Control of greenhouse tomato root rot [*Pythium ultimum*] in hydroponic systems, using plant-growth-promoting microorganisms

V. Gravel, C. Martinez, H. Antoun, and R.J. Tweddell

Abstract: Twenty-eight microorganisms showing in vitro antagonistic activity against *Pythium ultimum* were tested for their ability to reduce root rot [*P. ultimum*] on mature tomato plants grown in a greenhouse under hydroponic conditions. Of those, *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, and *Trichoderma atroviride* were shown to strongly reduce root rot severity, to improve the anchorage, and to increase the marketable yields of the plants grown in rockwool infested with *P. ultimum*. Experiments conducted in an organic medium containing peat, pine sawdust, and compost (*v/v/v*; 60:30:10) also revealed the capability of most of these microorganisms to reduce root rot severity and to improve the anchorage of the tomato plants. However, *Pseudomonas marginalis* was the only microorganism that significantly improved fruit production of infected plants grown in organic medium. Moreover, *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, and *T. atroviride* were shown to stimulate the growth of healthy tomato seedlings, suggesting that they act as PGPR (plant-growth-promoting rhizobacteria) or PGPF (plant-growth-promoting fungi). This study led to the selection of potential biocontrol agents against root rot of tomato caused by *P. ultimum* in hydroponic systems. This may open the way for new alternatives for the biological control of *Pythium* diseases in hydroponic systems that not only protect the crop but also have a beneficial effect on the plant growth and development in the absence of pathogens.

Key words: Pythium ultimum, root rot, greenhouse tomato, hydroponic, biological control.

Résumé : Vingt-huit microorganismes ayant une activité antagoniste in vitro envers le *Pythium ultimum* furent évalués en serre pour leur aptitude à réduire la pourriture pythienne [*P. ultimum*] sur des plants de tomate adultes en culture hydroponique en serre. Parmi ceux-ci, le *Penicillium brevicompactum*, la souche 1 du *Penicillium solitum*, la souche 2 du sous-groupe G du *Pseudomonas fluorescens*, le *Pseudomonas marginalis*, la souche 1 du sous-groupe B du *Pseudomonas putida*, la souche 1 du *Pseudomonas syringae* et le *Trichoderma atroviride* ont réduit considérablement l'intensité de la pourriture, amélioré l'ancrage et augmenté les rendements commercialisables des plantes cultivées dans la laine de roche contaminée avec le *P. ultimum*. Des expériences en substrat organique contenant de la tourbe, de la sciure de pin et du compost (*v/v/v*; 60:30:10) ont aussi montré la capacité de la plupart de ces microorganismes à réduire l'intensité de la pourriture et à améliorer l'ancrage des pieds de tomate. Cependant, seul le *Pseudomonas marginalis* a significativement augmenté la production de fruits des plantes infectées cultivées dans le substrat organique. En outre, le *Penicillium brevicompactum*, la souche 1 du *Penicillium solitum*, la souche 2 du sous-groupe G du *Pseudomonas fluorescens*, le *Pseudomonas marginalis*, la souche 1 du sous-groupe B du *Pseudomonas putida* et le *T. atroviride* ont stimulé la croissance de plants de tomate sains, ce qui suggère qu'ils agissent comme des rhizobactéries ou champignons promoteurs de croissance des plantes. La présente étude a permis la sélection d'agents de lutte biologique contre la pourriture des racines de la tomate causée par le *P. ultimum* dans des systèmes hydroponiques. De nouvelles alternatives pour la lutte biologique contre les maladies à *Pythium* dans des systèmes hydroponiques qui, non seulement protègent la culture, mais ont aussi un effet bénéfique sur la croissance et le développement des plantes en absence d'organismes pathogènes peuvent maintenant être explorées.

Mots clés : Pythium ultimum, pourriture des racines, tomate de serre, hydroponique, lutte biologique.

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V. Gravel, C. Martinez,[1] H. Antoun, and R.J. Tweddell.[2] Centre de recherche en horticulture, Université Laval, Québec, QC G1K 7P4, Canada.

[1]Present address: Premier Tech, 1 Premier Avenue, Rivière-du-Loup, QC G5R 6C1, Canada.
[2]Corresponding author (e-mail: russell.tweddell@crh.ulaval.ca).

Introduction

The use of hydroponic systems has become a standard for the greenhouse tomato industry in Canada. For many years, most greenhouse tomato growers have relied on systems, such as rockwool, that are relatively sterile at the beginning of a crop. Rockwool and other soilless systems eliminate the presence of soilborne pathogen inoculum at planting and are therefore often associated with a reduction of root diseases caused by fungi (Paulitz 1997). Nevertheless, most pathogens cannot be excluded from the greenhouse environment and are often introduced through the use of contaminated plant material or equipment (Jarvis 1992). Once the pathogens are introduced, the optimal environmental conditions for plant growth can also become conducive to the rapid development of plant diseases (Menzies and Bélanger 1996). The high water content in soilless systems is especially favourable to the zoosporic pathogenic species *Pythium* and *Phytophthora* (Zinnen 1988; Stanghellini and Rasmussen 1994). Moreover, soilless substrates lack a microbial diversity that provides a biological buffer, which generally suppresses the spread and the establishment of weak competitor pathogens (Hendrix and Campbell 1973; Paulitz 1997).

Pythium ultimum Trow, a pathogen with a weak competitive ability, is the causal agent of tomato root rot. This disease, characterized by premature weakening of the root system, has become an important disease of greenhouse tomato crops grown in soilless systems. In Canada, no chemical fungicide is currently registered for greenhouse vegetable crops in hydroponic systems. The control of tomato root rot is generally based on cultural practices that are often unreliable and unsuccessful.

In this context, the biocontrol of *P. ultimum* in hydroponic systems through the use of a biofungicide made of one or many microorganisms as a protective measure is viewed as a potential strategy. In general, the use of biocontrol approaches for disease management is highly desirable where environmental conditions are controlled, such as in greenhouses, and where the economic value of the crop is high (Paulitz and Bélanger 2001). In the case of pythium root rot of greenhouse tomato, the lack of competitive microflora in rockwool hydroponic systems further facilitates the establishment of a biocontrol agent, potentially increasing the efficacy of disease control (Paulitz 1997).

In the present study, microorganisms that were shown in vitro to have antagonistic activity against *P. ultimum* (Gravel et al. 2005) were tested for their ability to reduce root rot in greenhouse tomato plants growing in rockwool or organic medium under hydroponic conditions. The effect of these microorganisms on tomato plant growth and development was also investigated.

Materials and methods

Pathogen

A virulent strain of *P. ultimum* isolated from infected tomato roots was provided by the laboratory of Dr. Richard Bélanger (Université Laval, Québec, Que.). It was grown on potato dextrose agar (PDA; Difco Laboratories, Detroit, Mich.) at 24 °C. PDA disks colonized with *P. ultimum* mycelium suspended in sterile distilled water at 24 °C served as stock cultures. The propagule suspension of *P. ultimum*, which consisted of oospores and hyphae, was prepared in 1000 mL flasks containing 500 mL of potato dextrose broth (Difco Laboratories). Five PDA disks covered with actively growing mycelium of *P. ultimum* were used to inoculate the flasks, which were then incubated on a rotary shaker (150 r/min) (1 r = 2π rad) at 24 °C for 1 week. The liquid culture was then homogenized in a blender, and the propagule suspension was adjusted by dilution to 1×10^6 propagules/mL on the basis of hemacytometer counts.

Antagonistic microorganisms

The 28 microorganisms tested in this study are listed in Table 1; they were isolated from rockwool and organic media, where tomato plants were previously grown, and were shown to have an in vitro antagonistic activity against *P. ultimum* (Gravel et al. 2005). Selected bacteria were grown on tryptic soy agar (Sigma–Aldrich, Mississauga, Ont.), and stock cultures were kept in 80% glycerol at –80 °C. Bacterial suspensions were prepared in 500 mL flasks containing 250 mL of tryptic soy broth (Sigma–Aldrich). The flasks were incubated on a rotary shaker (150 r/min) at 24 °C for 24 h, after which the bacterial cells were removed by centrifugation (2000*g*, 10 min), resuspended, and diluted with sterile distilled water to 4×10^7 bacteria/mL. Selected fungi were grown on PDA, and stock cultures were freeze-dried and kept at –20 °C. The spore suspensions were prepared by scraping the surface of a 2-week-old culture on PDA with a glass rod. The concentration of each spore suspension was adjusted by dilution with sterile distilled water to 1×10^6 spores/mL on the basis of hemacytometer counts.

Effect of antagonistic microorganisms on pythium root rot severity on tomato plants grown in rockwool

The effect of the antagonistic microorganisms on the severity of tomato pythium root rot was evaluated according to a modified version of the method described by Rankin and Paulitz (1994). The first greenhouse assay was performed on a summer crop (May–August).

Tomato seeds (*Lycopersicon esculentum* Mill. 'Trust F1'; De Ruiter Seeds, Columbus, Ohio) were sown in multicellular blocks of rockwool and grown for 4 weeks at 25 °C and 80% relative humidity. The 10 cm tall plants were transplanted into rockwool slabs and drenched with 200 mL of either a bacterial (4×10^7 bacteria/mL) or a fungal spore (1×10^6 spores/mL) suspension of each antagonistic microorganism. Controls received 200 mL of sterile distilled water. A week later, the plants were drenched with 200 mL of a propagule suspension (1×10^6 propagules/mL) of *P. ultimum*. The plants were grown under typical greenhouse growing conditions (temperatures of 18 °C (night) and 24 °C (day), 80% relative humidity, natural daylight) and drip irrigated using a nutrient solution containing N–P–K 6:11:31 (0.51 g/L) and N–P–K 15.5:0:0 (0.69 g/L) (Plantprod, Brampton, Ont.). The 6:11:31 formulation 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%). The 15.5:0:0 formulation contained Ca (19%). Nutrient solution electrical conductivity (EC) and pH were monitored daily and were constant among the different experiments. The EC was kept at 3.0 mS/cm, whereas the pH was kept between 5.5 and 6.0

Table 1. Effect of different antagonistic microorganisms on the severity of root rot [*Pythium ultimum*] of tomato plants grown in rockwool in a greenhouse environment.

	Source of microorganism*	Root rot severity (% infected roots) Summer crop (trial No. 1)	Fall crop (trial No. 2)
Control†		4.2 m	3.3 g
Control (*Pythium ultimum*)‡		98.3 a	58.3 a
Acremonium potronii Vuill.	B	80.0 b	NT
Acrodontium griseum (Fassatiova) de Hoog	D	35.8 ijkl	35.0 bc
Enterobacter cloacae (Jordan) Hormaeche & Edwards	D	79.2 b	NT
Fusarium oxysporum Schlecht.:Fr. strain 1	C	68.3 bcd	NT
Fusarium tumidum Sherbakoff	D	48.3 efghi	28.9 cde
Fungus 5-12-6	B	62.5 cde	NT
Fungus 8-13-1	C	51.3 efgh	NT
Fungus 2-8-8	B	46.7 fghi	43.1 b
Fungus 2-6-2	A	30.8 jkl	34.4 bc
Penicillium brevicompactum Dierckx	B	27.5 l	23.8 cdeg
Penicillium griseofulvum Dierckx	C	51.7 efgh	45.0 b
Penicillium janthinellum Biourge strain 1	A	59.2 def	28.9 cde
Penicillium restrictum Gilman & Abbott	C	45.0 fghij	47.8 ab
Penicillium simplicissimum (Oudem.) Thom strain 1	B	57.5 defg	29.4 cd
Penicillium simplicissimum strain 2	A	79.2 b	NT
Penicillium solitum Westling strain 1	A	43.3 ghijk	15.0 efg
Penicillium solitum strain 2	B	93.0 a	NT
Pseudomonas corrugata Roberts & Scarlett strain 1	R	56.7 defg	NT
Pseudomonas fluorescens Migula	R	29.2 kl	18.8 def
Pseudomonas fluorescens F	C	73.3 bc	NT
Pseudomonas fluorescens G strain 2	R	53.3 efgh	22.8 cdef
Pseudomonas marginalis (Brown) Stevens	A	35.8 ijkl	22.8 cdef
Pseudomonas putida B (Trevisan) Migula strain 1	C	43.3 ghijk	15.0 efg
Pseudomonas resinovorans Delaporte	R	74.2 bc	NT
Pseudomonas syringae van Hall strain 1	R	34.2 ijkl	11.1 fg
Pseudomonas viridiflava (Burkholder) Dowson	R	68.3 bcd	NT
Trichoderma atroviride Karsten	A	29.2 kl	21.9 cdef
Trichoderma longibrachiatum Rifai	B	39.0 hijkl	25.6 cde

Note: Each value represents a mean of 2 (trial No. 1) or 3 (trial No. 2) replicates. Within a column, values followed by the same letter are not significantly different according to Fisher protected least significant difference ($P < 0.05$). NT, not tested.

*Microorganisms were isolated from: A, a mixture of peat and compost (9:1, *v/v*); B, a mixture of peat, pine bark, and compost (6:3:1, *v/v/v*); C, a mixture of peat, pine bark, and compost (3:6:1, *v/v/v*); D, a mixture of pine bark and compost (9:1, *v/v*); and (D) R, rockwool.

†Plants were drenched with sterile distilled water (noninfected control).

‡Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

through the addition of phosphoric acid. Four months after pathogen inoculation, the severity of pythium root rot was evaluated as the percentage of roots with at least one site of infection (infection rate), based on 100 roots for each plant. A complete randomized block design with two replicates was used. The experimental unit consisted of a rockwool slab, in which three plants were grown.

A second greenhouse assay was performed during a fall crop (September–December) with 17 selected isolates to evaluate the ability of the microorganisms to suppress root rot under different conditions. The tomato plants were treated and inoculated as described above. For this experiment, the rockwool slabs were placed on elevated gutters instead of directly on the greenhouse floor. The natural daylight was supplemented with high-pressure sodium (HPS) lamps (100 $\mu E \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation (PAR)) to maintain a photoperiod of 16 h. The experimental design was a complete randomized block design with three replicates. The experimental unit consisted of a rockwool slab, in which three plants were grown.

Effect of antagonistic microorganisms on root rot severity, root development, plant growth, and fruit yields in tomato plants infected with *P. ultimum*, grown in rockwool or organic medium

Greenhouse assays were conducted to evaluate the effect of the antagonistic microorganisms on root rot severity, root development, plant growth, and fruit yields in both rockwool and an 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 was a mixture of peat, pine sawdust, and compost (*v/v/v*; 60:30:10). Tomato plants were treated, inoculated, and watered

using a nutrient solution as described previously. For the organic-medium experiment, the natural daylight was supplemented with HPS lamps (100 $\mu E \cdot m^{-2} \cdot s^{-1}$ 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 five categories: No. 1, fresh mass (FM) of 114–450 g; No. 2, FM of 88–114 g; No. 3, FM less than 88 g; No. 4, blossom end rot; and No. 5, misshaped fruits, FM usually over 450 g. The marketable yield included the fruits from categories No. 1 and No. 2, whereas the total yield included all five categories. Throughout the crops, stem diameters were measured weekly to ensure that plants were developing normally. At the end of the crop (6 months), the severity of pythium root rot, the amount of roots, the anchorage of the plants, and the stem length were evaluated. Pythium root rot severity was evaluated as previously described. The anchorage of the plant in the growing media was evaluated qualitatively on a scale of 0–5. The evaluation scale was based on the force needed to manually remove the plants from the growing medium. The scale was as follows: 0, plant not anchored; 1, hardly anchored; 2, very weakly anchored; 3, weakly anchored; 4, well anchored; and 5, strongly anchored. The amount of roots at the point of anchorage was also evaluated on a scale of 0–5. The scale was based on the percentage of surface coverage by roots under the rockwool blocks. The scale was as follows: 0, 0% coverage; 1, 1%–20% coverage; 2, 21%–40% coverage; 3, 41%–60% coverage; 4, 61%–80% coverage; and 5, 81%–100% coverage. The experimental design was a complete randomized block design with three (rockwool experiment) or six replicates (organic-medium experiment). The experimental unit consisted of three plants in each slab and two plants in each 9 L plastic container for the rockwool and the organic-medium experiments, respectively. The biocontrol efficacy for each of the microorganisms was calculated using the following formula:

$$\frac{NRI_{nbt} - NRI_{bt}}{NRI_{nbt}} \times 100$$

where NRI_{nbt} is the number of roots infected by *P. ultimum* in the inoculated plants with no biological treatment with an antagonistic microorganism (controls) and NRI_{bt} is the number of roots infected by *P. ultimum* in the inoculated plants with biological treatment with an antagonistic microorganism.

Effect of antagonistic microorganisms on the growth of healthy tomato seedlings

Tomato seeds were soaked in sterile distilled water for 6 h and planted in pots (10 cm diameter) containing perlite (one plant per pot). These were treated with 1 mL of a bacterial suspension (4×10^7 bacteria/mL) or a fungal spore suspension (1×10^6 spores/mL) of one of the antagonistic microorganisms or with 1 mL of sterile distilled water (control). The seedlings were kept under typical greenhouse growing conditions (temperatures of 18 °C (night) and 24 °C (day), 80% relative humidity, 16 h photoperiod) for 30 d and watered daily with a nutrient solution of 20:20:20 (N–P–K; Plantprod) at a concentration of 1%. This fertilizer contained the following micronutrients: Fe (0.1%), Mn (0.05%), Zn (0.05%), Cu (0.05%), B (0.02%), and Mo (0.0005%). The seedlings were then gently removed from the pot, and the root system was washed with distilled water to remove the perlite. Roots and shoots were seperated, dried for 48 h at 75 °C, and weighed. A complete randomized design with four replicates was used. The experimental unit consisted of one pot containing one plant.

Statistical analysis

Data were analyzed using the general linear models (GLM) procedure of Statistical Analysis System (SAS Institute Inc. 1999). When significant ($P < 0.05$), treatment means were compared using Fisher's protected least significant difference (LSD) test.

Results

Effect of antagonistic microoganisms on pythium root rot severity on tomato plants grown in rockwool

Of the 28 antagonistic microorganisms tested in the first trial (summer crop), 27 significantly reduced root rot severity in plants inoculated with *P. ultimum* compared with the controls (plants inoculated with *P. ultimum* but not treated with an antagonistic microorganism) (Table 1). The microorganisms allowing an infection rate less than 60% were selected for the second trial (fall crop), with the exception of fungus 8-13-1 and *Pseudomonas corrugata* strain 1, which displayed irregular in vitro growth. Among the 17 microorganisms selected for further testing, only *Penicillium restrictum* failed to significantly suppress infection compared with the control treatment (no antagonistic microorganism) (Table 1). Eight microorganisms markedly reduced the severity of the root rot (infection rates to less than 25%): *Penicillium brevicompactum* (23.8%), *Penicillium solitum* strain 1 (15.0%), *Pseudomonas fluorescens* (18.8%), *Pseudomonas fluorescens* subgroup G strain 2 (22.8%), *Pseudomonas marginalis* (22.8%), *Pseudomonas putida* subgroup B strain 1 (15.0%), *Pseudomonas syringae* strain 1 (11.1%), and *Trichoderma atroviride* (21.9%). These microorganisms were selected for further study.

Effect of antagonistic microorganisms on root rot severity, root development, plant growth, and fruit yields in tomato plants infected with *P. ultimum*, grown in rockwool or organic medium

All the antagonistic microorganisms tested significantly reduced the rate of infection resulting from *P. ultimum* inoculation (Table 2). *Pseudomonas syringae* strain 1 (77.8%) and *Penicillium brevicompactum* (69.9%) exhibited the highest biocontrol efficacies in rockwool and in organic medium, respectively. In the rockwool, all the antagonistic microorganisms significantly improved the anchorage of the plants without significantly increasing the amount of roots under the blocks (Table 2). Plants treated with *Penicillium brevicompactum*, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, or *Pseudomonas syringae* strain 1 had significantly greater stem lengths than the control (not treated with an antagonistic microorganism). In the organic medium, plants treated with *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, or *T. atroviride* had significantly greater amounts of roots at the point of anchorage than the control (not treated with an

Table 2. Effect of selected antagonistic microorganisms on root rot [*Pythium ultimum*] severity, amount of roots, anchorage in the growing medium, and total length of the stem of infected tomato plants grown in rockwool and in an organic medium in a greenhouse environment.

	Root rot severity (% infected roots)	Biocontrol efficacy against root rot severity (%)	Amount of roots*	Anchorage of the plant†	Stem length (cm)
Rockwool (spring crop)					
Control‡	11.6 e		4.4 a	4.8 a	502 a
Control (*Pythium ultimum*)§	56.2 a	0.0	3.7 b	2.8 c	484 b
Penicillium brevicompactum	37.7 b	32.9	3.8 b	4.2 b	502 a
Penicillium solitum strain 1	25.4 bcde	54.8	4.0 ab	4.5 ab	499 ab
Pseudomonas fluorescens	13.7 de	75.6	3.6 b	4.0 b	505 a
Pseudomonas fluorescens G strain 2	17.9 cde	68.2	3.7 b	4.3 ab	500 ab
Pseudomonas marginalis	31.6 bc	43.7	3.9 ab	4.3 ab	506 a
Pseudomonas putida B strain 1	27.5 bcd	51.1	3.7 b	4.2 b	500 ab
Pseudomonas syringae strain 1	12.5 e	77.8	3.5 b	4.2 b	507 a
Trichoderma atroviride	27.9 bcd	50.4	3.9 ab	4.4 ab	499 ab
Organic medium (fall crop)					
Control‡	9.0 b		3.5 a	4.6 a	450 ab
Control (*Pythium ultimum*)§	42.9 a	0.0	2.4 b	3.2 c	433 a
Penicillium brevicompactum	12.9 b	69.9	3.5 a	3.9 ab	459 b
Penicillium solitum strain 1	16.3 b	62.1	3.4 a	3.8 abc	455 b
Pseudomonas fluorescens	14.1 b	67.2	3.8 a	3.9 ab	452 b
Pseudomonas fluorescens G strain 2	14.6 b	66.0	3.1 ab	3.8 abc	453 b
Pseudomonas marginalis	14.2 b	67.0	3.2 ab	3.5 bc	459 b
Pseudomonas putida B strain 1	14.6 b	66.0	3.9 a	4.2 ab	453 b
Pseudomonas syringae strain 1	13.8 b	68.0	3.8 a	3.8 abc	457 b
Trichoderma atroviride	14.6 b	66.0	3.6 a	3.9 ab	457 b

Note: Each value represents a mean of three (rockwool) and six (organic medium) replicates. For each experiment, values within a column followed by the same letter are not significantly different according to Fisher protected least significant difference ($P < 0.05$).

*The amount of roots at the point of anchorage was also evaluated on a scale of 0–5 defined as follows: 0, 0% coverage; 1, 1%–20% coverage; 2, 21%–40% coverage; 3, 41%–60% coverage; 4, 61%–80% coverage; and 5, 81%–100% coverage.

†The anchorage of the plant in the growing media was evaluated qualitatively on a scale of 0–5 based on the force needed to manually remove the plant from the growing medium. The scale was as follows: 0, plant not anchored; 1, hardly anchored; 2, very weakly anchored; 3, weakly anchored; 4, well anchored; and 5, strongly anchored.

‡Plants were drenched with sterile distilled water (noninfected control).

§Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

antagonistic microorganism). Except for *Penicillium solitum* strain 1 and *Pseudomonas syringae* strain 1, the improvement was associated with a significant increase in the anchorage of the plants. Plants treated with any one of the eight microorganisms had significantly longer stems as compared with the control. Stem diameters (average of 11 cm) were not significantly different between treatments throughout the crop.

In regard to fruit yields, tomato plants treated with *Penicillium solitum* strain 1, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, or *T. atroviride* had significantly greater individual-fruit mass and both marketable and total yields per plant compared with plants grown in rockwool, infected with *P. ultimum*, not treated with an antagonistic microorganism. In the organic medium, *Pseudomonas marginalis* was the only antagonistic microorganism, out of the eight tested, to significantly increase fruit production of plants infected with *P. ultimum*. Plants treated with *Pseudomonas marginalis* had significantly greater marketable fruit yield compared with the control (Table 3). This increase in fruit yield was associated with a significantly higher number of fruits per plant.

Effect of antagonistic microorganisms on the growth and root development of healthy tomato seedlings

The antagonistic microorganisms significantly influenced the development of both the shoot and the root system of the healthy tomato seedlings. Among the plants treated with an antagonistic microorganism, those treated with *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas marginalis*, or *T. atroviride* had both significantly greater shoot and root dry masses (DMs), whereas those treated with *Pseudomonas fluorescens* subgroup G strain 2 or *Pseudomonas putida* subgroup B strain 1 had significantly greater shoot DM only (Table 4).

Discussion

Tomato crops grown in hydroponic systems that use inert material as a growing medium are especially susceptible to pathogens such as *P. ultimum*, the causal agent of tomato root rot (Zinnen 1988; Stanghellini and Rasmussen 1994). The disease is characterized by a premature weakening of the root system and a poor anchorage in the growing medium, which affect water absorption. As a result, a gradual degeneration of the plant throughout the production period

Table 3. Effect of selected antagonistic microorganisms on marketable and total fruit yields of tomato plants infected with *Pythium ultimum*, grown in rockwool and in an organic medium in a greenhouse environment.

	Marketable			Total		
	Yield (g/plant)	No. of fruits/plant	Individual-fruit mass (g)	Yield (g/plant)	No. of fruits/plant	Individual-fruit mass (g)
Rockwool (spring crop)						
Control*	6003 ab	35.0 ab	172 bc	6662 ab	39.0 ab	171 ab
Control (*Pythium ultimum*)†	5262 c	33.3 abc	158 c	5973 b	37.7 abc	158 b
Penicillium brevicompactum	5991 ab	32.3 bc	186 a	6557 ab	36.5 bc	180 a
Penicillium solitum strain 1	6526 a	35.8 a	182 ab	7101 a	39.4 ab	180 a
Pseudomonas fluorescens	6406 a	35.4 ab	181 ab	6855 a	38.5 ab	178 a
Pseudomonas fluorescens G strain 2	5448 bc	31.3 c	174 ab	6003 b	35.1 c	171 ab
Pseudomonas marginalis	6453 a	36.2 a	179 ab	6894 a	39.0 ab	177 a
Pseudomonas putida B strain 1	6381 a	35.7 a	179 ab	6969 a	39.6 a	176 a
Pseudomonas syringae strain 1	6345 a	36.2 a	174 ab	6771 a	38.8 ab	173 a
Trichoderma atroviride	6428 a	36.0 a	178 ab	6968 a	40.0 a	174 a
Organic medium (fall crop)						
Control*	3695 ab	24.1 ab	153 ab	3998 ab	27.4 a	146 a
Control (*Pythium ultimum*)†	3359 b	20.8 b	162 ab	3877 ab	25.0 a	156 a
Penicillium brevicompactum	3544 ab	23.3 ab	152 b	3877 ab	27.1 a	144 a
Penicillium solitum strain 1	3833 ab	22.9 ab	166 a	4406 a	27.9 a	159 a
Pseudomonas fluorescens	3749 ab	24.0 ab	156 ab	4208 ab	28.1 a	150 a
Pseudomonas fluorescens G strain 2	3469 ab	20.6 b	155 ab	3654 b	24.7 a	148 a
Pseudomonas marginalis	4057 a	24.7 a	163 ab	4461 a	28.4 a	156 a
Pseudomonas putida B strain 1	3845 ab	23.1 ab	166 ab	4191 ab	26.3 a	160 a
Pseudomonas syringae strain 1	3449 b	22.6 ab	152 ab	3944 ab	27.7 a	143 a
Trichoderma atroviride	3765 ab	22.9 ab	165 ab	4123 ab	26.3 a	157 a

Note: Each value represents a mean of three (rockwool) and six (organic medium) replicates. For each experiment, values within a column followed by the same letter are not significantly different according to Fisher protected least significant difference ($P < 0.05$).

*Plants were drenched with sterile distilled water (noninfected control).

†Plants were drenched with sterile distilled water and were inoculated with a propagule suspension of *P. ultimum*.

Table 4. Effect of selected microorganisms on growth and root development of healthy tomato seedlings grown in perlite in a greenhouse environment.

	Tomato dry mass (g)	
	Shoot	Roots
Control (sterile distilled water)	0.96 c	0.09 c
Penicillium brevicompactum	2.88 ab	0.20 ab
Penicillium solitum strain 1	4.08 a	0.23 ab
Pseudomonas fluorescens	1.75 bc	0.15 bc
Pseudomonas fluorescens G strain 2	2.58 b	0.18 bc
Pseudomonas marginalis	2.60 b	0.19 b
Pseudomonas putida B strain 1	2.97 ab	0.18 bc
Pseudomonas syringae strain 1	1.68 bc	0.15 bc
Trichoderma atroviride	2.90 ab	0.29 a

Note: Each value represents a mean of four replicates. Within a column, values followed by the same letter are not significantly different according to Fisher protected least significant difference ($P < 0.05$).

is observed, leading to a decrease in plant growth and fruit yield.

In this study, several microorganisms previously shown to have in vitro antagonistic activity against *P. ultimum* (Gravel et al. 2005) were tested in greenhouse assays to evaluate their effect on tomato root rot development as well as on plant growth and fruit yield. The results showed that, except for *Penicillium solitum* strain 2, treatment with any of the 27 antagonistic microorganisms tested resulted in a significant suppression of disease severity. However, the extent of suppression depended on the microorganisms used. Among the microorganisms tested, *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens*, *Pseudomonas fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, and *T. atroviride* generally had the greatest effect as compared with the control (no antagonistic microorganism) during both growing seasons. These eight microorganisms were therefore further tested for their effect on anchorage, growth, and yield of plant infected with *P. ultimum*. Although *Pseudomonas syringae* is known as the causal agent of bacterial leaf spot in tomato (Richard and Boivin 1994), our strain did not cause this disease on plants throughout the experiments.

The anchorage of the plant in a growing medium infested with *Pythium* spp. can be decreased either by a reduction of the development of the root system or by a degradation of the anchoring roots (Hendrix and Campbell 1973). When plants were grown in rockwool in the presence of any of the eight antagonistic microorganisms tested further, the root system was healthier (less symptoms of root rot), but the amount of roots in the rockwool slab was not affected, suggesting that the improved anchorage resulted from a reduced degradation of the anchoring roots.

Among the microorganisms selected, *Penicillium brevicompactum*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *T. atroviride* have been reported as potential biocontrol agents against numerous diseases caused by *Fusarium* spp., such as crown and root rot on tomato (Menzies and Ehret 1997), cucumber root rot (Rankin and Paulitz 1994), sugar beet damping-off (Shah-Smith and Burns 1996), and apple ring rot (Kexiang et al. 2002), respectively. This study reports, for the first time, the biocontrol efficacy of those microorganisms against pythium root rot of mature greenhouse tomato crops grown in hydroponics. The possible mechanisms involved in the reduction of root rot in our experiment have not yet been identified. However, the suppressive activity of *Pseudomonas fluorescens* and *Pseudomonas putida* has, in the past, been associated with the production of antibiotic compounds (Thrane et al. 2000) and the induction of systemic resistance (Hoffland et al. 1996; Raupach et al. 1996; Ongena et al. 2000, 2002; Ramamoorthy et al. 2002). The biocontrol potential of *T. atroviride* has often been associated with its mycoparasitic activity (Olmedo-Monfil et al. 2002; Rocha-Ramírez et al. 2002; Lu et al. 2004) and its production of chitinases (Mach et al. 1999; Brunner et al. 2003; Hoell et al. 2005), whereas the antagonistic activity of *Penicillium brevicompactum* has been associated with its synthesis of active metabolites (Cantín et al. 1998; Moya et al. 1999).

Concerning their effect on tomato plant development, *Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, and *T. atroviride* improved the reproductive growth of infected tomato plants, which resulted in an increase in fruit yield per plant and individual-fruit mass. In this regard, they can be considered as plant-growth-promoting microorganisms with an indirect effect on plant growth (Antoun and Prévost 2005). Indeed, the beneficial effect of those microorganisms seems related, at least partially, to the suppression of *P. ultimum*, resulting in a generally better health of the plant. *Penicillium brevicompactum*, *Pseudomonas fluorescens*, *Pseudomonas marginalis*, and *Pseudomonas syringae* strain 1 also stimulated the vegetative growth of tomato plants in rockwool infested with *P. ultimum*. Such a beneficial effect on plant growth of *Pseudomonas fluorescens*, *Pseudomonas putida*, and *T. atroviride* has previously been reported in the presence of *Sclerotinia sclerotiorum* (Lib.) de Bary on alfalfa (Li et al. 2005), *Rhizoctonia solani* Kühn on wheat (de Freitas and Germida 1991), and *Fusarium culmorum* (W.G. Smith) Sacc. on rye (Kurek and Jaroszuk-Oecisel 2003), respectively.

Considering the results obtained, it is possible that the microorganisms could have a direct impact on the growth of healthy plants. To verify this hypothesis, the effect of these microorganisms on stem and root growth of healthy tomato seedlings was evaluated. The results obtained showed a stimulating effect of six microorganisms (*Penicillium brevicompactum*, *Penicillium solitum* strain 1, *Pseudomonas fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, and *T. atroviride*) on root DM, shoot DM, or both, strongly suggesting that these microorganisms act as PGPR (plant-growth-promoting rhizobacteria) or PGPF (plant-growth-promoting fungi). To the best of our knowledge, this is the first study to report the plant-growth-promoting effect of *Penicillium solitum*, *Pseudomonas marginalis*, and *Pseudomonas syringae*. The PGPR effect of *Pseudomonas putida* has been reported on cucumber seedlings (Utkhede et al. 1999). Other studies have shown that *Penicillium brevicompactum*, when used in combination with other microorganisms (Menzies and Ehret 1997), and *Pseudomonas fluorescens* (Gagné et al. 1993) have a stimulating effect on the vegetative growth of greenhouse tomato. Further studies will be undertaken to evaluate the capacity of these microorganisms to improve fruit yield and plant growth of mature plants under hydroponic conditions.

Penicillium brevicompactum, *Penicillium solitum* strain 1, *Pseudomonas fluorescens*, *Pseudomonas fluorescens* subgroup G strain 2, *Pseudomonas marginalis*, *Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1, and *T. atroviride* were shown to suppress root rot infection on tomato plants grown in an organic medium consisting of a mixture of peat, pine sawdust, and compost. The presence of an abundant microflora at planting in the organic medium did not appear to interfere with the biocontrol activity of the antagonistic microorganisms tested. The reduction in the disease severity was generally associated with a better anchorage in the growing medium, as was the case in the rockwool experiment, and probably resulted from a healthier and slightly more abundant root system. For all the microorganisms tested, the reduction in the disease resulted in a stimulation of the vegetative growth of the plant.

The greenhouse trials included in this study were done over a 2-year period and, therefore, were meant to test the microorganisms under a range of environmental conditions. Indeed, the development of root rot of greenhouse tomato crops is highly influenced by temperature, especially in the growing medium, and lighting conditions. The eight microorganisms selected were as effective in controlling root rot under spring (increasing temperature and natural light), summer (high temperature and optimal light), and fall (lower temperature and reduced natural light, which are conducive for disease development) conditions. Whereas most work on *P. ultimum* has concentrated on damping-off of seedlings (Hultberg et al. 2000; Carisse et al. 2003; Gravel et al. 2005) or on short-term crops (Caron et al. 2002), our study demonstrates the efficacy of these antagonistic microorganisms to reduce the deleterious effects of *P. ultimum* on mature tomato crops over several months.

This study led to the selection of antagonistic microorganisms that displayed biocontrol activity against tomato root rot under different environmental conditions (growing media; spring, summer, or fall conditions). This may open the way for new alternatives for the biological control of *Pythium* diseases in hydroponic systems that not only protect the crop but also have a beneficial effect on the plant growth and development. Further work will be conducted to develop formulations of these microorganisms to improve their efficacy. These will then be compared with commercially available biocontrol agents, including Rootshield® (*Trichoderma harzianum* Rifai) and Mycostop® (*Sreptomyces griseoviridis* Anderson et al.) that are currently registered for the control of root diseases in greenhouse vegetable crops. Further work will also be undertaken to identify the

mechanisms involved in the reduction of the disease and to confirm the PGPR and (or) PGPF effects of these microorganisms.

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