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Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid (IAA)

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Abstract

Five bacteria (*Pseudomonas fluorescens*, *P. fluorescens* subgroup G strain 2, *P. marginalis*, *P. putida* subgroup B strain 1 and *P. syringae* strain 1) and three fungi (*Penicillium brevicompactum*, *P. solitum* strain 1 and *Trichoderma atroviride*) were evaluated to determine their promoting effect on the growth of mature healthy tomato plants grown under hydroponic conditions. *P. putida* and *T. atroviride* were shown to improve fruit yields in rockwool and in organic medium. The production or degradation of indole acetic acid (IAA) by the two microorganisms was investigated as possible mechanisms for plant growth stimulation. Both *P. putida* and *T. atroviride* were shown to produce IAA. The production of IAA by the two microorganisms was stimulated *in vitro* by the addition of L-tryptophan, tryptamine and tryptophol (200 $\mu g\,ml^{-1}$) in the culture medium. *P. putida* and *T. atroviride* also increased the fresh weight of both the shoot and the roots of tomato seedlings grown in the presence of increasing concentrations of L-tryptophan (up to 0.75 mM). Both microorganisms showed partial degradation of IAA *in vitro* when grown in a minimal medium with or without sucrose. In addition, the capacity of these microorganisms to reduce the deleterious effect of exogenous IAA was investigated using tomato seedlings. The results showed that the roots of tomato seedlings grown in the presence of increasing concentrations of IAA (0–10 $\mu g\,ml^{-1}$) were significantly longer when seeds were previously treated with *P. putida* or *T. atroviride*. The reduction in the detrimental effect of IAA on root elongation could be associated with a reduced ethylene production resulting from a decrease of its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by microbial degradation of IAA in the rhizosphere and/or by ACC deaminase activity present in both microorganisms.

Keywords: Greenhouse tomato; Hydroponic; Plant growth-promoting microorganisms; Indole acetic acid; Plant growth-promotion; Fruit yields

1. Introduction

Beneficial effects of the introduction of specific microorganisms on plant growth have been reported for numerous crops, including tomato (*Lycopersicon esculentum* Mill.) grown under field (Kokalis-Burelle et al., 2002; Guo et al., 2004) or greenhouse conditions in organic media (Gagné et al., 1993). Such beneficial microorganisms referred as PGPR (plant-growth promoting rhizobacteria) or PGPF (plant-growth promoting fungi) enhance plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens (Whipps, 1997, 2001), enhancing the availability of nutrients to the host plant, lowering of the ethylene level within the plant or by the enhanced production of stimulatory compounds, such as plant growth regulators (Antoun and Prévost, 2005). Among plant growth regulators, indole-3-acetic acid (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 the soil (Vessey, 2003).

The involvement of IAA in the complex interaction between the rhizosphere microflora and the host plant, which relies on a constant exchange of materials and signals (Antoun and Prévost, 2005), has been the focus of numerous works (Costacurta and Vanderleyden, 1995;

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Patten and Glick, 1996; Persello-Cartieaux et al., 2003). Root tissues are especially sensitive to fluctuating concentrations of IAA and the development of the root system can be greatly affected by exogenous sources of this plant growth regulator, including microbial (Tanimoto, 2005). While the production of IAA by microorganisms commonly found in the rhizosphere of plants, such as *Pseudomonas* spp. and *Rhizobium* spp., is often associated with their potential to stimulate plant growth (Antoun et al., 1998; Barazani and Friedman, 1999; Patten and Glick, 2002), the synthesis of high amounts of IAA by rhizosphere bacteria has been shown to inhibit root growth rather than to promote it (Xie et al., 1996). In this context, rhizosphere microorganisms capable of degrading IAA might have a positive effect on plant growth. However, even though Leveau and Lindow (2005) reported the ecological role of such a microorganism, little is known on the specific impact of IAA-degraders on plant growth.

In the present study, eight microorganisms with a reported beneficial effect on the growth of *Pythium*-infected tomato plants (Gravel et al., 2006) were tested to evaluate their effect on the growth of mature healthy tomato plants grown in rockwool and in organic medium under hydroponic conditions, during a complete production period. The effect of selected microorganisms through the production or degradation of IAA on plant growth was also investigated.

2. Materials and methods

2.1. Microorganisms

The microorganisms tested in this study were isolated from the rhizosphere of tomato plants (Gravel et al., 2005). Bacteria tested were *P. fluorescens* Migula, *P. fluorescens* Migula subgroup G strain 2, *P. marginalis* (Brown) Stevens, *P. putida* (Trevisan) Migula subgroup B strain 1, and *P. syringae* van Hall strain 1. Bacteria were grown on tryptic soy agar (TSA, Sigma-Aldrich, Mississauga, ON, Canada) and stock cultures were kept in 80% glycerol at −80 °C. Bacterial suspensions used in the different experiments were prepared in 500 ml flask containing 250 ml of tryptic soy broth (TSB, Sigma-Aldrich). The flasks were inoculated with 1 ml of an overnight culture of the bacteria grown in TSB. The flasks were incubated on a rotary shaker (150 rev min^{-1}) at 24 °C for 24 h. Bacterial cells were removed by centrifugation (4000*g*, 10 min), resuspended and diluted in sterile distilled water to 4×10^7 CFU ml^{-1} as determined by plate count on TSA medium.

The fungi tested were *Penicillium brevicompactum* Dierckx, *P. solitum* Westling strain 1 and *Trichoderma atroviride* Karsten. All fungi were grown on potato dextrose agar (PDA, Difco Laboratories, Becton Dickinson, Sparks, MD, USA) and stock cultures were freeze-dried and kept at −20 °C. Spore suspensions used in the different experiments were prepared by scraping the surface of a 2-week-old mycelium grown on PDA using a glass rod. Each spore suspension was adjusted by dilution with sterile distilled water to 1×10^6 spores ml^{-1} based on hemacytometer counts.

2.2. Greenhouse assay on fruit yield and plant growth

Greenhouse assays were conducted to evaluate the effect of the microorganisms on plant growth and development in rockwool and in organic medium. The rockwool experiment was performed under spring conditions (January – June) while the organic medium experiment was conducted under fall conditions (July–December). The organic medium used was a mixture of peat, pine sawdust and compost (v/v/v, 60/30/10). Tomato seeds (cv. Trust F1, De Ruiter Seeds, Columbus, OH, USA) were sown in multicellular blocks of rockwool and grown for 4 weeks (25 °C and 80% relative humidity). The 4 week-old seedlings were placed on rockwool slabs or on top of 9-l plastic containers filled with the organic medium and were drenched with 200 ml of either a bacterial (4×10^7 bacteria ml^{-1}) or a fungal spore (1×10^6 spores ml^{-1}) suspension of each microorganism. Control plants received 200 ml of sterile distilled water. Plants were grown under typical greenhouse growing conditions [temperatures of 18 °C (night) and 24 °C (day), 80% relative humidity] and drip irrigated using a nutrient solution containing 0.51 g l^{-1} of 6–11–31 (N–P–K; Plantprod, Brampton, ON, Canada) and 0.69 g l^{-1} of 15.5–0–0 (N–P–K; Plantprod). The 6–11–31 formulation also contained Mg (3.0%), S (3.5%), Fe (0.3%), Mn (0.06%), Zn (0.02%), Cu (0.004%), B (0.027%) and Mo (0.009%). As for the 15.5–0–0 formulation, it contained Ca (19%). Nutrient solution conductivity (EC) was kept at 3.0 mS/cm. The pH of the nutrient solution was kept between 5.5 and 6.0 through the addition of phosphoric acid. For the organic medium experiment (fall conditions), the natural daylight was supplemented with HPS lamps (100 μE m^{-2} per second PAR) to maintain a photoperiod of 16 h. Fruit yield was measured throughout the crop. The fruits were harvested twice a week for a period of 11 weeks and were separated into marketable and total yields. The marketable yield included fruits with fresh weight between 88 and 450 g whereas the total yield included all fruits harvested including fruits with blossom end rot and misshaped fruits (usually over 450 g). Throughout the crops, stem diameter was measured weekly to assure that plants were developing normally. At the end of the crop (6 months), the length of the stem was measured. The experimental design was a randomized complete block design with 3 (rockwool experiment) and 6 replicates (organic medium experiment). The experimental unit consisted of 3 plants in each slab and 2 plants in each container for the rockwool and the organic medium experiments, respectively. Each plant represented a sampling unit. Microorganisms allowing the greatest increase in the reproductive growth of the tomato plants were further investigated.

In each of the two experiments, the 10th leaf from the apex was collected from each plant in every experimental unit after 6 months of culture. Leaves from the same experimental unit were pooled. Digestion of the leaves collected was done according to the method described by Isaac and Johnson (1976). Briefly, leaves were dried at 75 °C for 48 h, ground, and 100 mg were mixed with 2 ml of a mineralizing solution (97 g of H_2SeO_3 in 100 ml of H_2SO_4) and 2 ml of H_2O_2. The mixture was then placed on a digesting block at 400 °C for 20 min. Once the samples were cooled, 4 ml of distilled water, 1 ml of lanthane solution (5%) and 94 ml of distilled water were added, in that order. The amount of nitrogen and phosphorus present was evaluated colorimetrically by comparison with a standard curve of known concentrations (Tandon et al., 1968; Nkonge and Balance, 1982). The amount of potassium was evaluated by flame atomic emission spectroscopy whereas the amount of calcium and magnesium was measured by atomic absorption spectroscopy (Atomic Absorption Spectrometer 3300, Perkin Elmer, Überlingen, Germany) (Jones and Isaac, 1969).

2.3. Phosphate solubilization by P. putida and T. atroviride

The phosphate solubilization ability of *P. putida* and *T. atroviride* was evaluated qualitatively according to the method described by Mehta and Nautiyal (2001). This method is based on the decolourization of bromophenol blue (BPB) following a decrease in pH of the culture medium. Both microorganisms were grown in the NBRI-BPB medium described by Mehta and Nautiyal (2001) containing per liter: 10 g of sucrose, 5 g of $Ca_3(PO_4)_2$, 5 g of $MgCl_2 \cdot 6H_2O$, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of KCl, 0.1 g of $(NH_4)_2SO_4$ and 0.025 g of BPB. Prior to sterilization, the pH of the culture medium was adjusted to 7.0. Fifteen ml of NBRI-BPB medium in 50 ml tubes were inoculated with 100 µl of either a suspension of *P. putida* (4×10^7 bacteria ml^{-1}) or of *T. atroviride* (1×10^6 spores ml^{-1}). Controls consisted of the uninoculated culture medium. The cultures, in triplicates, were incubated for 96 h at 25 °C on a rotary shaker at 150 rev min^{-1} after which they were centrifuged (4000*g*, 10 min) and the optical density of the supernatants was measured at 600 nm.

2.4. Production of IAA and IAA-related compounds by P. putida and T. atroviride

The production of IAA and IAA-related compounds by the microorganisms was evaluated spectrophotometrically. Liquid cultures were prepared in 250 ml flasks containing 100 ml of half-strength TSB supplemented or not (control) with 200 µg ml^{-1} of either L-tryptophan, tryptamine or tryptophol (Sigma-Aldrich). For *P. putida*, the flasks were inoculated with 100 µl of an overnight culture (OD_{600} of 0.7). For *T. atroviride*, the culture medium was inoculated with 3 PDA disks covered with actively growing mycelium. After an incubation period of 72 h (*P. putida*) or 1 week (*T. atroviride*) on a rotary shaker (150 rev min^{-1}, 24 °C), bacterial cells and mycelium were removed by centrifugation (4000*g*, 10 min) and by filtration, respectively. One ml of each supernatant or each filtrate was mixed vigorously with 2 ml of Salkowski's reagent (150 ml of $HClO_4$, 250 ml of distilled water and 7.5 ml of 0.5 M $FeCl_3 \cdot 6H_2O$) (Gordon and Weber, 1951). The mixture was incubated at room temperature for 20 min and the absorbance was measured at 535 nm. The concentration of IAA and IAA-related compounds was evaluated by comparison with a standard curve prepared using serial dilutions of a 50 µg ml^{-1} IAA (Sigma-Aldrich) solution in half-strength TSB. Four replicates were used for each treatment. Following the same procedure, the effect on IAA production of increasing concentrations of L-tryptophan (from 0 to 800 µg/ml) in the culture medium was also evaluated.

The production of IAA by *P. putida* and *T. atroviride* was confirmed with thin-layer chromatography (TLC). Fifty ml of each supernatant (*P. putida*) or filtrate (*T. atroviride*) were adjusted to pH 2.0 with 1 M HCl and subsequently extracted with an equal volume of ethyl acetate. The ethyl acetate layer was recovered and evaporated with a Rotavapor R-200 (Büchi Analytical inc., New Castle, DE, USA). The residue was taken up in methanol and developed on TLC plates using isopropanol–ammonia–water (10/1/1, v/v/v) as a solvent. The plates were sprayed with a reagent (3% H_2SO_4 in methanol containing 50 mg $FeCl_3$) and heated until colour development. IAA appeared as a red colour under visible light and orange colour under UV light, with a R_f value ranging from 0.54 to 0.6.

2.5. Effect of L-tryptophan on the growth of seeds inoculated with P. putida or T. atroviride

Tomato seeds were surface sterilized by soaking in 70% ethanol for 5 min and subsequently in 2% hypochloric acid for 1 min. The seeds were then rinsed thoroughly three times with sterile distilled water. Seeds were inoculated by soaking for 1 h in a suspension of *P. putida* (4×10^7 bacteria ml^{-1}) or *T. atroviride* (1×10^6 spores ml^{-1}). Control seeds were soaked in sterile distilled water. Subsequently, two seeds were placed in individual growth pouches containing 20 ml of a sterile solution of 0, 0.25, 0.50 or 0.75 mM of L-tryptophan. Pouches, wrapped in aluminium foil, were placed in a growth chamber at 25 °C for 14 d after which the following parameters were measured: length of the shoot, fresh weight of the shoot and fresh weight of the roots. The experimental design was a complete randomized design with 5 replicates. The experimental unit consisted of a pouch in which 2 seedlings were grown. Each seedling was a sampling unit.

2.6. In vitro degradation of IAA by P. putida and T. atroviride

P. putida and *T. atroviride* were grown in 125 ml flasks containing 50 ml of M9 minimal medium (per liter: 6.02 g

of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, 2 ml of 1 M $MgSO_4 \cdot 7H_2O$, and 0.1 ml of 1 M $CaCl_2$) amended with 25 μg ml^{-1} of IAA with or without sucrose (2 mg ml^{-1}). For *P. putida* subgroup B strain 1, the flasks were inoculated with 50 μl of an overnight culture (OD_{600} of 0.7) whereas for *T. atroviride*, the flasks were inoculated with 2 PDA disks covered with actively growing mycelium. Control flasks were not inoculated (sterile cultures). Flasks were incubated on a rotary shaker (150 rev min^{-1}) for 72 h at 24 °C. Bacterial biomass was determined by measuring the optical density (OD_{600}) and bacteria were then removed by centrifugation (4000*g*, 10 min). Fungal biomass was determined by measuring the fresh weight of the mycelium in the culture medium collected by filtration. The amount of IAA remaining in the medium was evaluated as previously described. The percentage of IAA degradation was calculated as follows: 100−[(final concentration of IAA remaining in the culture medium expressed in μg ml^{-1}/25 μg ml^{-1} IAA) × 100].

2.7. Effect of exogenous IAA on root development of seeds inoculated with P. putida or T. atroviride

Tomato seeds were surface sterilized and inoculated with either *P. putida* or *T. atroviride* as described previously. Control consisted of uninoculated seeds. Subsequently, two seeds were placed in individual growth pouches containing 20 ml of a sterile solution of 0, 0.01, 0.1, 1 or 10 μg ml^{-1} of IAA. Pouches, wrapped in aluminium foil, were placed in a growth chamber at 25 °C for 7 d after which the length of the initial root was measured for each seedling. The experimental design was a complete randomized design with 5 replicates. The experimental unit consisted of a pouch in which 2 seedlings were grown. Each seedling was a sampling unit.

2.8. Evaluation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity in P. putida and T. atroviride

Lysates in four replicates of *P. putida* and *T. atroviride* cultures were prepared as described by Saleh and Glick (2001). Briefly, 25 μl of toluene was added to a 200 μl aliquot of the washed suspensions of *P. putida* or *T. atroviride* and was vortexed vigorously for 30 s. Then, 20 μl of 0.5 M ACC was added and after an incubation period of 15 min at 30 °C, 1 ml of 0.56 N HCl was added. The microorganisms lysates were centrifugated (10,000 g, 10 min) and 1 ml of the supernatant was mixed with 800 μl of 0.56 N HCl and 300 μl of 2,4-dinitrophenylhydrazine (0.2 g in 100 ml of 2N HCl). The mixtures were incubated for 30 min at 30 °C after which 2 ml of 2 N NaOH were added. The absorbance was measured at 540 nm. The ACC deaminase activity of *P. putida* and *T. atroviride* was evaluated quantitatively by measuring the amount of α-ketobutyrate produced by the deamination of ACC. ACC deaminase activity was expressed in μmol of α-ketobutyrate mg protein^{-1} h^{-1}.

2.9. Statistical analysis

When appropriate, analysis of variance (ANOVA) was performed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. When significant ($P<0.05$), treatment means were compared using the Fisher protected LSD test.

3. Results

3.1. Greenhouse assay on fruit yield and stem growth

Four of the microorganisms (*P. brevicompactum, T. atroviride, P. marginalis,* and *P. putida*) significantly increased the marketable fruit yield compared to the control in rockwool (Table 1). The total yield was also increased by the microorganisms as compared to the control, however not significantly. In the organic medium, the inoculation of the plants with *T. atroviride* or with *P. putida* lead to a significant increase in the marketable and total yields as compared to the control (Table 1). The length of the stems was not markedly affected by the inoculation of the microorganisms and ranged from 482 to 509 cm (rockwool) and 447 to 471 cm (organic medium).

Table 1
Effect of microorganisms on marketable and total fruit yield of mature tomato plants grown in rockwool and in organic medium for 6 months

	Fruit yield (g plant^{-1})	
Microorganisms	Marketable	Total
Rockwool (Spring crop)		
Control[a]	6003b	6662ab
Penicillium brevicompactum	6760a	7304a
Penicillium solitum strain 1	6542ab	7023ab
Trichoderma atroviride	6679a	7111ab
Pseudomonas fluorescens	6556ab	7047ab
Pseudomonas fluorescens G strain 2	5978b	6431b
Pseudomonas marginalis	6707a	7345a
Pseudomonas putida B strain 1	6881a	7350a
Pseudomonas syringae strain 1	6607ab	7167ab
Organic medium (Fall crop)		
Control	3695c	3998c
Penicillium brevicompactum	3889abc	4289bc
Penicillium solitum strain 1	3720bc	4069bc
Trichoderma atroviride	4218ab	4608ab
Pseudomonas fluorescens	4020abc	4301bc
Pseudomonas fluorescens G strain 2	3791bc	4238bc
Pseudomonas marginalis	3830abc	4234bc
Pseudomonas putida B strain 1	4329a	4931a
Pseudomonas syringae strain 1	4142abc	4504abc

Each value represents the mean of 3 (rockwool) or 6 (organic medium) replicates.
For each experiment, values within a column followed by a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).
[a]Plants were drenched with sterile distilled water.

The mineral content in the leaves of plants grown in rockwool was not affected by the presence of any of the microorganisms (data not shown). However, in the organic medium, the amount of leaves P was significantly higher when plants were grown in the presence of any of the eight microorganisms tested (Table 2). The N, K, Ca and Mg contents were, however, not affected significantly by the presence of the microorganisms, except in the case of *T. atroviride*, which reduced significantly the amount of Ca found in the leaves as compared to the control (Table 2).

3.2. Phosphate solubilization by *P. putida* and *T. atroviride*

The absence of decolourization of the culture medium indicated that neither *P. putida* nor *T. atroviride* were able to solubilize the sparingly soluble source of P in liquid cultures.

3.3. Production of IAA and IAA-related compounds by *P. putida* and *T. atroviride*

The capacity of *P. putida* and *T. atroviride* to produce IAA and IAA-related compounds was evaluated. In the absence of an indole derivative as a precursor in the culture medium (control), *P. putida* and *T. atroviride* produced low levels of IAA and IAA-related compounds (Table 3). The production of IAA by both microorganisms was stimulated by the addition ($200\,\mu g\,ml^{-1}$) of L-tryptophan, tryptamine and tryptophol. *P. putida* produced the highest level of IAA and IAA-related compounds ($23.4\,\mu g\,ml^{-1}\,OD_{600}^{-1}$) when grown in the presence of tryptamine whereas *T. atroviride* produced highest level of IAA and IAA-related compounds ($38.5\,\mu g\,ml^{-1}$) in the presence of tryptophol (Table 3). A constant increase in the production of IAA and IAA-related compounds by these two microorganisms was also observed as the concentration of L-tryptophan in the growth medium was increased from 0 to $800\,\mu g\,ml^{-1}$ (data not shown). In all cases, the production of IAA was confirmed by TLC analysis. Compounds with R_f values close to 0.56 (pure IAA) were considered as IAA (Table 3). Other unidentified IAA-related compounds but with different R_f values were also observed on the TLC plates indicating that they were also produced by *P. putida* and *T. atroviride*.

3.4. Effect of L-tryptophan on the growth of seeds inoculated with *P. putida* or *T. atroviride*

The length of the shoot and the fresh weight of both the shoot and the roots of control seedlings were not significantly stimulated by the increasing concentrations of L-tryptophan (Fig. 1). Whereas for seedlings inoculated

Table 2
Effect of microorganisms on mineral content of leaves of tomato plants grown for 6 months in organic medium (Fall crop)

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
Control[a]	3.32a	0.55d	7.44a	3.05a	0.40a
Penicillium brevicompactum	3.53a	0.73abc	8.13a	2.59ab	0.34a
Penicillium solitum strain 1	3.58a	0.75ab	7.93a	2.45ab	0.36a
Trichoderma atroviride	3.53a	0.73abc	8.11a	2.32b	0.33a
Pseudomonas fluorescens	3.65a	0.67bc	8.07a	2.92ab	0.37a
Pseudomonas fluorescens G strain 2	3.65a	0.67bc	7.99a	3.06a	0.33a
Pseudomonas marginalis	2.81a	0.81a	7.92a	2.63ab	0.36a
Pseudomonas putida B strain 1	3.51a	0.66c	7.57a	3.05a	0.37a
Pseudomonas syringae strain 1	3.57a	0.73abc	7.99a	2.50ab	0.34a

Values within a column followed by a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).
[a]Plants were drenched with sterile distilled water.

Table 3
Production of IAA and IAA-related compounds by *P. putida* subgroup B strain 1 and *T. atroviride* in liquid cultures containing either L-tryptophan, tryptamine or tryptophol

	IAA and IAA-related compounds[a]			
	Control	Tryptophan	Tryptamine	Tryptophol
P. putida B strain 1	1.5 ± 0.3^{b} (0.55)[c]	3.3 ± 0.6 (0.54)	23.4 ± 2.2 (0.59)	11.8 ± 0.9 (0.60)
T. atroviride	1.2 ± 0.1^{d} (0.54)	6.2 ± 0.1 (0.54)	9.8 ± 0.3 (0.60)	38.5 ± 9.2 (0.53)

Each value represents a mean of 4 replicates.
[a]The production of IAA and IAA-related compounds by *P. putida* and *T. atroviride* was evaluated in culture medium amended with $200\,\mu g\,ml^{-1}$ of tryptophan, tryptamine or tryptophol or without any IAA precursor (control).
[b]Expressed in μg of IAA equivalent $ml^{-1}\,OD_{600}^{-1}$.
[c]Values between brackets represent the R_f values for IAA present in the culture supernatant or filtrate.
[d]Expressed in μg of IAA equivalent ml^{-1}.

with either *P. putida* or *T. atroviride*, the fresh weight of the shoot and the roots increased as the concentration of L-tryptophan increased to 0.75 mM (Fig. 1). A similar effect was also observed for the length of the shoot (Fig. 1).

3.5. *In vitro degradation of IAA by P. putida and T. atroviride*

Both microorganisms partially degraded IAA in M9 minimal medium and in M9 minimal medium supplemented with sucrose. The degradation of IAA by *P. putida* in the medium containing no sucrose (38.3%) was significantly higher than that observed in the medium amended with sucrose (22.6%) (Fig. 2). The same tendency was observed with *T. atroviride*. Bacterial and fungal biomasses obtained in media amended with sucrose were not significantly different from those obtained in media unamended.

3.6. *Effect of exogenous IAA on root development of seeds inoculated with P. putida or T. atroviride*

Root development of non-inoculated (control) seedlings was significantly affected by the addition of IAA. The length of the root was reduced significantly as the concentration of IAA increased from 0 to 10 $\mu g\,ml^{-1}$ (Fig. 3). As compared to the control, seedlings inoculated with *P. putida* and *T. atroviride* developed a significantly longer root in the presence of 1 and 10 $\mu g\,ml^{-1}$ of IAA (Fig. 3). The increase in the IAA concentration from 0.1 to 10 $\mu g\,ml^{-1}$ did not affect significantly root elongation in seedlings treated with *P. putida* and *T. atroviride* (Fig. 3).

3.7. *Evaluation of ACC deaminase activity in P. putida and T. atroviride*

The ACC deaminase activities of *P. putida* and of *T. atroviride* were 23.7 ± 4.3 and 13.5 ± 2.2 µmol α-ketobutyrate mg protein^{-1} h^{-1}, respectively.

4. Discussion

In this study, the effect of eight microorganisms on the growth and development of healthy greenhouse tomato plants was investigated under hydroponic conditions using rockwool and organic medium as substrates. Greenhouse assays allowed the evaluation of the long-term effect of the microorganisms on the reproductive as well as on the vegetative growth of the tomato plants.

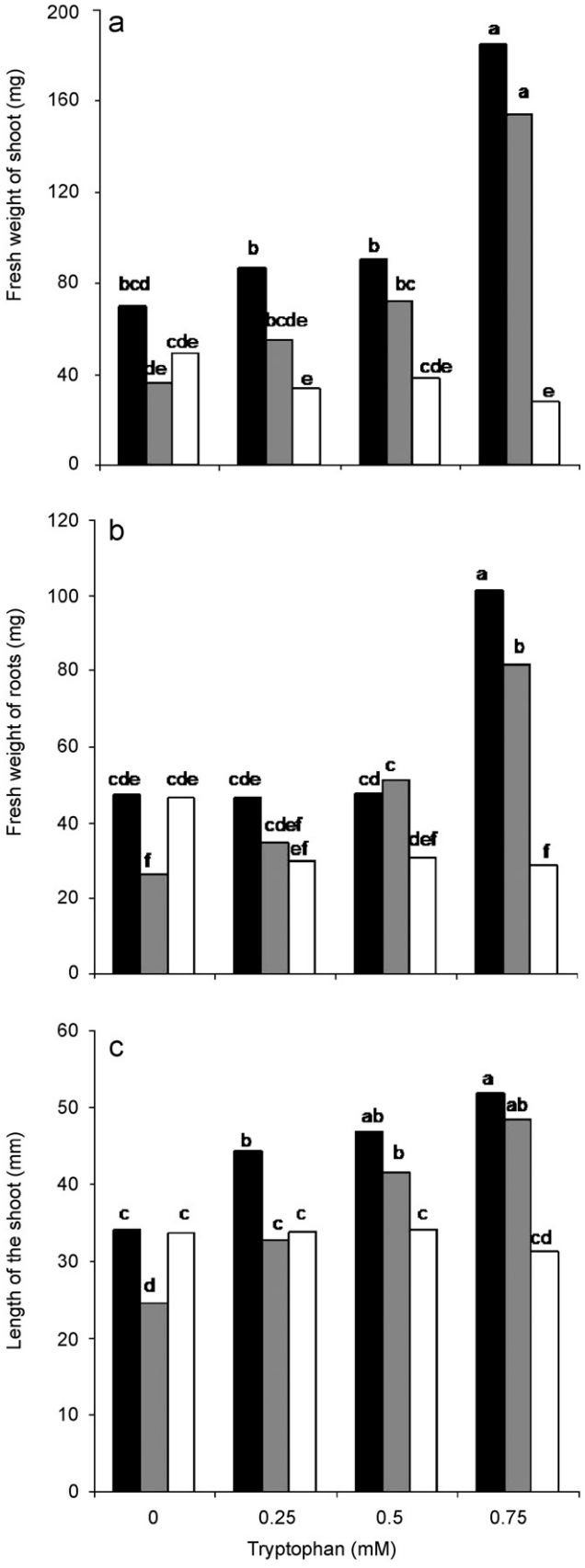

Fig. 1. Effect of tryptophan on fresh weight of shoot (a) and roots (b) and on shoot length (c) of tomato seedlings grown in pouches for 14 days in the presence of *P. putida* subgroup B strain 1 (4×10^7 bacteria ml^{-1}, ■) or *T. atroviride* (1×10^6 spores ml^{-1}, ▩). Control seedlings were grown in absence of *P. putida* and *T. atroviride* (□). Each value represents a mean of 5 replicates. Bars with a same letter are not significantly different according to Fisher protected LSD test ($P < 0.05$).

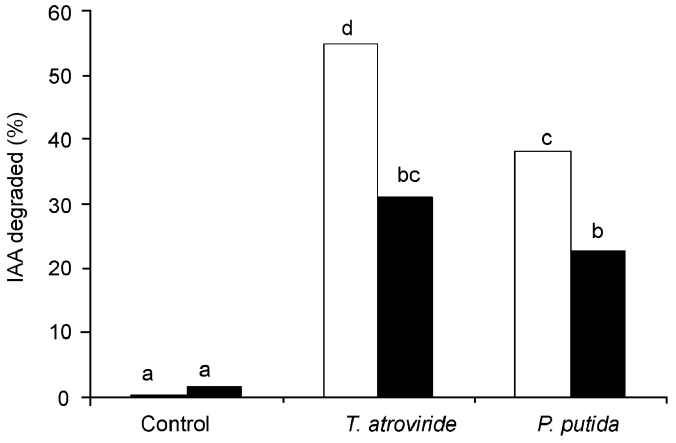

Fig. 2. *In vitro* degradation of IAA by *Trichoderma atroviride* and *Pseudomonas putida* subgroup B strain 1 in culture medium containing IAA ($25\,\mu g\,ml^{-1}$) amended ($2\,mg\ ml^{-1}$, ■) or not (□) with sucrose. Control flasks were not inoculated. Bars with a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

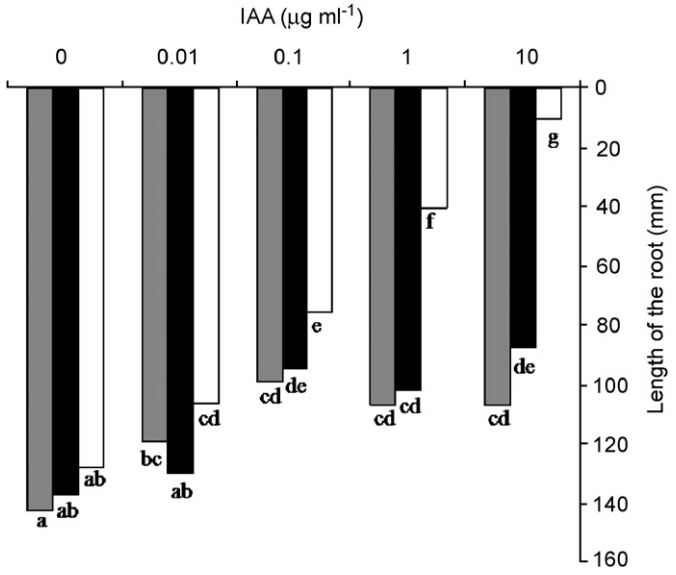

Fig. 3. Effect of different concentrations of exogenous IAA on the elongation of tomato roots grown in pouches in the absence (control, □) and in the presence of *P. putida* subgroup B strain 1 (1×10^8 bacteria ml^{-1}, ■) or *T. atroviride* (1×10^6 spores ml^{-1}, ▩). Bars with a same letter are not significantly different according to Fisher protected LSD test ($P<0.05$).

The results showed the stimulating effect of *P. putida* subgroup B strain 1 and *T. atroviride* on the reproductive growth of tomato plants in both growing media. Even though the organic medium experiment was performed under fall conditions (lower temperature and decreased natural light) which were less favourable to plant growth and fruit ripening, *P. putida* subgroup B strain 1 and *T. atroviride* increased fruit production, just as they did in the rockwool experiment performed under more favourable conditions (spring crop). Previous works have already reported the stimulating effect of *P. putida* on cucumber plants (Amer and Utkhede, 2000) and on canola seedlings (Xie et al., 1996). *Trichoderma* spp. have been also shown to exhibit plant growth-promoting activity on numerous cultivated plants (Kleifeld and Chet, 1992; Ousley et al., 1994; Altomare et al., 1999; Harman, 2000; Yedidia et al., 2001). However, this study reports for the first time the plant growth-promoting activity of *P. putida* and *T. atroviride* on the fruit yield of greenhouse tomato plants in hydroponic systems. *Penicillium brevicompactum* and *P. marginalis* were also shown to have a stimulating effect on the marketable fruit yield, but in plants grown in rockwool only.

The mechanisms by which *P. putida* subgroup B strain 1 and *T. atroviride* influenced plant growth were further investigated. In this study, the inoculation with *P. putida* subgroup B strain 1 and *T. atroviride* in the organic medium increased the P accumulation in the leaves as did the inoculation of the other tested microorganisms. Even though the solubilization of P is often associated with plant growth promotion (Richardson, 2001), in this particular experiment, the P in the nutrient solution was readily available for the plant, reducing the possibility that this mechanism played an important role in the stimulation observed. Furthermore, an *in vitro* assay showed that *P. putida* subgroup B strain 1 and *T. atroviride* were not P solubilizers under the conditions tested.

The production of plant growth regulators by the microorganisms is another important mechanism often associated with growth stimulation (Vessey, 2003). The balance between vegetative and reproductive growth is controlled by hormone signalling within the plant and can therefore be highly influenced by it (Taiz and Zeiger, 1991). At relatively high concentrations, natural auxins, such as IAA, stimulate shoot elongation and root induction while reducing root elongation (Tanimoto, 2005). IAA is also involved in tomato fruit development, especially during fruit setting and in the final phase of development (Srivastava and Handa, 2005). Previous works have reported that the synthesis of IAA is often associated with plant growth stimulation by microorganisms, including *P. putida* (Xie et al., 1996; Patten and Glick, 2002). In this study, results showed that *P. putida* subgroup B strain 1 and *T. atroviride* are able to synthesize IAA from different precursors *in vitro*, which supports the theory that microbial IAA could be involved in the growth stimulation observed in our greenhouse assay. Of particular interest, the results showed that the growth of seedlings inoculated with *P. putida* subgroup B strain 1 or *T. atroviride* increased as the concentration of L-tryptophan increased in the pouches. This suggests that the synthesis of IAA through tryptophan-dependent pathways by *P. putida* subgroup B strain 1 or *T. atroviride,* affected the growth of the tomato seedlings. Tryptophan is naturally secreted in root exudates of tomato plants and most of the auxin found in the rhizosphere is believed to come from the biosynthesis by microorganisms (Kamilova et al., 2006).

Exogenous sources of IAA, such as the one produced by microorganisms, are known to cause changes in the morphology of the root system which influence the uptake of nutrients by the plant (Arteca, 1996). In this regard, San-Francisco et al. (2005) showed that exogenous applications of IAA increased the amount of P in roots of pepper plants grown under hydroponic conditions. It is therefore possible that the increase in the level of P in the leaves of tomato plants grown in the organic medium might also be related, at least partially, to the production of IAA by *P. putida* subgroup B strain 1 and *T. atroviride*.

In addition to having a stimulating effect on plant growth, exogenous IAA in the rhizosphere can also have a detrimental effect on the elongation of roots over a wide range of concentrations. Such an effect has been associated with an increase in the level of ethylene in the plant (Glick et al., 1997, 1998). IAA can increase the activity of ACC synthase, which catalyses the conversion of *S*-adenosylmethionine to ACC, the precursor of ethylene in the plant (Kende, 1993). In this study, *P. putida* subgroup B strain 1 and *T. atroviride* were shown to reduce the inhibiting effect of relatively high concentrations of IAA on root elongation of tomato seedlings. The results from this study suggest the involvement of two possible mechanisms. First, both *P. putida* and *T. atroviride* were able to partially degrade IAA *in vitro*. The degradation of IAA by *P. putida* has been reported by Leveau and Lindow (2005) however, to the best of our knowledge, this is the first report of IAA degradation by *T. atroviride*. Such degradation could have reduced the concentration of IAA in the vicinity of the roots to a level which was not detrimental to the elongation. Also, previous studies have shown that ACC deaminase activity in PGPR, which hydrolyses ACC into ammonia and α-ketobutyrate, prevents the synthesis of inhibiting levels of ethylene (Penrose et al., 2001). This reduction in the level of ACC in the rhizosphere increases the exudation of ACC by the plant to maintain equilibrium, reducing the potential synthesis of ethylene since ACC is the immediate precursor of this compound in the plant (Glick et al., 1998). This study demonstrated that both *P. putida* subgroup B strain 1 and *T. atroviride* possess ACC deaminase activity when grown *in vitro*, suggesting that these microorganisms could also regulate the concentration of ethylene within the plant by reducing the amount of its precursor present. ACC deaminase has previously been reported for *Pseudomonas* spp. and its activity has been associated with an increase in root elongation due to the reduced inhibition caused by ethylene (Glick et al., 1997; Wang et al., 2000; Safronova et al., 2006). The synthesis of this enzyme has also been reported in fungi such as *Penicillium citrium* (Jia et al., 2000). However, to the best of our knowledge, this is the first report of an ACC deaminase activity in *T. atroviride*.

The effect of microbial production or degradation of IAA in the rhizosphere on fruit setting and tomato yield is most likely indirect, through an effect on the overall growth of the plant. Indeed, IAA, including microbial, can greatly influence the growth of the root system depending on the amount found in the rhizosphere, through root elongation and the formation of lateral or adventitious roots (Scott, 1972; Patten and Glick, 2002). In the case of *P. putida* and *T. atroviride*, their ability to produce and degrade IAA, combined with their ACC deaminase activity, may have promoted an optimal development of the root system and of the tomato plant in general, which could have resulted in the stimulation of the reproductive growth observed in this study.

Microbial production of IAA is known to result from different pathways (Persello-Cartieaux et al., 2003). Although tryptophan-independent biosynthesis pathways have been identified in numerous microorganisms, tryptophan remains the most common precursor of microbial IAA (Patten and Glick, 1996). The four main tryptophan dependent metabolic pathways are the followings: tryptophol, tryptamine, indole-3-pyruvic acid and indole-3-acetamide pathways (Bartel, 1997). In regard to the specific biosynthesis pathways of IAA in *P. putida* and *T. atroviride*, this study demonstrated that the enzymes responsible for the synthesis of IAA from both tryptophol and tryptamine are active. This suggests the existence of the tryptophol and tryptamine pathways in *P. putida* and *T. atroviride,* while previous works have reported the existence of the indole-3-pyruvic acid pathway in *P. putida* (Mordukhova et al., 2000; Patten and Glick, 2002).

This research demonstrated the capacity of two microorganisms, *P. putida* subgroup B strain 1 and *T. atroviride,* to promote the reproductive growth of tomato plants under typical hydroponic growing conditions. The plant growth stimulation reported in this study is, most likely, the synergic result of numerous modes of action exhibited by each microorganism tested, including a regulation in the concentration of IAA in the rhizosphere and a regulation of the concentration of ethylene within the roots. This study showed that *P. putida* subgroup B strain 1 and *T. atroviride* could be used as plant growth-promoting microorganisms to improve the productivity of greenhouse tomato crops under hydroponic conditions in inert or organic media. More specific works are, however, needed to further study the specific mechanisms involved in the growth stimulation by *P. putida* subgroup B strain 1 and *T. atroviride,* as well as to better understand the close interaction between the host plant and these two microorganisms.

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