fungi. Biocontrol agents may possess both antagonistic and plant growth-promoting traits, which are considered important for plant disease control as well as fruit yield (Sharma et al., 2018).

Furthermore, biological control agents may have a better chance of establishment and effective pathogen control if they are native to the soil, as compared to exotic microorganisms. Indeed, native microorganisms are already adapted to the local climate and edaphic conditions as well as to the soil microbiota (Gómez et al., 2016). Our group has previously created bacterial collections containing maize (Figueroa-López et al., 2016), tomato (Cordero-Ramírez et al., 2013) and *Datura* sp. rhizospheric bacteria (López-Rivera, 2011). Rhizobacteria isolated from native soils of Sinaloa will thus maintain their plant growth-enhancing effects as well as antagonistic activity against *Fol* R3 and *Forl*, which may contribute to tomato production. The aim of the present study was to select native rhizospheric bacteria with potential antagonism against *Fol* R3 and/or *Forl*, and that may promote plant growth under greenhouse conditions. We hypothesized that: 1) at least one bacterial isolate would be able to control *in vitro* both the *Fol* R3 and *Forl* pathogens; 2) bacterial isolates from the tomato rhizosphere would be a better source of antagonists against these two fungal phytopathogens than bacteria from the other two rhizospheres (i.e. *Datura* sp. or maize); and 3) the effect of different bacteria should vary according to tomato hybrid or developmental stage.

2. Materials and methods

2.1. Biological material and initial preparation for bioassays

We screened 1,875 out of 2,098 bacterial strains from three bacterial collections deposited at the Department of Agricultural Biotechnology at CIIDIR-Sinaloa, Instituto Politécnico Nacional (Mexico): 1) a sub-collection of the CIIDIR-003 collection (January 2009) containing 624 maize rhizospheric bacteria preselected as *Fusarium verticillioides* antagonists (Figueroa-López et al., 2016); 2) the CIIDIR-001 collection (March 2006) containing 706 tomato rhizospheric bacteria representing the bulk isolates collected from the mixed rhizosphere of five healthy plants from a tomato cv. Gabriela commercial field in Guasave, Sinaloa, Mexico (Cordero-Ramírez et al., 2013); and 3) CIIDIR-004 (January 2010) containing 768 *Datura* sp. rhizospheric bacteria from the mixed rhizosphere of 25 plants collected in groups of five plants per each of five quadrants at the ecological preservation zone La Uba in Guasave, Sinaloa, Mexico (López-Rivera, 2011). The bacteria collections showed 89.0–89.6% viability when maintained for 11 to 15 years at −80 °C.

Fol R3 (22) and *Forl* (1045) strains were previously identified molecularly, and *Fol* R3 or *Forl* identity was confirmed by tomato genotyping (Cordero-Ramírez et al., 2013; Fierro-Coronado et al., 2013). These fungal strains were taken from frozen stocks at −80 °C and grown in water agar (WA) medium supplemented with ten square pieces of a carnation leaf (~3–5 mm in length) and incubated at 25 °C for 14 days in darkness (Leslie and Summerell, 2006). Conidia were collected by adding 10 mL of sterile distilled water on top of the agar plate, which was then rubbed with a sterile glass triangle. The conidial suspensions were filtered through two layers of sterile gauze to discard the mycelium, and then conidia were counted under a light microscope (Zeiss, Axiostar, Göttingen, Germany) using a hematocytometer. Finally, the conidial suspensions were diluted to the various CFU mL^{-1} concentrations required in the different experiments. For mass production of conidia used in pot bioassays, two plugs of the fungus (1 cm in diameter) were grown for 14 days as previously described, and were used to inoculate a 250-mL flask containing 100 mL of PD broth and grown at 150 rpm for 7 days at 25 °C.

The indeterminate tomato hybrids SV4401TJ (Nunhems, Mexico) and Pai Pai (Enza Zaden, Mexico) and the determinate hybrid N6394 (Nunhems) were used for the antagonistic and growth promotion bioassays. These cultivars are susceptible to *Fol* R3 and *Forl*, and are resistant to *Fol* races 1 and 2, according to each individual company's information.

Cryopreserved bacteria used as inoculum were transferred onto Luria Bertani (LB, Sigma, No. Cat. L3022, USA) agar medium and incubated at 30 °C for 24 h before use in the different bioassays. After growing bacteria in plates, a single colony was transferred to 5 mL of LB broth and incubated at 30 °C for 24 h at 200 rpm to obtain the pre-inoculum bacterial suspension. Next, 1 mL of each bacterial suspension was transferred to 100 mL of LB medium and incubated at 30 °C for 9 h at 200 rpm in order to obtain the inoculum in the exponential growth phase. Bacteria were diluted to the optical density (OD) at 600 nm corresponding to 2×10^8 CFU mL^{-1} for the test on tomato hybrids N6394, SV4401TJ and Pai Pai.

2.2. Liquid antagonism assays and dual confrontation tests

As an initial screening, a high-throughput liquid antagonism assay using potato dextrose broth (PDB) was performed to determine the potential antagonistic bacteria as previously described by Figueroa-López et al. (2014).

Briefly, 25 μL (1×10^5 conidia) of a conidial suspension containing *Fol R3* and *Forl* and 5 μL of each bacterial suspension containing 1.1×10^6 CFU were added in a final volume of 500 μL PDB to 2-mL 96-well plates. The fungal and bacterial isolates were mixed and incubated for 36 h at 25 °C in an orbital shaker at 240 rpm. The fungal biomass was quantitated by staining the chitin residues of the fungal cell wall with wheat germ agglutinin lectin coupled to a fluorophore (WGA Alexa Fluor 488 conjugate), which was then measured using a multimodal fluorescence detector (Beckman, DTX800). Fungal growth inhibition percentages were calculated using the following previously described formulas (Quilambaqui-Jara et al., 2004; Revillini et al., 2016; Figueroa-López et al., 2014):

$$\text{Fungal growth } (\%) = \frac{\text{Total fluorescence (with bacteria)}}{\text{Fluorescencel of control (fungus without bacteria)}} \; x\; 100$$

Inhibition (%) = 100% - fungal growth (%)

The selection criterion was arbitrarily set at > 50% fungal growth inhibition.

A second screening step was conducted to confirm the antagonistic effect observed in the liquid antagonism assays. This was performed in 96-well plates by adding 0.2 mL of PDA medium to each well. The bacterial pellets were applied to one side of the well using a 10 μL tip, and then 3 μL of conidial suspension containing 1×10^4 conidia was added to the opposite side of the well. As a control, fungal isolates were inoculated on PDA without any antagonistic bacteria. All plates were incubated at 25 °C for 48 h. The inhibition efficacy of the bacteria on the selected fungal isolates was determined 48 h after inoculation, by measuring the percentage of the well covered by mycelial growth on the medium. Bacterial strains that inhibited mycelial growth by at least 21% from the point of inoculation were included in subsequent studies (Fig. S1C).

Third, a second dual confrontation test was performed to confirm bacterial efficacy against the fungi (Fig. S1A-B). This test consisted in challenging the fungus with the bacteria on Petri plates (10 cm in diameter) containing WA medium. A mycelial plug (8 mm in diameter) was placed at the center of the Petri plates, and 5 μL of bacterial suspension (1.1×10^6 CFU) was applied at four opposite edges, 2 cm from the center. Each fungus-bacterium dual confrontation test was repeated three times. The plates were incubated for 5 days at 25 °C, and the shortest radial growth of the fungal isolates at the center of the fungal growth was measured at the end of this period. Growth inhibition (GI) was calculated using the following formula (Whipps, 1987):

$$GI = \frac{R1 - R2}{R1} x100$$

where R1 is the radial growth (mm) of the fungus in control plates, and R2 is the radial growth (mm) of the fungus when confronted by the bacteria. The treatments were arranged in a completely randomized design with three replicate plates.

2.3. Hemolysis tests on blood agar medium

The hemolytic activity of 31 bacterial strains showing at least 21% inhibition in the second confirmatory dual confrontation assay was investigated. Bacteria were grown overnight in 5 mL of LB medium at 30 °C and 200 rpm. Briefly, 1-mL bacterial suspensions were transferred to a 1.6-mL microcentrifuge tube and centrifuged at room temperature for 20 min at 13,000 rpm. Next, 50 μL of supernatant was added to 5-mm circular wells (previously made using a sterile cork borer) in blood agar medium. Plates were then incubated for 24 h at 37 °C. The results were interpreted based on the length of the clear zone surrounding the wells: β-hemolysis was observed as a clear zone, demonstrating complete breakage of erythrocytes; α-hemolysis was revealed by a slight change in color surrounding the wells, indicating partial breakdown of erythrocytes; and γ-hemolysis (or no hemolysis) was indicated by the absence of any change in color or clearness of the medium surrounding the well (Misawa et al., 1995). Bacteria showing β-hemolysis were discarded, and only α- and γ-hemolytic bacteria were used in subsequent studies.

2.4. In vitro antagonism in seedling assays

Tomato seeds were surface-disinfected by submerging them in 70% ethanol for 2 min followed by treatment with a 0.3% sodium hypochlorite solution for 10 min, and rinsed five times with abundant sterile distilled water. Surface-disinfected tomato hybrid seeds (N6394) were soaked in a bacterial suspension containing 2×10^8 CFU mL^{-1} for 2 h at 25 °C. *Fol* R3 or *Forl* mycelial plugs actively growing on SNA (Spezieller Nährstoffarmer Agar) were placed on Petri plates (10 cm in diameter) containing WA medium. Five seeds per plate were placed around the fungal inoculum, and plates were set up in triplicate for each treatment. Plates were incubated for 1 week in a growth chamber on a 16-h light/8-h dark photoperiod. Seed germination rate of each seed batch used was > 90%. Disease severity caused by *Fol* R3 was determined following the scale previously described by Apodaca-Sánchez et al. (2004) with slight modifications. A scale from 0 to 5 was used in which: 0 = absence of damage in root and stem; 1 = slight darkening in root with root thinning; 2 = slight darkening in root with necrosis in stem; 3 = 1–5 mm necrotic lesion in root and necrosis in stem; 4 = 6–10 mm necrosis in root and decreased seedling development with necrosis in stem; and 5 = necrotic lesion > 11 mm in length, no germination or plant death. To determine *Forl* disease severity, we applied another scale from 0 to 5 reported by Cordero-Ramírez et al. (2013) with modifications, in which: 0 = absence of damage in root and stem; 1 = spots of slight brown necrotic tissue in roots or leaflets; 2 = brown necrotic spots at the base of the stem; 3 = brown necrosis of the crown from 1 to 5 mm in length; 4 = necrotic lesion 6–10 mm in length at the base of the stem and decreased seedling development; and 5 = necrotic lesion ≥ 11 mm in length, no germination or plant death. The treatments were arranged in a completely randomized experimental design.

2.5. PCR and sequencing of the 16S rRNA gene of potentially antagonistic bacteria

The most potentially antagonistic bacteria were selected and molecularly identified after the seedling bioassays. Briefly, the bacteria were grown in LB agar medium for 24 h at 30 °C. Total genomic DNA was collected in DNAzol (Invitrogen, Cat. No. 10503–027, USA) according to the manufacturer. Next, the genomic DNA was used for polymerase chain reaction (PCR). Primers F2C (5′-AGA GTT TGA TCC TGG CTC-3′) and C (5′-GTA CAC ACC GCC CGT-3′) were used to amplify a 1.4-kb product from the16S rDNA gene (Cordero-Ramírez et al., 2013). The PCR reaction for the 16S rDNA gene was performed in a total volume of 25 μL containing 1X PCR buffer, 1.5 mM of MgCl2, 0.2 μM of dNTPs, 0.2 μM of each oligonucleotide, 1 U of Taq DNA polymerase, and 10 ng of DNA. The PCR conditions included an initial denaturation step at 95 °C for 4 min, followed by 30 cycles at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min, and a final step at 72 °C for 5 min. PCR products were loaded and electrophoresed in a 1% agarose gel in 0.5 X Tris-acetate EDTA buffer, stained with ethidium bromide, and visualized on a Chemidoc XS (Biorad). 16S rDNA amplicons were purified using the QIAquick Gel Extraction kit (QIAGEN; Hilden, Germany) according to the manufacturer's instructions. The internal primer U1 (5′ CCA GCA GCC GCG GTA ATA CG 3′) and the primer C were used for sequencing the F2C/C amplicons (Cordero-Ramírez et al., 2013) with an ABI 3730 XL automated sequencer at the National Laboratory of Genomics and Biodiversity (LANGEBIO; Irapuato, Mexico).

2.6. Phylogenetic analysis

The sequence files were analyzed using Chromas freeware (ver. 2.01; Chromas lite Technelysium Pvt. Ltd.; South Brisbane, Australia). Reference sequences were obtained from the GenBank database (National Center for Biotechnology Information), and the sequences obtained in the present study were deposited in GenBank (Accession numbers MW714641-14645). The alignment was constructed using Muscle. The phylogenetic relatedness was estimated using the maximum likelihood method. The evolutionary distances were computed using the Kimura 2-parameter method and five gamma categories to model among-site rate vari-

ation. The bootstrap consensus tree inferred from 1,000 replicates represents the evolutionary history of the sequences analyzed. The phylogenetic analysis was performed using MEGA software version 10.2.4 (Kumar et al., 2016).

2.7. Compatibility test of potentially antagonistic bacteria

Before any greenhouse assays were performed, compatibility tests were conducted with one isolate from each of the following five bacteria: *B. siamensis Bsi*DA2, *B. subtilis Bs*TA16, *B. amyloliquefaciens Ba*MA26, *Acinetobacter calcoaceticus Ac*DB3, and *B. thuringiensis Bt*MB9. Briefly, 24-hour-old bacterial cultures were confronted with each other by streaking two strains longitudinally and in parallel 0.5 cm apart from each other at the center of LBA plates. Plates were then incubated at 30 °C for 48 h and the results were interpreted based on the presence or absence of an inhibition zone (Santiago et al., 2017).

2.8. Plate assays for antagonistic and plant growth promotion traits

*Bsi*DA2, *Bs*TA16, *Ba*MA26, *Ac*DB3 and *Bt*MB9 were tested for antagonistic traits such as siderophore production, and chitinase, glucanase, protease and lipase enzymatic activities. Briefly, siderophore production was determined after 24–48 h of incubation at 30 °C by the Chrome Azurol-S (CAS) agar assay (Fig. S2A). Bacteria were considered positive for siderophore production based on a blue to orange color change in the medium (Schwyn and Neilands, 1978). Bacterial chitinase activity was inspected on colloidal chitin agar medium after 5 days of growth at 30 °C. Chitinase activity was identified by the formation of a clear zone around the bacteria (Wen et al., 2002). *In vitro* β-1, 4-endoglucanase activity was assayed using carboxy-methyl cellulose (CMC) agar medium after 24 h of growth at 30 °C (Fig. S2B). The formation of a clear zone around the wells, resulting from β-1,4-endoglucanase activity, was revealed by adding 5 mL of Congo red solution (1% w/v) for 15 min as previously described (Teather and Wood, 1982). Protease activity was performed using skim milk (SM) agar medium (Fig. S2C) at 30 °C for 24 h as previously reported (Jones et al., 2007). Protease activity was then determined by the formation of a clear zone around the bacterial colonies. The lipase activity was assayed on tributyrin agar (TBA) medium at 30 °C for 24 h. Lipase activity was considered positive when a clear zone around the bacterial colonies was observed (Castro-Ochoa et al., 2005).

To identify phosphate solubilizing bacteria, 3 μL of bacterial suspension were placed on Pikovskaya's medium and incubated at 30 °C for 24–48 h (Pikovskaya, 1948). Phosphate-solubilizing bacteria formed a clear zone around the bacterial colony after 24–48 h of incubation (Fig. S2D). To evaluate IAA production, the bacterial supernatants were assayed with Salkowsky reagent (Bric et al., 1991). After 20 min of incubation at 25 °C, IAA production was detected as a color change in the supernatant from clear to pink. IAA concentration was evaluated using a standard IAA curve (Fig. S2E).

Biofilm assays were screened *in vitro* as previously reported (Mandhi et al., 2010). Briefly, an overnight culture grown in 5 mL of LB broth at 30 °C for 24 h at 200 rpm was diluted 1:100 (v/v) in LB broth with 2% glucose. Bacterial suspensions (200 μL) were transferred to a 96-well microtiter plate (Beckman Coulter, Cat. No. 609844) and incubated at 37 °C for 24 h. The bacterial suspensions were removed and washed three times with 200 μL of saline phosphate buffer and dried at 25 °C. Next, 200 μL of 95% ethanol was added for 10 min at 25 °C followed by staining with 100 μL of crystal violet (0.5% w/v) for 10 min. Excess stain was rinsed three times with sterile distilled water (300 μL) and the plate was dried at 25 °C. Optical density (OD) of biofilms was measured and the values were interpreted as strong ($OD_{600} \geq 1$), moderate ($0.1 \leq OD_{600} < 1$) or slight ($OD_{600} < 0.1$). Every plate assay was performed in triplicate and repeated twice (Fig. S2F).

2.9. Germination tray bioassays

A mixture of sterile sand and vermiculite (1:2, v/v) was used as substrate. Before sterilization, the sand was washed with running water (8 to 10 times). The vermiculite was moistened, and subsequently any excess water was discarded from the sand and vermiculite, which were then mixed and autoclaved three times for 1 h at 121 °C and 15 psi (pounds per square inch), with one day in between each autoclaving procedure. The mixture was then left for at least 48 h before use. *Bsi*DA2, *Bs*TA16 and *Ba*MA26 were used as antagonists against *Fol* R3, whereas *Ac*DB3 and *Bt*MB9 were used against *Forl*. Surface-disinfested tomato seeds were dipped in the bacterial suspension for 1 h at 25 °C, and the seeds were dried at room temperature under laminar flow hoods. The dried seeds (~4×10^5 –1×10^6 CFU of bacteria per seed) were subsequently placed in the germination tray cavities. Each treatment consisted of ten plants. The germination trays were kept under greenhouse conditions at 25 ± 2 °C until emergence of the seedlings (>50% germination), at which point the temperature was shifted to 30 ± 2 °C for *Fol* R3 and 20 ± 2 °C for *Forl*, under a natural daylight photoperiod. Plants were watered as needed and fertilized once per week (NPK 17–17–17: 1 g L^{-1}, 2 mL per plant at the beginning and up to 5 mL in the last month of the experiment; Vigoro, Lot No. VGT17230616-1 and Nutricel: 3 g L^{-1}, 3 mL per plant, Cosmocel®, Lot No. 123630).

N6394 and SV4401TJ hybrid seeds emerged at day 7 and were re-inoculated twice with bacterial suspensions (5 mL 2 x10^8 UFC mL^{-1}) applied to the soil by pipetting close to the plant base, at 1 and 3 weeks post-emergence. Pai Pai hybrid seeds emerged by day 10 and plants were re-inoculated (2 mL 2×10^8 UFC mL^{-1}) once, 10 days post-emergence (at the two true leaves stage).

Fungal infection for hybrids N639 and SV4401TJ was performed by adding 1×10^6 conidia per plant, two weeks after seed emergence. For the Pai Pai hybrid, fungal conidia were added at the beginning of the experiment by mixing conidia with the substrate (3×10^5 conidia per plant) prior to sowing the inoculated seeds in the germination tray. The Pai Pai hybrid was added with conidia (2 mL of conidia suspension for 1×10^4 conidia per plant) a second time, two days after the second bacterial inoculation, in order to facilitate the appearance of symptoms.

Disease severity (%) was measured at different times depending on the particular tomato hybrid, using the scales reported by Fierro-Coronado et al. (2013) and Cordero Ramirez et al. (2013) for *Fol* R3 and *Forl* respectively, and was calculated with the formula (Asran and Buchenauer, 2003):

$$Disease severity(\%) = \frac{[\sum (R \times N)] \times 100}{H \times T}$$

where R = disease rating, N = number of plants with this rating, H = highest rating category (i.e. 5), and T = total number of plants counted.

2.10. Pot bioassays

2.10.1. Non-sterile substrate pot assays

N6394 and SV4401TJ hybrid seeds (not surface-disinfested) were placed in a non-sterile sand-vermiculite mix (1:2 v/v) in germination trays. Plant growth, watering and temperature conditions were the same as those reported for the germination tray assays. Seeds germinated after 7 days (>50%). Forty days after seed emergence, plants were inoculated with bacterial suspensions by adding the suspension to the substrate with a pipette near the base of the plant (5 mL per plant, 1×10^8 UFC mL^{-1}). One week after

bacterial inoculation, plants were transferred to a pot (1-kg capacity) using 450 g final weight of a non-sterile soil-vermiculite mix (1:2 v/v) containing fungal conidia (4.24×10^4 and 8.13×10^4 per g of substrate for *Fol* R3 and *Forl*, respectively). Plants were damaged by cutting root tips at the bottom part of the root system and by inflicting a puncture with a 1-mL insulin syringe at the stem base. Potted plants were grown as previously indicated, watered every 2 days and fertilized once per week. A second bacterial re-inoculation using the same procedure and bacteria concentration was performed, either 3 days after fungal inoculation for *Fol* R3 or 2 days after for *Forl*. The assay was then evaluated at different times after disease was apparent, from 59 to 89 days post seed emergence (21 to 43 days after infection), depending on the hybrid and pathogen employed. A disease severity scale was used to evaluate disease in plants for *Fol* R3 (based on shoot damage) (Akkopru and Demir, 2005), in which 0 = 0%, 1 = 1–25%; 2 = 26–50%; 3 = 51–75% and 4 = 76–100% of shoot damage. Another disease severity scale was used for *Forl* (based on root damage) (Quilambaqui-Jara et al., 2004), with 1 = no root, crown or stem (RCS) lesions; 2 = less than five root lesions and absence of darkening in RCS; 3 = 5–10 root lesions and slight darkening of RCS; 4 = >10 lesions with darkening in RCS; 5 = complete root rot or plant death. Disease severity (%) was calculated by using the formula described in the previous section (Asran and Buchenauer, 2003). The treatments (ten replicate plants per treatment) were arranged in a completely randomized design.

2.10.2. Sterile substrate pot assays

The Pai Pai tomato hybrid seeds were surface-disinfested as described in Section 2.4. *In vitro* antagonism in seedling assays. Next, surface-disinfested seeds were placed in germination trays in a sterile sand-vermiculite mix (1:2 v/v). Plant growth, watering and temperature conditions were the same as those reported for the germination tray assays. Seeds germinated after 10 days (>50%). Thirty-eight days after seed emergence, plants were transplanted to 3-kg volume pots containing 2.4 kg of a sterile soil:vermiculite mix (1:2 v/v) with fungal conidia (1×10^5 per g of substrate). The sterilization procedure of the substrate was as described in Section 2.9. Germination tray bioassays. The dry soil was mixed with moist vermiculite and then sterilized as described before. At the time of transfer, plants were inoculated with bacterial suspensions by adding the suspension to the substrate with a pipette near the base of the plant (5 mL per plant, 1×10^8 UFC mL^{-1}). A second bacterial inoculation using the same concentration and procedure was performed two days after transfer of plants to pots. Potted plants were grown as previously indicated, watered every 2 days and fertilized once per week. The assay was then evaluated at both 59 and 72 days post seed emergence (21 and 32 days after inoculation) for *Fol* R3 and *Forl*, respectively. The treatments (ten replicate plants per treatment) were arranged in a completely randomized design. Disease severity was performed as described above for the non-sterile substrate pot assays.

2.11. Statistical analysis

Data regarding growth and disease severity were subjected to the Shapiro-Wilk test (Statistix software, version 8.1) in order to check whether or not the data were normally distributed. In the event of any homogeneous variance, the data were subjected to statistical analysis of variance (ANOVA) based on a linear model of fixed effects to detect differences among treatments, as well as an LSD test ($p = 0.05$) for mean comparisons. The disease severity percentage was arcsine-transformed and then analyzed using one-way ANOVA (Asran and Buchenauer, 2003; Cordero-Ramírez et al., 2013).

3. Results

3.1. In vitro selection of antagonistic rhizospheric bacteria against Fol R3 and Forl

A high-throughput liquid antagonism screen assay identified 424 strains capable of inhibiting *Fol* R3 growth as well as 154 strains inhibiting *Forl* growth by >50% out of a collection of 1,875 viable bacteria. Forty-two bacterial strains showing $\geq$ 21% growth inhibition were selected after performing a dual confrontation antagonism assay in 96-well microtiter plates, comprising 28 out of 424 strains against *Fol* R3 and 14 out of 154 strains against *Forl*. Dual confrontation tests in 10-cm Petri plates were performed to reconfirm their antagonistic effects (Table 1; Fig. S1). None of the bacteria displayed antagonism against both pathogens.

Thirty-one bacterial isolates that inhibited fungal growth by > 20% in both 96 well-plates and 10-cm Petri plates were selected for a hemolysis test (Table 1). Bacteria producing β-hemolysin were discarded to avoid possible human pathogenicity.

Seedling antagonistic assays in Petri plates were conducted on seventeen bacterial isolates, showing partial (α-) or no (γ-) hemolysis. Our results demonstrate that the γ-hemolytic *Bsi*DA2, *Bs*TA16 and α-hemolytic *Ba*MA26 strains displayed the highest antagonistic activity against *Fol* R3 (Table 1A), and the α-hemolytic *Ac*DB3 and *Bt*MB9 strains showed the highest antagonistic activity against *Forl* (Table 1B). These five bacteria were selected for greenhouse antagonism bioassays as well as characterization of their plant growth promotion and antagonistic traits. In order to use consortia of these five selected bacteria, we conducted confrontation assays in Petri plates, showing that all bacteria were compatible with each other (data not shown).

3.2. Molecular identification of potentially antagonistic bacteria

Five bacteria displaying the highest antagonistic effect were identified by phylogenetic analysis of the 16S rDNA gene. The phylogenetic tree in Fig. 1 demonstrates that one bacteria belongs to the genus *Acinetobacter* (*Ac*DB3) and groups close to the species *A. calcoaceticus*, while the other four bacteria group with members of the genus *Bacillus*. 16S rDNA gene sequences of *Bsi*DA2, *Bs*TA16, *Ba*MA26 and *Bt*MB9 group with isolates belonging to *B. siamensis*, *B. subtilis*, *B. amyloliquefaciens* and *B. thuringiensis*, respectively.

3.3. In vitro assays for antagonistic and plant growth promotion traits

The five bacterial strains selected from the previous screening steps were all positive for siderophore production, and negative for chitinase and lipase activity (Table 2). Only *Ba*MA26 was positive for glucanase and protease activities. All bacteria displayed a moderate biofilm capability, as defined by Mandhi et al. (2010). Finally, *Bsi*DA2 and *Bt*MB9 were able to solubilize phosphate, while only *Ac*DB3 and *Bsi*DA2 produced IAA (Table 2; Fig. S2).

3.4. Greenhouse bioassays against Fol R3

3.4.1. Bioassays in germination trays

Hybrid N6394 did not show any effect on disease control in response to any bacterial treatment (Table 3). *Bsi*DA2 and *Ba*MA26 decreased *Fol* R3 disease severity in the indeterminate tomato hybrids SV4401TJ and Pai Pai, respectively (Fig. S3). The combination *Fol* R3 + *Bsi*DA2 + *Bs*TA16 + *Ba*MA26 decreased *Fol* R3 disease severity in Pai Pai. Isolates *Bsi*DA2 and *Bs*TA16 were not effective against *Fol* R3 in Pai Pai, either individually or in any of their double combinations with other strains (Table 3).

Table 1
In vitro selection of potential bacterial antagonists against *Fol* R3 and *Forl*.

Isolate ID[a]/Pathogen	% of growth inhibition in 96-well titer plate assay[e]	% of growth inhibition in dual confrontation plate assays[e]	% of disease severity in seedling bioassay[f, g]	Hemolysis type
A) ***Fol* R3 antagonists**				
DA1	56.08 ± 4.06	43.24 ± 2.40	ND	β
***Bsi*DA2**	**32.62 ±** 12.18	**47.39** ± 4.79	**26.19** ± 14.80 **ef**	ɣ
*Bc*DA3	53.63 ± 22.35	34.93 ± 2.40	40.83 ± 10.00 bcdef	ɣ
*Bs*DA4	50.85 ± 17.30	32.17 ± 2.40	37.62 ± 14.40 bcdef	α
*B*TA5	57.94 ± 22.36	46.01 ± 0.00	51.90 ± 12.20 ab	ɣ
TA6	49.87 ± 5.20	5.86 ± 2.40	ND	ND
*Bs*TA7	49.93 ± 6.76	21.09 ± 8.31	61.17 ± 6.80 a	α
TA8	56.69 ± 6.67	15.56 ± 6.34	ND	ND
TA9	51.57 ± 1.70	18.32 ± 9.59	ND	ND
*Bs*TA10	61.40 ± 6.39	21.09 ± 10.99	30.83 ± 19.42 def	α
TA11	62.53 ± 5.58	14.17 ± 13.35	ND	ND
TA12	48.93 ± 12.78	5.86 ± 2.40	ND	ND
TA13	72.05 ± 4.93	47.39 ± 2.39	ND	β
TA14	67.47 ± 7.78	48.78 ± 2.39	ND	β
TA15	60.69 ± 2.18	41.86 ± 0.00	ND	β
***Bs*TA16**	**21.66** ± 4.10	**44.63** ± 6.34	**29.52** ± 9.10 **ef**	ɣ
*B*TA17	52.01 ± 17.52	51.55 ± 2.40	49.17 ± 17.56 abcd	ɣ
TA18	70.16 ± 4.27	44.62 ± 2.40	ND	β
TA19	57.35 ± 9.59	33.55 ± 4.15	ND	β
TA20	35.84 ± 6.21	7.25 ± 2.40	ND	ND
*B*TA21	61.64 ± 1.14	51.55 ± 2.40	31.67 ± 7.60 cdef	ɣ
TA22	61.23 ± 4.68	41.86 ± 4.15	ND	β
*B*TA23	70.65 ± 2.37	41.86 ± 4.15	50.12 ± 8.80 abc	α
MA24	32.66 ± 14.42	48.09 ± 2.08	ND	β
MA25	34.46 ± 7.53	48.09 ± 2.08	ND	β
***Ba*MA26**	**39.40** ± 5.12	**39.09** ± 4.79	**22.5** ± **6.60f**	**α**
MA27	31.13 ± 7.43	43.24 ± 2.39	ND	β
MA28	42.93 ± 10.10	47.39 ± 2.40	ND	β
Fol R3 CTL[c]	–––	–––	42.88 ± 10.67 abcd	–––
B) ***Forl* antagonists**				
DB1	78.3 ± 0.80	40.82 ± 2.07	ND	β
DB2	77.4 ± 4.20	39.56 ± 0.00	ND	β
***Ac*DB3**	**68.4** ± 10.00	**27.39** ± 4.57	**32.5** ± 13.92 **de**	**α**
MB4	69.5 ± 4.30	17.49 ± 3.18	ND	ND
MB5	57.8 ± 0.00	6.27 ± 16.49	ND	ND
*Pc*MB6	68.3 ± 0.00	26.07 ± 2.29	80 ± 0.00 a	ɣ
MB7	72.6 ± 5.90	40.81 ± 2.04	ND	ND
MB8	63.7 ± 3.30	0.33 ± 2.39	ND	ND
***Bt*MB9**	ND[b]	**21.08** ± 2.36	**18.67** ± 16.17 **ef**	**α**
MB10	ND	6.12 ± 4.08	ND	ND
MB11	ND	8.84 ± 4.71	ND	ND
*Pp*MB12	ND	30.61 ± 2.04	50 ± 6.61 cd	ɣ
*Bc*MB13	ND	25.17 ± 2.36	50.33 ± 11.38 cd	ɣ
*B*MB14	ND	40.82 ± 2.04	76.33 ± 19.76 ab	ɣ
Forl CTL[d]	–––	–––	57.78 ± 13.47 bc	–––

[a]Isolate identification (ID): B = Bacillus sp.; Ba = B. amyloliquefaciens; Bsi = B. siamensis; Bs = B. subtilis; Bt = B. thuringiensis; Bc = B. cereus; Pp = Paenibacillus polymyxa; Ac = Acinetobacter calcoaceticus; and Pc = Pseudomonas corrugata. The bacterial isolates are ordered numerically. The letters D, M, and T refer to the rhizospheric origin of the isolate: D = Datura, M = maize, and T = tomato. The digits refer to the consecutive isolate number assigned in this work. [b]ND: Not determined. [c]Fol R3 and [d]Forl CTL indicate the pathogenicity controls used for the seedling antagonistic bioassay. [e]The bacteria were initially selected based on the Fol R3 and Forl growth inhibition percentage only. Values in bold are for the five bacterial isolates that were selected for further characterization based on a significant decrease in disease severity during the seedling bioassay. [f]Disease severity was measured after 9 days of inoculating seeds from tomato hybrid N6394. [g]Statistically significant differences ($p \leq 0.05$) are indicated by mean values which do not share lower case letters. Data are presented as mean values ± standard errors (SE).

No plant growth promotion was observed in germination tray assays in the N6394 and SV4401TJ hybrids. The *Fol* R3 + *Ba*MA26 (Fig. S3) and *Fol* R3 + *Bs*TA16 + *Ba*MA26 treatments increased all growth parameters in the Pai Pai hybrid (Table 3). The combination of *Fol* R3 + *Bsi*DA2 + *Bs*TA16 + *Ba*MA26 effectively decreased disease severity, but only promoted slightly growth rate. The combination *Fol* R3 + *Bs*TA16 + *Ba*MA26 promoted all growth parameters, but did not reduce disease severity (Table 3).

3.4.2. Bioassays in pots

In pot experiments, *Bsi*DA2 and *Bs*TA16 significantly decreased the disease severity caused by *Fol* R3 in the determinate N6394 and indeterminate SV4401T tomato hybrids under non-sterile substrate conditions. *Ba*MA26 was the only bacterium tested against all three tomato hybrids, and was only effective in the indeterminate hybrid SV4401TJ (Table 4).

In similar experiments, *Bs*TA16 and *Ba*MA26 increased all biomass parameters, while *Bsi*DA2 only increased shoot and total dry biomass in the tomato hybrid N6394 as compared to the *Fol* R3-treated plants. All three bacterial strains promoted growth rate, shoot dry weight, and total biomass in the tomato hybrid SV4401TJ with respect to the *Fol* R3-treated plants. *Ba*MA26 increased growth rate with respect to the control and the *Fol* R3 treatment in the Pai Pai tomato hybrid under sterile conditions (Table 4; Fig. S4).

3.5. Greenhouse bioassays against Forl

3.5.1. Bioassays in germination trays

The combination of *Ac*DB3 or *Bt*MB9 with *Forl* did not decrease disease severity in the determinate tomato hybrid N6394 (Table 5). *Bt*MB9 significantly reduced *Forl* disease severity in both indeter-

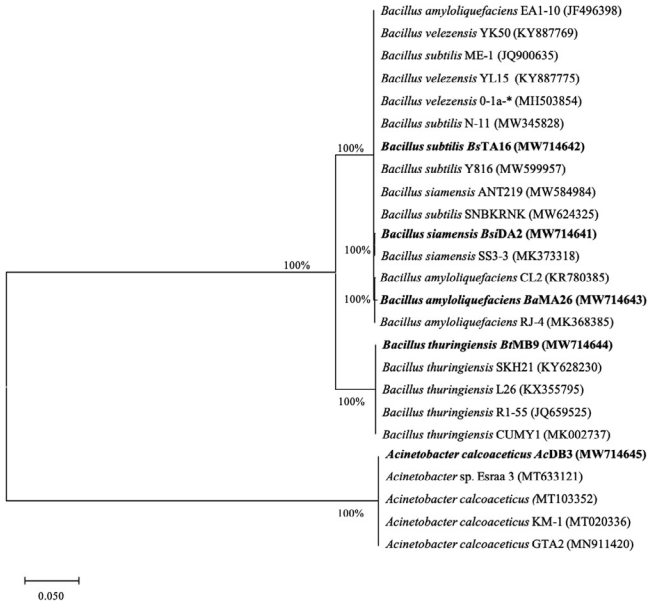

Fig 1. Maximum likelihood tree showing the position of five antagonistic bacteria among the related taxa based on 16S rRNA gene sequences. The tree was constructed with MEGA 10.2.4 using the Kimura 2-parameter model and five gamma categories to model among-site rate variation. The numbers at branch points were the significant bootstrap values (expressed as percentages based on 1000 replicates). The scale bar indicates the number of nucleotide substitutions per site. Accession numbers of the strains are presented in parentheses. Bold accessions indicate the strains identified in the present study.

minate tomato hybrids SV4401TJ and Pai Pai (Fig. S5). Furthermore, *Ac*DB3 was effective in the SV4401TJ hybrid but not in Pai Pai, except when in combination with *Bt*MB9 (Table 5).

*Ac*DB3 increased all growth and biomass parameters (dry weight of shoots and roots, and total biomass) in hybrid N6394 seedlings inoculated with *Forl*, while *Bt*MB9 only improved growth rate but not the biomass parameters (Table 5). *Ac*DB3 also increased all growth and biomass parameters in the SV4401TJ tomato hybrid, whereas it only increased shoot and total biomass in Pai Pai in comparison to *Forl* (Fig S5). An increase in biomass parameters was observed in the Pai Pai hybrid when *Bt*MB9 was applied to seedlings inoculated with *Forl* (Fig. S5). Interestingly, the combination of *Ac*DB3 + *Bt*MB9 in Pai Pai increased all biomass parameters in comparison to the water control in the absence of *Forl* (Table 5).

3.5.2. Bioassays in pots

Our pot bioassays show that *Ac*DB3 reduced the disease severity caused by *Forl* in all three varieties in comparison to *Forl*-treated plants (Table 6), irrespective of substrate sterility. *Bt*MB9 decreased *Forl* disease severity in the determinate N6394 under non-sterile substrate conditions as well as in indeterminate Pai Pai (Fig. S6) under sterile substrate conditions. In the Pai Pai hybrid, the combination of both bacterial strains decreased disease severity, similar to the effect of *Ac*DB3 alone (Table 6).

Both *Ac*DB3 and *Bt*MB9 promoted significantly different growth parameters, depending on the tomato hybrid (for Pai Pai see Fig. S6). Strikingly, using *Bt*MB9 to control *Forl* disease in Pai Pai hybrid pot assays increased all growth parameters as compared to *Forl*-inoculated plants (Table 6; Fig. S6).

4. Discussion

In this study, we explored the biological control of *Fol* R3 and *Forl* in tomato using rhizospheric bacteria associated with different plant species in different soils in Sinaloa, Mexico (López-Rivera, 2011; Cordero-Ramírez et al., 2013; Figueroa-López et al., 2016). Although *in vitro* Petri dish antagonistic assays are fast, inexpensive, and allow continuing the selection process at the plant level, it was previously shown that introducing the plant host will not necessarily mirror the results obtained when only the bacteria and the fungus are included (Kamilova et al., 2007). Our tomato seedling antagonism bioassays confirm previous reports indicating that the bacterial isolates respond differentially as antagonists to fungi when analyzed either *in vitro* or *in planta*. The tomato seedling antagonistic bioassay was the final *in vitro* selection step, which allowed us to select three antagonists against *Fol* R3 (*Bsi*DA2, *Bs*TA16 and *Ba*MA26) and two against *Forl* (*Ac*DB3 and *Bt*MB9) in tomato. Cordero-Ramírez et al. (2013) similarly reported isolates exhibiting *Forl* antagonism in tomato seedling germination assays. Although they used the same bacterial collection as in our study, their screening strategy started with dual confrontation assays in Petri dishes. In contrast, our screening strategy in the present work started with a liquid antagonistic assay. The antagonism mechanisms in a dual confrontation assay involve antibiosis or nutrient competition, while in the liquid assay other mechanisms involving direct contact assays such as the attachment of bacteria to the fungus (biofilm formation) and fungal cell wall degradation enable the identification of different types of antagonists. This suggests that different strategies for screening potential antagonistic bacteria *in vitro* may result in the identification of different isolates from the same bacterial collection, as previously reported by Stefani et al. (2015).

We were unable to find any bacterial isolate that could control both the *Fol* R3 and *Forl* pathogens (Table 1), thus rejecting our first hypothesis. To date, no resistance to *Forl* has been described in tomato (Ates et al., 2019), and even though the Frl resistance gene that confers partial resistance to *Forl* was identified in 2001 (Staniaszek et al., 2014), the nature of this gene is still unknown. It is possible that the antagonistic mechanisms required to inhibit growth of both pathogens could be very different, which would explain why we could not find a common isolate able to control both fungal tomato pathogens.

Table 2
Screening of activities associated with plant growth promotion or biocontrol in the selected rhizospheric bacteria.

Isolate ID[a]	Bacteria	Siderophore[b]	Chitinase	β-*endo*-1, 4-glucanase	Protease	Lipase	Pi IS[c]	IAA μg mL^{-1}	Biofilm
*Bsi*DA2	*Bacillus siamensis*	+	–	–	–	–	1.22	0.04	M[d]
*Bs*TA16	*B. subtilis*	+++	–	–	–	–	–	–	M
*Ba*MA26	*B. amyloliquefaciens*	+++	–	+	+	–	–	–	M
*Ac*DB3	*Acinetobacter calcoaceticus*	++	–	–	–	–	–	1.25	M
*Bt*MB9	*B. thuringiensis*	+++	–	–	–	–	1.53	–	M

[a]ID: identification. All enzymatic tests were recorded as either positive (+) or negative (-). For glucanase and protease activities, (+) indicates positive results based on the presence of clear halos surrounding the bacterial colonies. [b]For siderophores, (+) = <2 mm, (++) = 2.01 to 4 mm, and (+++) = >4 mm halos surrounding the bacterial colonies. [c]Pi IS: index of phosphate solubilization. [d]M indicates a moderate capability of biofilm formation ($0.1 \leq OD_{600} < 1$).

Table 3
Antagonistic and plant growth promotion effects of three rhizospheric bacteria assayed against *Fol* R3 in three different tomato hybrids grown in germination trays.

Treatment	Antagonism	Growth promotion effects			
	Percentage of disease severity	Growth rate (cm day^{-1})	DW[b] of roots (mg)	DW of shoots (mg)	Total DW biomass (mg)
***Hybrid N6394 (determinate/48 days)**					
CTLa	0.00 ± 0.00 **c	0.20 ± 0.004 a	85.20 ± 2.47 ab	257.92 ± 7.24 ab	343.12 ± 7.41 a
Fol R3	16.67 ± 5.2 ab	0.20 ± 0.008 a	94.83 ± 3.66 a	238.10 ± 3.79 ab	332.93 ± 6.22 ab
Fol R3 + *Bsi*DA2	20.83 ± 4.16 ab	0.19 ± 0.009 a	88.78 ± 4.32 ab	264.47 ± 15.16 a	353.25 ± 11.34 a
Fol R3 + *Bs*TA16	29.17 ± 4.16 a	0.20 ± 0.003 a	82.23 ± 4.97b	228.20 ± 10.26b	310.43 ± 9.09b
Fol R3 + *Ba*MA26	12.5 ± 5.59 bc	0.19 ± 0.005 a	95.17 ± 4.20 a	255.85 ± 11.66 ab	351.02 ± 12.15 a
Hybrid SV4401TJ (indeterminate/43 days)					
CTL	20.83 ± 4.16c	0.28 ± 0.016 a	84.22 ± 3.43 a	191.05 ± 9.92 ab	275.27 ± 12.36 a
Fol R3	50.00 ± 0.00 a	0.28 ± 0.016 a	75.95 ± 1.91b	183.53 ± 5.26b	259.48 ± 6.69 a
Fol R3 + *Bsi*DA2	***33.00 ± 5.27**b**	0.24 ± 0.013b	62.20 ± 1.26c	213.92 ± 12.92 a	276.12 ± 11.86 a
Fol R3 + *Bs*TA16	41.67 ± 5.27 ab	0.31 ± 0.010 a	63.50 ± 1.81c	205.88 ± 9.92 ab	269.38 ± 11.28 a
Fol R3 + *Ba*MA26	50.00 ± 0.00a	0.29 ± 0.013 a	61.40 ± 4.03c	208.93 ± 10.35 ab	270.33 ± 8.01 a
Hybrid Pai Pai (indeterminate/40 days)					
CTL	0.00 ± 0.00 d	0.09 ± 0.002 bc	18.00 ± 1.03 bc	67.25 ± 0.75 bc	85.25 ± 2.04 bc
*Bsi*DA2	0.00 ± 0.00 d	0.10 ± 0.003 ab	**28.10** ± 4.46 **a**	**105.93** ± 2.49 **a**	**134.02** ± 4.88 **a**
*Bs*TA16	0.00 ± 0.00 d	**0.12** ± 0.007 **a**	22.75 ± 1.49 ab	98.25 ± 5.21 ab	121.00 ± 6.98 ab
*Ba*MA26	0.00 ± 0.00 d	**0.12** ± 0.005 **a**	24.75 ± 2.28 ab	**101.25** ± 0.47 **a**	**126.00** ± 2.39 **a**
Fol R3	81.25 ± 18.75 a	0.02 ± 0.004 ef	3.42 ± 0.91f	10.73 ± 2.46 e	14.15 ± 2.72f
Fol R3 + *Bsi*DA2	75.00 ± 25 a	0.04 ± 0.008 de	7.20 ± 2.23 ef	25.85 ± 6.40 de	33.05 ± 9.20 ef
Fol R3 + *Bs*TA16	50.00 ± 28.86 abc	0.03 ± 0.011 ef	6.32 ± 1.48 ef	29.33 ± 9.28 de	35.65 ± 10.75 ef
Fol R3 + *Ba*MA26	**12.50** ± 12.5 **cd**	**0.06** ± 0.007 **cd**	**11.50** ± 2.16 **cde**	**43.10** ± 7.82 **cd**	**54.60** ± 9.73 **cde**
Fol R3 + *Bsi*DA2 + *Bs*TA16	87.50 ± 7.21 a	0.04 ± 0.011 de	**14.62** ± 0.46 **cd**	36.95 ± 5.85 cde	**51.58** ± 6.20 **cde**
Fol R3 + *Bsi*DA2 + *Ba*MA26	62.50 ± 23.93 ab	0.04 ± 0.009 de	9.20 ± 2.10 def	**49.18** ± 4.54 **cd**	**58.38** ± 6.29 **cde**
Fol R3 + *Bs*TA16 + *Ba*MA26	43.75 ± 18.75 abcd	**0.07** ± 0.009**c**	**12.32** ± 3.67 **cde**	**61.05** ± 7.25.38**c**	**73.38** ± 11.25 **cd**
Fol R3 + *Bsi*DA2 + *Bs*TA16 + *Ba*MA26	**25.00** ± 7.21 **bcd**	**0.06** ± 0.006 **cd**	9.90 ± 1.76 def	39.00 ± 8.02 cde	48.90 ± 8.84 def

[a]CTL: water control; [b]DW: dry weight; *Bsi*DA2: *B. siamensis*; *Bs*TA16: *B. subtilis*; *Ba*MA26: *B. amyloliquefaciens.*
*Statistical treatment of the data was performed with each hybrid separately. Hybrid growth habit type and plant age (in days) when the experiment was evaluated are provided in parentheses; >50% seed germination was reached at day 7 after sowing for N6394 and SV4401TJ and at day 10 for Pai Pai. **Statistically significant differences ($p \leq 0.05$) are indicated by mean values which do not share lower case letters. ***Values in bold indicate a significant increase relative to the respective controls (CTL or *Fol* R3 = fungus without any antagonist). Data are presented as mean values ± standard errors (SE).

Table 4
Antagonistic and plant growth promotion effects of three rhizospheric bacteria assayed against *Fol* R3 in different tomato hybrids grown in pots under non-sterile (N6394 and SV4401TJ) and sterile (Pai Pai) conditions.

Treatment	Antagonism	Growth promotion effects			
	Percentage of disease severity	Growth rate (cm day^{-1})	DW[b] of roots (g)	DW of shoots (g)	Total DW biomass (g)
***Hybrid N6394 (determinate/75 days)**					
CTL[a]	0.00 ± 0.00 **c	1.29 ± 0.04b	0.64 ± 0.01 ab	2.70 ± 0.06 bc	3.34 ± 0.12b
Fol R3	40.00 ± 0.00 a	1.37 ± 0.05 ab	0.50 ± 0.03c	2.51 ± 0.08c	3.01 ± 0.11c
Fol R3 + *Bsi*DA2	***20.00 ± 0.00**b**	1.43 ± 0.03 ab	0.57 ± 0.02 bc	**2.84** ± 0.08**b**	**3.40** ± 0.10**b**
Fol R3 + *Bs*TA16	**6.67** ± 6.66**c**	1.51 ± 0.08 a	**0.74** ± 0.07 **a**	**3.15** ± 0.13 **a**	**3.89** ± 0.08 **a**
Fol R3 + *Ba*MA26	33.33 ± 6.66 a	1.41 ± 0.04ab	**0.65** ± 0.04 **ab**	**2.86** ± 0.08**b**	**3.50** ± 0.09**b**
Hybrid SV4401TJ (indeterminate/89 days)					
CTL	4.00 ± 2.26b	1.26 ± 0.01 a	1.15 ± 0.08 a	5.27 ± 0.06 a	6.42 ± 0.06 a
Fol R3	24.00 ± 2.66 a	1.16 ± 0.02b	0.98 ± 0.04 a	4.51 ± 0.07c	5.49 ± 0.06c
Fol R3 + *Bsi*DA2	**8.00** ± 3.26**b**	**1.31** ± 0.03 **a**	0.93 ± 0.02 a	**4.93** ± 0.08**b**	**5.86** ± 0.10**b**
Fol R3 + *Bs*TA16	**10.00** ± 3.33**b**	**1.27** ± 0.02 **a**	1.00 ± 0.05 a	**4.87** ± 0.07**b**	**5.88** ± 0.10**b**
Fol R3 + *Ba*MA26	**8.00** ± 3.20**b**	**1.25** ± 0.03 **a**	1.15 ± 0.05 a	**5.20** ± 0.06 **a**	**6.36** ± 0.10 **a**
Hybrid Pai Pai (indeterminate/59 days)					
CTL	0.00 ± 0.00b	1.27 ± 0.02b	0.63 ± 0.27 a	2.68 ± 0.26 a	3.30 ± 0.29 a
*Ba*MA26	0.00 ± 0.00b	**1.67** ± 0.05 **a**	0.58 ± 0.64 a	3.10 ± 0.13 a	3.68 ± 0.16 a
Fol R3	65.00 ± 6.12 a	0.97 ± 0.04c	0.33 ± 0.28b	1.64 ± 0.16b	1.97 ± 0.19b
Fol R3 + *Ba*MA26	45.00 ± 14.57 a	**1.31** ± 0.09**b**	0.31 ± 0.75b	1.31 ± 0.24b	1.62 ± 0.32b

[a]CTL: water control; bDW: dry weight; BsiDA2: B. siamensis; BsTA16: B. *subtilis*; *Ba*MA26: *B. amyloliquefaciens.*
*Statistical treatment of the data was performed with each hybrid separately. Hybrid growth habit type and plant age (in days) when the experiment was evaluated are provided in parentheses; >50% seed germination was reached at day 7 after sowing for N6394 and SV4401TJ and at day 10 for Pai Pai. **Statistically significant differences ($p \leq 0.05$) are indicated by mean values which do not share lower case letters. ***Values in bold indicate a significant increase relative to the respective controls (CTL or *Fol* R3 = fungus without any antagonist). Data are presented as mean values ± standard errors (SE).

The five bacterial isolates exhibited a differential set of potential biocontrol mechanisms (Table 2). All five bacteria in this study produced siderophores. Bacteria that produce siderophores can rescue iron by converting inorganic Fe^{3+} to organic Fe^{2+} forms in the rhizosphere, thus making it unavailable for pathogens and available for plant uptake (Venkat et al., 2017). Previous reports have also identified siderophore production as a biocontrol mechanism against fungal pathogens in other plant species (El-Sayed et al., 2014; Vinayarani and Prakash, 2018). *Bacillus subtilis*, *B. siamensis* and *B. amyloliquefaciens* are able to produce siderophores, which may help to decrease *Fusarium* wilt caused by *Fol* as reported with other isolates of the *B. subtilis* group (Kumari et al., 2021). The bacteria in this study did not show any chitin degradation properties. However, *Ba*MA26 exhibited protease and β-1,4-glucan degrada-

Table 5
Antagonistic and plant growth promotion effects of three rhizospheric bacteria assayed against *Forl* in three different tomato hybrids grown in germination trays.

Treatment	Antagonism	Growth promotion effects			
	Percentage of disease severity	Growth rate (cm day^{-1})	DW[b] of roots (mg)	DW of shoots (mg)	Total DW biomass (mg)
***Hybrid N6394 (determinate/42 days)**					
CTL[a]	0.00 ± 0.00 **b	0.10 ± 0.005 a	44.22 ± 1.87 ab	126.03 ± 7.44 a	170.25 ± 9.14 a
Forl	37.00 ± 5.59 a	0.06 ± 0.004b	28.96 ± 1.99c	66.57 ± 4.62c	95.53 ± 4.15b
Forl + *Ac*DB3	50.00 ± 6.45 a	**0.10***** ± 0.008 **a**	**45.80** ± 5.07 **ab**	**102.74** ± 7.05**b**	**148.54** ± 11.68 **a**
Forl + *Bt*MB9	50.00 ± 9.12 a	**0.11** ± 0.005 **a**	36.40 ± 2.57 bc	78.15 ± 6.69c	114.55 ± 8.05b
Hybrid SV4401TJ (indeterminate/34 days)					
CTL	0.00 ± 0.00c	0.16 ± 0.008b	42.65 ± 5.04 a	118.78 ± 7.92 a	161.43 ± 10.96 a
Forl	67.00 ± 5.27 a	0.16 ± 0.006b	23.06 ± 1.20c	55.28 ± 8.07b	78.35 ± 8.27c
Forl + *Ac*DB3	**29.00** ± 4.16**b**	**0.19** ± 0.006 **a**	**32.73** ± 2.26**b**	**100.38** ± 6.16 **a**	**133.12** ± 4.69**b**
Forl + *Bt*MB9	**42.00** ± 5.27**b**	0.17 ± 0.005b	**31.88** ± 2.33**b**	76.08 ± 8.89b	**107.97** ± 7.99**b**
Hybrid Pai Pai (indeterminate/50 days)					
CTL	0.00 ± 0.00b	0.12 ± 0.007 a	27.44 ± 1.12 bc	112.70 ± 4.37b	140.14 ± 6.13b
*Ac*DB3	0.00 ± 0.00b	0.10 ± 0.004 ab	33.18 ± 2.41 ab	119.60 ± 7.94b	152.78 ± 10.95b
*Bt*MB9	0.00 ± 0.00b	0.11 ± 0.004 ab	27.56 ± 2.45 bc	119.56 ± 5.15b	147.12 ± 6.73b
*Ac*DB3 + *Bt*MB9	0.00 ± 0.00b	0.10 ± 0.002 ab	**40.26** ± 0.89 **a**	**143.02** ± 7.74 **a**	**183.28** ± 9.87 **a**
Forl	50.00 ± 11.18 a	0.11 ± 0.005 ab	23.06 ± 3.66c	73.62 ± 9.49c	96.68 ± 10.97c
Forl + *Ac*DB3	60.00 ± 16.95 a	0.10 ± 0.007 ab	23.76 ± 3.84c	**116.30** ± 4.50**b**	**140.06** ± 9.47**b**
Forl + *Bt*MB9	**20.00** ± 12.24**b**	0.11 ± 0.004 ab	**35.38** ± 1.38 **a**	**116.48** ± 5.09**b**	**151.86** ± 5.91**b**
Forl + *Ac*DB3 + *Bt*MB9	**10.00** ± 6.12**b**	0.10 ± 0.010 ab	26.24 ± 3.80 bc	**105.06** ± 8.42**b**	**131.30** ± 16.31**b**

[a]CTL: water control; [b]DW: dry weight; *Ac*DB3: *Acinetobacter calcoaceticus*; *Bt*MB9: *B. thuringiensis*.
*Statistical treatment of the data was performed with each hybrid separately. Hybrid growth habit type and plant age (in days) when the experiment was evaluated are provided in parentheses; >50% seed germination was reached at day 7 after sowing for N6394 and SV4401TJ and at day 10 for Pai Pai. **Statistically significant differences ($p \leq 0.05$) are indicated by mean values which do not share lower case letters. ***Values in bold indicate a significant increase relative to the respective controls (CTL or *Forl* = fungus without any antagonist). Data are presented as mean values ± standard errors (SE).

Table 6
Antagonistic and plant growth promotion effects of three rhizospheric bacteria assayed against *Forl* in three different tomato hybrids grown in pots under non-sterile (N6394 and SV4401TJ) and sterile (Pai Pai) conditions.

Treatment	Antagonism	Growth promotion effects			
	Percentage of disease severity	Growth rate (cm day^{-1})	DW[b] of roots (g)	DW of shoots (g)	Total DW biomass (g)
***Hybrid N6394 (determinate/75 days)**					
CTL	12.00 ± 3.27 **c	1.09 ± 0.04 a	1.59 ± 0.12 a	4.34 ± 0.07c	5.93 ± 0.12b
Forl	54.00 ± 3.06 a	0.97 ± 0.03b	0.83 ± 0.04c	4.34 ± 0.14c	5.18 ± 0.15c
Forl + *Ac*DB3	***40.00** ± 2.98**b**	**1.07** ± 0.02 **a**	**1.15** ± 0.06**b**	**5.27** ± 0.09**b**	**6.41** ± 0.11 **a**
Forl + *Bt*MB9	**36.00** ± 4.00**b**	0.95 ± 0.01b	**1.14** ± 0.02**b**	**5.58** ± 0.08 **a**	**6.72** ± 0.13 **a**
Hybrid SV4401TJ (indeterminate/89 days)					
CTL	6.00 ± 3.05 d	0.95 ± 0.02 a	1.61 ± 0.07 a	5.32 ± 0.06c	6.93 ± 0.10 ab
Forl	54.00 ± 3.06 a	0.89 ± 0.01b	1.37 ± 0. 06b	5.39 ± 0.06 bc	6.75 ± 0.07b
Forl + *Ac*DB3	**28.00** ± 3.26**c**	**0.99** ± 0.03 **a**	1.17 ± 0.04c	**5.87** ± 0.10 **a**	**7.04** ± 0.10 **a**
Forl + *Bt*MB9	44.00 ± 2.66b	0.97 ± 0.02 a	1.48 ± 0.09 ab	**5.63** ± 0.11**b**	**7.11** ± 0.10 **a**
Hybrid Pai Pai (indeterminate/72 days)					
CTL	0.00 ± 0.00 d	0.91 ± 0.11 bc	0.84 ± 0.10 bc	4.76 ± 0.08b	5.61 ± 0.09b
*Ac*DB3	0.00 ± 0.00 d	1.12 ± 0.03 ab	1.13 ± 0.10b	**6.41** ± 0.04 **a**	**7.54** ± 0.04 **a**
*Bt*MB9	0.00 ± 0.00 d	**1.37** ± 0.05 **a**	**1.43** ± 0.08**a**	**7.13** ± 0.03 **a**	**8.56** ± 0.03 **a**
Forl	90.00 ± 6.12 a	0.34 ± 0.08 e	0.11 ± 0.03 e	0.87 ± 0.02 d	0.98 ± 0.02 d
Forl + *Ac*DB3	**50.00** ± 7.90**b**	0.46 ± 0.06 de	**0.40** ± 0.09 **d**	2.18 ± 0.04 cd	2.59 ± 0.05 cd
Forl + *Bt*MB9	**25.00** ± 7.90**c**	**1.08** ± 0.14**b**	**0.80** ± 0.15**c**	**4.83** ± 0.06**b**	**5.62** ± 0.07**b**
Forl + *Ac*DB3 + *Bt*MB9	**50.00** ± 11.18**b**	**0.66** ± 0.8 **cd**	**0.44** ± 0.07 **d**	**3.09** ± 0.04**c**	**3.53** ± 0.04**c**

[a]CTL: water control; [b]DW: dry weight; *Ac*DB3: *Acinetobacter calcoaceticus*; *Bt*MB9: *B. thuringiensis*.
*Statistical treatment of the data was performed with each hybrid separately. Hybrid growth habit type and plant age (in days) when the experiment was evaluated are provided in parentheses; >50% seed germination was reached at day 7 after sowing for N6394 and SV4401TJ and at day 10 for Pai Pai. **Statistically significant differences ($p \leq 0.05$) are indicated by mean values which do not share lower case letters. ***Values in bold indicate a significant increase relative to the respective controls (CTL or *Forl* = fungus without any antagonist). Data are presented as mean values ± standard errors (SE).

tion activities, which may represent additional mechanisms for inhibiting *Fol* R3 growth (Lin et al., 2012; Ennouri et al., 2013). Phosphate solubilization (Mehta et al., 2014) and IAA production (Chandra et al., 2018) are considered growth-promoting mechanisms in plants. In our study, both *Bsi*DA2 and *Bt*MB9 solubilized phosphate, while *Ac*DB3 and *Bsi*DA2 produced IAA. Indeed, IAA-producing bacteria promote plant growth directly by developing the plant root system, which allows the plant to absorb more nutrients from rhizospheric soil (Fierro-Coronado et al., 2014; Vinayarani and Prakash, 2018). In our study, *Ac*DB3 produced IAA and also enhanced tomato growth (root and shoot length as well as total biomass) under greenhouse conditions, in agreement with previous reports on *Acinetobacter* sp. isolates (Kwon and Song, 2014). It has been reported that *Acinetobacter calcoaceticus* displays IAA and siderophore production that promotes wheat plant growth, with antagonistic activity to different phytopathogens such as *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger* (Sarode Prashant et al., 2009). *Bacillus thuringiensis* isolate has also been reported to promote growth in *Abelmoschus esculentus* plants by phosphate solubilization (Bandopadhyay, 2020). In our study, all five bacteria were capable of forming biofilms. *Bacillus* sp. (Escamilla-Montes et al., 2015) and *Acinetobacter* sp. (Jang et al., 2016) isolates form biofilms, and they can fix themselves to both living and non-living surfaces. Importantly, bacteria utilize biofilm

formation for survival and stress tolerance. This ability also helps them attach to the root surface of host plants, allowing colonization of specific niches in the plant rhizosphere (Chen et al., 2013).

Native rhizobacteria are always desirable for the biological control of fungal phytopathogens since they are pre-adapted to the local crops, climate and edaphic conditions (Revillini et al., 2016). Our present work is in line with this paradigm, since we describe novel rhizobacteria native to the soils of northern Sinaloa that may be useful for controlling *Fol* R3 and *Forl* as well as promoting growth in tomato.

Our greenhouse bioassay results (Tables 3–6) indicate that *Bs*TA16 and *Ac*DB3 have the most promising antagonistic effects against *Fol* R3 and *Forl*, respectively, in addition to their potential as growth-promoting agents. Since there was no clear advantage to using bacterial isolates from the tomato rhizosphere showing antagonism against the *Forl* and *Fol* R3 pathogens as opposed to isolates from *Datura* or maize in the *in vitro* selection stage, our second hypothesis was also rejected. Although *Bs*TA16, an isolate from tomato, was the most promising antagonist against *Fol* R3, this is not consistent with the most promising bacterial antagonist in *Forl*, which originated from the *Datura* rhizosphere. In general, it is accepted that the best source of bacterial antagonists comes from the soil/rhizosphere of the same plant species (Jangir et al., 2018), although several examples exist of antagonistic bacteria able to control a fungal pathogen originating from a different plant species (Aydi-Ben-Abdallah et al., 2016).

The three *Fol* R3 antagonistic species *B. subtilis Bs*TA16, *B. siamensis Bsi*DA2, and *B. amyloliquefaciens Ba*MA26 belong to the *Bacillus subtilis* group. On the other hand, *Forl* antagonists include the strain *B. thuringiensis Bt*MB9 from the *Bacillus cereus* group and *Acinetobacter calcoaceticus Ac*DB3. To the best of our knowledge, this is the first report of *Acinetobacter calcoaceticus* (*Ac*DB3) as a potential antagonist against *Forl* in tomato. Our results are in agreement with Gadag and Krishnaraj (2017) who reported that *Acinetobacter* significantly suppresses *Fusarium* wilt caused by *Fol* and improves tomato plant growth under greenhouse conditions. Previous reports demonstrated that *Bacillus subtilis* and *Bacillus* sp. isolates show an antagonistic effect against *Fol* (Aydi-Ben-Abdallah et al., 2020; Kamali et al., 2021), similar to the three antagonists identified in our study. Furthermore, *B. subtilis* was observed to inhibit *Forl* growth by 60% during *in vitro* seedling assays (Cordero-Ramírez et al., 2013). *Bacillus* spp. isolates suppressed *Fol* R3 disease severity by 36% in greenhouse bioassays (Jangir et al., 2018), in agreement with our findings here. *Bacillus siamensis* significantly decreased tobacco brown spot disease caused by *Alternaria alternata*, in greenhouse conditions (Xie et al., 2021). *Bacillus amyloliquefaciens* has been demonstrated to control *Fol* growth and significantly reduce disease severity of *Fusarium* wilt under greenhouse conditions (Wan et al., 2017). Nevertheless, we did observe that the tomato hybrids responded differentially to the bacterial isolates. This observation is important since it opens the possibility of using the other three bacterial isolates (*Bt*MB9, *Bsi*DA2 and *Ba*MA26) as antagonists/growth promoters in specific tomato hybrids where *Ac*DB3 or *Bs*TA16 do not have any effect.

Our results in germination trays (22–31 days post-inoculation) and pot assays (~35 days of growth on germination trays plus 24–54 days post-inoculation in pots) reveal that bacteria responses regarding plant growth promotion or a decrease in disease severity caused by *Fol* R3 and *Forl* in tomato differ depending on the tomato hybrid as well as the tomato plant developmental stage. These findings therefore verify our third hypothesis. This is in agreement with other studies showing a differential response in the biological control of fungal diseases depending on the hybrid or variety used (López et al., 2018) and on the plant developmental growth stage (Fauzi, 2009).

This work was conducted in germination trays using sterile substrates to ensure the correct evaluation of the interaction between the plant host, the fungal phytopathogen, and the bacterial isolate. Our results from pot assays conducted under sterile (Pai Pai hybrid) and non-sterile (N6394 and SV4401TJ hybrids) soil conditions show that some isolates may exert growth induction and control these fungal pathogens irrespective of the presence of live microbiota. A previous study reported that the effect of plant growth-promoting bacteria (*Bacillus velezensis*, formerly described as *Bacillus amyloliquefaciens*) as an antagonist against the fungal phytopathogen *Fusarium oxysporum* f. sp. *physali* in cape gooseberry (*Physalis peruviana* L.) may depend on whether sterile or non-sterile substrate is used (Moreno-Velandia et al., 2019). Since each soil type will be different in its physical–chemical properties and will contain different microbiota, we are currently directing future studies at testing all five selected bacteria under field conditions.

5. Conclusions

No single bacterial isolate in our study displayed a control effect against both the *Fol* R3 and *Forl* phytopathogens. While the origin of the antagonistic bacteria that control these fungi may not be limited to a particular plant rhizosphere, any plant rhizosphere may carry antagonistic bacteria that can suppress *Fol* R3 or *Forl* in tomato, as well as enhance tomato plant growth. Moreover, bacterial antagonism and plant growth enhancement depend on the bacterial strain interacting with specific tomato hybrids as well as on the tomato developmental stage.

The findings presented in this work lead us to suggest that strains *B. subtilis Bs*TA16 and *Acinetobacter calcoaceticus Ac*DB3 have the most potential as biocontrol agents against *Fol* R3 and *Forl* in tomato, respectively. However, it must be noted that tomato growers switch from one hybrid to another every year. Nevertheless, we did observe biological control responses specific to tomato hybrids with the other three tested bacteria. These observations have led us to propose testing all five bacteria in currently ongoing field trials.

Funding

The financial support for this study was provided by grants SIP-20170939, SIP-20181778, SIP 2019–6353 and SIP 2020–1685 from the Instituto Politécnico Nacional (Mexico), and from Consejo Nacional de Ciencia y Tecnología (CONACyT), Mexico (Programa de Estímulos a la Innovación, Proinnova, Grant No. 230186).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dr. Brandon Loveall from Improvence editing services for English proofreading of the manuscript. M.M.R. Khalil acknowledges the Consejo Nacional de Ciencia y Tecnología (CONACyT) of Mexico, and the BEIFI program from Instituto Politécnico Nacional (IPN) for the Ph.D. fellowships.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.08.043.

References

Akkopru, A., Demir, S., 2005. Biological control of *Fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some rhizobacteria. J. Phytopathology. 153 (9), 544–550. https://doi.org/10.1111/jph.2005.153.issue-910.1111/j.1439-0434.2005.01018.x.

Apodaca-Sánchez, M.A., Zavaleta-Mejía, E., Osada, K.S., García-Espinoza, R., 2002. Frecuencia de campos infestados con *Fusarium oxysporum* f. sp. *radicis-lycopersici* en Sinaloa, México, y su control. Rev. Mex. Fitopatol. 20, 1–7 https://www.redalyc.org/articulo.oa?id=61220101.

Apodaca-Sánchez, M.A., Zavaleta-Mejía, E., Osada, K.S., García-Espinoza, R., 2004. Pudrición de la corona del chile (*Capsicum annuum* L.) en Sinaloa, México. Rev. Mex. Fitopatol. 22, 22–29 https://www.redalyc.org/pdf/612/61222104.pdf.

Asran, M.R., Buchenauer, H., 2003. Pathogenicity of *Fusarium graminearum* isolates on maize (*Zea mays* L.) cultivars and relation with deoxynivalenol and ergosterol contents. J. Plant Dis. Protect. 110, 209–219. https://doi.org/10.2307/43215506.

Ates, C.A., Fidan, H., Karacaoglu, M., Dasgan, H.Y., 2019. The identification of the resistance levels of *Fusarium oxysporum* f. sp. *radicis - lycopersici* and Tomato Yellow Leaf Curl viruses in different tomato genotypes with traditional and molecular methods. Appl. Ecol. Environ. Res. 17 (2), 2203–2218. https://doi.org/10.15666/aeer/1702_22032218.

Aydi-Ben-Abdallah, R., Jabnoun-Khiareddine, H., Daami-Remadi, M., 2020. *Fusarium* wilt biocontrol and tomato growth stimulation, using endophytic bacteria naturally associated with *Solanum sodomaeum* and *S. bonariense* plants. Egypt J. Biol. Pest Control 30, 113. https://doi.org/10.1186/s41938-020-00313-1.

Aydi-Ben-Abdallah, R., Jabnoun-Khiareddine, H., Nefzi, A., Mokni-Tlili, S., Daami-Remadi, M., 2016. Endophytic bacteria from *Datura stramonium* for *Fusarium* wilt suppression and tomato growth promotion. J. Microb. Biochem. Technol. 8, 030–041. https://doi.org/10.4172/1948-5948.1000259.

Bandopadhyay, S., 2020. Application of plant growth promoting *Bacillus thuringiensis* as biofertilizer on *Abelmoschus esculentus* plants under field condition. J. Pure Appl. Microbiol. 14 (2), 1287–1294. https://doi.org/10.22207/JPAM10.22207/JPAM.14.210.22207/JPAM.14.2.24.

Bric, J.M., Bostock, R.M., Silverstone, S.E., 1991. Rapid *in situ* assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl. Environ. Microbiol. 57, 535–538. https://doi.org/10.1128/AEM.57.2.535-538.1991.

Castro-Ochoa, L.D., Rodríguez-Gómez, C., Valerio-Alfaro, G., Oliart Ros, R., 2005. Screening, purification and characterization of the thermoalkalophilic lipase produced by *Bacillus thermoleovorans* CCR11. Enzyme Microb. Tech. 37 (6), 648–654. https://doi.org/10.1016/j.enzmictec.2005.06.003.

Chandra, S., Askari, K., Kumari, M., 2018. Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. J. Genet. Eng. Biotechnol. 16 (2), 581–586. https://doi.org/10.1016/j.jgeb.2018.09.001.

Chen, Y., Yan, F., Chai, Y., Liu, H., Kolter, R., Losick, R., Guo, J.H., 2013. Biocontrol of tomato wilt disease by *Bacillus subtilis* isolates from natural environments depends on conserved genes mediating biofilm formation. Environ. Microbiol. 15, 848–864. https://doi.org/10.1111/j.1462-2920.2012.02860.x.

Chow, Y.Y., Rahman, S., Ting, A.S.Y., 2018. Interaction dynamics between endophytic biocontrol agents and pathogen in the host plant studied via quantitative real-time polymerase chain reaction (qPCR) approach. Biol. Control 125, 44–49. https://doi.org/10.1371/journal.pone.0128766.

Cordero-Ramírez, J.D., López-Rivera, R., Figueroa-López, A.M., Mancera-López, M.E., Martínez-Álvarez, J.C., Apodaca-Sánchez, M.A., Maldonado-Mendoza, I.E., 2013. Native soil bacteria isolates in Mexico exhibit a promising antagonistic effect against *Fusarium oxysporum* f. sp. *radicis-lycopersici*. J. Basic Microbiol. 53, 838–847. https://doi.org/10.1002/jobm.201200128.

Debbi, A., Boureghda, H., Monte, E., Hermosa, R., 2018. Distribution and genetic variability of *Fusarium oxysporum* associated with tomato diseases in Algeria and a biocontrol strategy with indigenous *Trichoderma* spp. Front. Microbiol. 9, 1–11. https://doi.org/10.3389/fmicb.2018.00282.

Elanchezhiyan, K., Keerthana, U., Nagendran, K., Prabhukarthikeyan, S.R., Prabakar, K., Raguchander, T., Karthikeyan, G., 2018. Multifaceted benefits of *Bacillus amyloliquefaciens* strain FBZ24 in the management of wilt disease in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. Physiol. Mol. Plant Pathol. 103, 92–101. https://doi.org/10.1016/j.pmpp.2018.05.008.

El-Sayed, W.S., Akhkha, A., El-Naggar, M.Y., Elbadry, M., 2014. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. Front. Microbiol. 5, 1–11. https://doi.org/10.3389/fmicb.2014.00651.

Ennouri, K., Ben Khedher, S., Jaoua, S., Zouari, N., 2013. Correlation between delta-endotoxin and proteolytic activities produced by *Bacillus thuringiensis* var. kurstaki growing in an economic production medium. Biocontrol Sci. Technol. 23 (7), 756–767. https://doi.org/10.1080/09583157.2013.791364.

Escamilla-Montes, R., Luna-González, A., Flores-Miranda, M. del C., Álvarez-Ruiz, P., Fierro-Coronado, J.A., Sánchez-Ortiz, A.C., Ávila-Leal, J., 2015. Isolation and characterization of potential probiotic bacteria suitable for mollusk larvae cultures. Thai J. Vet. Med. 45, 11–21. https://he01.tci-thaijo.org/index.php/tjvm/article/view/32129

FAOSTAT - Food and Agriculture Organization Corporate Statistical Database, United Nations, 2019. http://www.fao.org/faostat/en/#home (Accessed 15 February 2021).

Fauzi, Mohamad Taufik, 2009. Biocontrol ability of *Puccinia abrupta* var. *partheniicola* on different growth stages of Parthenium weed (*Parthenium hysterophorus* L.). Hayati J. Biosci. 16 (3), 83–87. https://doi.org/10.4308/hjb.16.3.83.

Fierro-Coronado, R.A., Castro-Moreno, M.G., Ruelas-Ayala, R.D., Apodaca-Sánchez, M.Á., Maldonado-Mendoza, I.E., 2013. Induced protection by *Rhizophagus intraradices* against *Fusarium* wilt of tomato. Interciencia 38, 48–53 https://www.interciencia.net/wp-content/uploads/2017/12/048-c-MALDONADO-6.pdf.

Fierro-Coronado, Rosario Alicia, Quiroz-Figueroa, Francisco Roberto, García-Pérez, Luz María, Ramírez-Chávez, Enrique, Molina-Torres, Jorge, Maldonado-Mendoza, Ignacio Eduardo, 2014. IAA-producing rhizobacteria from chickpea (*Cicer arietinum* L.) induce changes in root architecture and increase root biomass. Can. J. Microbiol. 60 (10), 639–648. https://doi.org/10.1139/cjm-2014-0399.

Figueroa-López, Alejandro Miguel, Cordero-Ramírez, Jesús Damián, Martínez-Álvarez, Juan Carlos, López-Meyer, Melina, Lizárraga-Sánchez, Glenda Judith, Félix-Gastélum, Rubén, Castro-Martínez, Claudia, Maldonado-Mendoza, Ignacio Eduardo, 2016. Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticillioides*. SpringerPlus. 5 (1). https://doi.org/10.1186/s40064-016-1780-x.

Figueroa-López, Alejandro Miguel, Cordero-Ramírez, Jesús Damián, Quiroz-Figueroa, Francisco Roberto, Maldonado-Mendoza, Ignacio Eduardo, 2014. A high-throughput screening assay to identify bacterial antagonists against *Fusarium verticillioides*. J. Basic Microbiol. 54 (S1), S125–S133. https://doi.org/10.1002/jobm.v54.S110.1002/jobm.201200594.

Gadag, A.S., Krishnaraj, P.U., 2017. Effect of Actinobacteria and Glomus fasciculatum against Fusarium oxysporum f. sp. lycopersici in tomato plant. Int. J. Curr. Microbiol. App. Sci. 6, 488–498. 10.20546/ijcmas.2017.609.059

Gómez, P., Paterson, S., De Meester, L., Liu, X., Lenzi, L., Sharma, M.D., McElroy, K., Buckling, A., 2016. Local adaptation of a bacterium is as important as its presence in structuring a natural microbial community. Nat. Commun. 7, 12453. https://doi.org/10.1038/ncomms12453.

Hibar, K., Daami-Remadi, M., El Mahjoub, M., 2007. Induction of resistance in tomato plants against *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Trichoderma* spp. Tunis. J. Plant Prot. 2, 47–58.

Hu, Ruifa, Huang, Xusheng, Huang, Jikun, Li, Yifan, Zhang, Chao, Yin, Yanhong, Chen, Zhaohui, Jin, Yanhong, Cai, Jinyang, Cui, Fang, Arias-Carrion, Oscar, 2015. Long- and short-term health effects of pesticide exposure: a cohort study from China. PLoS ONE 10 (6), e0128766. https://doi.org/10.1371/journal.pone.0128766.

Jang, I.A., Kim, J., Park, W., 2016. Endogenous hydrogen peroxide increases biofilm formation by inducing exopolysaccharide production in *Acinetobacter oleivorans* DR1. Sci. Rep. 6, 21121. https://doi.org/10.1038/srep21121.

Jangir, M., Pathak, R., Sharma, S., Sharma, S., 2018. Biocontrol mechanisms of *Bacillus* sp., isolated from tomato rhizosphere against *Fusarium oxysporum* f. sp. *lycopersici*. Biol. Control 123, 60–70. https://doi.org/10.1016/j.biocontrol.2018.04.018.

Jarvis, W.R., Shoemaker, R.A., 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68, 1679–1680. https://doi.org/10.1094/Phyto-68-1679.

Jones, B.V., Sun, F., Marchesi, J.R., 2007. Using skimmed milk agar to functionally screen a gut metagenomic library for proteases. Lett. Appl. Microbiol. 45, 418–420. https://doi.org/10.1111/j.1472-765X.2007.02202.x.

Kamali, M., Ahmadi, J., Naeimi, S., Guo, D., 2021. Characterization of *Bacillus* isolates from the rhizosphere of tomato suppressing fusarium wilt disease. Acta Phytopathol. Entomol. Hung. 54 (1), 53–68. https://doi.org/10.1556/038.54.2019.006.

Kamilova, Faina, Leveau, Johan H.J., Lugtenberg, Ben, 2007. *Collimonas fungivorans*, an unpredicted in vitro but efficient in vivo biocontrol agent for the suppression of tomato foot and root rot. Environ. Microbiol. 9 (6), 1597–1603. https://doi.org/10.1111/emi.2007.9.issue-610.1111/j.1462-2920.2007.01263.x.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.

Kumari, P., Bishnoi, S.K., Chandra, S., 2021. Assessment of antibiosis potential of *Bacillus* sp. against the soil-borne fungal pathogen *Sclerotium rolfsii* Sacc. (*Athelia rolfsii* (Curzi) Tu & Kimbrough). Egypt J. Biol. Pest Control 31, 1–11. https://doi.org/10.1186/s41938-020-00350-w.

Kwon, Hyeok-Do, Song, Hong-Gyu, 2014. Interactions between Indole-3-acetic acid producing *Acinetobacter* sp. SW5 and growth of tomato plant. Kor. J. Microbiol. 50 (4), 302–307. https://doi.org/10.7845/kjm.2014.4050.

Leslie, J.F., Summerell, B.A.I., 2006. The *Fusarium* laboratory manual. Blackwell, USA. https://doi/book/10.1002/9780470278376.

Lin, L., Kan, X., Yan, H., Wang, D., 2012. Characterization of extracellular cellulose-degrading enzymes from *Bacillus thuringiensis* strains. Electron. J. Biotechnol. 15, 1–7. https://doi.org/10.2225/vol15-issue3-fulltext-1.

López, S.M.Y., Pastorino, G.N., Franco, M.E.E., Medina, R., Lucentini, C.G., Saparrat, M. C.N., Balatti, P.A., 2018. Microbial endophytes that live within the seeds of two tomato hybrids cultivated in Argentina. Agronomy. 8, 136. https://doi.org/10.3390/agronomy8080136.

López-Rivera, R., 2011. Biodiversidad de la microbiota rizosférica de dos especies solanáceas: (Solanum lycopersicon, L.) y Datura spp. (tomate y toloache). M. Sc. Thesis Dissertation, National Polytechnic Institute, CIIDIR-Sinaloa, México.

Mandhi, A., Habri, B., Ángeles-Esteban, M., Chaieb, K., Kaumon, F., Bakhrouf, A., 2010. Using mixture design to construct consortia of potential probiotic *Bacillus*

strains to project gnotobiotic *Artemia* against pathogenic *Vibrio*. Biocontrol Sci. Technol. 20, 983–996. https://doi.org/10.1080/09583157.2010.495185.
Mehta, Preeti, Walia, Abhishek, Kakkar, Nitin, Shirkot, C.K., 2014. Tricalcium phosphate solubilisation by new endophyte *Bacillus methylotrophicus* CKAM isolated from apple root endosphere and its plant growth-promoting activities. Acta Physiol. Plant. 36 (8), 2033–2045. https://doi.org/10.1007/s11738-014-1581-1.
Misawa, N., Hirayama, K., Itoh, K., Takahashi, E., 1995. Detection of alpha- and beta-hemolytic-like activity from *Campylobacter jejuni*. J. Clin. Microbiol. 33 (3), 729–731.
Moreno-Velandia, C.A., Izquierdo-García, L.F., Ongena, M., Kloepper, J.W., Cotes, A. M., 2019. Soil sterilization, pathogen and antagonist concentration affect biological control of *Fusarium* wilt of cape gooseberry by *Bacillus velezensis* Bs006. Plant Soil 435 (1-2), 39–55. https://doi.org/10.1007/s11104-018-3866-4.
Pikovskaya, R.E., 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology 17, 362–370.
Quilambaqui-Jara, M., Zavaleta-Mejía, E., Mora-Aguilera, G., Delgadillo-Sánchez, F., Marín-Jarillo, A., 2004. Patogenicidad de tres especies de *Fusarium* asociadas con el declinamiento del espárrago (*Asparagus officinalis L.*) en Guanajuato, México. Rev. Mex. Fitopatol. 22, 30–36. https://www.redalyc.org/pdf/612/61222105.pdf.
Ramírez-Estrada, F.J., Leyva-Mir, S.G., 1990. Etiología de la pudrición de la corona y raíz del tomate (*Lycopersicon esculentum* Mill) en Sinaloa y San Luis Potosí y búsqueda de fuentes de resistencia al patógeno. Revista Chapingo 15, 13–16.
Revillini, Daniel, Gehring, Catherine A., Johnson, Nancy Collins, 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant-soil feedback systems. Funct. Ecol. 30 (7), 1086–1098. https://doi.org/10.1111/fec.2016.30.issue-710.1111/1365-2435.12668.
Salim, H.A., Jasim, B.N., Kadhim, A.D., Salman, I.S., Abdalbaki, A.A., 2017. Effect of biocontrol, physical control and compost on tomato plants that infected with *Fusarium* wilt under greenhouse conditions. World J. Agric. Res. 5, 5–8. https://doi.org/10.12691/wjar-5-1-2.
Santiago, C.D., Yagi, S., Ijima, M., Nashimoto, T., Sawada, M., Ikeda, S., Asano, K., Orikasa, Y., Ohwada, T., 2017. Bacterial compatibility in combined inoculations enhances the growth of potato seedlings. Microbes Environ. 32, 14–23. https://doi.org/10.1264/jsme2.ME16127.
Sarode Prashant, D., Rane Makarand, R., Chaudhari Bhushan, L., Chincholkar Sudhir, B., 2009. Siderophoregenic *Acinetobacter calcoaceticus* isolated from wheat rhizosphere with strong PGPR activity. Mal. J. Microbiol. 5, 6–12. https://doi.org/10.21161/mjm.13508.
Schwyn, B., Neilands, J.B., 1978. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 160, 47–56. https://doi.org/10.1016/0003-2697 (87)90612-9.
Sharma, A., Kashyap, P.L., Srivastava, A.K., Bansal, Y.K., Kaushik, R., 2018. Isolation and characterization of halotolerant bacilli from chickpea (*Cicer arietinum L.*) rhizosphere for plant growth promotion and biocontrol traits. Eur. J. Plant Pathol. 153, 787–800. https://doi.org/10.1007/s10658-018-1592-7.
SIAP-SAGARPA - Servicio de Información Agroalimentaria y Pesquera - Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación) 2019. https://nube.siap.gob.mx/cierreagricola/ (Accessed 15 February 2021)
Staniaszek, M., Szczechura, W., Marczewski, W., 2014. Identification of a new molecular marker C2–25 linked to the *Fusarium oxysporum* f.sp. *radicis-lycopersici* resistance *Frl* gene in tomato. Czech J. Genet. Plant Breed. 50, 285–287. https://doi.org/10.17221/70/2014-cjgpb.
Stefani, F.O.P., Bell, T.H., Marchand, C., de la Providencia, I.E., El Yassimi, A., St-Arnaud, M., Hijri, M., 2015. Culture-dependent and -independent methods capture different microbial community fractions in hydrocarbon-contaminated soils. PLoS ONE 10. https://doi.org/10.1371/journal.pone.0128272 e0128272.
Teather, R.M., Wood, P.J., 1982. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl. Environ. Microbiol. 43, 777–780. PMID: 7081984.
Valenzuela-Ureta, J.G., Lawn, D.A., Heisey, R.F., Zamudio-Guzman, V., 1996. First report of *Fusarium* wilt race 3, caused by *Fusarium oxysporum* f. sp. *lycopersici* of tomato in Mexico. Plant. Dis. 80, 105. https://doi.org/10.1094/PD-80-0105A.
Venkat, K.S., Menon, S., Agarwal, H., Gopalakrishnan, D., 2017. Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. Resour.-Effic. Technol. 3, 434–439. https://doi.org/10.1016/j.reffit.2017.04.004.
Vinayarani, G., Prakash, H.S., 2018. Growth promoting rhizospheric and endophytic bacteria from *Curcuma longa* L. as biocontrol agents against rhizome rot and leaf blight diseases. Plant Pathol. J. 34, 218–235. https://doi.org/10.5423/PPJ.OA.11.2017.0225.
Wan, T., Zhao, H., Wang, W., 2017. Effect of biocontrol agent *Bacillus amyloliquefaciens* SN16-1 and plant pathogen *Fusarium oxysporum* on tomato rhizosphere bacterial community composition. Biol. Control 112, 1–9. https://doi.org/10.1016/j.biocontrol.2017.05.014.
Wen, C.M., Tseng, C.S., Cheng, C.Y., Li, Y.K., 2002. Purification, characterization and cloning of chitinase from *Bacillus* sp. NCTU2. Biotechnol. Appl. Biochem. 35, 213–219. https://doi.org/10.1042/0885-4513:0350213.
Whipps, J.M., 1987. Effect of media on growth and interactions between a range of soil-borne glasshouse pathogens and antagonistic fungi. New Phytol. 107, 127–142. https://doi.org/10.1111/j.1469-8137.1987.tb04887.x.
Xie, Z., Wang, D., Wang, F., Shen, H., Sun, G., Feng, C., Wang, X., Chen, D., Sun, X., 2021. Biocontrol efficacy of *Bacillus siamensis* LZ88 against brown spot disease of tobacco caused by *Alternaria alternata*. Biol. Control 154,. https://doi.org/10.1016/j.biocontrol.2020.104508 104508.