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Fusarium wilt biocontrol and tomato growth stimulation, using endophytic bacteria naturally associated with *Solanum sodomaeum* and *S. bonariense* plants

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Abstract

Background: Fusarium wilt biocontrol using endophytic microorganisms may represent a potentially attractive and environmentally safe alternative since endophytes could better limit disease incidence and severity through inhibition of the systemic fungus progress.

Main body of the abstract: Twenty-three endophytic bacterial isolates, naturally associated with *Solanum sodomaeum* and *Solanum bonariense*, were evaluated for their ability to control Fusarium wilt of tomato induced by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and to promote plant growth. Selected endophytic isolates were screened in vivo, using the root dipping and the culture substrate drenching methods. The most bioactive isolates were subjected to morphological and biochemical characterization and subsequent identification through 16S rDNA sequencing genes. Seven isolates (*Stenotrophomonas maltophilia* S23, S24, S26 and S28; *Bacillus* sp. SV81; *Azotobacter chroococcum* S11; and *Serratia marcescens* S14) were found to be the most efficient in reducing disease severity by 82–96% over control. Treatments with these isolates led to a significant enhancement in growth parameters, estimated at 45.5–61 and 24.2–70.5% than the control, in tomato plants infected or not with FOL, respectively. Diffusible and volatile metabolites released from bacterial cultures had significantly limited FOL radial growth. All isolates were positive for indole-3-acetic acid (IAA) production. *S. marcescens* S14, *S. maltophilia* S28, and *Bacillus* sp. SV81 exhibited a positive phosphate solubilization activity. Production of chitinase, protease, pectinase, and hydrogen cyanide were also investigated.

Short conclusion: This study clearly demonstrated that endophytic bacteria recovered from these 2 *Solanum* species can be explored as promising biocontrol agents active against FOL and are able to enhance tomato growth.

Keywords: Biocontrol, Endophytic bacteria, *Fusarium oxysporum* f. sp. *lycopersici*, Tomato growth, *Solanum* spp

Background

Tomato (*Solanum lycopersicum* L.) is an economically important vegetable crop worldwide. However, it is threatened by various soilborne fungal diseases such as Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hans (FOL). This fungus is responsible for considerable crop losses (Hussain et al. 2016). Main disease symptoms include chlorosis and progressive leaf wilting, vascular tissue discoloration, stunting, and, in most severe cases, eventual wilt of the entire plant (Aydi Ben Abdallah et al. 2016b).

Disease management is difficult due to the soilborne nature of the causal agent and its ability to colonize the vascular tissues of infected plants and the emergence of new and aggressive pathogen physiological races. Therefore, the use of resistant tomato varieties lost its

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effectiveness as the mutation in *Fusarium* species. Furthermore, chemical control is now losing its ground due to adverse effects of chemicals on environment and soil microbiota that calls for alternative inputs with low dependency on chemicals for sustainable agriculture (Fatima and Anjum 2017).

The use of endophytic microorganisms as a biological control agent has become an attractive, promising, and eco-friendly alternative since this agent could inhibit the vascular progress of the target pathogen, limiting more efficiently disease incidence and severity (de Lamo et al. 2018; Constantin et al. 2019). These endophytes colonize plant parts without causing any adverse effects. On many hosts, they act as plant growth-promoting, and/or biocontrol agents by direct antagonism or via the host by triggering induced resistance (Constantin et al. 2019; Passari et al. 2019). For instance, endophytic bacteria namely, *Bacillus mojavensis* was shown able to promote growth of maize plants infected with *Fusarium verticillioides* (Kalai-Grami et al. 2014). *Collimonas arenae* Cal35 showed the highest in vitro antifungal activity against FOL, the causal agent of Fusarium wilt of tomato. In field trial, this bacterial isolate was found to be more active when combined with *Bacillus*-based biofungicide in reducing disease severity and the relative abundance of *F. oxysporum* in the root endosphere of tomato plants challenged with FOL (Doan et al. 2020).

Various mechanisms, such as antibiotics synthesis, cell wall-degrading enzymes, competition for nutrients and minerals, and/or systemic resistance induction, are implicated in pathogen inhibition (Lugtenberg et al. 2013), while plant growth stimulation was achieved through indole-3-acetic acid (IAA) and siderophore production, phosphate solubilization, and nitrogen fixation (Rosenblueth and Martínez-Romero 2006).

More attention was given to wild *Solanum* species as attractive sources of biologically active molecules with antifungal and antibacterial activities as previously shown for *S. torvum* (Bari et al. 2010), *S. trilobatum* and *S. surattense* (Tuba et al. 2016), and *Solanum linnaeanum* (Nefzi et al. 2018a). In addition, *Solanum* spp. were extensively used as potent isolation sources of endophytic bacteria with plant growth-promoting and/or biocontrol properties, as demonstrated for *Solanum elaeagnifolium* (Aydi Ben Abdallah et al. 2016a), *S. trilobatum* (Bhuvaneswari et al. 2013), and *S. melongena* and *S. torvum* (Achari and Ramesh 2014). Furthermore, fungi naturally associated with *S. linnaeanum* are used for the biocontrol of Fusarium crown root rot disease and for the enhancement of tomato growth (Nefzi et al. 2018b).

In the present study, bacterial isolates collected from surface-sterilized stems of *Solanum sodomaeum* and *Solanum bonariense* were evaluated for their ability to stimulate growth of tomato plants and to control Fusarium wilt.

Material and methods

Tomato seedling preparation

Tomato cv. Rio Grande cultivar, susceptible to FOL races 2 and 3, was used in this study. Seedlings were grown in 7 × 7 cm-alveolus plates, filled with sterilized peat® (Floragard Vertriebs GmbH für gartenbau, Oldenburg), regularly watered and maintained under 16 h photoperiod, 60–70% RH, and 20–30 °C air temperatures. For all trials, seedlings were used at two-true-leaf growth stage.

Pathogen growth conditions

F. oxysporum f. sp. *lycopersici* isolate used in this study was originally recovered from tomato plants showing Fusarium wilt symptoms and severe vascular discoloration. The pathogen was re-isolated from artificially infected plants, fulfilling Koch's postulates, and identified in a previous study (Aydi Ben Abdallah et al. 2016b). It was grown on potato dextrose agar (PDA) medium at 25 °C for 7 days.

Plant sampling and isolation of endophytic bacteria

Symptomless wild *S. sodomaeum* and *S. bonariense* plants, growing nearby tomato-cropping fields with long history of soilborne diseases, were targeted as a source of endophytic bacteria. Stem, leaf, and fruit samples (5 each) were individually disinfected. Twenty pieces (1 cm in length) of sterilized organ tissues were aseptically placed onto nutrient agar (NA) medium (Aydi Ben Abdallah et al. 2016b). Three stem pieces were also pressed using a sterile-nipper and the released liquid was spread on NA medium. The disinfection process efficiency was checked as previously detailed (Aydi Ben Abdallah et al. 2016a). Plates were maintained for 48 h at 25 °C. Developing bacterial colonies showing distinct macro-morphological traits were picked separately onto NA. Before their use in different assays, stock cultures maintained at − 20 °C in nutrient broth (NB) added 40% glycerol were cultured at 25 °C for 48 h onto NA.

Endophytic colonization ability

Collected bacterial isolates were cultured to NA supplemented with 100 μg/ml (w/v) of streptomycin sulfate and 100 μg/ml (w/v) of rifampicin (Chen et al. 1995). Those behaving as resistant to both antibiotics were further investigated for their endophytic potential when applied onto tomato seedlings. The wild-type ones were used for tomato cv. Rio Grande inoculation using the root dipping method. Control seedlings were soaked in sterile distilled water (SDW) only. Seedlings were transferred to individual pots (12.5 cm × 14.5 cm) filled with commercialized peat. Five seedlings were used for each individual treatment. At 60 days post-inoculation, inoculated isolates were re-isolated on NA amended with both

antibiotics. After incubation at 25 °C for 48 h, bacterial colonies showing similar morphological traits as the wild-type ones were selected and classified as endophytes.

Hypersensitivity reaction and hemolytic activity

Hypersensitivity test was carried out according to Nawangsih et al. (2011) method. Isolates leading to the formation of chlorotic and/or necrotic zones on inoculated leaf areas were considered phytopathogens and removed from the next trials. Endophytic isolates were tested for their capacity to degrade hemoglobin according to Murray et al. (2003) protocol. Isolates showing a positive hemolytic activity, as determined by the development of clear zones around their colonies, were classified as human pathogens and excluded from further investigations.

Assessment of plant growth-promoting ability

Selected endophytic isolates were evaluated for their plant growth-promoting ability. Treated and control seedlings (two-true-leaf stage) were root dipped for 30 min into bacterial cell suspensions (~ 10^8 cells/ml) and SDW, respectively. They were potted in sterile peat, maintained under the same greenhouse conditions as described above for about 30 and 60 days, and regularly watered with tap water. Five seedlings were used for each individual treatment. At the end of the trial, tomato plants were uprooted and washed for removing adhering peat. Various growth parameters were noted for all tomato plants (namely, height, aerial parts and roots fresh weights, and maximum root length and fresh weight).

Assessment of disease suppression ability

Tested endophytic isolates were challenged to tomato cv. Rio Grande seedlings (two-true-leaf stage) using the culture substrate drenching method with 25 ml of a bacterial cell suspensions containing 10^8 cells/ml. Pathogen inoculation was applied, 6 days after bacterial challenge, through substrate drench with equal volume of a conidial suspension (10^6 conidia/ml). Un-inoculated control seedlings were watered by an equal volume of SDW (negative control). Positive control plants were FOL-inoculated but treated with SDW only. Each individual treatment was replicated five times (five seedlings per treatment). Fusarium wilt severity was evaluated, 30 and 60 days post-inoculation (DPI), on FOL-infected tomato plants based on intensity of leaf yellowing and necrosis using a 0–4 scale (Amini 2009). The vascular browning extent (from collar), plant height, fresh weight of the whole plant, roots' fresh weight, and FOL re-isolation frequency were also noted. FOL re-isolation frequency was calculated. The most active bacterial isolates, showing significant ability to efficiently control tomato Fusarium wilt, were selected for a series of characterizations.

Characterization and identification of efficient endophytes

Colonies of selected isolates were morphologically characterized based on their form, margin, elevation, surface, opacity, and color on NA medium (Patel et al. 212). Gram staining was determined using light microscopy. They were also characterized using conventional biochemical tests according to Schaad et al. (2001) protocols. Molecular identification was undertaken after extraction of the genomic DNA. The PCR conditions and the 2 universal eubacterial primers 27f and1492r used for amplification of 16S rDNA gene were detailed in a previous work (Aydi Ben Abdallah et al. 2016a). The homology of the 16S rDNA sequence of a given isolate was performed, using BLAST-N program from GenBank database (www.ncbi.nlm.gov/BLAST/). Alignment of sequences was performed using the ClustalX (1.81).

Antifungal activity of selected endophytic isolates

The antifungal activity of selected endophytic isolates against FOL was assessed using the streak and the disk diffusion methods on PDA medium (Aydi Ben Abdallah et al. 2015). SDW was used as control. Each individual treatment was replicated 4 times. After 4 days of incubation at 25 °C, effects of tested treatments were evaluated based on FOL colony diameter and the diameter of the inhibition zone. For the assessment of the antifungal activity of volatile metabolites, a sealed plate method was used (Aydi Ben Abdallah et al. 2015). The plates were incubated at 25 °C for 7 days. Three plates were used for each individual treatment. The effect of tested treatments was assessed based on pathogen mycelial growth inhibition (Tiru et al. 2013).

Hydrolytic enzyme production

Chitinase, pectinase, and protease production of selected isolates was tested according to Tiru et al. (2013). Treatments were performed in triplicate. After 48 h to 72 h of incubation at 28 ± 2 °C, isolates showing the presence of clear zones around their colonies were classified as chitinase-, pectinase-, and protease-producing agents.

Hydrogen cyanide (HCN) production

Hydrogen cyanide (HCN) production was determined qualitatively (Sgroy et al. 2009). Treatments were performed in triplicate. Plates were sealed with parafilm and incubated at 25 °C for 4 days. Change in color from yellow to light-reddish brown indicates positive HCN production.

Phosphate solubilization capacity

Phosphate solubilization ability was determined according to Sgroy et al. (2009) protocol. The test was performed in triplicate. After 7 days of incubation at 28 ± 2 °C, the clearing zone formed around colonies was noted.

Indole-3-acetic acid (IAA) production

IAA production was determined according to Sgroy et al. (2009) protocol. Treatments were performed in triplicate. Absorbance was read at 530 nm. IAA concentration was determined based on a standard curve performed from IAA dilution series at 100 μg/ml (w/v) in LB medium.

Statistical analysis

A one-way analysis of variance (ANOVA) was used for data analysis. The software used is the Statistical Package for the Social Sciences (SPSS) for Windows version 16.0. Experiments were undertaken according to a completely randomized design. Means were compared using Student-Newman-Keuls test at $P \leq 0.05$. Correlations between disease severity and plant growth parameters were carried out using bivariate Pearson's test at $P \leq 0.05$.

Results and discussion

Endophytic behavior of bacterial isolates was collected from *S. sodomaeum* and *S. bonariense*.

Twenty-five bacterial isolates, recovered from stems, leaves, and fruits of *S. sodomaeum* and *S. bonariense*, showed double-resistance to streptomycin sulfate and rifampicin (100 μg/ml w/v). When inoculated to tomato cv. Rio Grande plants, only 23 isolates were re-isolated from tomato stem tissues when plated on NA medium amended with both antibiotics. These 23isolates were selected for their growth-promoting and disease suppression abilities. All plants were inhabited by diverse bacteria known as endophytes.

Wild solanaceous plants species, i.e., *Datura* spp. (Aydi Ben Abdallah et al. 2016b and c), *Nicotiana glauca* (Aydi Ben Abdallah et al. 2016d), *Withania somnifera* (Aydi Ben Abdallah et al. 2016e), *S. elaeagnifolium* (Aydi Ben Abdallah et al. 2016a), and *S. linnaeanum* (Nefzi et al. 2018b) have been widely investigated as isolation source for beneficial endophytic bacteria and/or fungi. In the present study, *S. sodomaeum* and *S. bonariense* were explored for their naturally associated endophytic bacteria, with tomato growth promotion and Fusarium wilt control abilities.

Currently, microbial endophytic communities are the focus of several studies aiming unraveling and clarifying of their roles as plant growth promoters and their significant involvement in plant health (Vurukonda et al. 2018). Bacteria that colonize the internal tissues of plants seem to have an ecological advantage over bacteria than can only colonize plants epiphytically. The internal tissues of plants possibly provide a more uniform and protective environment for microorganisms than plant surface (Wang et al. 2013).

Growth-promoting potential noted on pathogen-free tomato plants

All tomato cv. Rio Grande plants separately inoculated with the 23 endophytic isolates remained symptomless until the end of the trial (30 and 60 days post-inoculation). Their growth-promoting ability was assessed based on various parameters.

At 30 and 60 days post-inoculation, all noted growth parameters (height, maximum root length, aerial part fresh weight, and root fresh weight), varied significantly (at $P \leq 0.05$) depending on tested bacterial treatments. As presented in Fig. 1, treating tomato plants with 18 strains out of the 23 tested resulted in significant increment of plant height, by 22.2–36.9%, than the controls. The highest increase (36.9%) was achieved, using SV81, and followed by 35.3% using S11 treatment. The aerial part fresh weight recorded on plants treated with all tested bacterial isolates was also significantly enhanced by 34.1–70.5% versus control. The highest weight increase (69.5–70.5%) was noted, following S14 and S11 treatments and at a lesser extent S24 (63.3%). The root development, as estimated based on the maximum root length, was also significantly increased by 22.2 to 38.7% versus control, following the treatments with 10 tested isolates (namely S11, S14, S23, S24, S26, S28, S29, SV71, SV77, and SV81; Fig. 1). The root fresh weight was also significantly augmented by 30.9 to 52.2% over control, following the treatments with S11, S14, S23, S24, S26, S28, SV77, and SV81 isolates. The greatest increase of this parameter, of about 51.5–52.2% over control, was achieved at S14 and S11 isolates, followed by 39.6–40.9% noted, using S24 and S23.

These endophytic microorganisms provide real advantages to the host plants, for example, by enhancing the physiological activity of the plant or facilitating the uptake of nutrients from the soil. Thus, they may serve as plant growth promoters (Vurukonda et al. 2018). Some bacterial endophytes provide an advantage to the host they colonize over non-infected plants. These organisms are capable of promoting plant growth both directly and indirectly. Endophytes can promote growth directly by the production and/or the regulation of plant growth hormones, the nitrogen fixation, and the phosphate solubilization. Indirectly, bacterial endophytes promote plant growth by protecting the plant against pathogens (Aydi Ben Abdallah et al. 2019 and Ben Slama et al. 2019). The inoculation of mung beans (*Vigna radiata*) with *Streptomyces thermocarboxydus* S3 showed a significant increase in fresh weight, root length, and total length in the presence of IAA production as also state by Lasudee et al. (2018).

Growth-promoting potential noted on pathogen-inoculated tomato plants

When tested onto FOL-inoculated tomato plants, bacterial treatments had significantly (at $P \leq 0.05$) affected all growth parameters noted 30 and 60 days post-treatment. As shown in Fig. 2, treatments with S10, S11, S14, S23, S24, S26, S27, S28, S29, and SV81 isolates had significantly improved plant height and fresh weight by

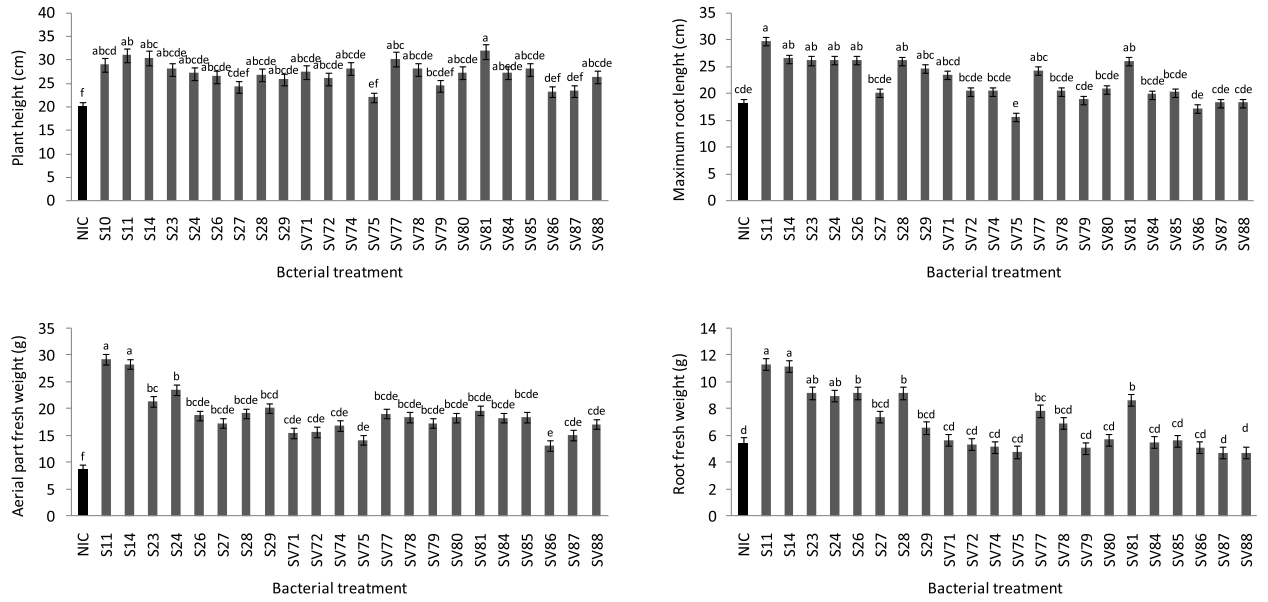

Fig. 1 Plant growth-promoting potential of various endophytic bacterial isolates, recovered from *Solanum sodomaeum* and *S. bonariense*, noted on pathogen-free tomato cv. Rio Grande plants 60 days post-treatment. S11, S14, S26, S27, S28, and S29 are isolates issued from *S. sodomaeum*. S23, S24, SV71, SV72, SV74, SV75, SV77, SV78, SV79, SV80, SV81, SV84, SV85, SV86, SV87 and SV88 are bacterial isolates recovered from *S. bonariense*. NIC, untreated control. Bars sharing the same letter are not significantly different according to Student-Newman-Keuls test at $P \leq 0.05$

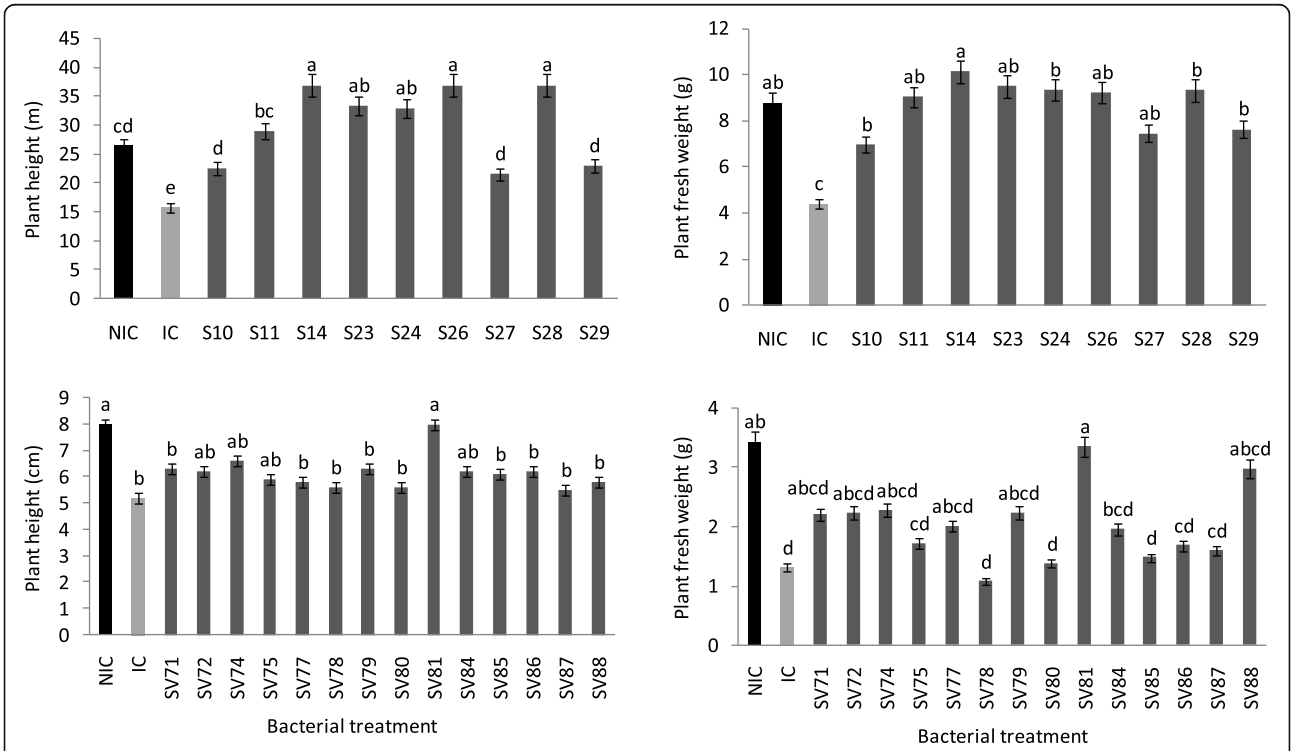

Fig. 2 Effect of endophytic bacteria recovered from *Solanum sodomaeum* and *Solanum bonariense* on growth promotion of tomato cv. Rio Grande plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici* compared to the untreated controls. S11, S14, S26, S27, S28, and S29 are bacterial isolates recovered from *S. sodomaeum*. S23, S24, SV71, SV72, SV74, SV75, SV77, SV78, SV79, SV80, SV81, SV84, SV85, SV86, SV87, and SV88 are bacterial isolates recovered from *S. bonariense*. NIC, un-inoculated with pathogen and untreated control. Bars sharing the same letter are not significantly different according to Student-Newman-Keuls test at $P \leq 0.05$

26.8–57.3 and 36.7–61%, respectively, over pathogen-inoculated and untreated control. It should be also noted that tomato plants infected with FOL and treated with these isolates showed significantly similar growth as disease-free and untreated controls. Furthermore, tomato plants inoculated with FOL and subjected to S14-, S26-, S28-, S23-, and S24-based treatments were significantly longer than the un-inoculated and the untreated control plants.

Obtained results clearly indicated that the majority of tested bacterial isolates enhanced the aerial part growth and root development on pathogen-free and FOL-inoculated tomato plants. Similar results were previously reported on plant growth-promoting ability displayed by endophytic *Pseudomonas* spp. (*P. aeruginosa* HR7 and *Pseudomonas* sp.) recovered from symptomless tomato roots and stems (Patel et al. 2012). An endophytic *B. amyloliquefaciens* JK-SD002, recovered from tomato stems, augmented also height of treated tomato plants (Nawangsih et al. 2011). In Selvakumar et al. (2008) study, growth improvement was also observed on wheat seedlings treated with endophytic *S. marcescens* KR-4, *Bacillus thuringiensis* KR-1, and *Enterobacter asburiae* KR-3, isolated from *Puerariathun bergiana* nodules. In the recent findings, diverse endophytic *Bacillus cereus* S42, *Alcaligenes faecalis* S18, *B. mojavensis* S40, *Stenotrophomonas maltophilia* S37, *Pseudomonas* sp. S85, *Bacillus* sp. SV101, and *Bacillus tequilensis* SV104 isolates, obtained from various wild *Solanaceae* species, also exhibited growth-promoting ability, when used for the treatment of tomato plants inoculated or not with FOL (Aydi Ben Abdallah et al. 2016a, 2016b, 2016d). Seed coating with endophytic bacteria promoted tomato plant growth and quality of tomato production in plants challenged or not with FOL (Koohakan et al. 2020).

Consequently, intimate and non-harmful associations can be established between bacteria and their host plants. This is the biggest difference between endophytes and plant growth-promoting rhizobacteria (PGPR) (Timmusk et al. 2017). There are several mechanisms by which endophytic bacteria offer several benefits to their host plants, particularly via the growth promotion, and their protection against biotic and abiotic stresses (Kha et al. 2018). The beneficial effect of plant growth-promoting bacteria (PGPB), including endophytic bacteria, on plant physiology may be also attributed to their ability to tolerate biotic stresses such as infections by pathogenic bacteria and fungi, insect attacks, and native plants colonization (Egamberdieva et al. 2017). Several questions regarding the association of endophytic microorganisms with host plants, however, are still unanswered, including how long do they reside in the host and do they have any impact on photosynthesis. It appears evident that microbes can augment biotic stress tolerance in plants through the PGPB process (Passari et al. 2019). Indeed, PGPB are known to produce various enzymes including amylase, chitinase, cellulase, invertase, lipase, keratinase, peroxidase, pectinase, protease, phytase, and xylanase which transform the complex nutrients into simple mineral forms. This nutrient cycling capacity makes them ideal candidates for natural fertilizers (Vurukonda et al. 2018).

Disease suppression potential of selected endophytic isolates

At 30 and 60 DPI with FOL, disease severity, noted on tomato plants varied significantly (at $P \leq 0.05$) upon bacterial treatments tested. Significant decreases in the leaf damage and in the vascular browning extent by 35.3–94.1 and 19.5–96.3% over control, respectively, were noted on tomato plants infected with FOL and treated with S10, S11, S14, S23, S24, S26, S27, S28, S29, and SV81 (Fig. 3). The highest decreases in disease severity and in the vascular browning extent of about 83.3–94.1 and 82–96.3%, respectively, were achieved, using S11, S14, S23, S24, S26, S28, and SV81 treatments. It should be noted that tomato plants inoculated with FOL and treated with these seven isolates were significantly similar to disease-free and untreated controls. FOL re-isolation frequency from tomato stems of FOL-inoculated plants treated with S11, S14, S23, S24, S26, S28, and SV81 isolates was lowered by 90% than the control (Fig. 3). These 7 isolates were selected for further characterizations.

Later discovery of the metabolic potential of such endophytes in planta, their ability to efficiently compete with other endophytes including plant pathogens, and their role in stimulating the expression or over-expression of plant genomic sequences involved in tolerance to plant stresses indicated that selected endophytes may be considered a very promising agent to control plant pests and diseases (Vurukonda et al. 2018). Multiple reports demonstrated the capacities of *Bacillus* strains to control *Fusarium*-induced plant diseases. The antagonistic capacities of endophytic bacteria are associated to their plant growth-promoting abilities, either directly and indirectly under normal and stress conditions (Ben Slama et al. 2019). Biocontrol ensured by endophytic bacteria along the different steps of the infection process may be achieved through antibiosis, competition with the target pathogens for nutrients and space and through the induction of disease resistance in the host plant that they colonize. Therefore, protection of plants from biotic stresses may be the result of one or more microbe-microbe or plant-microbe interactions (Vurukonda et al. 2018).

In this study, during pathogenic infection, endophytic association mitigated the disease and improved the growth and biomass of tomato; this may be due to inhibition of pathogenic infection, stimulation of plant

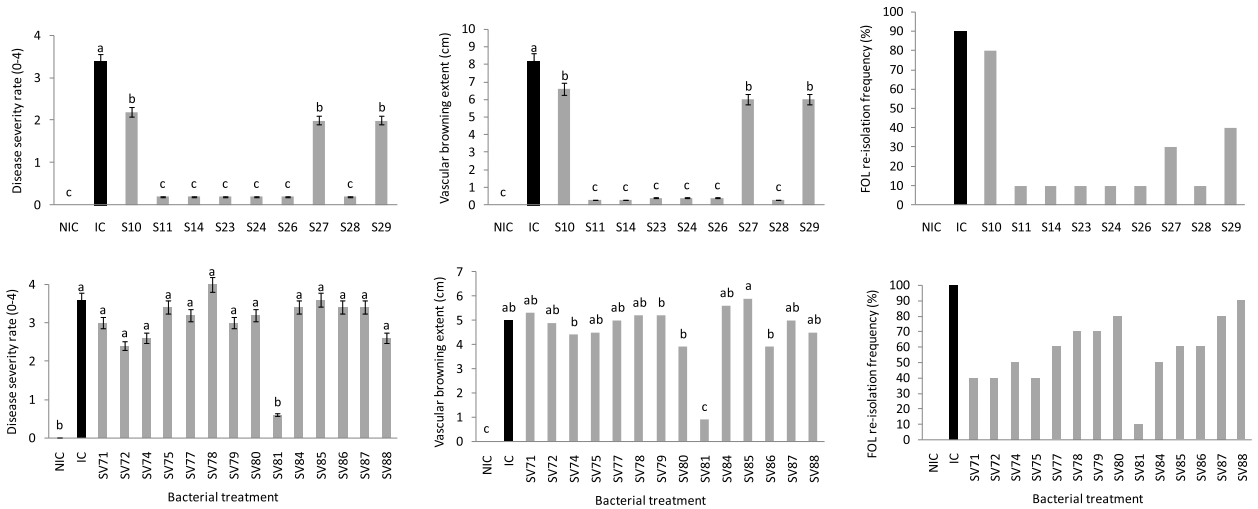

Fig. 3 Effect of various endophytic bacterial isolates, recovered from *Solanum sodomaeum* and *Solanum bonariense*, on Fusarium wilt severity noted on tomato cv. Rio Grande plants 60 days post-treatment compared to the untreated controls. S11, S14, S26, S27, S28, and S29 are isolates issued from *S. sodomaeum*. S23, S24, SV71, SV72, SV74, SV75, SV77, SV78, SV79, SV80, SV81, SV84, SV85, SV86, SV87, and SV88 are isolates issued from *S. bonariense*. IC, inoculated with *Fusarium oxysporum* f. sp. *lycopersici* and untreated; NIC, un-inoculated with pathogen and untreated control. Bars sharing the same letter are not significantly different according to Student-Newman-Keuls test at $P \leq 0.05$

resistance, high nutrient uptake, and promotion of plant growth. Waqas et al. (2015) showed that endophytes able to produce siderophores and organic acids are helpful in combating pathogenic effects in sunflower plants. Furthermore, the defense hormones regulation might be correlated with the ability of endophytic bacterial strain RWL-1 to produce organic acids (succinic acid, acetic acid, propionic acid, and citric acid) during the inoculation and infection of tomato plants with FOL (Shahzad et al. 2017). Tomato plants challenged or not with FOL and treated with endophytic *B. amyloliquefaciens* subsp. *plantarum* SV65 showed significant expression of PRs and lipooxygenase genes than in the untreated ones. This bacterial strain was also able to produce chitinase, protease, organic acids, siderophores, and fengycines. These metabolites may be involved in bio-protection against FOL by direct inhibition of the pathogen and/or by inducing tomato systemic resistance (Aydi Ben Abdallah et al. 2017). Further studies are needed to ascertain secondary metabolites produced by bacterial entophytes and determine their role in plant defense against pathogenic infection-induced stresses. Furthermore, the use of a biocontrol agent as one of the natural enemy of soilborne pathogens besides the enhancement of the crop production potential can provide a complementary tactic for sustainable integrated pest management (Fatima and Anjum 2017).

Correlation between disease severity and plant growth parameters

Pearson's correlation analysis indicated that the lowered disease severity, estimated based on the leaf damage index and the vascular browning extent, was associated by significant increases in all plant growth parameters. In fact, height was negatively correlated to the leaf damage index ($r = -0.893$, $P = 2.111E-4$) and to the vascular browning extent ($r = -0.888$, $P = 2.620E-4$). The plant fresh weight was negatively linked to the leaf yellowing score ($r = -0.934$, $P = 2.537E-5$) and to the vascular browning extent ($r = -0.918$, $P = 6.877E-5$). Similar trend was observed between FOL re-isolation frequency and plant growth parameters where negative correlations were recorded between pathogen re-isolation frequency, plant height ($r = -0.781$, $P = 0.005$), and whole plant fresh weight ($r = -0.913$, $P = 8.614E-5$).

In addition to growth promotion, an important Fusarium wilt-suppressive ability was reached, in the current study, using the seven selected bacterial isolates (namely S11, S14, S23, S24, S26, S28 and SV81) comparatively to the control. Fusarium wilt biocontrol was associated with a reduced colonization of the vascular tissues thus leading to indirect growth improvement. Similar results were cited by Ramyabharathi and Raguchander (2014) showing that *Bacillus subtilis* EPC016-treated and FOL-infected tomato plants exhibited reduced disease severity and improved plant growth. Aydi Ben Abdallah et al. (2016a, b, d and e) found that the following treatments with endophytic *Bacillus*, *Stenotrophomonas*, *Pseudomonas*, and *Alcaligenes* isolated from *N. glauca*, *Datura metel*, *S. elaeagnifolium*, and *W. somnifera*, Fusarium wilt-suppressive effect was associated to tomato growth improvement. Similarly, growth promotions were also recorded on maize plants bacterized with an endophytic

B. mojavensis and infected with *F. verticillioides* (Kalai-Grami et al. 2014). Tests on infested soil with *F. oxysporum*, tomato plants treated with endophytic bacteria *B. subtilis* SV41, and *B. amyloliquefaciens* subsp. *plantarum* SV65 showed diversity on soil microbial community structures, enhancement on plant height, and fruit production accompanied with a decrease on Fusarium wilt severity compared to untreated ones (AydiBenAbdallah et al. 2019). Enhanced growth may also be exerted by improved nutrient acquisition. This increased capacity for nutrient uptake could contribute to diminishing the deleterious effect of the pathogen (Deketelaer et al. 2017). Plant growth promotion and productivity stimulated by microbial endophytic communities are often associated with increased plant health, achieved through direct and/or plant-mediated control of plant pathogens (Vurukonda et al. 2018).

Characterization and identification of the seven selected isolates

The 7 bioactive isolates selected above (namely S11, S14, S23, S24, S26, S28, and SV81) on the base of their effectiveness in Fusarium wilt suppression and tomato growth promotion even in FOL-inoculated plants were morphologically and biochemically characterized (Table 1). These isolates were also checked for their hypersensitivity reaction and their hemolytic activity and were found negative for these both tests.

Blast-N analysis of sequenced 16S rDNA gene homology indicated that S28, S23, S24, and S26 isolates were affiliated to the genus of *Stenotrophomonas* with 99.9% similarity to *S. maltophilia* RPS 4, 99.5% to *S. maltophilia* PPA N3, and 99.1–99.8% to *S. maltophilia* Ysm, respectively. The isolate SV81 was identified as *Bacillus* sp. with 99.7% of similarity to *Bacillus* sp. hb122. The isolate S14 shared identical similarity to *Serratia marcescens* 35dr and the isolate S11 belonged to *Azotobacter* with 98.4% of similarity to *Azotobacter chroococcum* ABA-1 (Fig. 4). Sequences of S11, S14, S23, S24, S26, S28, and SV81 were submitted to GenBank and attributed the following accession numbers KR818078 to KR818083 and KU994900, respectively.

Table 1 Biochemical characterization and Gram staining of selected endophytic bacterial isolates recovered from *Solanum sodomaeum* and *S. bonariense* plants

Solanum spp.	*S. sodomaeum*				*S. bonariense*		
Bacterial isolates	**S11**	**S14**	**S28**	**S26**	**S23**	**S24**	**SV81**
Catalse	+	+	+	+	+	+	+
Lecithinase	+	−	−	−	+	−	−
Urease	+	−	+	−	+	−	+
Nitrate reductase	+	+	+	+	+	+	+
Tryptophane deaminase	−	−	−	+	−	−	−
Lysine decarboxylase	−	−	−	−	−	−	−
Indole	+	+	+	+	+	+	+
Mannitol	+	+	+	+	−	+	+
Simmons Citrate	+	−	+	−	−	−	−
Red of methyl	+	−	−	−	+	−	−
Vosges-Proskauer	−	+	+	+	−	+	+
King A	−	−	−	−	−	−	−
Gram staining	−	−	−	−	−	−	+

"+", positive test; "−", negative test

Zhu et al. (2012) pointed out that the significant role of play by the endophyte *S. maltophilia* in agricultural production, as a plant growth-promoting agent. Algam et al. (2005) reported that endophytic *Brevibacillus brevis* B2 and *B. subtilis* strains B5, B7, and B8, isolated from tomato rhizosphere, also were able to promote growth and to suppress bacterial wilt induced by *Ralstonia solanacearum*. Mrkova-Ki et al. (2001) demonstrated also that *A. chroococcum* displayed growth and yield-promoting abilities of field-grown beets. Gyaneshwar et al. (2001) showed also the similar potential of endophytic *S. marcescens* recovered from rice plants. In the same sense, cyclamen plants, bacterized with *S. marcescens* B2 and inoculated with *Rhizoctonia solani* sclerotia and/or *Fusarium oxysporum* f. sp. *cyclaminis* conidia, did not develop any disease symptoms and were found to be healthy (Someya et al. 2000).

Antifungal activity of the selected bacterial isolates

Tested using the streak method, the 7 isolates had significantly ($P \leq 0.05$) reduced FOL radial growth compared to control (Fig. 5a). The highest decrease in FOL mycelial growth, by 38.4 and 45.4%, was noted on pathogen cultures co-cultured with *S. maltophilia* S23 and *Bacillus* sp. SV81, respectively. Also, *S. maltophilia* S23, *S. maltophilia* S24, and *Bacillus* sp. SV81 formed inhibition zones of 11.5–12.75 mm in diameter, which were significantly ($P \leq 0.05$) higher than those induced by the remaining tested isolates (7.75–8.5 mm in diameter; Fig. 5b). Using the sealed plate method, only *Bacillus* sp. SV81 exhibited a significant inhibitory effect ($P \leq 0.05$) against the target pathogen as expressed by 31.8% lower FOL growth versus control. This revealed the ability of *Bacillus* sp. SV81 to inhibit pathogen at distance through its antifungal volatile compounds (Fig. 5a).

Assessed in vitro for their antifungal activity against FOL growth, the 7 selected isolates had inhibited pathogen radial growth through the release of diffusible and volatile metabolites. Similar results were noted by Aydi Ben Abdallah et al. (2015) using others bacterial isolates. In fact, they showed that endophytic *Bacillus* spp., isolated from wild *Solanaceae* stems, released diffusible and volatile compounds which are bioactive against FOL growth. Chaurasia et al. (2005) demonstrated the

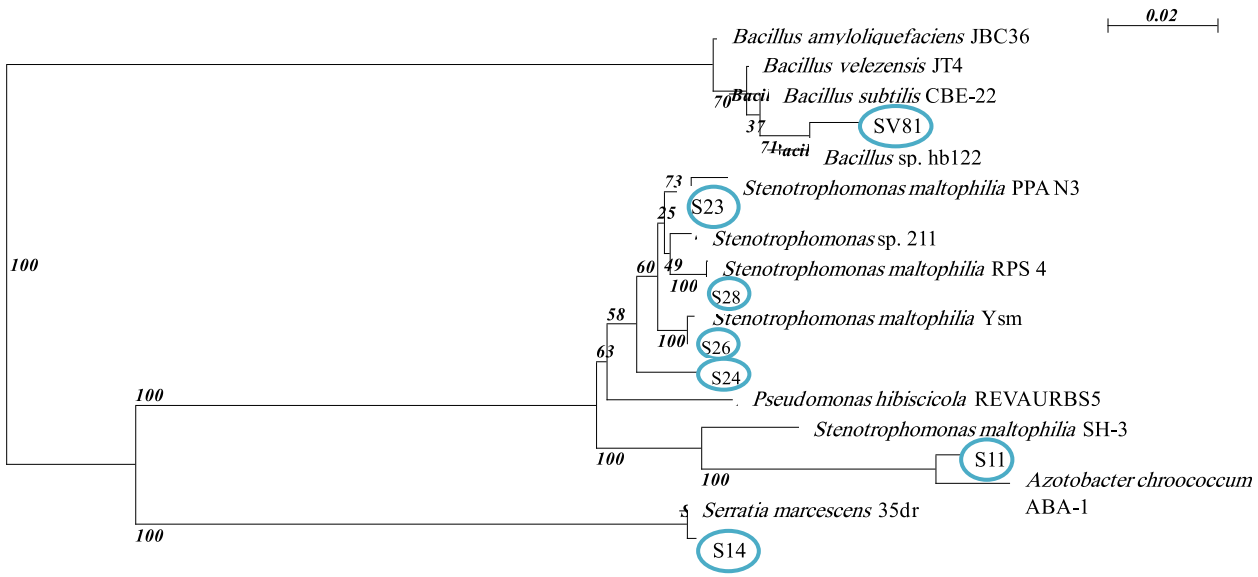

Fig. 4 Neighbor-joining phylogenetic tree of 16S rDNA sequences of SV81, S23, S28, S26, S24, S11, and S14 isolates and their closest phylogenetic relatives. The nucleotide sequences used of representative isolates were obtained from Genbank database under the following accession numbers: KF494187 (*Azotobacter chroococcum* ABA-1), GQ884174 (*Stenotrophomonas maltophilia* SH-3), MT555732 (*Pseudomonas hibiscicola* REVAURBS5), KJ729606 (*Serratia marcescens* 35dr), JQ308605 (*Stenotrophomonas maltophilia* RPS 4), KF278963 (*Stenotrophomonas maltophilia* Ysm), JQ308603 (*Stenotrophomonas maltophilia* PPA N3), KT034429 (*Stenotrophomonas* sp. 211), KF863899 (*Bacillus* sp. hb122), JX418320 (*Bacillus amyloliquefaciens* JBC36), MK928419 (*Bacillus velezensis* JT4), MT026940 (*Bacillus subtilis* CBE-22), and for the bacterial isolates tested KR818078 (S11), KR818079 (S14), KR818080 (S23), KR818081 (S24), KR818082 (S26), KR818083 (S28), and KU994900 (*Bacillus* sp. SV81). The tree topology was constructed using ClustalX (1.81)

inhibitory effect of diffusible and volatile metabolites from *B. subtilis* against some plant fungi including *F. oxysporum*. The basis for the observed activity of selected bacterial isolates against FOL has been linked to secondary metabolites that strongly interfere with fungal pathogens.

Antifungal activity features of the selected bacterial isolates

Enzymatic activity

All tested bacterial isolates displayed pectinolytic activity when grown on pectin-agar medium. As for chitinase activity, 6 isolates, except *S. maltophilia* S26, showed clear zones around colonies when cultured on chitin-agar medium and were found to be chitinase-producing agents. For protease production, except *Bacillus* sp. SV81, the remaining 6 isolates displayed positive activity (Table 2). The greatest activity was noted at *A. chroococcum* S11, *S. maltophilia* S23, and *S. maltophilia* S24 cultures, where the clear zone formed around their colonies reached 30–45.83 mm in diameter. In the present study, the selected bacterial agents were evaluated in vitro for their traits implicated in their antifungal potential. All selected isolates were found to be pectinase-producing agent. In the present study, 6 bacterial isolates are chitinase- and protease-producing agent, except *S. maltophilia* S26 and *Bacillus* sp. SV81. Therefore, their antifungal potential against Fusarium wilt agent may be attributed in part to their capacity to produce chitinases and/or proteases. Antagonistic bacteria such as *Bacillus*, *Burkholderia*, *Serratia*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, and *Stenotrophomonas* were widely known by their chitinase, protease, and β-1,3-glucanase activities (Berg et al. 2005). Microorganisms with extracellular enzyme activities are not only helpful in organic matter decomposition and plant growth-promoting, but also play an important role in disease suppression by antagonizing soilborne pathogens and facilitating intracellular colonization and development of endophytism (Ben Slama et al. 2019).

HCN production

Only *A. chroococcum* S11 and *S. maltophilia* S28 were shown to be able to modify the filter paper color from yellow to light-reddish color which indicated their HCN production potential when grown on NA medium amended with glycine (Table 2).

The production of HCN plays an important role in disease suppression of crop plants (Ben Slama et al. 2019). The registered antifungal activity displayed by *S. maltophilia* S28 and *A. chroococcum* S11 may due, in part, to the production of HCN. This volatile antibiotic, frequently produced by Gram-negative bacteria, such as *P. fluorescens*, *S. maltophilia* S37, and *A.*

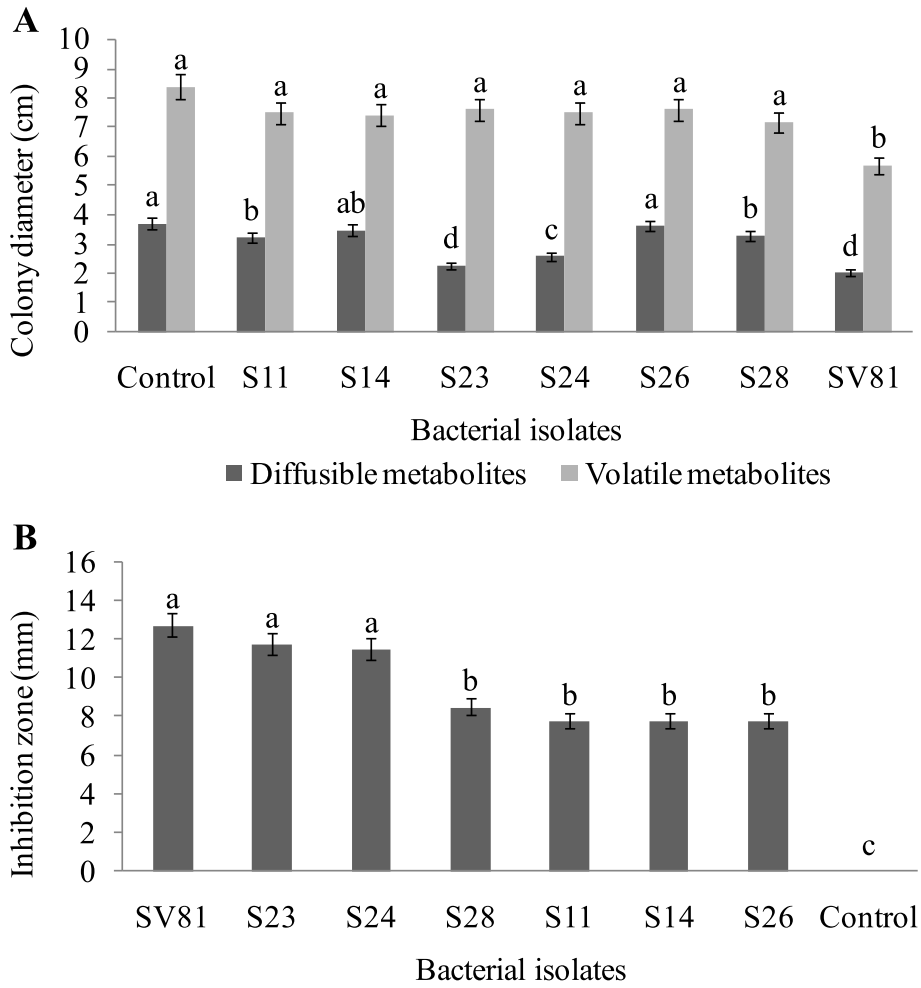

Fig. 5 Antifungal activity of the seven selected bacterial isolates, recovered from *Solanum sodomaeum* and *Solanum bonariense*, toward *Fusarium oxysporum* f. sp. *lycopersici* noted after 4 and 7 days of incubation at 25 °C. S11, *Azotobacter chroococcum*; S14, *Serratia marcescens*; S23, S24, S26 and S28, *Stenotrophomonas maltophilia*; SV81: *Bacillus* sp. For each test, values with the same letter are not significantly different according to Student-Newman-Keuls test at $P \leq 0.05$

faecalis S8, proved to be deployed in *R. solani* (Nagarajkumar et al. 2004) and/or FOL (Aydi Ben Abdallah et al. 2016c and d) biocontrol.

Plant growth-promoting traits of the selected isolates

Phosphate solubilization

Serratia marcescens S14, *Bacillus* sp. SV81, and *S. maltophilia* S28 were able to solubilize phosphate as determined by the formation of a clear zone of about 8.33 and 13.17 mm in diameter around their colonies, respectively (Table 2).

As phosphorus mostly occurs in the soil in an insoluble form, the intervention of phosphate-solubilizing endophytic bacteria becomes important for plants (Ben Slama et al. 2019). In this study, 3 isolates (*S. marcescens* S14, *Bacillus* sp. SV81, and *S. maltophilia* S28) out of the7 tested were phosphate producers. Therefore, the plant growth-promoting potential induced by these 3 isolates in tomato plants inoculated or not with the pathogen may be due to their ability to solubilize the phosphate and facilitate its uptake by plant. *Bacillus* species, i.e., *Bacillus velezensis*, *B. mojavensis*, and *B. methylotrophicus*, were shown able to solubilize phosphate as previously demonstrated by Kalai-Grami et al. (2014). Furthermore, *Pseudomonas* spp., *Serratia* spp., *Yokenella regensburgei*, and *Stenotrophomonas* were capable to produce phosphatase (Ngamau et al. 2012; Ngoma et al. 2013).

IAA production

The 7 selected endophytic isolates were able to produce the IAA after 48 h of incubation (Table 2). The highest IAA production, of about 20.5–24.8 μg/ml, was recorded on *S. maltophilia* S23 and *S. maltophilia* S24 cultures, followed by *S. marcescens*

Table 2 Antagonistic and plant growth-promoting (PGP) mechanisms of the seven selected endophytic bacterial isolates recovered from *Solanum sodomaeum* and *Solanum bonariense*

Solanum spp.	Bacterial isolates	Antagonistic traits				PGP ability	
		Hydrolytic enzymes			Volatile antibiotic		
		Chitinase[a]	Protease[b]	Pectinase[c]	HCN[d]	Phosphate solubilization[e]	IAA[f]
S. sodomaeum	S11	+	++	+	+	−	+
	S14	+	+	+	−	+	++
	S28	+	+	+	+	++	++
	S26	-	+	+	−	−	++
S. bonariense	S23	+	++	+	−	−	+++
	S24	+	++	+	−	−	+++
	SV81	+	−	+	−	+	+

S11, *Azotobacter chroococcum*; *S14*, *Serratia marcescens*; *S23*, *S24*, *S26*, and *S28*, *Stenotrophomonas maltophilia*; *SV81*, *Bacillus sp.*
[a]Tested on chitin-agar (0.5% w/v) medium and incubated at 28 ± 2 °C for 72 h; "+", presence of clear zone; "−", absence of clear zone
[b]Tested on skim milk agar (3% v/v) medium and incubated at 28 ± 2 °C for 48 h; "+" and "++", presence of clear zone (15.67 to 25 and 30 to 45.83 mm in diameter, respectively); "−", absence of clear zone
[c]Tested on pectin-agar (0.5% w/v) medium and incubated at 28 ± 2 °C for 48 h; "+", presence of clear zone
[d]*HCN*, hydrogen cyanide production on glycine-agar (4.4 g/L w/v) medium and incubated at 25 °C for 4 days; "+", modification on the filter paper color (light-reddish color) compared to the control (yellow); "−", no modification on the filter paper color (yellow)
[e]Tested on Pikovskaya agar medium and incubated at 28 ± 2 °C for 7 days; "+" and "++", presence of clear zone (8.33–9.8 and 13.17 mm in diameter, respectively); "−", absence of clear zone
[f]*IAA*, indole-3-acetic acid production after 48 h of incubation at 28 ± 2 °C in Luria-Broth medium; "+", "++", and "+++", production of IAA (5–9.4, 14.38–18.71, and 20.5–24.8 μg/ml, respectively)

S14, *S. maltophilia* S28, *S. maltophilia* S26 (14.38–18.71 μg/ml), and *A. chroococcum* S11 and *Bacillus* sp. SV81 (5–9.4 μg/ml). Many scientific reports have explained the ability of endophytic bacteria to stimulate the secretion of plant growth hormones and enhance their growth-promoting activity. In this study, remarkably efficient growth promotion was stimulated by the 7 selected bacterial isolates in tomato-free pathogen plants and those inoculated with FOL, which was due to their ability to produce IAA. Many previous studies reported the ability of endophytic bacteria to produce IAA such as *B. thuringiensis*, *B. subtilis*, *Bacillus arbutinivorans*, *Bacillus fusiformis*, *Bacillus megaterium* (Wang et al. 2013), *S. maltophilia* (Ngoma et al. 2013), *S. marcescens* (Selvakumar et al. 2008), and *Serratia nematodiphila* (Dastager et al. 2011).

Conclusions

The present study emphasized the importance of the wild solanaceous species, *S. sodomaeum* and *S. bonariense*, as potential sources for isolation of beneficial endophytic bacteria acting as plant growth-promoter and a biocontrol agent on both pathogen-free and FOL-inoculated tomato plants. Seven isolates were identified as the most effective bio-agent. Their plant growth-promoting abilities were confirmed via IAA production and phosphate solubilization activities. The 7 selected bacterial isolates displayed interesting enzymatic activity and 3 of them were shown able to produce HCN. The diffusible and volatile metabolites produced by these isolates were effective against FOL mycelial growth. These isolates will be further tested for their potential to suppress Fusarium wilt and to promote growth and production of tomato grown under naturally infested and non-infested soils.

Abbreviations

F. oxysporum: *Fusarium oxysporum*; *F. verticillioides*: *Fusarium verticillioides*; *R. solani*: *Rhizoctonia solani*; *S. maltophilia*: *Stenotrophomonas maltophilia*; *A. chroococcum*: *Azotobacter chroococcum*; *S. marcescens*: *Serratia marcescens*; *A. faecalis*: *Alcaligenes faecalis*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *B. thuringiensis*: *Bacillus thuringiensis*; *B. cereus*: *Bacillus cereus*; *B. mojavensis*: *Bacillus mojavensis*; *B. tequilensis*: *Bacillus tequilensis*; *B. subtilis*: *Bacillus subtilis*; *B. arbutinivorans*: *Bacillus arbutinivorans*; *B. fusiformis*: *Bacillus fusiformis*; *B. megaterium*: *Bacillus megaterium*; *B. velezensis*: *Bacillus velezensis*; *S. sodomaeum*: *Solanum sodomaeum*; *S. bonariense*: *Solanum bonariense*; *W. somnifera*: *Withania somnifera*; *S. elaeagnifolium*: *Solanum elaeagnifolium*; *S. linnaeanum*: *Solanum linnaeanum*; *D. metel*: *Datura metel*; *N. glauca*: *Nicotiana glauca*; PDA: Potato dextrose agar; NB: Nutrient broth; NA: Nutrient agar; SDW: Sterile distilled water; DPI: Days post-inoculation; FOL: *Fusarium oxysporum* f. sp. *lycopersici*; IAA: Indole-3-acetic acid; HCN: Hydrogen cyanide

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Authors' contributions

The concept and design of the experiments were prepared by all authors. RAB conducted the experiments, analyzed the results, and wrote the manuscript. HJK coordinated the laboratory as well as in greenhouse works. MDR supervised the results analysis and corrected the manuscript draft. All authors have read and approved the manuscript.

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Availability of data and materials
All data generated and/or analyzed during the present study are available in the manuscript, and the corresponding author has no objection to the availability of data and materials.

Ethics approval and consent to participate
The study was conducted on plant-pathogen fungus and beneficial entophytic bacteria that are abundant in the environment and do not require ethical approval.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no conflict of interest.

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