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Characterization of rhizosphere bacteria for control of phytopathogenic fungi of Tomato

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ABSTRACT

Fluorescent *Pseudomonas* spp., isolated from rhizosphere soil of tomato and pepper plants, was evaluated *in vitro* as potential antagonists of fungal pathogens. Strains were characterized using the API 20NE biochemical system, and tested against the causal agents of stem canker and leaf blight (*Alternaria alternata* f. sp. *lycopersici*), southern blight (*Sclerotium rolfsii* Sacc.), and root rot (*Fusarium solani*). To this end, dual culture antagonism assays were carried out on 25% Tryptic Soy Agar, King B medium, and Potato Dextrose Agar to determine the effect of the strains on mycelial growth of the pathogens. The effect of two concentrations of $FeCl_3$ on antagonism against *Alternaria alternata* f. sp. *lycopersici* was also tested. In addition, strains were screened for ability to produce exoenzymes and siderophores. Finally, the selected *Pseudomonas* strain, PCI2, was evaluated for effect on tomato seedling development and as a potential candidate for controlling tomato damping-off caused by *Sclerotium rolfsii* Sacc., under growth chamber conditions. All strains significantly inhibited *Alternaria alternata* f. sp. *lycopersici*, particularly in 25% TSA medium. Antagonistic effect on *Sclerotium rolfsii* Sacc. and *Fusarium solani* was greater on King B medium. Protease was produced by 30% of the strains, but no strains produced cellulase or chitinase. Growth chamber studies resulted in significant increases in plant stand as well as in root dry weight. PCI2 was able to establish and survive in tomato plants rhizosphere after 40 days following planting of bacterized seeds.

1. Introduction

Tomato (Lycopersicon esculentum Mill.) is the second leading vegetable crop worldwide, next to potato. World production is $\sim 1 \times 10^6$ tonnes from 3.7×10^6 ha (Food Agricultural Organization, 2010). In Argentina, it is the vegetable occupying the most greenhouse area. The percentage of total production going to industry is 35–40% and the rest is sold as fresh produce domestically. The area dedicated to tomato in field and greenhouse is 1.2×10^4 ha and 3×10^3 ha, respectively; average yield in both cases is ~35–40 tonnes per ha (Nakama and Fernández Lozano, 2006). Due to increasing demand, tomato has a great potential for increased commercialization. More efficient tomato production requires better knowledge of its pathogens and control methods.

The fungus *Alternaria alternata* f. sp. *lycopersici*, frequently isolated from diseased tomato plants, is the cause of stem canker (Gilchrist and Grogan, 1975) and leaf blight (Akhtar et al., 2004). *Sclerotium rolfsii* Sacc. is a soilborne fungus that causes southern blight disease in a wide variety of agricultural and horticultural crops (Flores-Moctezuma et al., 2006). *Fusarium solani* causes root rot in several crops. Penconazole [1-(2,4-dichloro-β-propylphenethyl)-1*H*-1,2,4-triazole], penthachloronitrobenzene (PCNB), and idropione [3-(3,5-dichlorophenyl)-N-(1-methylethyl)2,4-dioxo-1-imidazole-carboxamide] are three chemical fungicides commonly used to control the above pathogenic fungi. Nevertheless, increasing public concern regarding use of chemical pesticides that damage human health or the environment is driving the search for more environmentally "friendly" methods to control plant disease. A realistic alternative, or supplement, to chemical fungicides for management of plant diseases is the use of soilborne, non-

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pathogenic bacteria that inhibit fungal phytopathogens. Such bacteria are known by several generic names, including "biological control agents" (BCAs) and "plant growth promoting rhizobacteria" (PGPR). Soilborne, fluorescent pseudomonads have received particular attention because of their catabolic versatility, excellent root-colonizing abilities, and production of a wide range of antifungal metabolites (Walsh et al., 2001).

The objectives of this study were (1) to biochemically characterize fluorescent *Pseudomonas* strains, (2) to evaluate their antagonistic activities against phytopathogenic fungi of vegetables *in vitro*, and (3) to determine the effect of a strain, PCI2, on tomato growth as well as to evaluate its potential for controlling tomato damping-off caused by *S. rolfsii* Sacc.

2. Materials and methods

2.1. Isolation and characterization of fluorescent Pseudomonas

Fluorescent *Pseudomonas* spp. were isolated from the rhizosphere of healthy tomato (*L. esculentum* Mill.) and pepper (*Capsicum annuum* L.) plants from four regions of the province of Córdoba, Argentina: Colonia Caroya (20° 36′ N, 102° 13′ W), Embalse (32° 12″ S, 64° 23″ W), Mattaldi (34° 49′ 16″ S, 64° 34′ 22″ W) and Río Cuarto (33° 04′ S, 64° 38′ W). 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 (KB) (King et al., 1954) supplemented with ampicillin (100 μg ml^{-1}) and cycloheximide (75 μg ml^{-1}) (Simon and Ridge, 1974). 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. All 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 of acids from 1% glucose in Oxidation/Fermentation (OF) basal medium (Hugh and Leifson, 1953), and analysis with the API 20NE biochemical test plus computer software (bioMèrieux S.A., Marcy l'Etoile, France).

2.2. Phytopathogenic fungi and reference bacteria

Fungal phytopathogens used were *Sclerotium rolfsii* Sacc., *Fusarium solani* (from the fungal collection of the Laboratory of Plant–Microbe Interactions, Universidad Nacional de Río Cuarto), and *Alternaria alternata* f. sp. *lycopersici* (kindly supplied by the Laboratory of Mycology, Universidad Nacional de Río Cuarto), all isolated from diseased tomato and pepper plants. Fungi were kept in potato dextrose agar (PDA) plates at room temperature or at 4 °C, and replicated monthly.

Reference bacteria were used in this research. *Pseudomonas fluorescens* CHA0 and *P. aurantiaca* SR1 were grown on KB and 25% Tryptic Soy Agar (TSA). *Serratia marcescens* WF was grown on 25% TSA. *Bradyrhizobium* spp. C 145 and *Sinorhizobium meliloti 3DOh13* were maintained on Yeast Mannitol Agar (Vincent, 1970). All the bacteria were routinely cultured at 28 °C.

2.3. Evaluation of strains for in vitro biological control

2.3.1. Antagonism in dual culture

The fluorescent *Pseudomonas* were tested against *S. rolfsii* Sacc., *A. alternata* and *F. solani* in plate bioassays. *A. alternata* and *F. solani* were cultivated in PDA at 28 °C. Conidia were harvested from the surface of plates by flooding the 10-day-old cultures with 9 ml of sterilized distilled water and gently scraping with a sterilized glass rod; conidial concentration was determined with a Neubauer chamber (Cota et al., 2007). Plates containing the media to be tested (KB, PDA) were prepared. Then, an agar over-layer containing the target fungus, immobilized at a concentration of 10^4–10^5 conidia ml^{-1}, was placed on the medium. The methodology described by Montesinos et al. (1996) was followed in order to prepare the overlay, using 0.7% agar. Four ml of the medium was placed in screw-capped test tubes that, once sterilized, were kept inside of a bath of water at 40 °C. Next, 100 μl of a target conidia suspension was added to each test tube, which were vortexed and the content of each tube was then homogeneously distributed on a plate containing the same culture medium. The bacterial strains tested were sown by gently touching the agar surface with a sterile toothpick, previously inoculated by touching the surface of a single colony. Plates were incubated for 72 h at 28 °C. The degree of inhibition in each medium was determined by measuring the halo around the bacterial strain with no fungal growth. The average of six replicates was considered for the value of the inhibition halo. For screening for potential antagonism against *S. rolfsii* Sacc., mature sclerotia were removed from the surface of 15-day-old cultures with sterile forceps and four were immediately placed around the edges and one in the center of a plate 24 h after the stab-inoculation of four bacterial strains. The experiment was conducted twice.

2.3.2. Mycelial growth inhibition

The bacterial strains were streaked on 1/3 of a Petri plate containing 25% TSA, KB or PDA. A mycelial disc (9 mm diameter) of a 8–15 day-old-culture of an actively growing target fungus was equidistantly placed on the opposite side of the Petri plate 48 h after inoculation of the strain. Plates were incubated for 7 days at 28 °C. The plates with fungal pathogens on one side that were not inoculated with bacterial strains served as controls. For each fungal colony, two diameters, measured at right angles to one another, were averaged to find the mean diameter for that colony. The mean diameter of fungal growth in the presence of each strain was compared to that of the control cultures in order to determine the inhibition percentage. All fungal colony diameters were determined by using three replicates for each strain on each medium. *P. aurantiaca* SR1 (Rosas et al., 2001; Rovera et al., 2008) and *P. fluorescens* CHA0 were used as positive controls.

In addition, each strain was tested on both 25% TSA and KB supplemented with two concentrations of $FeCl_3$ (50 and 100 μM) in order to evaluate the influence of iron on the ability of the strains to control *A. alternata*. Plates were incubated for 7 days at 28 °C. The fungal colony diameter was determined by using three replicates for each strain on each medium. The plates with *A. alternata* on one side that were not inoculated with bacterial strains served as controls. Experiments were conducted twice.

2.3.3. Production of hydrolytic enzymes

Proteolytic activity was detected by inoculating the strains on a medium composed of 1% casein and 2.3% agar dissolved in Castañeda medium (Castañeda-Agulló, 1956). Plates were incubated for 48 h at 28 °C. Casein hydrolysis was detected by the formation of a whitish, opaque halo (coagulated casein) around a translucent area (totally hydrolyzed casein), surrounding the colony. Strains were also tested for its ability to produce extracellular chitinases in a liquid medium; assay medium was prepared with 2% chitin from crab shells (w:v) in tap water (Rojas Avelizapa et al., 1999). *S. marcescens* WF was used as a positive control. Tests were performed twice. To determine cellulolytic activity, carboxymethyl cellulose (CMC) was incorporated at 0.1% into the YEMA–0.2% mannitol agar plates. Colonies were grown for 3 days at 28 °C and

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washed off with water. The plates were then flooded with 0.1% (wt:vol) Congo Red in water for 15 min, washed for 10 min with 1 M NaCl, and then washed for 5 min with 5% acetic acid. Degradation of CMC was observed as clearings (reduction of staining) (Zorreguieta et al., 1999). Test was performed twice. *Bradyrhizobium* spp. C 145 was used as the positive control.

2.3.4. Siderophores production

The chrome azurol S (CAS) method described by Alexander and Zuberer (1991) was used for screening strains for siderophore production. Plates were incubated at 28 °C for 5 days, and microorganisms exhibiting an orange halo were considered to be producers of siderophores. *S. meliloti 3DOh13* (Rosas et al., 2006) was used as the positive control.

2.4. Identification and quantification of indole-3-acetic acid (IAA) in culture supernatant of strain PCI2

Strain PCI2 was grown in nutrient broth (NB). Then, 20 ml were taken during the late exponential growth phase (24 h) for identification and quantification of IAA, which were carried out by High Performance Liquid Chromatography HPLC–Mass spectrometry (HPLC–MS). A 100 ng 2H5-IAA (OlChemIm, Czech Republic) deuterated internal standard was included.

2.5. Evaluation of selected strain PCI2 for growth promotion and biological control

2.5.1. Preparation of fungal and bacterial inocula and treatment of seeds

Cultures of *S. rolfsii* Sacc. were maintained on PDA, on which brown sclerotia formed within 8–10 days. Pathogen inoculum added to sterile mixture consisted of 30-day-old sclerotia which were dislodged from the surface of plates and used immediately (Papavizas and Lewis, 1989). Plastic pots (15 cm diameter; 25 cm height) were filled with 600 g of sterile mixture (soil:sand:perlite at 2:1:1 w/w/w), previously sterilized by heating at 180 °C for 2 h on four consecutive days. Each pot was then moistened with sterile distilled water and infested in the mixture surface with 30 mg of sclerotia. Pots were kept for 8 days in a growth chamber under controlled conditions: 16 h light at 28 ± 2 °C, 8 h dark at 16 ± 2 °C (light intensity of 220 μE m^{-2} s^{-1}).

After incubation, tomato seeds (*L. esculentum* Mill.) cv. Platense Italiano (Asociación Cooperativa INTA La Consulta, Mendoza, Argentina) were surface-disinfected for 10 min in 5% sodium hypochlorite solution (60 g l^{-1} of active chlorine), washed ten times in sterilized distilled water, and air dried (Tsahouridou and Thanassoulopoulos, 2002). Then, 10 g of seeds were soaked for 30 min in 2.5 ml of a 10^9 CFU ml^{-1} aqueous cell suspension of strain PCI2. The bacterium was prepared by growing by shaking (80 rpm) in KB broth for 48 h at 28 °C (Jayaraj et al., 2007). Then, eight inoculated seeds were placed into the mixture surface in each pot. The four treatments were: (1) non-infested, non-bacterized healthy control (treated with sterile distilled water), (2) infested with *S. rolfsii* Sacc., non-bacterized control, (3) infested with *S. rolfsii* Sacc. and bacterized with PCI2, and (4) bacterized with PCI2 alone. Pots were incubated in a growth chamber under the conditions described above. Damping-off was determined by counting the total healthy stand after 40 days, compared to non-infested control plants. Shoot and root dry weights (72 h at 70 °C) were recorded from twenty randomly selected plants from each treatment. Pots were arranged in a completely randomized design. The experiment was performed twice, each with six replicates per treatment.

2.5.2. Tomato rhizosphere colonization

Survival of strain PCI2 in the rhizosphere of tomato plants from treatments 1 and 4 was determined according to a modification of the procedure described by Landa et al. (2004) at 10, 25 and 40 days after sowing. Briefly, 1 g of rhizosphere mixture was collected at 10 days from the surroundings of a seedling from each treatment and placed into 9 ml of sterile 0.9% NaCl solution. Also, a seedling from each treatment was carefully removed from a pot at 25 and 40 days and roots were gently shaken to remove all but the tightly adhering potting mixture. One gram of the adhering rhizosphere mixture was collected and placed into 9 ml of sterile 0.9% NaCl solution. Serial dilutions of the suspension were vortexed and plated onto 25% TSA medium. Plates were incubated for 48 h at 28 °C. The developed colonies from each treatment were counted and the number of CFU g^{-1} of mixture was calculated.

2.6. Statistical analyses

The data were analyzed by using analysis of variance (ANOVA). When ANOVA showed treatment effect, the Least Significant Difference (LSD) test was applied to make comparisons between the means at $P < 0.05$. A non parametric Kruskall-Wallis variance analysis was applied to evaluate the differences between the inhibitory capacities of the strains in media supplemented with different concentrations of iron. All data were subjected to statistical analysis using Statgraphics plus software for Windows *V 4.1* (Statistical Graphics Corp., Maryland, USA).

3. Results

Ten bacterial strains were obtained from tomato and pepper roots. All of the strains were Gram-negative rods, oxidase-positive and capable of metabolizing glucose in an oxidative form. The API 20NE test revealed that the strains belong to the species *Pseudomonas fluorescens* (four strains), *P. putida* (four strains), *P. aeruginosa* (one strain) and *Ralstonia pickettii* (one strain).

Recovered bacterial strains were tested for their antagonistic ability against the phytopathogenic fungi *A. alternata*, *S. rolfsii* Sacc. and *F. solani*. As a result, the bacterial antagonistic effect in the dual culture assay depended both on the target pathogen and the culture media used; moreover, the influence of the composition of the medium was observed against all fungi. The strongest *in vitro* antagonism against *A. alternata* was observed on 25% TSA, while the higher inhibitory activity against *F. solani* and *S. rolfsii* Sacc. was observed on KB.

Similarly, the observed *in vitro* inhibition of mycelial growth also varied with the culture medium and the target pathogen. The inhibitory effect on the mycelial growth of *A. alternata* was higher on 25% TSA. All the tested strains resulted in >60% inhibition on 25% TSA, >40% on KB and ≤20% on PDA. As observed for the germination inhibition assay, mycelial growth inhibition of *F. solani* and *S. rolfsii* Sacc. by all the recovered strains was more effective on KB. However, the growth of the fungal pathogens was barely inhibited in the presence of the strains on PDA (Table 1).

Addition of iron to 25% TSA affected the antagonistic activity of the strains against *A. alternata*. A 50 μM $FeCl_3$ concentration significantly decreased the effectiveness of seven strains (P1, P8, Tbr2, TR1, P12, Pbr3 and PCI2). Addition of 100 μM $FeCl_3$ inhibited the antagonistic activity of all of the isolated strains. Moreover, addition of iron to KB caused a stronger decrease in the antagonistic activity of the strains against *A. alternata*. A 50 μM concentration of $FeCl_3$ significantly decreased the effectiveness of strain P1. Addition of 100 μM $FeCl_3$ produced a significant decrease in the antagonistic activity of four strains (P1, TR1, P12 and PCI2) (Table 2). Three

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Table 1
Inhibition percentage of mycelial growth of *S. rolfsii* Sacc., *A. alternata* f. sp. *lycopersici* and *F. solani* on three different media.

Bacterial strain	Inhibition percentage of mycelial growth								
	TSA			KB			PDA		
	S. r.	*A. a.*	*F. s.*	*S. r.*	*A. a.*	*F. s.*	*S. r.*	*A. a.*	*F. s.*
P. putida P1	12.39b	66.65b	2.62de	9.01cd	41.43ns	12.55bcde	7.43bc	20.31b	3.60d
P. fluorescens P8	17.19b	77.48ab	3.05cde	8.19cd	55.82ns	11.05cdef	5.78bc	13.54bc	5.40d
P. putida TBR2	4.39c	73.86ab	4.79c	2.45d	47.98ns	10.55def	2.88cd	9.89c	8.10c
P. fluorescens TEI1	1.99c	72.84ab	4.36cd	11.47cd	50.58ns	12.06cdef	4.95bc	14.57bc	5.40d
P. fluorescens TR1	1.79c	82.11a	4.79c	20.07bc	54.50ns	9.54ef	5.37bc	11.98bc	8.10c
P. aeruginosa P4	1.00c	75.93ab	3.48cde	24.58bc	53.19ns	13.56bcd	7.43bc	16.14bc	7.66c
R. pickettii P6	15.99b	75.93ab	4.79c	18.02cd	41.62ns	15.57b	3.71cd	9.89c	4.95d
P. putida P12	1.79c	75.40ab	1.74f	3.68d	44.05ns	14.06bc	2.47cd	15.10bc	9.01c
P. putida PBR3	1.59c	80.04ab	1.74f	1.63d	41.43ns	9.54ef	6.61bc	7.81c	5.40d
P. fluorescens PCI2	2.39c	71.80ab	2.17ef	25.81bc	50.58ns	9.04f	7.02bc	15.62bc	7.66c
P. aurantiaca SR1	44.40a	78.50ab	12.65b	58.93a	50.58ns	15.57b	19.42a	29.68a	13.51b
P. fluorescens CHA0	51.19a	79.00ab	28.38a	59.00a	57.71ns	43.21a	19.90a	13.54bc	20.27a

S. r.: *S.* rolfsii Sacc.; *A. a.*: *A. alternata* f. sp. *lycopersici*; *F. s.*: *F. solani*.
Percentages with the same letter within the same column are not significantly different according to the LSD ($P < 0.05$) test.
ns: not significantly different.

strains (P4, P6 and P8) showed protease activity, whereas none of them produced cellulase or chitinase. Additionally, all of the strains were able to respond to iron limitation producing siderophores in CAS medium.

Of the ten strains isolated from the root system of tomato and pepper plants, strain PCI2 was selected for further study based on its *in vitro* inhibitory activity in the antagonism in dual culture as well as in the mycelial growth inhibition assays against phytopathogenic fungi of tomato, in particular against *S. rolfsii* Sacc. Thus, PCI2 was evaluated for growth promotion of tomato plants and biological control of *S. rolfsii* Sacc. *in vivo*. In *S. rolfsii* Sacc. infested mixture, inoculating tomato seeds with strain PCI2 improved seedling stand by 29% and increased shoot and root dry weight of plants over the untreated pathogen controls by 84.7 mg and 59.9 mg, respectively (Fig. 1). No evident differences between bacterized seeds and control seeds were observed in non-infested potting mixture when recording plant stand; however, inoculation of seeds with PCI2 increased ($P < 0.05$) root dry weight by 71.8 mg. The increase in root dry weight may be due to phytohormone-like substances, since strain PCI2 produces indole-3-acetic acid (IAA) at 4.71 μg ml^{-1} (without addition of tryptophan to culture medium) after 24 h of incubation. Although inoculation with strain PCI2 increased shoot dry weight by 33.6 mg, when compared to healthy controls, differences were not significant (Fig. 1). Fluorescent *Pseudomonas* morphologically similar to PCI2 reached a population density of 10^7–10^8 and 10^6–10^7 CFU g^{-1} mixture after ten and forty days of experimentation, respectively, in the bacterized with PCI2 alone treatment (Fig. 2). Colony counts performed from non-infested, non-bacterized control plants revealed absence of colonies morphologically similar to PCI2.

4. Discussion

The aim of this study was the isolation, characterization, and selection of *Pseudomonas* spp. with antagonistic activity against phytopathogenic fungi, but harmless to vegetable crops. The strains used were initially isolated from rhizosphere of healthy tomato and pepper plants from four regions of Córdoba province. Williams and Asher (1996) concluded that methods employed to isolate rhizobacteria play an important role in identification of potential biocontrol agents, and that the strains should be from the rhizosphere of the target crop.

Antagonistic properties of strains tested *in vitro* were influenced by culture medium composition, the fungal pathogen, and its growth stages. These results were consistent with those of Borowicz and Saad Omer (2000), who proposed that differences between media could result in alterations of metabolites produced, or their relative concentrations. Also, the type of medium used to grow both bacteria and fungi in studies of biological control affects the interaction of the organisms (Benko and Highley, 1990).

Enzymatic degradation of the cell wall of fungal pathogens by biocontrol agents has been reported (Bar-Shimon et al., 2004; Compant et al., 2005). In this work, protease, cellulase and chitinase production were assayed. Protease production proved to be the

Table 2
Inhibition percentage of *A. alternata* f. sp. *lycopersici* mycelial growth in media supplemented with different iron concentrations.

Bacterial strain	Inhibition percentage of*A. alternata* f. sp. *lycopersici* mycelial growth					
	25% TSA			King's B medium		
	No $FeCl_3$	$FeCl_3$ 50 μM	$FeCl_3$ 100 μM	No $FeCl_3$	$FeCl_3$ 50 μM	$FeCl_3$ 100 μM
P. putida P1	66.66a	48.14b	43.95b	40.94a	13.63b	12.69b
P. fluorescens P8	66.66a	56.07b	54.39b	35.42a	37.27a	42.85a
P. putida TBR2	65.98a	54.91b	51.65b	29.91a	20.09a	17.45ab
P. fluorescens TEI1	58.50a	56.07ab	46.85b	30.57a	21.83b	18.26b
P. fluorescens TR1	61.90a	42.20b	42.85b	44.87a	33.65a	13.50b
P. aeruginosa P4	68.02a	56.07ab	50.55b	46.35a	29.91b	30.16b
R. pickettii P6	65.98a	54.91ab	47.25b	29.12ab	30.92a	40.47a
P. putida P12	58.50a	39.30b	48.35b	35.42a	24.54a	7.00b
P. putida PBR3	67.34a	52.60b	53.14b	26.76a	25.47a	12.69b
P. fluorescens PCI2	64.62a	37.57b	48.90b	31.49a	26.54a	9.52b
P. aurantiaca SR1	70.74a	60.69b	60.44b	73.54a	72.72a	49.21b
P. fluorescens CHA0	72.78a	71.67a	75.37a	69.71a	71.81a	75.39a

Percentages with the same letter within the same row (in each medium) are not significantly different according to the non parametric Kruskall-Wallis variance analysis.

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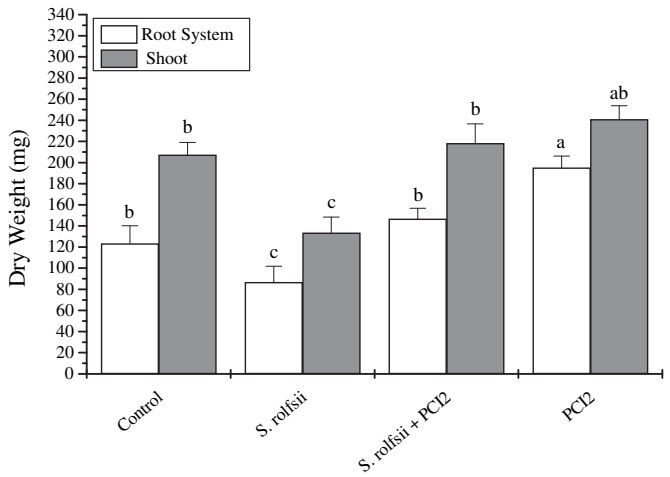

Fig. 1. Biocontrol activity of strain PCI2 against *S. rolfsii* Sacc. Tomato root and shoot dry weights were measured after 40 days of experimentation. Data represent the average of two experiments ± standard deviation. Bars for each plant fraction with different letters are significantly different according to the LSD test ($P < 0.05$).

only exoenzymatic activity detected in the strains. Antagonistic activity of the strains against *A. alternata* declined as iron concentration in both 25% TSA and KB increased, suggesting involvement of siderophores in this system. Based on significant *in vitro* antagonistic effect against leaf pathogen *A. alternata* and root pathogen *S. rolfsii* Sacc., a potential biocontrol agent, strain PCI2, was selected for a future evaluation of ability to suppress fungal pathogens *in vivo*.

A growth chamber assay was performed to evaluate tomato plants response to strain PCI2. Walsh et al. (2001) emphasized the need to investigate *in situ* colonization in the rhizosphere to determine the potential of a *Pseudomonas* strain as an effective BCA. Forty days after sowing of inoculated seeds, PCI2 reached a population density of 10^6–10^7 CFU g^{-1} mixture under growth chamber conditions. In this system, strain PCI2 did not appear to negatively affect development of tomato plants, but it also enhanced growth of the root system. Several reports have indicated that IAA synthesis is related to plant growth stimulation by microorganisms, including *P. putida* (Patten and Glick, 2002). IAA is the most common natural auxin found in plants and its positive effect on root growth and morphology is believed to increase the access to more nutrients in

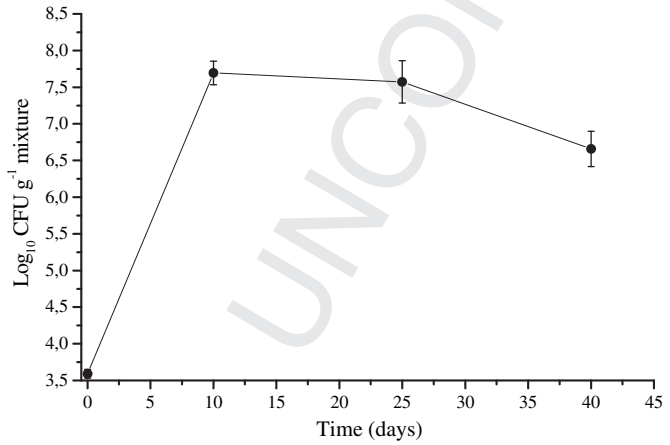

Fig. 2. Persistence of strain PCI2 in the rhizosphere of tomato plants. Data represent the average of two experiments ± standard deviation. For determining colony count at 1 h (zero time), 1 g of rhizosphere mixture was collected from the surroundings of a seed and placed into 9 ml of sterile 0.9% NaCl solution. Serial dilutions of the suspension were vortexed and plated onto 25% TSA medium. Plates were incubated for 48 h at 28 °C; the developed colonies were counted and the number of CFU g^{-1} of mixture was calculated.

the soil (Vessey, 2003). Thus, production of IAA is a characteristic that may enhance PCI2 use as an effective biological control agent to contribute to the control of tomato damping-off caused by *S. rolfsii* Sacc.

5. Conclusions and future perspectives

The use of BCA and/or PGPR fluorescent *Pseudomonas* as bioformulations for sustainable horticulture requires a thorough understanding of their functioning in the complex rhizosphere environment as well as of the response of vegetable crops to introduced microorganisms.

Strain PCI2 showed *in vitro* inhibition of three fungal phytopathogens, it enhanced growth of tomato root system and it showed promise to control tomato damping-off caused by *S. rolfsii* Sacc. by increasing plant stand by 29%. Further work is underway in order to elucidate the specific factors involved in both growth stimulation and protection of tomato plants by PCI2.

To conclude, the potential biocontrol activity of strain PCI2 must be confirmed in long-term greenhouse assays before its development into a commercial formulation for control of vegetables diseases.

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