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The Pgpr as Elicitors of Plant Defence Mechanisms and Growth Stimulants on Tomato (*Lycopersicum esculentum* Mill.)

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Abstract: In this study, the CAS agar plate assay for siderophores was used to screen bacteria from the rhizospheric soil of tomato in Annamalai nagar, Tamilnadu. As a result, *Bacillus subtilis* and *Pseudomonas fluorescens*, which produced a large orange halo, was obtained. A dual-culture test showed that both organisms strongly antagonized the growth of *Fusarium oxysporum* plant fungal pathogens, which rates of inhibition ranging from 25.59 to 93.45%. Analysis of the siderophore production by CAS agar plate assays showed that both organisms produced the siderophore. A pot culture experiment was analyzed to study the effects of *P. flourecens* and *B. subtilis* on pathogen development and plant growth. Both organisms reduced the incidence of Fusarium wilt in tomato significantly, by 79.69-75.24% which indicated that those organisms induced systemic resistance to Fusarium wilt in tomato. Iron supplementation reduced this biocontrol effect. There were significant increases in plant height, no. of fruits per plant, yield per plant and average fruit weight. This research showed that *P. fluorescens B. subtilis* has great potential for plant growth promotion and biological control for tomato disease.

Key words: Tomato · Siderophore · IAA · *Fusarium* wilt · *Pseudomonas* · *Bacillus*

INTRODUCTION

The recognition of plant growth-promoting rhizobacteria (PGPR), a group of beneficial plant bacteria, as potentially useful for stimulating plant growth and protection of minor and major pathogen, increasing crop yields has evolved over the past several years. Increased growth and yields of potato, sugar beet, radish and sweet potato have been reported by Farzana *et al.* [1]. PGPR, root-colonizing bacteria are known to influence plant growth by various direct or indirect mechanisms. Several chemical changes in soil are associated with PGPR. Plant growth-promoting bacteria (PGPB) are reported to influence the growth, yield and nutrient uptake by an array of mechanisms. Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral and nitrogen availability in the soil as a way to augment growth. The isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically Yasmin *et al*. [2].

Pseudomonas is one of the well-known solubilizer of phosphate, to produce plant growth hormones like IAA. They have broad spectrum antagonistic activity against plant pathogens such as antibiosis [4], siderophore production [5] and nutrition or site competition [6]. Some *Pseudomonas* species produce higher levels of hydrogen cyanide that is toxic to certain pathogenic fungi [7]. These characteristics make *Pseudomonas* species good candidate for use as seed inoculants and root dips for biological control of soil borne plant pathogens.

Bacillus spp. especially *Bacillus subtilis*, *Bacillus cereus* and *Bacillus amyloliquefaciens* are effective for the control of plant diseases caused by soil borne, foliar and post harvest fungal pathogens [8, 9]. Microorganisms that grow in the rhizosphere are ideal as biocontrol agents, since this region provides the first line of defence. In the soil, plant roots normally coexist with bacteria and fungi that may produce siderophores capable of sequestering the available soluble iron, which could interfere with plant growth and function. However, plant roots are sometimes capable of taking up ferric complexes

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of siderophores and using these as sources of iron [10]. Thus, siderophores may play an important role in the competition between microorganisms and may also act as growth promoters [11].

Tomato (*Lycopersicum esculentum* Mill.) is one of the most important vegetable crops grown extensively throughout the world, especially in temperate countries [12]. Fusarium wilt of tomato is caused by the soil borne fungus *Fusarium oxysporum* Schl. f. sp. Radicis-lycopersici. The fungus causes tremendous yield loss to tomato [13]. It may result in a severe loss of tomato quality and quantity and it persists indefinitely in most soils because of its ability to colonize the roots of a number of weeds and to produce resistant spore structures. The aims of the present study were to screen for plant growth promoting microbes and to evaluate their potentials in the biological control of *F. oxysporum*, the causal agent of *Fusarium* wilt of tomato and the promotion of plant growth.

MATERIALS AND METHODS

Isolation and Characterization of PGPR: *Pseudomonas fluorescens* and *Bacillus subtilis* were isolated from the rhizosphere soil of healthy tomato and pepper plants from Annamalai nagar, Tamilnadu, India. Non rhizosphere soil was removed from the root system of the plants. Roots were then excised and placed into 10 ml of sterile 0.9% NaCl solution and vortexed for 10 min in order to detach the associated rhizosphere soil. Serial dilutions of the resulting root wash were plated on King B medium (KM) supplemented with amphicillin (100µg ml-1) and cyclohexamide (75µg ml-1) described by King *et al* [14]. Plates were incubated at 28°C for 24-48 h, at which time the fluorescent colonies were observed under UV light (354 nm). To obtain the most abundant bacteria from each sample, selection of strains showing fluorescence and different colony morphology was performed from the highest dilutions, both bacterial cultures were stored at -20°C in Tryptic Soy Broth (TSB) supplemented with 20% (v:v) glycerol.

Bacterial characterization was carried out on the basis of colony morphology, gram stain, oxidase test, production acid from 1% glucose in oxidation/fermentation (OF) basal medium [15].

Antagonistic Activity of *P. Flourecens* and *B. Subtilis* under *in Vitro* Condition: A dual-culture test was conducted to examine, whether *P. fluorescens* and *B. subtilis* could antagonize the growth of plant fungal pathogens (Table 1). A mycelial disc, F0.5 cm in diameter, of a pure culture of fungal pathogen was placed in the centre of a PDA (Potato Dextrose Agar) plate and then *P. fluorescens* and *B. subtilis* was inoculated in four symmetrical spots around the mycelial disc. The plates were incubated for 7-10 days at 28°C. The plates were scanned once a day to monitor the formation of an inhibition zone and the growth of fungal pathogens. The width of the inhibition zones were measured and then averaged. A cell-free supernatant of *P. fluorescens and B. subtilis* was prepared by inoculating in the improved SM medium (Sodium Succinate Medium) [16] for 48 h at 37°C and then centrifuging at 12,000 rpm for 10 min. After mixing with PDA medium in ratios of 1:10, 1:20, 1:50, 1:100, 1:200, 1:500 and 1:1000, the mixture was poured onto plates. After solidification, fungal mycelial disc of □ 0.5 cm were placed in the centres of each petri dish. PDA medium containing 500µg ml^{-1} of 75% chlorothalonil wettable powder was used as a positive control (CK^{+}). The plates were incubated at 28°C and observed once a day to monitor the mycelial growth of the fungal pathogens. The fungal colony diameters were measured and then compared to the diameters of the same pathogens grown on the control plates. The inhibition percentage was calculated using the following formula:

$$\% \text{ Inhibition} = (1 - \text{diameter of treatment/diameter of control}) \times 100$$

Siderophore Assay: Production of siderophore was determined using chrome azurol sulphonate (CAS) agar method [17]. Briefly, the bacterial inoculum was

Table 1: Evaluation of the ability of the microorganisms to exhibit some PGP properties *in vitro* condition

	Phosphate solubilization		IAA production		
Treatments	Zone clarification	Zone diameter (cm)	Colour intensity	µg/ml	Siderophore production Colour intensity
Control	-	-	-	-	-
Pseudomonas fluorescens	+++	2.93±0.0879	+++	19.08±0.594	+++
Bacillus subtilis	+++	2.69±0.0807	++	16.40±0.492	+++

The values are mean ± SD for three samples in each group

dropped onto the centre of a CAS plate. After incubation at 28°C for 5 days, siderophore production was assessed by change in the colour of the medium from blue to orange.

Determination of indole-3-acetic acid (IAA): The production of IAA was determined using LB-tryptophan agar [18]. Single colony was streaked onto LB agar amended with 5 mM L-tryptophan, 0.06% sodium dodecyl sulphate and 1% glycerol. Plates were overlaid with Whatman no. 1 filter paper (82 mm diameter) and the bacteria were allowed to grow for a period of 3 days. After the incubation period, the paper was removed and treated with Salkowski's reagent [19] with the formulation of 2% ferric chloride (0.5 M) in 35% perchloric acid. Membranes were saturated in a Petri dish by soaking directly in Salkowski's reagent and the production of IAA was identified by the formation of a characteristic red halo within the membrane immediately surrounding the colony.

Phosphate Solubilisation Test: Phosphate-solubilization test was conducted quantitatively by plating the bacteria in agar containing precipitated tricalcium phosphate. The medium was a modification of Pikovskaya medium [20], consisted of 10 g glucose, 5 g tribasic phosphate ($Ca_5HO_{13}P_3$), 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, trace of $MnSO_4$ and $FeSO_4$, 0.5 g yeast extract and 15 g agar in 1,000 ml distilled water. Bacterial culture was streaked on the surface of replicated agar plates. The presence of clearing zone around bacterial colonies after overnight incubation was used as indicator for positive P-solubilization.

Effect on Spore Germination of *Fusarium Oxysporum* Schl. F. Sp. Lycopersici: Bacterial suspensions were made by suspending cells cultured on NA plates in 10 mM $MgSO_4$. The density of the bacterial suspensions was adjusted to 108 cfu ml^{-1} and suspensions of 10^7, 10^6, 10^5, 10^4, 10^3 cfu ml^{-1} were obtained by serial dilution. The spores of *F. oxysporum* obtained from 10-day-old cultures on PDA medium were collected, suspended in distilled sterile water and mixed with appropriated aliquots of stock aqueous suspension to obtain a density of 106 spores ml^{-1}. Three drops (about 50 µl) of each suspension were then placed on a microscopic slide and kept at 20°C on moistened, sterilized filter paper placed in petridishes for 24 h. The germination percentages of conidia (50 conidia for each treatment) were measured using the Olympus Cx40 microscope. The conidium was considered when the length of the germ tube length was at least equal to the diameter of the conidium.

Pot Culture Experiment

Preparation of Inoculum of Antagonists: The bacteria were grown for 24 h at 28°C on KB-agar plate's and then suspended in sterile 10 mM $MgSO_4$. The bacterial strains were introduced into an autoclaved (2-20 min at 121°C with 24 h interval) potting soil-sand mixture [21] to a density of approximately 7×106 cfu g^{-1}. The inocula for the induced systemic resistance experiments were prepared by mixing the bacterial suspensions with talcum (1:1, v/w) to a final density of 5×107 cfu g^{-1} [22].

Preparation of Inoculum of *F. Oxysporum:* *F. oxysporum* which was collected from *Fusarium*-diseased tomato stems was cultured in aerated PDA medium at 22°C. After 14 days of growth, cultures were g5 filtered through sterile glass wool to remove mycelial mats. Microconidia were harvested by centrifugation at 8000 g for 20 min, resuspended in 10 mM $MgSO_4$ and mixed through a potting soils and mixture (12:5, v/v) to a density of 3.75×10^5 cfu g^{-1}. The infested soil was incubated in polyethylene bags for 3-5 days at 20°C before use in the potting soil bioassays to allow colonization of the soil by the pathogen. The inoculum for the induced systemic resistance experiments was prepared as described above, except that *F. oxysporum* was mixed through a peates and (1:1, v/v) mixture to a density of 3×10^4 cfu g^{-1}.

Suppression of *Fusarium* Wilt in Potting Soil: The effects of single bacterial strains on *Fusarium* wilt in tomato were tested in a potting soil bioassay [23]. For this bioassay, the *F. oxysporum* infested soil, bacterized soil, additional autoclaved soil and the non-autoclaved sand were mixed in order to obtain final densities of 10^4 conidia per g of soil of *F. oxysporum* and 10^6 cfu g^{-1} of soil of the bacterial strain being tested. Pre-treatment, 9 pots (30 cm high and 30 cm in diameter) were filled with 1.5 kg of the soils and mixture, in which 10 tomato seeds were sown. The plants were grown in acclimatized greenhouse at 20°C and 70% relative humidity with a photoperiod of 16 h. Plants were watered with tap water once a week and once a week they received half-strength Hoagland's solution [24]. In order to study the influence of iron on

the suppression of *Fusarium* wilt in tomato, the solution was supplemented with 10 mM Fe-EDDHA (ethylenediaminodi (0-hydroxyphenyl) acetic acid). After approximately 28 days the percentage of diseased plants per pot was scored on the basis of both external wilting and internal browning symptoms [25].

Suppression of *Fusarium* Wilt by Induced Systemic Resistance: This bioassay was conducted as described by Leeman *et al*. [21]. Ten tomato seeds were sown in sand and after 5 days, the seedlings were transferred to rock wool cubes, in such a way that the root system was divided between two cubes. The biocontrol bacteria were inoculated on the lower part of the root system at the root tips and two days later, *F. oxysporum* was delivered in the peat and sand mixture at the part of the root system near the stem. The populations of the biocontrol bacteria and *F. oxysporum* remained spatially separated throughout the experiment, avoiding direct interactions between the biocontrol strains and the pathogen. The plants were grown in acclimatized greenhouse as described above. Plants were watered with deionized water. Twenty-eight days after transplanting, plants were scored for both external wilting and internal browning symptoms [25].

Root Colonization by Introduced Bacterial Strain: To investigate root colonization, both microorganisms was obtained by inoculation into LB (Luria-Bertani) medium. In the studies of the suppression of *Fusarium* wilt in potting soil, colonization by *P. fluorescens and B. subtilis* was determined at four time points (20,40, 60 and 80 days). When sampling plants for root colonization, the soil surrounding the root system was loosened and two root systems, randomly chosen from the ten plants per pot, were harvested. The samples were suspended in 10 ml of sterile solution 10 mM $MgSO_4$ and shaken vigorously for 30sec in glass tubes containing 0.5 g of glass beads (0.56-0.80 mm in diameter). Aliquots (100 ml) of serial dilutions of these rhizosphere samples were thoroughly mixed with 300 ml of not-yet-solidified selective LB agar (LB supplemented with kanamycin at 100 mg per ml and also rifampin at 100 mg per ml for *B. subtilis*) at 45°C in 24 well tissue culture plates on a reciprocal shaker. After incubation for 24 h at 28°C, the number of bacterial colonies that had developed inside the agar was determined. The rhizosphere populations of introduced bacterial strains approximate a log normal distribution along the root system [26]. Therefore, the numbers of cfu were transformed to $\log_{10}$ (cfu+1).

Plant Growth Promotion and Yield Enhancement: Seeds of tomato were incubated at 25°C for germination after soaking in the cell-free supernatant (prepared as described in the section on antagonistic activity) of *P. fluorescens and B. subtilis* for 8-10 h, seeds soaked in water were used as the control. The seeds were sown in pots of soil when the embryo root emerged and then incubated in acclimatized greenhouse at 20°C and 70% relative humidity. The culture liquid of *P. fluorescens and B. subtilis* was applied by irrigating two times, at 10 and 30 days after sowing; water was used to irrigate the control. The plant height was recorded at 80th day after sowing. The yield of tomatoes was determined at the end of crop duration. At harvesting time under the experimental conditions, the yield expressed as the average fruit weight (g), number of fruits per plant and yield per plant (g) was recorded.

Statistical Analysis: All data are reported as Mean±SD of the mean for three replicates.

RESULTS

The two bacterial strains were obtained from tomato roots. Both strains were Gram negative rods, oxidase-positive and capable of metabolizing glucose in an oxidase form. The API 20NE test revealed that the strains belong to the species *P. fluorescens* and *B. subtilis*.

The *P. fluorescens and B. subtilis* strain significantly inhibited the mycelial growth tested and the zone of inhibition ranged from 4.5 mm to 13.5 mm, indicating strong antagonistic activity against plant fungal pathogens. Seven concentrations of both strains cell-free supernatants were tested *in vitro* against linear growth of *F. oxysporum*. As shown in Table 2, the cell-free supernatant of *P. fluorescens and B. subtilis* significantly inhibited the mycelial growth, with inhibition rates ranging from 19.26% to 94.07%. Increasing conc. of the cell-free supernatant caused a decrease in the linear growth rate of the pathogen. Treatment with 500 mg ml^{-1} of 75% chloro thalonil wettable powder gave an inhibition rate between those of 1:20 and 1:50 dilutions of these organisms cell-free supernatant.

To quickly and efficiently screen for siderophore-producing soil bacteria, we used the CAS agar plate assay. After culture and purification by streaking 3 or 4 times, two bacterial isolates were obtained. Clones producing orange halos were observed on the preliminary screening plates.

Table 2: Effects of PGPR inoculations on growth, yield and disease incidence of tomato

Treatments	Plant height (cm)	No. of fruits per plant	Yield per plant	Average fruit weight per plant	Disease incidence (%)
Control	55.85±1.676	11.33±0.339	321.05±9.631	23.64±0.709	55.14±1.654
Pseudomonas fluorescens	83.56±2.506	21.33±0.639	460.74±13.82	31.82±0.954	20.31±0.609
Bacillus subtilis	74.5±2.235	19.33±0.579	414.81±12.44	26.98±0.809	24.76±0.742

The values are mean ± SD for three samples in each group

Table 3: Inhibition percentage of mycelial growth of *P. fluoresces* and *B. subtilis*

		Inhibition percentage of mycelial growth						
Bacterial strain	CK^+	1:10	1:20	1:50	1:100	1:200	1:500	1:1000
P. fluorescens	79.15±2.37	93.45±2.80	83.06±2.49	71.37±2.14	61.48±1.84	54.06±1.62	41.61±1.25	3.10±0.90
B. subtilis	78.07±2.34	92.03±2.76	82.98±2.49	65.17±1.95	61.44±1.84	57.16±1.71	38.83±1.16	25.59±0.77

The values are mean ± SD for three samples in each group

Table 4: Effect of *P. fluorescens* and *B. subtilis* on spore germination of *F. oxysporum* Schl. F. sp. *Lycopersi*

	Concentration of cell suspensions (cfu ml-1)												
	P. fluorescens							B. subtilis					
	0	10^3	10^4	10^5	10^6	10^7	10^8	0	10^3	10^4	10^5	10^6	10^7	10^8
Germinated spores	50.0	46.0±1.38	45.7±1.37	35.4±1.06	28.53±0.86	19.8±0.59	17.9±0.54	50.0	46.1±1.38	43.9±1.32	37.5±1.13	24.1±0.72	20.7±0.62	19.9±0.60
Germination percent (%)	100.0	92.3±2.76	87.3±2.62	70.3±2.12	58.33±1.75	40.2±1.21	36.9±1.11	100.0	91.9±2.76	88.3±2.65	72.4±2.17	56.3±1.69	39.1±1.17	33.9±1.02
Reduction (%)	-	8.7±0.26	12.7±0.38	29.7±0.39	41.67±1.25	51.8±1.55	63.1±1.89	-	8.1±0.24	11.7±0.35	27.6±0.83	43.7±1.31	61.0±1.83	66.1±1.98

The values are mean ± SD for three samples in each group

Table 5: Population densities of *P. fluorescens* and *Bacillus subtilis* on tomato roots

	Population densities (10g 10 CFU/g root)			
Treatments	20 days	40 days	60 days	80 days
P. fluorescens	6.62±1.198	6.61±0.198	6.36±0.190	6.35±0.190
B. subtilis	6.57±1.197	6.06±1.18	6.24±0.187	6.16±0.184

The values are mean ± SD for three samples in each group

When spotted and streaked on the fresh CAS agar plates, they produced bright orange halo. However, *P. fluorescens* appeared to be largest size of halo compared with *B subtilis*.

Results showed that P-solubilisation is the common feature of all tested microorganism grown on synthetic media as expressed by halo clarification zone formed around their colonies (zone diameter ranged from 2.69 to 2.93 cm). However, *P. fluorescens* appeared to be superior to the *B. subtilis*, as it produced 2.93 cm of clear zone and *B. subtilis* displayed the lowest capacity (2.69 cm) (Table 3).

The results originated from both qualitative and quantitative assays of IAA reflected the ability of all tested microorganisms to produce indole compounds. The two tested microorganisms exhibited a pink to red colour with a little variation in intensity. In the quantitative measurements, the highest value of auxin production was obtained by *P. fluorescens* followed by *B. subtilis* as they produced 19.8 µg/ml, while *B. subtilis* produced nearly lower amount of IAA being 16.4 µg/ml. The effects of six concentrations of *P. fluorescens and B. subtilis* cell suspensions on spore germination of *F. oxysporum* were tested *in vitro*. As shown in Table 1, increasing concentrations caused a decrease in the spore germination percentage.

The data in Table 2 show that both treatments significantly reduced the incidence of *Fusarium* wilt in tomato seedlings compared with the control (non-treated seedlings). The reduction of disease reached at *P. fluorescens* (79.69%) and *B. subtilis* (75.24%). The bacteria were applied prior to inoculation by *F. oxysporum* which indicated that *P. fluorescens and B. subtilis* could suppress *Fusarium* wilt of tomato by induced systemic resistance. The results also showed that after treatment the suppression by *P. fluorescens and B. subtilis* of *Fusarium* wilt in tomato was significantly reduced to 20.31% and 24.76% respectively. The data in Table 5 show that the two strains colonized well on tomato roots. W hen applied at 20, 40, 60 and 80 days,

the population densities of those organisms were not significantly different which indicated that *P. fluorescens and B. subtilis* stably colonized tomato roots after application. an average fruit weight

The plant height was different between the treated and control plants (83.56 cm and 55.85 cm respectively). The data in Table 2 show that the fresh weight of fruits from *P. fluorescens* and *B. subtilis* treated plants was significantly different than the control, with an average fruit weight of 31.82, 26.98 and 23.64 g (control) respectively. The *P. fluorescens* and *B. subtilis* treated plants also showed yield per plant 460.74, 414.81 and 321.05 g (control) and no. of fruits per plant by 21.33, 19.33 than that of the control (11.33 g).

DISCUSSION

PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots which can improve the extent or quality of plant growth directly and/or indirectly. In last few decades, a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, Enterobacter, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth [27].

In the CAS agar plate assay, the ternary complex CAS/iron III/hexadecyltri-methyl ammonium bromide serves as an indicator. When a strong chelator such as a siderophore, removes the iron from the dye, its colour turns from blue to purple or orange. When this CAS complex is incorporated into agar plates, halos around the colonies were formed, indicating the production of a siderophore [28]. In this study, siderophore-producing isolates (*P. fluorescens and B. subtilis*) were screened from rhizospheric soil which has strong siderophore production and produced the largest size of orange halo confirmed the high efficiency of screening by the CAS assay method. Both has strong siderophore induction and produced the largest size of orange halo which selected for further study.

To study the antagonistic activity of *P. fluorescens and B. subtilis* on plant fungal pathogens, belonging to the species of *F. oxysporum* were used in the dual culture test *in vitro* condition. They strongly antagonized the growth of the fungal pathogens which provided the basis for the study of the biocontrol effect in a pot culture experiment. Larkin and Fravel [29] reported that non-pathogenic isolates of *F. oxysporum* and *Fusarium solani* were the most effective antagonist of fusarium wilt of tomato among numerous fungi and bacteria that were tested using identical revaluation methods. Several microorganism produce lytic enzymes, which can degrade cell wall of other organism. The production of lytic enzymes by bacteria has been shown to be one of the mechanisms of biocontrol of plant diseases Kaur *et al.* [30].

Siderophores are iron-specific compounds that are secreted under low iron stress and capture iron from the environment. The biosynthesis and secretion of siderophores are strictly regulated by environmental factors of which iron concentration is the most important [31]. Microbial siderophores may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly by competitively inhibiting the growth of plant pathogens with less efficient iron-uptake systems [32]. Kloepper *et al*. [33] were the first to demonstrate the importance of siderophore in the growth-promoting *P. fluorescens* strains A1, BK1, TL3B1 and B10. A direct correlation was established *in vitro* between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of *F. oxysporum* [34]. Taking into account this factor, we studied the effect of Fe (III) on the *P. fluorescens and B. subtilis* siderophore production by the DHB (G) assay.

Phosphate solubilisation is the common feature of two tested microorganism grown on synthetic media as expressed by halo clarification zone formed around their colonies. The clear zone caused by the tested microorganisms may indicate excretion of particular groups of organic acids, which have high affinity to chelate the calcium ions. The ability of bacteria and fungi to dissolve the precipitated phosphorus depends on its efficiency to produce inorganic and organic acids and/or CO2 [35 and 36]. In this concern, Altomare *et al.* [37] reported that the capability of the plant-growth-promoting and biocontrol bacteria to solubilize *in vitro* some insoluble or sparingly soluble minerals via three possible mechanisms: acidification of the medium, production of chelating metabolites and redox activity. These bacteria may also solubilize inorganic phosphate, making soil phosphorus otherwise remaining fixed available to the plants. Therefore, the unavailable forms of phosphorus can be partially dissolved and enhance its availability against the adverse conditions by the action of phosphate dissolving microorganism naturally occurring or introduced into the soil [38].

Indeed, a high proportion of rhizo-microorganisms are able to produce plant growth hormone, i.e. indole acetic acid, which acts to stimulate root growth and provides it with more branching and larger surface area. In fact, many investigators consider the indole secretion, by PGPRs, as a vital mechanism to clarify plant promotion [39]. The present communication was carried out to evaluate the biocontrol effect of the siderophore-producing bacterium *P. fluorescens and B. subtilis* on *Fusarium* wilt of tomato and on plant growth promotion in tomato. Before the pot culture experiment, the suppression of these organisms on the *F. oxysporum* spore germination was experimentally determined. These two strains were shown to significantly suppress the spore germination on *P. flourescens* (8.7% to 63.1%) and *B. subtilis* (8.1% to 66.1 %) which is in accordance with its antagonistic activity.

In the present study, the incidence of *Fusarium* wilt, plant height (cm), average fruit weight (g), number of fruits per plant and yield per plant (g) were recorded. The plant height (cm), average fruit weight (g), number of fruits per plant and yield per plant (g) were significantly higher in treated plants than in the control. The disease incidence was much lower in the treated plants than in the control, with an inhibition of 12.5-56.9% (Table 4), indicating that *P. fluorescens and B. subtilis* induced systemic resistance to *Fusarium* wilt in tomato plants. Fe decreased the inhibitory effect of *P. fluorescens and B. subtilis* on *Fusarium* wilt in tomato.

CONCLUSION

Biological control of plant pathogens has been the subject of much research in recent years. It can potentially help us limit the use of chemical pesticides that are harmful to the environment. For this aspect, we use of plant growth-promoting rhizobacteria such as siderophore-producing bacteria, represents a potentially attractive and alternative disease management approach, since they have the capacity to increase yield and protect crops simultaneously. Both strains are stably colonized on tomato roots and the population densities of *P. fluorescens* and *B. subtilis* in tomato roots were quite stable as the plants grew.

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