Single and combined effects of Pythium oligandrum Po37 and a consortium of three rhizobacterial strains on Sclerotinia stem rot severity and tomato growth promotion

Nada Ouhaibi-Ben Abdeljalil (nadouhaibi@hotmail.fr)
LR21AGR03-Production and Protection for a Sustainable Horticulture (2-PHD), IRESA- University of Sousse
Jessica Vallance
Bordeaux Sciences Agro, INRAE, SAVE
Jonathan Gerbore
Bordeaux Sciences Agro, INRAE, SAVE
Mejda Daami-Remadi
LR21AGR03-Production and Protection for a Sustainable Horticulture (2-PHD), IRESA- University of Sousse
Patrice Rey
UPPA-CNRS) I-Site Energy Environment Solutions (E2S) University of Pau

Research Article

Keywords: Biocontrol, Microbial community, Plant growth, Sclerotinia stem rot, Strain mixture, Tomato

Posted Date: June 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1745667/v1

Abstract

Individual plant growth-promoting rhizobacterial (PGPR) strains (*B. thuringiensis* B2, *B. subtilis* B10 and *E. cloacae* B16) and their combination were used, with or without *Pythium oligandrum*, against *Sclerotinia sclerotiorum*. *In vitro*, all BCAs reduced successfully hyphal growth of the targeted pathogenic fungus. They displayed antifungal activity by more than 50% compared to controls. *In planta* trials were conducted two years in the row and led to a significant decrease in stem rot severity two months after the antagonist's application onto infected tomato plants: the reduction reached 75% using rhizobacterial mixtures and 72% using *P. oligandrum solo*, compared to controls. The plant growth-promoting potential of the three-strain consortium and *P. oligandrum* was also assessed. Increased height in disease-free plants was obtained with rhizobacterial mixtures (60%) compared to *P. oligandrum* (47%). The BCA's mixture increased the height of treated plants inoculated with *S. sclerotiorum* (up to 80%) compared to inoculated and untreated plants. A 42% and 30% increase in fresh weight of aerial parts and roots was also observed on disease-free plants respectively for those treated with mixtures of rhizobacteria and *P. oligandrum* alone.

On plants inoculated with *S. sclerotiorum* in both trials, the highest growth-enhancing effect was observed using the combined treatment of *P. oligandrum* and the three-strain rhizobacterial consortium (Po37+B2+B10+B16).

Rhizospheric microbial communities were assessed using Single Strand Conformational Polymorphism (SSCP). Differences in the genetic structure of the fungal and bacterial communities were observed following treatments applied in both trials.

1. Introduction

Several diseases affect tomato plants, some causing great damage such as anthracnose, fusariosis, and white mold. The latter one is caused by *Sclerotinia sclerotiorum* (Lib.) and has a wide host range consisting of approximately 278 genera and 408 plant species (Boland and Hall 1994; Gupta et al. 2020).

The pathogen resting structures, myceliogenically germinating sclerotia, are the initial source of infection of tomato plants leading to the development of a soft rotting of the aerial parts in contact with the soil line (Gao et al. 2014; Mamani-Gonzáles et al. 2015; Baturo-Cieśniewska et al. 2018). Disease management may be accomplished using conventional cultural practices of different cropping systems (no-tillage, crop rotation, intercropping), and intensive use of chemicals (Juliatti et al. 2019). This last approach is incredibly expensive and associated with serious ecological impacts attributed to taster toxic residues (Figueiredo et al. 2010; Sabaté et al. 2018). These constraints have increased the interest to develop safer alternatives such as biological control. This approach relies on the use of beneficial microbes as alternatives to chemical pesticides and fertilizers (Ab-Rahman et al. 2018; Niu et al. 2020).

Successful disease suppression was achieved using various microbial agents or formulations including bacteria (Kamal et al. 2015; Ouhaibi Ben Abdeljalil et al. 2016a; Schmidt et al. 2021), fungi (Juliatti et al. 2019; Schmidt et al. 2021), and bio fungicides (Woo et al. 2014; Sun et al. 2016; Macena et al. 2020).

It is necessary to look for organisms suitable for use in biological control and to study their mechanisms of action and the optimal conditions for their application in plant protection and integrated management systems. One of such agents is the oomycete, *Pythium oligandrum* with worldwide distribution (Gerbore et al. 2014) that has received considerable attention as a potential biocontrol agent because of its ability to destroy a wide range of fungal and oomycete disease-causing pathogens (Takenaka et al. 2008; Gerbore et al. 2014; Baturo-Cieśniewska et al. 2018; Yacoub et al. 2020).

Microbial formulations based on *Bacillus*, *Enterobacter* and *P. oligandrum* strains are attractive for commercialization due to their sustainable shelf life, their capacity to promote plant growth (PGPR) and their ability to produce antibiotics that are

effective against various plant pathogens including *S. sclerotiorum* (Stefan et al. 2013; Ouhaibi et al. 2016a; Sabaté et al. 2017; Torres et al. 2017; Goussous et al. 2019; Farzand et al. 2019; Yacoub et al. 2020).

Biocontrol agents should be as effective and reliable as chemical pesticides in the myriad microenvironments that exist in soil and on plant surfaces where disease control interactions occur (Hu et al. 2019). Most biocontrol approaches have used single biocontrol agents as antagonists to control a single (Miethling et al. 2000) or various pathogens (Berg and Smalla 2009; Saraf et al. 2014). This may partially account for the reported inconsistent performances of biocontrol formulations because single bioagents are not likely to be active against various bio-aggressors in all soil environments in which they are released.

It is thought that collectively a combination of microbes, with different environmental adaptations and mechanisms of action against target pathogens, is more likely to express important traits for more effective disease control over many different environmental conditions and agricultural ecosystems than an individual microbe (reviewed in Roberts and Kobayashi 2011; Latha et al. 2011; Ouhaibi et al. 2016b; Guijarro et al. 2019, Ouhaibi-Ben Abdeljalil et al. 2021).

The success associated with the integration of biocontrol agents is attributed to their possible synergistic effects (Pylak et al. 2019; Ouhaibi-Ben Abdeljalil et al. 2021). In past trials, a microbial consortium composed of *Bacillus subtilis* str. B10 (KT921327), *B. thuringiensis* str. B2 (KU158884) and *Enterobacter cloacae* str. B16 (KT921429) had efficiently controlled *S. sclerotiorum*-induced stem rot on tomatoes (Ouhaibi et al. 2016c, 2016b). This same consortium exhibited interesting potential in a previous study when mixed with *P. oligandrum* against Rhizoctonia root rot on two tomato cultivars based on two consecutive years of testing (Ouhaibi-Ben Abdeljalil et al. 2021).

In the present study, our objective was to assess the ability of this bacterial consortium mixed or not with *P. oligandrum*, to control Sclerotinia stem rot and to stimulate the growth of tomato-based on trials of two consecutive years. Possible changes occurring in the rhizospheric microbial community following these biocontrol treatments were also investigated using the Single-Stranded Conformational Polymorphism (SSCP) technique.

2. Material And Methods

2.1. Plant growth-promoting rhizobacteria (PGPR) strain cultures

A mixture of three PGPR strains, *i.e. Bacillus subtilis* str. B10 (KT921327), *B. thuringiensis* str. B2 (KU158884) and *Enterobacter cloacae* str. B16 (KT921429), was used in the present study. They were originally recovered from the rhizosphere of visibly healthy tomato plants. They were previously subjected to biochemical characterization and molecular identification (Ouhaibi-Ben Abdeljalil et al. 2016a). They were selected based on their antifungal and/or plant growth-promoting abilities (Ouhaibi-Ben Abdeljalil et al. 2016b). Their specific traits are summarized in Table 1. They were routinely cultured on Nutrient Agar medium and stock cultures were maintained at -20°C in Luria Bertani (LB) broth amended with 15% glycerol.

2.2. *Pythium oligandrum* strain inocula

Inocula of the *P. oligandrum* Po37 strain (oospores-mycelium homogenates) used in *planta* biocontrol assays were prepared and kindly provided by Biovitis (Saint Etienne de Chomeil, France). Their concentration was adjusted to 10^4 oospores/mL before being applied *in planta*.

2.3. *Sclerotinia sclerotiorum* strain and culture conditions

The target pathogen was originally recovered from tomato plants exhibiting typical symptoms of white mold and/or stem rot disease at the Plant Pathology Laboratory of the Regional Research Centre on Horticulture and Organic Agriculture of Chott-Mariem in Tunisia, as previously described in Ouhaibi et al. (2016a).

For *planta* assays, the *S. sclerotiorum* isolate was cultured on Potato Dextrose Agar (PDA) medium amended with Streptomycin sulsulfate00 mg/L) (w/v) and incubated at 28°C duriforays. Mycelia were scraped at the surface of 10 Petri dishes and then mixed in 1 L of sterile distilled water (SDW) (Ouhaibi el al. 2016b). Mycelial fragment density was assessed using a hemacytometer and adjusted to 10^8 mycelial fragments/mL.

2.4. Assessment of the *in vitro* antifungal activity of tested microbial agents

2.4.1. Potential of PGPR strains

The ability of the three rhizobacterial strains to suppress *S. sclerotiorum* mycelial growth was evaluated using the dual culture technique. A loopful of each bioagent (48 hr old culture) was addedina 100 mL Nutrient Broth (NB) medium, then placed on a rotary shaker (150 rpm) and incubated at 28 ± 2°C for 2 days. A 5-day-old fungal plug (6 mm in diameter) was placed on the side of a 90 mm-diameter Petri plate containing a PDA medium. Then, 10 µL taken from 48-h-old bacterial suspension (~10^8 cells/mL) was deposited into a well (6 mm in diameter, 3 mm in depth) on the opposite side. Plates inoculated with fungal agar plugs and treated with the same volume of SDW served as control. The assays were performed in triplicate.

After incubation at 28°C for 7 days, the diameter of the pathogen colony and the inhibition zone were measured and compared with the untreated control. The percentage of fungal inhibition (FI) was calculated according to Rostami et al. (2013) as follows: GI (%) = (C- t)/ C ×100; where C is the diameter of the pathogen colony in control plates and t is the colony diameter in treated plates.

2.4.2. Potential of *Pythium oligandrum*

The dual culture technique was also used in this antagonism study. A mycelial plug (6 mm in diameter) taken from 7-day-old *P. oligandrum* Po37 culture was placed at one side of the Petri plate (90 mm in diameter) and another of *S. sclerotiorum* (6 mm in diameter), removed from a 5-day-old culture, was placed at the opposite side. For control plates, only pathogen plugs were placed on the PDA medium. Then, the plates were incubated at 25 °C for 5 days.

The diameter of *S. sclerotiorum* colony was measured and the mycelial growth inhibition percentage was calculated as described above. For the elucidation of hyphal interactions between the antagonist and the target pathogen, samples of mycelium were taken from the zone of interaction of the two agents and examined under an optical microscope followed by the taking of micrographs (Horner et al. 2012).

1.

2.5. Combined biocontrol treatment in pot experiments

2.5.1. Plant material and growth condition

Tomato cv. Rio Grande seedlings, a cultivar known for its susceptibility to *S. sclerotiorum* infection, were used for all the *in vivo* trials. Seeds were surface sterilized with 3% sodium hypochlorite for 3 min and immediately rinsed with SDW three times. Next, they were sowed in alveolus plates (7 × 7 cm) filled with sterilized peat. Plates were placed under controlled conditions with day and night photoperiod and temperatures ranging between 21–18 ± 2°C, respectively. They were watered regularly, to avoid water stress, until reaching the two-true-leaf growth stage.

2.5.2. Combined treatment preparation and co-inoculation assessment

Stock cultures of rhizobacteria were cultured onto Nutrient Agar (NA) medium and incubated at 28°C for 48 h. After the incubation period, a bacterial colony of each strain was suspended in NB (300 mL) and incubated in a rotary shaker (175 rpm) for two days at 28°C. Then, the 48h-old cell culture was diluted in 1L of SDW and adjusted to 10^8cells/mL (Wu et al.

2014). For the preparation of mixed biocontrol formulations, equal volumes of each rhizobacterial cell suspension were mixed, and the consortium obtained (3B) was tested alone or in a mixture with the *P. oligandrum* inoculum (3B+Po37).

Tomato seedlings used for the trial were deprived of water two days before the bioassay. Seedling treatment was performed as substrate drench around the stem using 30 mL of the rhizobacterial consortium alone or in a mixture with *P. oligandrum*. After one week, *S. sclerotiorum* inoculum (30 mL) was sprayed at the same level on each seedling. The next day, seedlings were transplanted into pots (16 cm in diameter) containing pathogen-infected peat (Benchabane et al. 2000; Le Foch et al. 2003).

Negative controls (uninoculated control seedlings) were treated with SDW only, while positive control plants were inoculated with *S. sclerotiorum* and treated with SDW or with a commercial fungicide, *i.e.* Previcur® (Bayer, France, propamocarb hydrochloride 722 g/L) applied at 0.5 mL/L. Uninoculated seedlings challenged with the rhizobacterial consortium and/or *P. oligandrum* were also used for comparison and the elucidation of their plant growth-promoting potential. This method of inoculation was chosen to avoid any trauma to tomato seedlings following root or stem injury.

Pots were grown under controlled conditions (60-70% relative humidity, 13/11 h light/dark photoperiod at 21/18 ± 2°C light/dark temperature) and the whole experiment was repeated for two consecutive years (2012 and 2013).

2.5.3 Experimental layout

For the experiment, 135 tomato seedlings were used and distributed between nine treatments. The experimental design consisted of a randomized complete block design with 15 seedlings per individual treatment, under the two trials (2012 and 2013). The different treatments were: (i) C: untreated control, (ii) Sc: inoculated with *S. sclerotiorum* and untreated, (iii) Sc+f: *S. sclerotiorum*-inoculated and treated with a commercial fungicide, *i.e.* Previcur®, (iv) Po37: uninoculated and treated with *P. oligandrum* Po37, (v) Sc+Po37: inoculated with *S. sclerotiorum* and treated with *P. oligandrum,* (vi) 3B: uninoculated and treated with the rhizobacterial consortium, (vii) 3B+Po37: uninoculated and treated with *P. oligandrum* and the rhizobacterial consortium, (viii) Sc+3B: inoculated with *S. sclerotiorum* and treated with the rhizobacterial consortium, (ix) Sc+3B+Po37: inoculated with S. *sclerotiorum* and treated with *P. oligandrum* and the rhizobacterial consortium.

2.5.4. Assessment of the disease suppression ability and the plant growth-promoting potential of tested treatments

At the end of the experiment (two months after the pathogen challenge), plants were uprooted, washed under running tap water to remove peat traces, and air-dried on filter papers. Parameters measured were plant height (cm) and fresh weight of aerial parts and roots (g) (Hassen and Labuschagne 2010). Disease severity on roots was scored using a 0-5 scale where 0= no symptoms, 1= 0-25% root browning, 2 = 26-50% root browning, 3 = 51-75% root browning, 4= 76-100% root browning and 5= plant dieback (Takenaka et al. 2008). Disease incidence (DI) percentage was determined using the following equation:

DI % = (Number of symptomatic plants /The total number of scored plants) ×100.

2.5.5. Assessment of the shifts in the microbial communities following treatments

Characterization of the microbial (fungi and bacteria) communities' structure and diversity was performed using profiles obtained by the CE-SSCP method, as previously described by Gerbore et al. (2014).

2.5.6. Sampling and DNA extraction

At the end of the experiment and the scoring of growth and severity parameters, 15 plants of each treatment were used for the characterization of the microbial community of roots following tested treatments.

Plants were uprooted gently from each pot to preserve the small feeder roots and were shaken to remove clumps of peat around the roots. Roots were cut into small fragments and crushed until further use for microbial and molecular analyses.

Total DNA was extracted as reported by Godon et al. (1997) with slight modifications. Briefly, root fragments were transferred into 2 ml polypropylene microcentrifuge tubes and kept frozen in a -80°C freezer rack, then lyophilized for 12 h before DNA extraction.

DNA was extracted from 60 mg of ground lyophilized root fragments. A volume of 600 µL of lysis buffer CTAB (1x) was added to each 2 mL tube and incubated at 65°C/1 h. To remove proteins, 400 µL of chloroform-isoamyl alcohol (24 :1, v/v) were added and tubes were shaken at 200 rpm for10 min, then centrifuged at 13,000 rpm for 10 min at 4°C. Aqueous phases were transferred to new 2 ml tubes. Nucleic acids were precipitated by the addition of 330 mL of cold isopropanol and then kept at -20°C overnight. Nucleic acids were recovered by centrifugation at 13,000 rpm at 4°C for 10 min.

Supernatants were discarded and DNA finally were pellets washed with 800 µL of ethanol 70%. After centrifugation at 13,000 rpm at 4°C for 10 min, ethanol was discarded. Then DNA pellets were air-dried and re-suspended into 50 µL of SDW. DNA extracts were then quantified with a nanodrop (ND-1000, Thermo scientific, Labtech) and normalizedat 10 ng/ µL.

2.5.7. PCR-SSCP Analyses

For fingerprinting analyses using Single-Strand Conformation Polymorphism (SSCP), pairs of primers recognizing the V5–V6 region of the 16S rRNA gene, *i.e.* 799f /1115r (Redford et al. 2010), and the mitochondrial large subunit rDNA gene, *i.e.*ML1/ML2 (White et al.1990) were used respectively for bacteria and fungi.

DNA was amplified by PCR in a reaction mixture (25 µL final volume) consisting of 1 µL of DNA template (10 ng/µL), 2.5 µL of Pfu buffer (10x), 2.5 µL of BSA (Bovine Serum Albumin) at 10 µg/µL (BioLabs), 1 µL of dNTP (10 mM), 0.5 µL of each primer, 0.5 µL of PfuTurboo (Stratagene) and 16.5 µl of sterile distilled water.

The cycling conditions for bacteria were: enzyme activation at 95°C for 2 min; 25 cycles of denaturation at 95°C for 45 s; hybridization at 54°C for 30 s; extension at 72°C for 1 min; and a final extension at 72°C for 10 min. For fungi, the cycling parameters were 95°C for 2 min, followed by 35 cycles at 95°C for 30 s, 58°C for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products were visualized by 2% Tris-Borate-EDTA (TBE) agarose gel electrophoresis before SSCP analysis. The lengths of the fragments yielded by amplification were 250 bp and 350 pb for fungi and bacteria, respectively.

Single-Strand Conformation Polymorphism analyses were performed on an ABI PRISM 3130 genetic analyser (Applied Biosystems) equipped with four 36-cm-long capillaries. One microliter of a PCR product was mixed with 18.8 µl Hi-Di formamide (Applied Biosystems) and 0.2 µl of the Genescan 400 HD ROX standard internal DNA molecular size marker (Applied Biosystems). The sample mixture was denatured at 95°C for 5 min, instantly iced (10 min) and then placed onto the instrument. SSCP is based on the electrophoretic mobility of single-stranded DNA fragments. This mobility is different according to their three-dimensional conformation. Samples were allowed to co-migrate with the fluorescent size standard (GeneScan 400 ROX) to allow the comparison of migration profiles between samples.

2.6. Statistical analyses

Analysis of experimental data was achieved by using one-way analysis of variance (ANOVA) with Statistical Package for the Social Sciences (SPSS) software for Windows version 16.0.

Each of the in vitro or in vivo experiments was repeated twice in time. Data were analyzed according to a completely randomized design in which 9 treatments were tested (i.e. 15 seedlings per individual treatment).

The means were separated using the Student-Newman-Keuls test to identify significant pair-wise differences at $P \leq 0.05$. Correlations between disease severity and plant growth parameters were analyzed using the bivariate Pearson's test at $P < 0.01$.

SSCP patterns were aligned with Stat Fingerprints (version 2.0) (Michelland et al. 2009) and were gathered in a single numerical database before being statistically described by a global PCA using R software (version 2.15.2).

3. Results

3.1. Antifungal potential of tested microbial agents

3.2. Inhibitory effects of PGPR strains

The inhibitory effect against *S. sclerotiorum* hyphal extension was examined after its dual culture with the tested PGPR strains as compared to control plates (Figure 1). After 5 days of incubation at 25°C, a significant ($P \leq 0.05$) decrease in the growth of the pathogen was observed following its confrontation with PGPR strains compared to the untreated control (Table 2). The *in vitro* growth of the pathogen was inhibited by 41, 46 and 54% respectively by *B. thuringiensis* str. B2, *B. subtilis* str. B10, and *E. cloacae* str. B16. When confronted with *S. sclerotiorum* colonies, tested bacterial strains led to the formation of growth-inhibition zones with radial dimensions (distance between pathogen and bioagent colony) estimated at respectively 7.6, 9, and 10.6 mm for B16, B2, and B10 (Table 2).

3.3. Inhibitory effects of *Pythium oligandrum*

Macroscopic evaluation. ANOVA analysis revealed a significant variation ($P \leq 0.05$) in the diameter of *S. sclerotiorum* colony, measured after 5 days of incubation at 25°C. *P. oligandrum* significantly inhibited the pathogen radial growth by more than 50% compared to untreated control cultures (Figure 2).

After one week of incubation, the dual culture technique revealed that *P. oligandrum* was capable of overgrowing the pathogen colony.

Microscopic evaluation. At the light microscope level, the whole process of antagonism formation was observed. Microscopic examination showed that *P. oligandrum* (P) densely coiled around hyphae of *S. sclerotiorum* (S). *P. oligandrum* hyphae often coiled around the host (Figure 3a), they grew parallel to the host and attached themselves to mycelium by forming hooks (Figure 3a, arrow). In tip-to-host side interactions, the mycoparasite tips continued to grow after contact, they grew over the host hyphae, depending on the angle of contact (Figure 3b).

After contact with *P. oligandrum*, the mycoparasite sometimes penetrated *S. sclerotiorum* mycelium (Figure 3b, arrow). Then, at a later stage of the antagonistic process, several hyphae of *S. sclerotiorum* were strongly degraded and cytoplasm of pathogenic hyphae became empty, as well as pathogenic hyphae appeared as abnormally shaped, empty pleiomorphic shells.

3.4. Effect of the tested microbial treatments on Sclerotinia stem rot severity and plant growth

3.4.1. Comparative disease suppression ability

Tested rhizobacterial consortium, applied alone or mixed with *P. oligandrum*, significantly (at $P \leq 0.05$) reduced the severity of *S. sclerotiorum*-induced stem rot compared to the untreated and pathogen-infected tomato plants (control). However, all tomato plants not challenged with the pathogen remained symptomless and healthy throughout the two-month experiment duration.

Disease incidence, estimated on the presence of root browning, ranged from 27 to 100% in 2012 and from 73 to 100% in 2013 (Table 3).

The rhizobacterial consortium, applied singly or in combination with *P. oligandrum*, was found to be more efficient in reducing disease severity than the fungicide, during the two consecutive years of assays. Results given in Table 3 showed that disease severity (DS) was lowered by 72 to 93% in 2012 and by 72 to 75% in 2013 compared to respectively 52 and 49 % using the fungicide control.

In 2012, the treatment composed of the three-strain consortium combined with *P. oligandrum* exhibited significantly higher effectiveness in decreasing Sclerotinia stem rot severity compared to the treatment with *P. oligandrum* alone. No such difference was observed in 2013.

3.4.2. Comparative plant growth-promoting ability

Enhancement of plant height

Results shown in Table 4 indicated that the treatment with the three-strain consortium and *P. oligandrum* had significantly ($P \leq 0.05$) enhanced the plant growth during the two consecutive years of trials compared to the uninoculated and untreated control. The height increase varied from 47-62% and 49-60% depending on the treatments tested in the 2012 and 2013 trials respectively, with the microbial consortium (Po37+B2+B10+B16) being the most efficient (62% in 2012 and 60% in 2013).

Data provided in Table 4 showed that all treatments tested in both trials significantly ($P \leq 0.05$) augmented the height of *S. sclerotiorum*-inoculated and treated plants compared to the inoculated and untreated ones. For both years, the increase in plant height was 80-82% compared to 51 (2012) and 65% (2013) observed with the commercial fungicide Previcur®. For both trials, the highest height-increasing effect was obtained using the combined treatment based on *P. oligandrum* and the three-strain rhizobacterial consortium (Po37+B2+B10+B16).

Enhancement of aerial part growth

Based on their comparative ability to enhance the growth of the aerial part, the rhizobacterial consortium and/or *P. oligandrum* significantly ($P \leq 0.05$) increased the aerial part's fresh weight (APFW) compared to the untreated and pathogen-free plants. Results illustrated in Table 5 showed that the increment in this growth parameter ranged from 5 to 42% during the first trial (2012) and from 18 to 55% in the second one (2013) where the combined treatment (Po37+B2+B10+B16) led to the highest parameter increase.

Data given in Table 5 also revealed that, in both trials, the APFW varied significantly ($P \leq 0.05$) depending on the treatments tested. Indeed, all tomato plants inoculated with the pathogen and treated with the rhizobacterial consortium applied singly or in combination with *P. oligandrum*, showed a significant increase in their APFW compared to *S. sclerotiorum*-inoculated and untreated control plants. The recorded APFW increment ranged from 34 to 36 % in 2012 and from 38 to 45 % in 2013 compared to 28% noted on Previcur®-treated and pathogen-inoculated plants.

Enhancement of root growth

Data provided in Table 6 indicated that the root fresh weight (RFW), measured two months post-planting, varied significantly ($P \leq 0.05$) depending on the treatments tested. For disease-free plants, RFW was improved by 30 to 51% in 2012 and by 35 to 50% in 2013 with the different treatments applied compared to the untreated control. The highest RFW increase was noted on plants treated with the three-strain rhizobacterial consortium in 2012 (B2+B10+B16) (51%) and the combined treatment (B2+B10+B16+ Po37) (50%) in 2013.

Regarding the comparative capacity to increase the RFW of tomato *S. sclerotiorum*-inoculated plants, the three-strain mixture combined with *P. oligandrum* (Po37+B2+B10+B16) or *P. oligandrum* alone were the most effective treatments compared to pathogen-inoculated and untreated control in both trials. Results illustrated in Table 6 indicated that the root growth-promoting effect varied from 24 to 58% in 2012 and 55 to 68% in 2013 respectively versus 40 and 48% recorded on plants treated with the fungicide. Overall and in both trials, the greatest root growth-promoting effect was induced by the mixed treatment based on the rhizobacterial consortium and *P. oligandrum* (Po37+B2+B10+B16).

3.3. Shifts occurring in the structure of the fungal and bacterial communities in the rhizosphere following tested treatments

A total of 54 SSCP profiles were generated from the root samples collected from cv. Rio Grande tomato plants (27 in 2012 and 27 in 2013). Based on the number of peaks and the relative height of the baseline, the SSCP profiles revealed complex microbial communities (data not shown).

Principal Component Analyses (PCAs) were studied to assess the genetic structure of the rhizospheric bacterial and fungal communities of tomato plants inoculated with *S. sclerotiorum* and treated with the rhizobacterial consortium applied singly or in combination with *P. oligandrum* strain.

3.3.1. SSCP Analyses of the Fungal Communities. The distributions of the samples on the principal plans generated by PCAs of fungal communities are represented in Figure 4. Differences in fungal communities' genetic structure were observed with the P. oligandrum treatment applied alone or in combination with rhizobacteria during the 2013 trial. PCA eigenvalues indicate that the first two principal components, Dim 1 and Dim 2, account for 69% of the total fungal variability in 2013.

These results allowed three main types of community structure to be delineated: (i) rhizobacteria applied as a consortium in association with *P. oligandrum,* (ii) *P. oligandrum* treatment applied alone; and (iii) *S. sclerotiorum* inoculated and untreated plants.

3.3.2. SSCP Structure of Bacterial Communities. The distribution of samples on the principal plans generated by PCAs of bacterial communities is represented in Figure 5. Differences in the genetic bacterial structure were observed during the two trials (2012 and 2013) depending on the rhizobacterial treatment.

PCA eigenvalues indicated that the first two principal components, Dim1 and Dim2, explained 75% and 77% of the total data variance respectively for 2012 and 2013. For both trials the, ellipses do not overlap when comparing plants inoculated with *S. sclerotiorum* only (sc_-) and treated by the consortium of rhizobacteria (sc_3b).

In 2012, bacterial communities differed between uninoculated and untreated plants (-) and those on which the three rhizobacteria (-_3b) had been applied; while in 2013, a shift occurred in the genetic structure of the bacterial communities of plants treated by the three rhizobacteria (-_3b) and then inoculated with *S. sclerotiorum* (sc_3b).

4. Discussion

Plant-pathogen interaction and disease development have always been a big challenge (Jeger et al. 2021). With time, farmers have applied various strategies to control pathogens' growth. Physical and chemical strategies are important to control devastating plant diseases. However, the environmental pollution caused by excessive use of agrochemicals and the development of resistance in pathogens to certain fungicides led to considerable changes in people's attitudes towards the use of pesticides in agriculture (Egüen et al. 2016). Biological control strategies are frequently reported as being much safer compared to other methodologies from the point of view of environmental protection (Singh et al. 2020). The best

biological control is thought to be that one originated from the natural-occurring organisms since these organisms are adapted to the environmental conditions (Goussous et al. 2019). Microorganisms under natural habitats live in communities and some provide benefits to plants Further, microbes when introduced to the soil as a consortium and interact with a host plant, partially mimic the natural soil conditions. The current research trend has therefore oriented toward investigating the role of microbial consortia in promoting plant growth and health against various invading pathogens (Sarma et al. 2015).

In our previous studies, we determined that three bacterial strains identified as *B. thuringiensis* B2 (KU158884), *B. subtilis* B10 (KT921327), and E. cloacae B16 (KT921429), whether or not associated with *P. oligandrum*, have the potential to control Rhizoctonia root rot and to promote growth for tomato plants (Ouhaibi-Ben Abdeljalil et al. 2021). On this basis, the present work aimed at gaining more insight into the effect of the combination of antagonists (oomycete and rhizobacteria) to evaluate their ability to coexist in the tomato rhizosphere (cv. Rio Grande), to suppress white mold disease caused by *S. sclerotiorum* and to promote plant growth during two consecutive years' assays.

The biological control of *S. sclerotiorum* has been demonstrated in numerous studies (Kamal et al. 2015; Sabaté et al. 2018; Macena et al. 2020). One of these microorganisms is the oomycete *P. oligandrum*, which is known as a biocontrol agent against many plant pathogens (Rey et al. 2008; Benhamou et al. 2012; Gerbore et al. 2014; Pisarik et al. 2021). Besides, the use of Bacillus strains as biocontrol agents to inhibit *S. sclerotiorum* has been previously tested in different crops (Chen et al. 2014; Hu et al. 2014; Ouhaibi-Ben Abdeljalil et al. 2016; Karthika et al. 2020). To improve the consistency of disease control, mixtures of bacteria and fungi as biocontrol agents (BCAs) are generally more effective than single BCAs, as published in many previous researches (Liu et al. 2018; Karthika et al. 2020; Ouhaibi-Ben Abdeljalil et al. 2021). However, development of new active compounds with low phytotoxicity, reduced environmental impact and broad spectrum of activity are still required (Cabrefiga and Montesinos, 2017).

Ideally, the biocontrol activity of candidate BCAs are evaluated using *in vitro* and *in vivo* screenings (Köhl et al. 2020). A dual culture technique was used for our two trials and the inhibitory activity of the isolated *B. thuringiensis* B2, *B. subtilis* B10, and *E. cloacae* B16 strains was evaluated against *S. sclerotiorum*. The overall results demonstrate that the three strains significantly inhibited the pathogen growth *in vitro*, compared to the untreated control, with values ranging from 41 to 54%. The distorted areas and mycelium anomalous formations of pathogens due to bacterial antagonists were frequently reported by many studies. The distorted areas and mycelium anomalous formations of pathogens due to bacterial antagonists were frequently reported by many studies (Li et al. 2014; El Arbi et al. 2016; Torres et al. 2016; 2017; Sabaté et al. 2018; Ouhaibi-Ben Abdeljalil et al. 2021). Regarding *P. oligandrum*, dual antagonist-pathogen cultures revealed that the oomycete significantly inhibited the growth of *S. sclerotiorum* by more than 50% compared to the untreated control. The results of the *in vivo* investigation also showed that after contact between *P. oligandrum* and *S. sclerotiorum* mycelia, hyphae of the pathogen were degraded and cytoplasm became empty. *P. oligandrum* has also the capacity to parasite other fungi such as *Fusarium oxysporum* and *Verticillium albo-atrum*, by producing enzymes (cellulases or chitinases) that degrade the cell walls of target pathogens (Benhamou et al.1999). Numerous studies have shown that *P. oligandrum* also secretes other extracellular enzymes, *i.e.* lipases, proteases and β-1,3-glucanases, which affect pathogenic fungi (Picard et al. 2000; Yacoub et al. 2020).

Velandia et al. (2021) suggested that the mechanism of *in vitro* mycelial growth suppression and inhibition success is mostly based on the production of antagonistic secondary metabolites, mainly the non-ribosomal cyclic lipopeptides (CLPs), which can affect phytopathogens directly (*e.g.* iturins and fengycins) or indirectly (*e.g.* surfactins…). In our previous experiments, we found that the three bacterial strains were able to produce Fengycin A and/or Bacillomycin D (Ouhaibi-Ben Abdeljalil et al. 2016a).

In general, primary selection of potential BCAs via *in vitro* dual culture assays has proven to be a valuable strategy to identify, on a higher throughput scale, BCAs with further confirmed *in vivo* biocontrol activity (Huang et al. 2012; Wang et

al. 2015; Sharifazizi et al. 2017).

The majority of published reports on plant disease biocontrol evaluate single strains against a specific pathogen (Liu et al. 2018). Despite the positive results reported, single BCAs have not been used on a wide range of host plants and have typically often exhibited inconsistent performances in the field (Pal and Gardener, 2006). To solve this inconsistency issue, mixtures of BCAs are used because this strategy combines multiple modes of action of BCAs to control plant diseases (Liu et al. 2018; Alfiky and Weisskopf, 2021). We proved the efficacy of such microbial mixtures after having tested the association of the antagonistic oomycete *P. oligandrum* with the 3 rhizobacteria*, B. thuringiensis* B2, *B. subtilis* B10, and *E. cloacae* B16, against *R. solani* on tomato plants (Ouhaibi-Ben Abdeljalil et al. 2021).

Many works initiated so far have concentrated on the effects of BCAs on the inhibition of fungal mycelial growth under *in vitro* conditions (Ettayebi et al. 2000; Liu et al. 2015). But very few studies have been conducted under *in vivo* conditions to show the properties of combined antagonistic oomycete with the rhizobacterial strains against *S. sclerotiorum.* This is why in the present work we tried to confirm the potential of microbial mixtures (*P. oligandrum* and 3 rhizobacteria) in the *in vivo* biological control of the pathogen *S. sclerotiorum* during two consecutive years of greenhouse assays.

Tomato is an important crop that is grown worldwide and is an excellent model for studying plant-microbe interactions (Romero et al. 2015). A single organism may fail during adverse environmental conditions; therefore, a combination of more than one BCA is more likely to resist and help to reduce disease incidence through synergistic action. Moreover, microbial consortia can easily colonize the rhizosphere. Kannan and Sureendar (2009) proved the efficiency of consortia treatment in growth promotion and wilt resistance of tomatoes (Karthika et al. 2020). This study adds support to this strategy because it has provided evidence that tomato root treatment with the three-strain consortium, mixed or not with *P. oligandrum*, ameliorated plant growth parameters compared to the uninoculated and untreated control. Interestingly, our results showed a significant increase in plant growth, roots and aerial part fresh weight when we applied microbial mixtures. In the two consecutive years of trials, the microbial consortium (Po37+B2+B10+B16) was the most efficient in enhancing plant growth by up to 60%, aerial part growth by more than 40% and increasing by 50% roots fresh weight. In summary, the results reported here show that the PGPR strains mixture associated or not with *P. oligandrum,* exhibited both biological control of *S. sclerotiorum* disease and plant-growth promotion. The magnitude of these results was better with mixtures than with individual BCA strains and it revealed that combined application of oomycetal and bacterial biocontrol agents resulted in a synergistic effect on disease suppression. These results are partly supported by the studies of many authors (Pylak et al. 2019; Trotel-Aziz et al. 2019; Zhang et al. 2019, Attia et al. 2020; Ouhaibi-Ben Abdeljalil et al. 2021) who showed that PGPR and *P. oligandrum*,applied individually, or in combinations, as biological agents can stimulate plant growth, improve plant health and productivity, and also soil health. The authors suggested that the observed increase in plant biomass may be due to the production of plant growth promoters and antibiotics. In agreement with this suggestion, the strains tested in our study previously exhibited multiple traits related to plant-growth promotion and broad-spectrum biocontrol activity (Ouhaibi-Ben Abdeljalil et al. 2016 b).

Besides, recent research by Attia et al. (2020) showed that the combined application of *B. subtilis*, *Lysinibacillus fusiformis* and *Achromobacter xylosoxidans* improved several growth parameters of tomatoes such as plant height, shoot biomass, root length, and leaf area. According to our results, the treatment with the mixture of BCAs was recording the highest percentage of protection. Indeed, the rhizobacterial consortium tested, applied alone or mixed with *P. oligandrum*, significantly reduced the severity of *S. sclerotiorum*-induced stem rot compared to the untreated and pathogen-infected tomato plants. For combined BCAs (*P. oligandrum* + 3 rhizobacteria), disease suppression ranged from 72 to 93% in 2012 and from72 to 89% in 2013 compared to respectively 52 and 49 % using the fungicide control. This result is similar to those obtained in previous studies, showing that BCAs are generally recognized as important tools for more sustainable disease management and represent valuable alternatives to classical pesticides (Liu et al. 2018; McDougall 2018). Therefore, results of our*in planta* experiments indicate that the three-strain consortium, mixed or not with *P. oligandrum*, could be a promising alternative to the commercial fungicide Previcur®.

Following the plant treatment with BCAs, a change may occur within the native microbial community structure as indicated in the study of Buyer et al. (2010). On this basis, to assess the influence of the inoculated strains on native rhizospheric microbial communities of tomato cv. Rio Grande, Single Strand Conformational Polymorphism (SSCP) profiles of amplified 16S rRNA genes, and internal transcribed spacers (ITS) were compared. In summary, in this study, we focused on the microbial communities colonizing the root systems of tomato plants after a combination of treatments. We found that native fungal and bacterial communities responded differently when microbial BCAs were inoculated individually or in a mixture in the rhizosphere. Data available in the literature reported that the biocontrol agents can establish in different soil environments without perturbing the bacterial and fungal communities (Edel-Hermann et al. 2009). Vallance et al. (2009 and 2012) and Renault et al. (2012) did not report shifts in the native fungal community of the rhizosphere after root inoculation by *P. oligandrum*, while they noticed a temporary shift in the native bacterial community (Vallance et al. 2012). These results may be compared with the investigation observed in the literature (Attia et al. 2017; Raymaekers et al. 2020) reporting that the BCAs group showed synergistic and antagonistic interactions with microbes present in the rhizosphere by indirectly boosting plant growth rate through the production of phytohormones.

To conclude, our data suggest that the tested individual PGPR strains (*B. thuringiensis* B2, *B. subtilis* B10 and *E. cloacae* B16) and their mixtures with or without the antagonistic oomycete *P. oligandrum*, exhibited both biological control of Sclerotinia stem rot and plant-growth promotion. The magnitude of these results was better with the synergistic effect obtained with mixtures of *P. oligandrum* and the three rhizobacteria, than with individual PGPR strains. Overall, the present study provides an important data resource for further application of combination BCAs in tomato protection and production. Future studies should evaluate whether PGPR mixtures provide similar enhanced biological control and growth promotion in field tests.

Declarations

Acknowledgments

The authors would like to thank the Tunisian Ministry of Higher Education and Scientific Research for its financial support (funds allocated to the research laboratoryLR21AGR03-Production and Protection for a Sustainable Horticulture (2-PHD), IRESA-University of Sousse, Regional Research Centre on Horticulture and Organic Agriculture, Chott Mariem, Tunisia), as well as the French Ministry of Agriculture for its financial support (UMR SAVE/INRA Bordeaux-France and CFBP/INRA Angers-Nantes) and Biovitis.

Funding

This study was funded by the Tunisian Ministry of Higher Education and Scientific Research for its financial support (funds allocated to the research laboratory LR21AGR03-Production and Protection for a Sustainable Horticulture (2-PHD), IRESA-University of Sousse, Regional Research Centre on Horticulture and Organic Agriculture, Chott Mariem, Tunisia), as well as the French Ministry of Agriculture for its financial support (UMR SAVE/INRA Bordeaux-France and CFBP/INRA Angers-Nantes) and Biovitis.

Competing interests

The authors confirm that there is no conflict of interests and are also liable for the content and writing of this article.

Authors' contributions

OBN, VJ, GJ, DM and RP conceived, designed, analyzed, wrote, corrected and approved the final draft.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author, Dr. Nada OUHAIBI BEN ABDELJALIL on request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

References

1. Ab Rahman SFS, Eugenie S, Corné MJP, Peer MS (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant science 267:102-111. https ://doi.org/10.1016/j.plant sci.2017.11.012.
2. Alfiky A, Weisskopf-Deciphering L (2021) *Trichoderma*–plant–pathogen interactions for better development of biocontrol applications. Journal of Fungi 7(1):61. https://doi.org/10.3390/jof7010061.
3. Andreia MF, Macena N, Kobori N, Mascarin GM, Vida JB, Hartman GL (2020) Antagonism of *Trichoderma*-based biofungicides against Brazilian and North American isolates of *Sclerotinia sclerotiorum* and growth promotion of soybean. BioControl 65: 235-246. https://doi.org/10.1007/s10526-019-09976-8.
4. Attia MS, El-Sayyadb GS, Abd Elkodousd M, El-Batal A (2020) The effective antagonistic potential of plant growth-promoting rhizobacteria against *Alternaria solani*-causing early blight disease in tomato plant. Scientia Horticulturae 266: 109-289.
5. Atti, MS, Sharaf AE-MM, Zayed AS (2017) Protective action of some bio-pesticides against early blight disease caused by *Alternaria solani* in tomato plant. International Journal of Innovative Science, Engineering and Tech 4:67-94.
6. Baturo-Cieśniewska A, Łukanowski A, Koczwara K, Lenc L (2018) Development of *Sclerotinia sclerotiorum* (Lib.) de Bary on stored carrot treated with *Pythium oligandrum* Drechsler determined by qPCR assay. Acta Sci. Pol. Hortorum Cultus 17(5) : 111–121. DOI: 10.24326/asphc.2018.5.10.
7. Benchabane M, Bakour R, Toua D, Boutekrabt A (2000) Mise en évidence de l'effet antagoniste de *Pseudomonas fluorescens* vis-à-vis de la fusariose vasculaire de la tomate. OEPP/EPPO Bulletin 30:243-246.
8. Benhamou N, Rey P, Picard K, Tirilly Y (1999) Ultrastructural and cytochemical aspects of the interaction between the mycoparasite *Pythium oligandrum* and soilborne pathogens. Phytopathology 89:506-517, https://doi.org/10.1094/PHYTO.1999.89.6.506.
9. Benhamou N, le Floch G, Vallance J, Gerbore J, Grizard D, Reyn P (2012) *Pythium oligandrum*: an example of opportunistic success. Microbiol-SGM 158: 2679-2694.
10. Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology 68:1-13.
11. Boland GJ, Hall R. (1994) Index of plant hosts of *Sclerotinia sclerotiorum*. Canadian Journal of Plant Pathology 16: 93-108. https://doi.org/10.1080/07060669409500766.
12. Buyer JS, Teasdale JR, Roberts DP, Zasada IA, Maul JE (2010) Factors affecting soil microbial community structure in tomato cropping systems. Soil Biology and Biochemistry 42:831-841.
13. Cabrefiga J, Montesinos E (2017) Lysozyme enhances the bactericidal effect of BP100 peptide against *Erwinia amylovora*, the causal agent of fire blight of rosaceous plants. BMC Microbiology. 17:39. DOI 10.1186/s12866-017-0957-y.
14. Chen A, Tian X, Jiang D, Yuan G, Xiong Y, Wan Y, Li G (2014) Control stem white rot of rapeseed caused by *Sclerotinia sclerotiorum* with *Coniothyrium minitans*, focused on application time. Plant Protection: Diseases 69-74.

15. Edel-Hermann V, Brenot S, Gautheron N, Aimé-Alabouvette CS, Steinberg C (2009 Ecological fitness of the biocontrol agent *Fusarium oxysporum* Fo47 in soil and its impacton the soil microbial communities. FEMS Microbiology Ecology. 68:37-45.
16. Egüen B, Melgarejo P, Cal A (2016) The effect of fungicide resistance on the structure of *Monilinia laxa* populations in Spanish peach and nectarine orchards. European Journal of Plant Pathology 145:815-827.
17. El Arbi A, Rochex A, Chataign G, Béchet M, Lecouturier D, Arnauld S, Gharsalla N, Jacques P (2016) The Tunisian oasis ecosystem is a source of antagonistic *Bacillus* spp. producing diverse antifungal lipopeptides. Research in Microbiology167:46-57.
18. Ettayebi K, El Yamani J, Rossi-Hassani BD (2000) Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. FEMS Microbiology Letters 183(1):190-195. doi:10.1016/s0378-1097(99)00665-5
19. Figueiredo MdVB, Seldin L, Araujo FF, de Mariano RLR (2010) Plant Growth Promoting Rhizobacteria: Fundamentals and Applications. D.K. Maheshwari (ed.), Plant Growth and Health Promoting Bacteria, Microbiology Monographs 18, DOI 10.1007/978-3-642-13612-2-2.
20. Juliatti FC, Rezende AA, Juliatti MBC, Morai TP (2019) *Trichoderma* as a Biocontrol Agent against Sclerotinia Stem Rot or White Mold on Soybeans in Brazil: Usage and Technology. In book: Trichoderma: The Most Widely Used Fungicide (1-24). IntechOpen. DOI:10.5772/intechopen.84544.
21. Farzand A, Moosa A, Zubair M, Rashid Khan A, Ayaz M, Colman Massawe V, Gao X (2019) Biological Control 110:317-326. https://doi.org/10.1094/PHYTO-05-19-0156-R.
22. Gao X, Han Q, Chen Y, Qin H, Huang L, Kang Z (2014) Biological control of oilseed rape Sclerotinia stem rot by *Bacillus subtilis* strain Em7, Biocontrol Science and Technology 24:1, 39-52, DOI: 10.1080/09583157.2013.844223.
23. Gerbore J, Vallance J, Yacoub A, Delmotte F, Grizard D, Regnault RC, Rey P (2014) Characterization of *Pythium oligandrum* populations that colonize the rhizosphere of vines from the Bordeaux region. FEMS Microbiology Ecology 90:153-167, https://doi.org/10.1111/1574-6941.12380.
24. Godon JJ, Zumstein E, Dabert P, Habouzit F, Moletta A (1997) Molecular Microbial Diversity of an Anaerobic Digestor as Determined by Small-Subunit rDNA Sequence Analysis. Applied and Environmental Microbiology. 63(7):2802-2813
25. Goussous SJ, Ibrahim MI, Tahhan RA, Al Antary TM (2019) Biological control of stem rot, *Sclerotinia sclerotiorum* by indigenous *Bacillus subtilis* isolates. Fresenius Environmental Bulletin 28 (12):9517-9525.
26. Guijarro, B, Larena, I, Casals, C, Teixidó, N, Melgarejo, P, Cal, AD (2018) Compatibility interactions between the biocontrol agent *Penicillium frequentans* Pf909 and other existing strategies to brown rot control. Biological Control. 129:45-54. doi: https://doi.org/10.1016/j.biocontrol.2018.11.011.
27. Gupta NC, Sharma P, Rao M, Kumar-Rai P, Kumar-Gupta A (2020) Evaluation of non-injury inoculation technique for assessing Sclerotinia stem rot (*Sclerotinia sclerotiorum*) in oilseed Brassica. Journal of Microbiological Methods 175:105983. DOI: 10.1016/j.mimet.2020.105983.
28. Hassen AI, Labuschagne N (2010) Root colonization and growth enhancement in wheat and tomato by rhizobacteria isolated from the rhizoplane of grasses. World Journal of Microbiology and Biotechnology 26:1837-1846. DOI 10.1007/s11274-010-0365-z.
29. Hu X, Robertsb DP, Xiea L, Qina L, Lia Y, Liaoa X, Hana P, Yua C, Liaoa X (2019) Seed treatment containing *Bacillus subtilis* BY-2 in combination with other *Bacillus* isolates for control of *Sclerotinia sclerotiorum* on oilseed rape. Biological Control 133:50-57. https://doi.org/10.1016/j.biocontrol.2019.03.006.
30. Huang X, Zhang N, Yong X, Yang X, Shen Q (2012) Biocontrol of *Rhizoctonia solani* damping-off disease in cucumber with *Bacillus pumilus* SQR-N43. Microbiological Research. 167 (3):135-143. https://doi.org/10.1016/j.micres.2011.06.002.

31. Horner NR, Grenville-Briggs LJ, van West P (2012) The oomycete *Pythium oligandrum* expresses putative effectors during mycoparasitism of *Phytophthora infestans* and is amenable to transformation. Fungal Biology. 116(1):24-41. 10.1016/j.funbio.2011.09.004.
32. Jeger M, Beresford R, Bock C, Brown N, Fox A, Newton A, Vicent A, Xu X, Yuen J (2021) Global challenges facing plant pathology: multidisciplinary approaches to meet the food security and environmental challenges in the mid-twenty-first century. CABI Agric Biosci.2,:20. https://doi.org/10.1186/s43170-021-00042-x.
33. Kamal MM, Lindbeck KD, Savocchia S, Ash GJ, (2015) Biological control of sclerotinia stem rot of canola using antagonistic bacteria. Plant Pathology 64 (6):1375-1384. Doi: 10.1111/ppa.12369.
34. Kannan V, Sureendar R (2009) Synergistic effect of beneficial rhizosphere microflora in biocontrol and plant growth promotion. Journal of Basic Microbiology. 49(2):158-164. doi:10.1002/jobm.200800011.
35. Karthika S, Varghese S, Jisha MS (2020 Exploring the efficacy of antagonistic rhizobacteria as native biocontrol agents against tomato plant diseases. 3 Biotech. 10:320. https://doi.org/10.1007/s13205-020-02306-1.
36. Köhl J, Medeiros F HV, Plas CLVD, Lia de Haas G, Bosch TVD (2020) Efficacies of bacterial and fungal isolates in biocontrol of *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* and growth promotion in tomato do not correlate. Biological Control 150:104375. https://doi.org/10.1016/j.biocontrol.2020.104375.
37. Latha, P., Anand, T., Prakasam, V., Jonathan, EI., Paramathma, M., 2011. Combining *Pseudomonas*, *Bacillus* and *Trichoderma* strains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut. Applied Soil Ecology 49: 215-223.
38. Le Floch G, Rey P, Déniel F, Benhamou N, Picard K, Tirilly Y (2003) Enhancement of development and induction of resistance in tomato plants by the antagonist, *Pythium oligandrum*. Agronomie 23:455-460, https://doi.org/10.1051/agro:2003018.
39. Liu K, McInroy JA, Hu CH, Kloeppe JW (2018) Mixtures of plant-growth-promoting rhizobacteria enhance biological control of multiple plant diseases and plant-growth promotion in the presence of pathogens. Plant Disease 102:67-72. https://doi.org/10.1094/PDIS-04-17-0478-RE.
40. Liu J, Hagberg I, Novitsky L, Hadj-Moussa H, Tyler JA (2014) Interaction of antimicrobial cyclic lipopeptides from *Bacillus subtilis* influences their effect on spore germination and membrane permeability in fungal plant pathogens. Fungal biology. 118, 855-861.
41. Macena AMF, Kobori NN, Mascarin GM, Vida JB, Hartman GL (2020) Antagonism of *Trichoderma*-based biofungicides against Brazilian and North American isolates of *Sclerotinia sclerotiorum* and growth promotion of soybean. BioControl 65:235-246. https://doi.org/10.1007/s10526-019-09976-8.
42. Mamaní-Gonzáles SY, Vizgarra ON, Mendez DE, Espeche CM, Jalil AC, Ploper LD, de poroto C, (2015) 2015 bean season: results of field trials and analysis. Agroindustrial Report. Statistics and margins of Tucumán crops EEAOC Nº108.
43. McDougall JA, Helmick CG, Lim SS, Johnston JM, Gaddy JR, Gordon C, Ferucci ED (2018) Differences in the diagnosis and management of systemic lupus erythematosus by primary care and specialist providers in the American Indian/Alaska Native population, Lupus. 27 :1169-1176. https://doi.org/10.1177/0961203318763529.
44. Michelland RJ, Dejean S, Combes S, Fortun-Lamothe L, Cauquil L (2009) StatFingerprints: a friendly graphical interface program for processing and analysis of microbial fingerprint profiles. Molecular Ecology Resources. 9:1359-1363.
45. Miethling R, Wieland G, Backhaus H, Tebbe CC (2000) Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L33. Microbial Ecology 40: 43-56, https://doi.org/10.1007/s002480000021.
46. Niu B, Wang W, Yuan Z, Sederoff RR, Sederoff H, Chiang VL, Borriss R (2020) Microbial Interactions Within Multiple-Strain Biological Control Agents Impact Soil-Borne Plant Disease. Frontiers in Microbiology 11:585404. doi:

10.3389/fmicb.2020.585404.

47. Ouhaibi-Ben Abdeljalil N, Vallance J, Gerbore J, Rey P, Daami-Remadi, M (2016a) Bio-suppression of Sclerotinia stem rot of tomato and biostimulation of plant growth using tomato-associated rhizobacteria. Journal of Plant Pathology and Microbiology 7:1-11, http://doi.org/10.4172/2157-7471.1000331.
48. Ouhaibi-Ben Abdeljalil N, Renault D, Gerbore J, Vallance J, Rey P, Daami-Remadi, M (2016b) Comparative efficacy of tomato-associated rhizobacteria strains used singly or in combination in suppressing Rhizoctonia root rot and enhancing tomato growth. Journal of Microbial & Biochemical Technology 8:110-119. http://doi.org/10.4172/1948-5948.1000272.
49. Ouhaibi-Ben Abdeljalil N, Renault D, Gerbore J, Vallance J, Rey P, Daami-Remadi M (2016c) Evaluation of the effectiveness of tomato-associated rhizobacteria applied singly or as three-strain consortium for biosuppression of Sclerotinia stem rot in tomato. Journal of Microbial & Biochemical Technology 8: 312-320. doi: 10.4172/1948-5948.1000302.
50. Ouhaibi-Ben Abdeljalil N, Vallance J, Gerbore J, Yacoub A, Daami-Remadi M, Rey P (2021) Combining potential oomycete and bacterial biocontrol agents as a tool to fight tomato Rhizoctonia root. Biological Control 155:104521. https://doi.org/10.1016/j.biocontrol.2020.104521.
51. Pal KK, Mc B, Gardener S (2006) Biological Control of Plant Pathogens. Plant Health Instructor. 1-25. DOI: 10.1094/PHI-A-2006-1117-02.
52. Picard, K., Ponchet, M., Blein, JP., Rey, P., Tirilly, Y., Benhamou, N., 2000. Oligandrin. A proteinaceous molecule produced by the mycoparasite *Pythium oligandrum* induces resistance to *Phytophthora parasitica* infection in tomato plants. Plant Physiology. 124(1),379-395.
53. Pisarcik M, Hakl J, Szabo O, Hrevusov Z (2021) Efficacy of variable timing of *Pythium oligandrum* applications on red clover grown under field conditions. Crop Protection 149:105780. https://doi.org/10.1016/j.cropro.2021.105780.
54. Pylak M, Oszust K, Frac M (2019) Review report on the role of bioproducts, biopreparations, biostimulants and microbial inoculants in organic production of fruit. Reviews in Environmental Science and Biotechnology. 18:597-616. https://doi.org/10.1007/s11157-019-09500-5.
55. Raymaekers K, Ponet L, Holtappels D, Berckmans B, Cammue BPA (2020) Screening for novel biocontrol agents applicable in plant disease management - A review. Biological Control 144:104240. https://doi.org/10.1016/j.biocontrol.2020.104240.
56. Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N (2010) The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leaves. Environmental microbiology 12:2885-2893.
57. Renault D, Vallance J, Déniel F, Wery N, Godon JJ, Barbier G, Rey P(2012) Diversity of Bacterial Communities that Colonize the Filter Units Used for Controlling Plant Pathogens in Soilless Cultures. Microbial Ecology 63:170-187. DOI 10.1007/s00248-011-9961-1
58. Rey P, Floch GL, Benhamou N, Tirilly Y (2008) *Pythium oligandrum* biocontrol: its relationships with fungi and plants. In: Ait Barka, E., Clément, C. (Eds.), Plant-Microbe Interactions. Research Signpost, Kerala, India, pp. 43-67.
59. Roberts DP, Kobayashi DY (2011) Impact of spatial heterogeneity within spermosphere and rhiziosphere environments on performance of bacterial biological control agents. In: Maheshwari, D.K. (Ed.), Bacteria in Agrobiology: Crop Ecosystems.
60. Romero FM, Marina M, Pieckenstain FL (2015) Novel components of leaf bacterial communities of field-grown tomato plants and their potential for plant growth promotion and biocontrol of tomato diseases. Research in Microbiologoy. 167(3):222-33 doi: 10.1016/j.resmic.2015.11.001.
61. Rostami S, Maleki M, Shahriari D (2013) The use of *Bacillus amyloliquefaciens* to control of Sclerotinia stem rot (*Sclerotinia Sclerotiorum*) of cucumber. International Journal of Farming and Allied Sciences. 22: 965-970.

62. Schmidt CS, Mrnkal L, Lovecká P, Frantík T, Fenclová M, Demnerova K, Vosátkal M (2021) Bacterial and fungal endophyte communities in healthy and diseased oilseed rape and their potential for biocontrol of *Sclerotinia* and *Phoma* disease. Scientific Reports 11:3810.| https://doi.org/10.1038/s41598-021-81937-7.
63. Sun G, Yao T, Feng C, Chen L, Li J, Wang L (2016) Identification and biocontrol potential of antagonistic bacteria strains against *Sclerotinia sclerotiorum* and their growth-promoting effects on *Brassica napus*. Biologicol control 104 :35-43. doi: http://dx.doi.org/10.1016/j.biocontrol.2016.10.008.
64.
65. Sabaté DC, Pérez Brandan C, Petroselli G, Erra-Balsells R, Audisio MC (2018) Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary on common bean by native lipopeptide-producer *Bacillus* strains. Microbiological Research 211:21-30. DOI: 10.1016/j.micres.2018.04.003.
66. Saraf M, Pandya U, Thakkar A (2014) Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. Microbiological Research 169:18-29.
67. Sarma BK, Yadav SK, Singh S, Singh HB (2015) Microbial consortium-mediated plant defense against phytopathogens: Readdressing for enhancing efficacy. Soil Biology and Biochemistry, 87: 25-33, https://doi.org/10.1016/j.soilbio.2015.04.001.
68. Sharifazizi M, Harighi B, Sadeghi A (2017) Evaluation of biological control of *Erwinia amylovora*, causal agent of fire blight disease of pear by antagonistic bacteria. Biological Control. 104: 28-34. http://dx.doi.org/10.1016/j.biocontrol.2016.10.007.
69. Singh A, Singh VK, Dwivedy AK, Tiwari-Dwivedi A, Dubey NK (2020) Biological Control of Plant Diseases: Opportunities and Limitations. Plant Microbiome Paradigm. https://doi.org/10.1007/978-3-030-50395-6_7.
70. Stefan M, Munteanu N, Stoleru V, Mihasan M, Hritcu L (2013) Seed inoculation with plant growth promoting rhizobacteria enhances photosynthesis and yield of runner bean (*Phaseolus coccineus* L.). Scientia Horticulturae. 151: 22-29.
71. Takenaka S, Sekiguchi H, Nakaho K, Tojo M, Masunaka A, Takahashi H (2008) Colonization of *Pythium oligandrum* in the tomato rhizosphere for biological control of bacterial wilt disease analyzed by Real-Time PCR and confocal laser-scanning microscopy. Biological Control. 98: 2-187. doi:10.1094/PHYTO-98-2-0187.
72. Torres MJ, Pérez C, Brandan-Sabaté DC, Petroselli G, Erra-Balsells MC, Audisio R (2017) Biological activity of the lipopeptide-producing Bacillus amyloliquefaciens PGPBacCA1 on common bean Phaseolus vulgaris L., pathogens. Biological Control 105:93-99. https://doi.org/10.1016/j.biocontrol.2016.12.001.
73. Torres MJ, Pérez-Brandan C, Petroselli G, Erra-Balsells R, Audisio MC (2016) Antagonistic effects of *Bacillus subtilis* subsp. *subtilis* and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. Microbiological Research. 182:31-39.
74. Trotel-Aziz P, Abou-Mansour E, Courteaux B, Rabenoelina F, Clément C, Aziz A (2019) *Bacillus subtilis* PTA-271 counteracts Botryosphaeria dieback in grapevine, triggering immune responses and detoxification of fungal phytotoxins. Frontiers in Plant Science. 10. DOI: 10.3389/fpls.2019.00025.
75. Vallance J, Le Floch G, Déniel F, Barbier G, Lévesque CA, Rey P (2009) *Pythium oligandrum* biocontrol in the rhizosphere: influence on fungal and oomycete population dynamics. Applied and Environmental Microbiology 75:4790-4800, https://doi.org/10.1128/AEM.02643-08.
76. Vallance J, Deniel F, Barbier G, Guerin-Dubrana L, Benhamou N, Rey P (2012) Influence of *Pythium oligandrum* on the bacterial communities that colonize the nutrient solutions and the rhizosphere of tomato plants. Canadian Journal of Microbiology 58(9):1124-1134.
77. Velandia CAM, Ongena M, Kloepper JW, Cotes AM (2021) Biosynthesis of Cyclic Lipopeptides by *Bacillus velezensis* Bs006 and its Antagonistic Activity are Modulated by the Temperature and Culture Media Conditions. Current Microbiology 78:3505-3515. https://doi.org/10.1007/s00284-021-02612-8.

78. Wang X, Zhang X, Liu L, Xiang M, Wang W, Sun X, Che Y, Guo L, Liu-Wang G, Yin, C, WB M, Zhang X, Liu X (2015) Genomic and transcriptomic analysis of the endophytic fungus Pestalotiopsis fici reveals its lifestyle and high potential for synthesis of natural products. BMC Genomics 16:28. doi: 10.1186/s12864-014-1190-9.
79. White TJ, Bruns TD, Lee S. Taylor J (1990) Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal RNA genes. PCR Protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ & White TJ, eds), pp. 315-322. Academic Press, New York, NY.
80. Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G, Lorito M (2014) Trichoderma-based products and their widespread use in agriculture. The Open Mycology Journal. 8:71-126. doi: 10.2174/1874437001408010071.
81. Wu Y, Yuan J, Raza W, Shen Q, Huang Q (2014) Biocontrol traits and antagonistic potential oaf *Bacillus amyloliquefaciens* strain NJZJSB3 against *Sclerotinia sclerotiorum*, a causal agent of canola stem rot. Journal of Microbiology and Biotechnology 24: 1327-1336. http://dx.doi.org/10.4014/jmb.1402.02061.
82. Yacoub A, Magnin N, Gerbore J, Haidar R, Bruez E, Compant S, Guyoneaud R, Rey P (2020) The biocontrol root-Oomycete, *Pythium oligandrum*, triggers grapevine resistance and shifts in the transcriptome of the trunk pathogenic fungus, *Phaeomoniella chlamydospora*. International Journal of Molecular Sciences 21:6876. doi:10.3390/ijms21186876.
83. Zhang W, Yan J, Li X, Xing Q, Chethana KWT, Zhao W (2019) Transcriptional response of grapevine to infection with the fungal pathogen *Lasiodiplodia theobromae*. Scientific Report. 9:5387. DOI: 10.1038/s41598-019-41796-9.

Tables

Table 1

Main traits of the selected rhizobacterial strains explored in the present investigation.

Molecular identification	**Biochemical characterization**				**Detection of antibiotic biosynthesis genes**			**PGPR traits**	
Strain	Accession No.	Gram	Oxidase activity	Catalase activity	Fen A	Bac D	IAA	Phosphate solubilization	Siderophore production
Bacillus thuringiensis **str.B2**	KU158884	+	+	+	-	+	+	+	+
Bacillus subtillis **str.B10**	KT921327	+	+	+	+	+	+	+	+++
Enterobacter cloacae **str.B16**	KT921429	-	-	+	+	+	+	+	+++

Positive reaction (+); Negative reaction (–); Fen A: Fengycin A, Bac: Bacillomycin D, IAA: Indole-3- acetic acid. All isolates were negative for hypersensitive reaction (HR) on tobacco leaves.

PGPR: Plant Growth Promoting Rhizobacteria.

Table 2

Antifungal potential displayed by tomato-associated rhizobacteria against *Sclerotinia sclerotiorum* mycelial growth under *in vitro* conditions using dual culture plate test, noted after 5 days of incubation at 25°C.

Biological treatments	Strains	Colony diameter (mm)	Growth inhibition (%)	Inhibition zone (mm)
Untreated control	C	90.00 a	0	0.00 c
Bacillus thuringiensis	B2	53.40 b	41	10.60 a
B. subtilis	B10	45.60 c	49	9.00 ab
Enterobacter cloacae	B16	41.80 c	54	7.60b

For each column, values followed by the same letter are not significantly different according to Student–Newman–Keuls test (at $P \leq 0.05$).

Table 3

Effect of a three-strain bacterial consortium mixed or not with *Pythium oligandrum* Po37 on Sclerotinia stem rot disease incidence and severity on tomato cv. Rio Grande plants compared to fungicide and untreated controls, noted 60 days post-planting, based on two consecutive years of testing.

	2012		2013	
Biological treatments tested	DI* (%)	DS **	DI (%)	DS
Untreated control	0	0 d	0	0 c
Pythium oligandrum Po37	0	0 d	0	0 c
B2+B10+B16 [a]	0	0 d	0	0 c
Po37+B2+B10+B16	0	0 d	0	0 c
S. sclerotiorum-inoculated control	100	4.00 a (0)[c]	100	4.00 a (0)[c]
S. sclerotiorum + Po37	80.00	1.13 c (72)	80.00	1.13 b (72)
S. sclerotiorum +B2+B10+B16	26.67	0.27 d (93)	86.66	1.00 b (75)
S. sclerotiorum+ Po37+B2+B10+B16	41.67	0.40 d (90)	73.33	1.00 b (75)
S. sclerotiorum + Fungicide[b]	100	2.00 b (50)	80.00	1.27 b (68)

*DI: Disease incidence; **DS: Disease severity

[a] B2: *Bacillus thuringiensis* str. B2 (KU158884); B10: *Bacillus subtilis* str. B10 (KT921327) and B16: *Enterobacter cloacae* str. B16 (KT921429).

[b] Fungicide-based treatment using Previcur® (Bayer, France, propamocarb hydrochloride 722 g/L) applied at 0.5 mL/L.

[c] Values in brackets (in %) indicate the percentage of disease severity decrease compared to the inoculated and untreated control plants.

For each column, values followed by the same letter are not significantly different according to Student–Newman–Keuls test (at $P \leq 0.05$).

Table 4

Efficacy of the three-strain bacterial consortium, mixed or not with *Pythium oligandrum* Po37, on the height of tomato cv. Rio Grande plants noted 60 days post-planting, based on two consecutive years of testing.

	2012		2013	
Biological treatments tested	Plant height (cm)	%[c]	Plant height (cm)	%[c]
Untreated control	28.13 c	0	28.13 e	0
P. oligandrum Po37	53.27 b	47	55.47 bc	49
B2+B10+B16[a]	54.13 b	48	58.87 b	52
Po37+B2+B10+B16	74.53 a	62	69.80 a	60
S. sclerotiorum-inoculated control	9.60 e	0	9.60 f	0
S. sclerotiorum + Po37	48.47 b	80	49.13 d	80
S. sclerotiorum +B2+B10+B16	52.07 b	82	48.47 d	80
S. sclerotiorum+ Po37+B2+B10+B16	50.00 b	81	52.13 cd	82
S. sclerotiorum + Fungicide[b]	19.67 d	51	27.40 e	65

[a]B2: *Bacillus thuringiensis* str. B2 (KU158884); B10: *Bacillus subtilis* str. B10 (KT921327) and B16: *Enterobacter cloacae* str. B16 (KT921429).

[b]Fungicide-based treatment using Previcur® (Bayer, France, propamocarb hydrochloride 722 g/L) applied at 0.5 mL/L.

[c]Values (in %) indicate the percentage of plant height increase as compared to *S. sclerotiorum*-inoculated and untreated controls.

For each column, values followed by the same letter are not significantly different according to Student–Newman–Keuls test at $P \leq 0.05$.

Table 5

Effect of the three-strain bacterial consortium, mixed or not with *Pythium oligandrum* Po37, on the aerial part fresh weight of tomato cv. Rio Grande plants, compared to fungicide and untreated controls, noted 60 days post-planting based on two consecutive years of testing.

	2012		2013	
Biological treatments tested	APFW[c](g)	%[d]	APFW (g)	%[d]
Untreated control	29.38 c	0	24.86 c	0
P. oligandrum Po37	30.96 bc	5	30.44 b	18
B2+B10+B16[a]	50.94 a	42	51.94 a	52
Po37+B2+B10+B16	35.57 b	17	55.06 a	55
S. sclerotiorum-inoculated control	17.21 e	0	16.69 d	0
S. sclerotiorum + Po37	26.4 cd	35	26.80 bc	38
S. sclerotiorum +B2+B10+B16	26.24 cd	34	27.62 bc	40
S. sclerotiorum+ Po37+B2+B10+B16	26.80 cd	36	30.26 b	45
S. sclerotiorum+ Fungicide[b]	23.85 d	28	23.33 c	28

[a]B2: *Bacillus thuringiensis* str. B2 (KU158884); B10: *Bacillus subtilis* str. B10 (KT921327) and B16: *Enterobacter cloacae* str. B16 (KT921429).

[b]Fungicide-based treatment using Previcur® (Bayer, France, propamocarb hydrochloride 722 g/L) applied at 0.5 mL/L.

[c]APFW: Aerial Part Fresh Weight.

[d]Values (in %) indicate the percentage of the aerial part fresh weight increase compared to *S. sclerotiorum*-inoculated and untreated controls.

For each column, values followed by the same letter are not significantly different according to Student–Newman–Keuls test at $P \leq 0.05$.

Table 6

Effect of the three-strain bacterial consortium, mixed or not with *Pythium oligandrum* Po37, on the root fresh weight of tomato cv. Rio Grande plants, compared to fungicide and untreated controls, noted 60 days post-planting based on two consecutive years of testing.

	2012		2013	
Biological treatments tested	RFW[c] (g)	%[d]	RFW (g)	%
Untreated control	3.03 c	0	4.41 de	0
P. oligandrum Po37	4.32 b	30	6.77 b	35
B2+B10+B16[a]	6.21 a	51	8.25 a	47
Po37+B2+B10+B16	5.21 ab	42	8.78 a	50
S. sclerotiorum-inoculated control	1.78 d	0	1.78 g	0
S. sclerotiorum + Po37	2.92 c	39	4.01 ef	55
S. sclerotiorum +B2+B10+B16	2.36 cd	24	4.97 cd	64
S. sclerotiorum+ Po37+B2+B10+B16	4.23 b	58	5.51 c	68
S. sclerotiorum+ Fungicide[b]	2.97 c	40	3.41 f	48

[a]B2: *Bacillus thuringiensis* str. B2 (KU158884); B10: *Bacillus subtilis* str. B10 (KT921327) and B16: *Enterobacter cloacae* str. B16 (KT921429).

[b]Fungicide-based treatment using Previcur® (Bayer, France, propamocarb hydrochloride 722 g/L) applied at 0.5 mL/L.

[c]RFW: Root Fresh Weight.

[d]Values (in %) indicate the percentage of the root fresh weight increase as compared to *S. sclerotiorum*-inoculated and untreated control.

For each column, values followed by the same letter are not significantly different according to Student–Newman–Keuls test at $P \leq 0.05$.

Figures

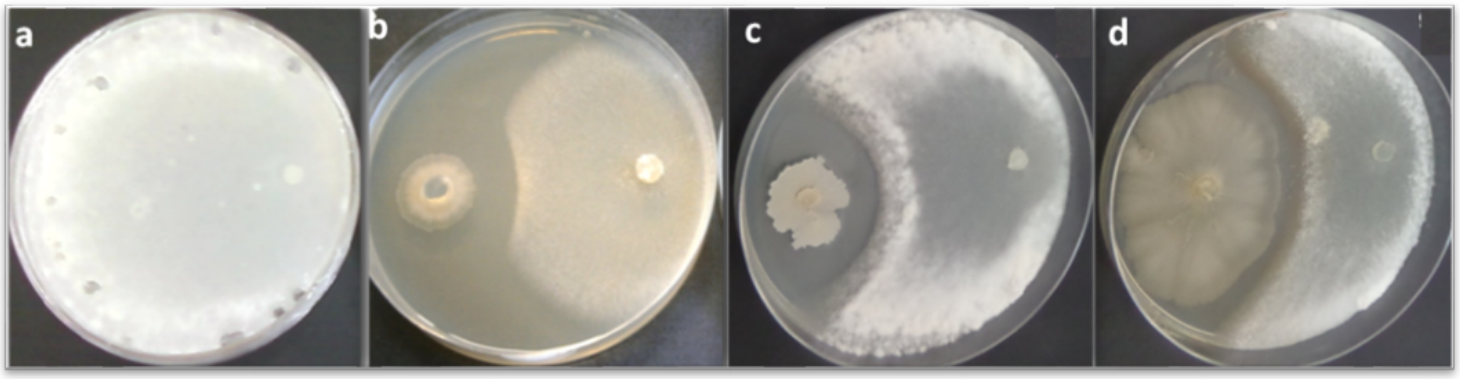

Figure 1

In vitro growth inhibition of *Sclerotinia sclerotiorum* due to diffusible metabolites from tomato-associated rhizobacterial strains compared to untreated controls.

a) *Sclerotinia sclerotiorum* control plate, b) B10: *Bacillus subtilis* str. B10 (KT921327), c) B16: *Enterobacter cloacae* str. B16 (KT921429) and d) B2: *Bacillus thuringiensis* str. B2 (KU158884).

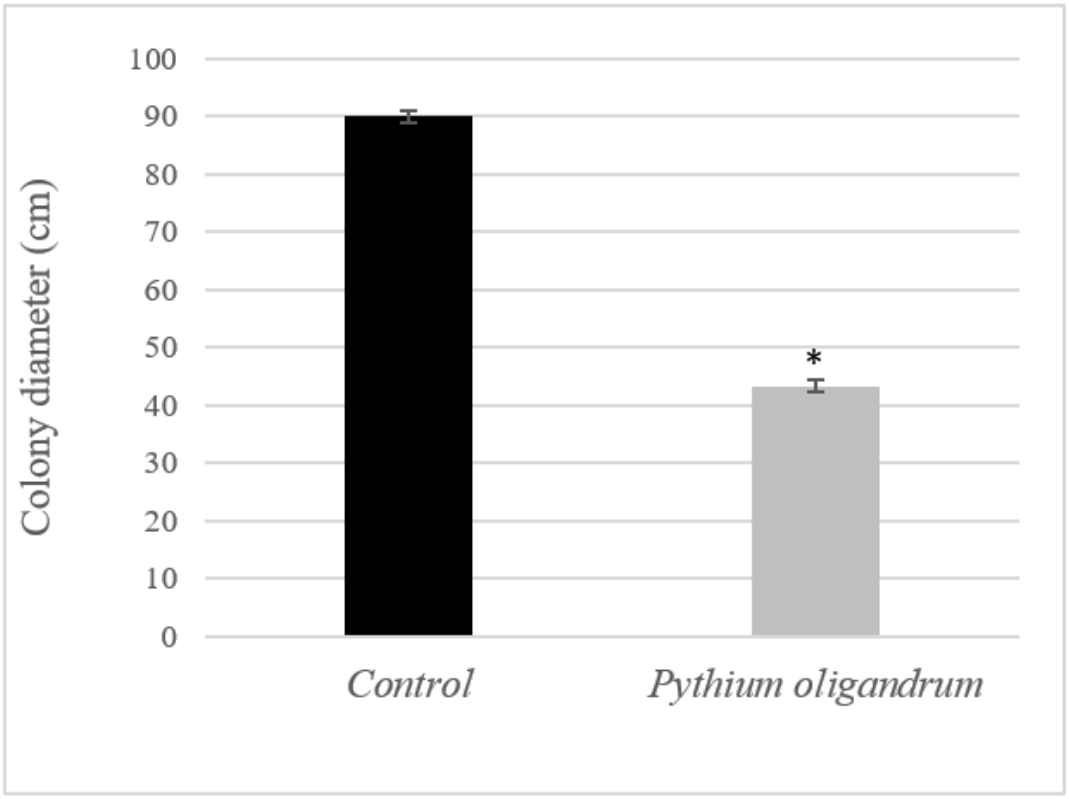

Figure 2

Antifungal potential of *Pythium oligandrum* Po37 against the mycelial growth of *Sclerotinia sclerotiorum* compared to control cultures measured after 5 days of incubation at 25°C.

*: Indicates means that are, within the trial, significantly different between the two treatments at $P < 0.05$ (ANOVA).

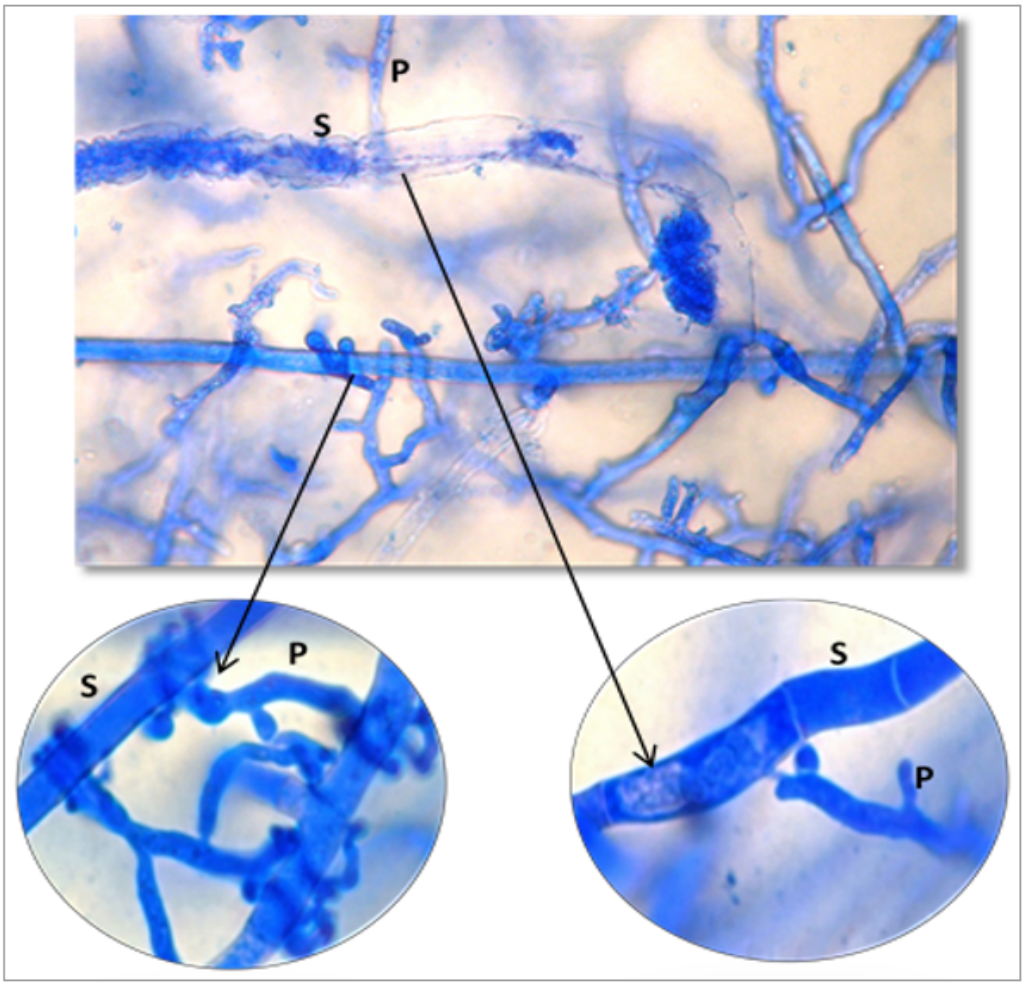

Figure 3

Scanning light micrograph of *Pythium oligandrum* hyphae interacting with those of *Sclerotinia sclerotiorum*.

a) Condensed coiling of *P. oligandrum* around a hypha of *S. sclerotiorum*. Then *P. oligandrum* hypha formed hooks and attached itself to *S. sclerotiorum* hypha (arrow). b) Mycelial abnormality of *S. sclerotiorum* caused by the antimicrobial activity of *P. oligandrum.* The arrow shows digested zone with penetration sites.

P: *Pythium oligandrum* Po37; S: *Sclerotinia sclerotiorum*

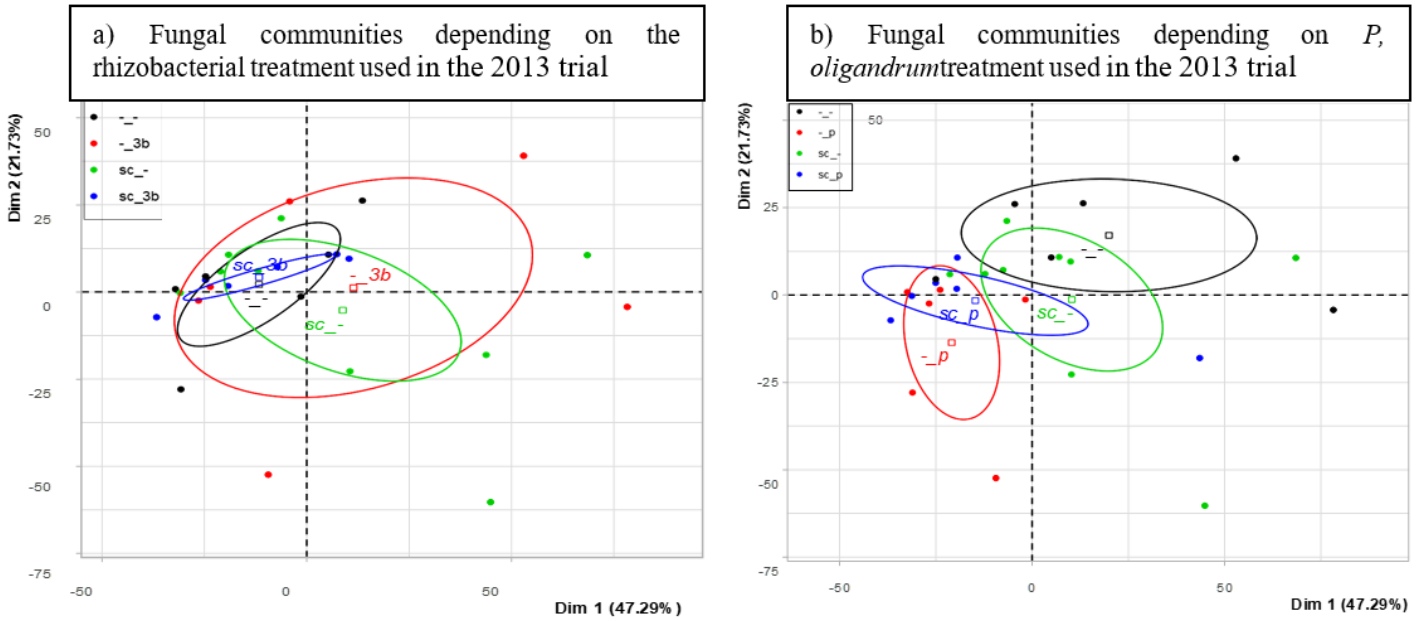

Figure 4

Distribution of the fungal communities on the principal plans defined by the first two axes obtained in the principal component analysis (PCA) of SSCP profiles depending on rhizobacterial treatments **(a)** and *Pythium oligandrum* treatments **(b)** tested singly or as microbial consortium during 2013 trial.

(a) The colors used here represent the profiles depending on tested treatments (3b: 3 tomato-associated rhizobacteria, applied as consortium (red); 3bpsc: 3 rhizobacterial consortium mixed with *P. oligandrum*, against *S. sclerotiorum* (blue); inoculated and untreated plants (green) and -: uninoculated and untreated plants (black)).

(b) The colors used here represent (-p: *P. oligandrum* (red); psc: *P. oligandrum* tested against *S. sclerotiorum* (blue); sc-: inoculated and untreated plants (green) and -: uninoculated and untreated plants (black)).

The variation (%) explained by each PCA axis is given in brackets. Ellipsoids draw the center of factors with 95% confidence.

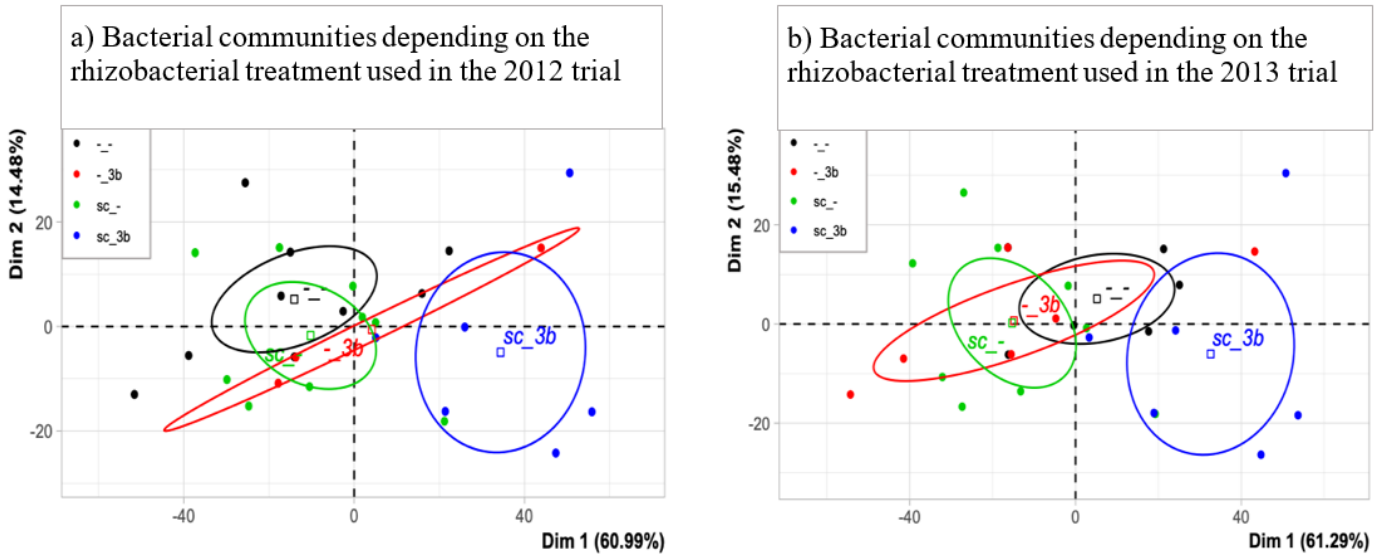

Figure 5

Principal Component Analysis (PCA) of the bacterial communities colonizing the rhizosphere of tomato plants (cv: Rio Grande) during two consecutive years, 2012 (a) and 2013 (b), based on SSCP profiles.

The colors used here represent the profiles depending on the rhizobacterial treatment used (_3b: 3 tomato-associated rhia zobacteria, applied as caonsortium (red); sc_3b: 3 tomato-associated rhizobacteria, applied as consortium against *S. sclerotiorum* (blue); sc_-: *S. sclerotiorum* inoculated and untreated plants (green); and -_-: uninoculated and untreated plants (black)).

The variation (%) explained by each PCA axis is given in brackets. Ellipsoids draw the center of factors with 95% confidence