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Antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds in rockwool

Valerie GRAVEL[1], Carole MARTINEZ[1], Hani ANTOUN[2] and Russell J. TWEDDELL[1,]*
[1]*Centre de recherche en horticulture, Université Laval, Québec, Qc, Canada G1K 7P4;*
[2]*Dépt. des sols et génie agroalimentaire, Université Laval, Québec, Qc, Canada G1K 7P4*
**Author for correspondence: e-mail: russell.tweddell@crh.ulaval.ca*

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Abstract. A total of 237 microorganisms were isolated from five different greenhouse tomato growing media. Of those, 40 microorganisms reduced the *in vitro* mycelial growth of both *Pythium aphanidermatum* and *Pythium ultimum*. The ability of these microorganisms to control damping-off was then tested in rockwool. As a result, *Pseudomonas corrugata* strains 1 and 3, *Pseudomonas fluorescens* subgroup F and G strains 1, 2, 3, 4 and 5, *Pseudomonas marginalis, Pseudomonas putida* subgroup B strain 1, *Pseudomonas syringae* strain 1 and *Pseudomonas viridiflava* significantly reduced damping-off caused by *P. ultimum* or *P. aphanidermatum*. *Pseudomonas marginalis* was the only microorganism that significantly reduced damping-off caused by both pathogens.

Key words: biological control, greenhouse culture, hydroponic, seed decay

Introduction

Damping-off is an important disease of greenhouse tomato, causing important losses in nurseries where young susceptible transplants are produced. In Canada, this disease is mainly caused by *Pythium ultimum* and *Pythium aphanidermatum*, which are responsible for seed decay as well as pre- and post-emergence damping-off of tomato seedlings. Presently, none of the tomato cultivars used in greenhouse crops are resistant to *Pythium* spp. and in Canada no chemical fungicides are registered to control this pathogen in greenhouse tomato crops (Paulitz and Bélanger, 2001). Consequently, control and management of the disease rely on cultural practices such as proper control of irrigation and UV sterilization of the nutritive solution, but the effectiveness is often questionable (Paulitz, 1997). Since cultural practices alone are not always sufficient to effectively control the disease, alternative strategies are needed.

In Europe and Canada, the rockwool system of production is widely used by commercial greenhouse tomato growers. In such a system, the number of resident potentially competitive microorganisms is extremely low, facilitating dissemination of the pathogen through the nutritive solution (Stanghellini and Rasmussen, 1994) and allowing biocontrol agents with a low competitive ability to become established (Rankin and Paulitz, 1994). Few works have reported on successful biocontrol of *Pythium* in greenhouse hydroponic production systems. Rankin and Paulitz (1994) published the first report of reduction of *Pythium* disease with rhizobacteria in rockwool. They showed that application of specific strains of *Pseudomonas corrugata* and *P. fluorescens* reduced *P. aphanidermatum* root rot of cucumbers. Beneficial effects of application of *P. corrugata* (strain 13) and *P. fluorescens* (strains 63-49, 63-28, 15) on rockwool-grown cucumbers was subsequently reported by McCullagh et al. (1996). *P. fluorescens* 63-28 was also reported to increase the marketable tomato fruit yield and to inhibit efficiently *P. ultimum* in peat-based substrates (Gagné et al., 1993). To our knowledge, no specific microorganisms were reported for their ability to control *Pythium* disease on rockwool-grown tomato.

As part of ongoing research aimed to test different organic materials such as peat, barks and composts as alternatives to rockwool for greenhouse production, the objectives of this study were (1) to isolate microorganisms from these materials and (2) to test their ability to protect tomato seedlings against *Pythium* damping-off.

Materials and methods

Fungi

Virulent strains of *Pythium ultimum* and *P. aphanidermatum* isolated from infected tomato roots were provided by the laboratory of Dr. Richard Bélanger (Université Laval, Québec, QC, Canada). The fungi were grown on potato dextrose agar (PDA; Difco Laboratories, Becton Dickinson, Sparks, MD) at 24 °C. PDA disks covered with *P. ultimum* or *P. aphanidermatum* mycelium conserved in sterile distilled water at 24 °C served as stock cultures. Considering that, at room temperature, zoospores are not produced by *P. ultimum* (van der Plaats-Niterink, 1981), the propagule suspension of *P. ultimum* consisted of hyphae and oospores. The propagule suspension of *P. aphanidermatum* consisted of zoospores, hyphae and oospores. They were both prepared

from liquid cultures (250 ml flasks) containing 100 ml of potato dextrose broth (PDB; Difco Laboratories) incubated on a rotary shaker (150 rpm) at 24 °C for 1 week. PDA disks covered with actively growing mycelium of either *P. ultimum* or *P. aphanidermatum* were used to inoculate the flasks.

Isolation of microorganisms

Microorganisms were isolated, using serial dilutions and spread on selective media in Petri dishes, from rockwool, a mixture of peat and compost (9:1, v:v), a mixture of peat, pine bark and compost (6:3:1, v:v:v), a mixture of peat, pine bark and compost (3:6:1, v:v:v) and a mixture of pine bark and compost (9:1, v:v) in which tomato was previously cultivated. Isolation of microoganisms was performed according to Michaud et al. (2002). One gram of each substrate was added to 10 ml of distilled sterile water. Six dilutions 1/10 were made following homogenization of the mixture. Fungi were isolated on malt agar (Difco Laboratories) containing streptomycin (0.3 g l^{-1}; Sigma–Aldrich, Mississauga, Ontario) and on czapek agar (Sigma–Aldrich). Bacteria were isolated on the following media: tryptic soy agar (TSA; Sigma–Aldrich), TSA amended with crystal violet (0.002 g l^{-1}; Sigma–Aldrich), and pseudomonas agar F (Difco Laboratories) amended with chloramphenicol (0.125 g l^{-1}; Sigma–Aldrich) and ampicillin (0.5 g l^{-1}; Sigma–Aldrich). Crystal violet was added to TSA to facilitate the isolation of Gram-negative bacteria, while ampicillin and chloramphenicol were added to pseudomonas agar F to improve *Pseudomonas* spp. bacteria isolation. Petri dishes were incubated at 24 °C following the spread of the dilutions. Morphologically distinct microorganisms were isolated and grown in pure culture on TSA (bacteria) or PDA (fungi).

Effect of microorganisms on mycelial growth of P. ultimum *and* P. aphanidermatum

The effect of microorganisms on the growth of the pathogens was performed *in vitro* according to Brown et al. (1987). A drop of each bacterial suspension (4×10^7 bacteria ml^{-1}) was placed on PDA at one side of a Petri dish. A mycelial disk (7 mm in diameter) of either *P. ultimum* or *P. aphanidermatum* was placed on the opposite side of the Petri dish, 3 cm away from the bacteria. Bacterial suspensions were obtained from pure cultures grown in 10 ml of tryptic soy broth (TSB; Sigma–Aldrich) for 24 h on a rotary shaker at 24 °C. Fungal isolates were grown on PDA in pure culture. A PDA disk covered with actively

growing mycelium of each fungus (7 mm in diameter) was placed on PDA at one side of a Petri dish 24 h prior to adding *P. ultimum* or *P. aphanidermatum* as described before. The five following controls were used: *P. ultimum* against *P. ultimum, P. ultimum* against *P. aphanidermatum, P. aphanidermatum* against *P. aphanidermatum, P. ultimum* against a drop of TSB medium and *P. aphanidermatum* against a drop of TSB medium. Triplicate Petri dishes were incubated at 24 °C for 48 h. The presence (+) or the absence (−) of an inhibition zone between the pathogen and the microorganism tested was then recorded.

Effect of microorganisms on damping-off incidence

Microorganisms which inhibited the *in vitro* mycelial growth of *P. ultimum* and *P. aphanidermatum* were tested for their effect on the incidence of damping-off of tomato seedlings. Tomato seeds (cv. Trust F1; De Ruiter Seeds, Columbus, OH) were soaked in distilled water for 6 h, planted in multicellular blocks of rockwool and subsequently received either 1 ml of a bacterial suspension (4×10^7 bacteria ml^{-1}) or 1 ml of a fungal spore suspension (1×10^4 spores ml^{-1}). The seeds received subsequently 1 ml of either propagule (hyphae and oospores) suspension (1×10^4 propagules ml^{-1}) of *P. ultimum* or propagule (zoospores, hyphae and oospores) suspension (1×10^4 propagules ml^{-1}) of *P. aphanidermatum* (prepared from liquid cultures) and were then covered with vermiculite and placed in a growth chamber (16–20 °C, 80% relative humidity and 16 h photoperiod) for 2 weeks. During that period, the rockwool blocks were watered daily with a nutritive solution (1%) of 20–20–20 (N–P–K). Non-infected (non-infected control) and infected (infected control) seeds treated with distilled water were used as controls. Two weeks after sowing, seeds were rated for emergence. The rate of emerged seedlings was then calculated as follows: (number of emerged seedlings/number of seeds planted) × 100. The rate of decayed seeds was also evaluated and expressed in percent. A completely randomized experimental design with four replicates was used.

Antagonist identification

Bacteria and fungi which inhibited the *in vitro* mycelial growth of both *P. ultimum* and *P. aphanidermatum* were identified using the BIOLOG Identification System (BIOLOG Inc., Hayward, CA) or by 16S rDNA sequencing by Dr. Anne-Marie Simao-Beaunoir (Université de Sherbrooke, Sherbrooke, Québec, Canada).

Statistical analysis

Data on the effect of microorganisms on damping-off incidence were analyzed with SAS (SAS Institute, Cary, NC), using the general linear models procedure. Data percent values were arcsin transformed before analysis. When significant ($P < 0.05$), treatment means were compared using the Fisher protected LSD test. Each experiment was performed twice. Considering that same trends were obtained in both experiments, results of both experiments were pooled.

Results

A total of 237 microorganisms (160 bacteria and 77 fungi) were isolated from five tomato growing media. They were tested for their ability to inhibit the *in vitro* mycelial growth of the pathogens. In dual cultures on agar, 40 microorganisms (19 bacteria; 21 fungi) were shown to reduce the mycelial growth of both *P. ultimum* and *P. aphanidermatum,* as indicated by the presence of an inhibition zone (Table 1). Most of the antagonistic bacteria and fungi were from the genus *Pseudomonas* and *Penicillium,* respectively. No Gram-positive bacteria isolates were shown to reduce the mycelial growth of either *P. ultimum* or *P. aphanidermatum.*

The isolates that presented an inhibiting effect *in vitro* on both pathogens were then tested for their ability to reduce damping-off of tomato seedlings under growth chamber conditions. Compared to infected-control seeds, *Fusarium oxysporum* strain 2, *Pseudomonas corrugata* strains 1 and 3, *P. fluorescens* subgroup F, *P. fluorescens* subgroup G strains 1, 2, 3, 4 and 5, *P. marginalis, P. putida* subgroup B strain 1, *P. resinovorans, P. syringae* strain 1, *P. viridiflava* and *Trichoderma longibrachiatum* reduced significantly the rate of decayed seeds (Table 2) resulting from *P. ultimum* inoculation. Among these microorganisms, *P. corrugata* strain 3, *P. fluorescens* subgroup F, *P. fluorescens* subgroup G strains 1, 2 and 4, *P. marginalis, P. syringae* strain 1 and *P. viridiflava* significantly increased the percentage of emerged tomato seedlings (Table 2).

The application of microorganisms also influenced significantly the percentage of *P. aphanidermatum*-decayed seeds (Table 3). *F. oxysporum* strain 2, *Penicillium janthinellum* strain 4, *P. corrugata* strains 1 and 3, *P. fluorescens* subgroup G strain 3, *P. marginalis, P. putida* subgroup B strain 1, *P. resinovorans, P. syringae* strain 3 and *P. viridiflava* were shown to reduce significantly the rate of decayed seeds. Among these

Table 1. Effect of microorganisms isolated from different tomato growth substrates on the *in vitro* mycelial growth of *Pythium ultimum* and *Pythium aphanidermatum*

Microorganisms	Source[a] medium	Inhibiting zone[b]	
		P. ultimum	*P. aphanidermatum*
Acremonium potronii	B	+[c]++	+++
Acrodontium griseum	D	++[d]+	+++
Enterobacter cloacae	D	+++[e]	+++
Fungus 2-8-8[f]	B	+++	+++
Fungus 5-12-6[f]	B	+++	+++
Fungus 8-13-1[f]	C	+++	+++
Fusarium oxysporum strain 1	C	+++	+++
Fusarium oxysporum strain 2	B	+++	+++
Fusarium tumidum	D	+++	+++
Penicillium brevicompactum	B	+++	+++
Penicillium griseofulvum	C	+++	+++
Penicillium janthinellum strain 1	A	+++	+++
Penicillium janthinellum strain 2	C	+++	+++
Penicillium janthinellum strain 3	C	+++	+++
Penicillium janthinellum strain 4	B	+++	+++
Penicillium janthinellum strain 5	B	+++	+++
Penicillium restrictum	C	+++	+++
Penicillium simplicissimum strain 1	B	+++	+++
Penicillium simplicissimum strain 2	A	+++	+++
Penicillium solitum	A	+++	+++
Pseudomonas corrugata strain 1	R	+++	+++
Pseudomonas corrugata strain 2	A	+++	+++
Pseudomonas corrugata strain 3	R	+++	+++
Pseudomonas fluorescens subgroup F	C	+++	+++
Pseudomonas fluorescens subgroup G strain 1	C	+++	+++
Pseudomonas fluorescens subgroup G strain 2	R	+++	+++
Pseudomonas fluorescens subgroup G strain 3	D	+++	+++
Pseudomonas fluorescens subgroup G strain 4	C	+++	+++
Pseudomonas fluorescens subgroup G strain 5	D	+++	+++
Pseudomonas fluorescens subgroup G strain 6	R	+++	+++
Pseudomonas marginalis	A	+++	+++
Pseudomonas putida subgroup B strain 1	C	+++	+++
Pseudomonas putida subgroup B strain 2	C	+++	+++

Table 1. (Continued)

Microorganisms	Source[a] medium	Inhibiting zone[b]	
		P. ultimum	*P. aphanidermatum*
Pseudomonas resinovorans	R	+ + +	+ + +
Pseudomonas syringae strain 1	R	+ + +	+ + +
Pseudomonas syringae strain 2	B	+ + +	+ + +
Pseudomonas syringae strain 3	C	+ + +	+ + +
Pseudomonas viridiflava	R	+ + +	+ + +
Trichoderma atroviride	A	+ + +	+ + +
Trichoderma longibrachiatum	B	+ + +	+ + +
Pythium aphanidermatum		− − −	− − −
Pythium ultimum		− − −	

[a]Microorganisms were isolated from rockwool (R), a mixture of peat and compost (9:1, v:v) (A), a mixture of peat, pine bark and compost (6:3:1, v:v:v) (B), a mixture of peat, pine bark and compost (3:6:1, v:v:v) (C) and a mixture of pine bark and compost (9:1, v:v) (D).
[b]Presence (+) or absence (−) of an inhibition zone between the pathogen and the microorganism.
[c,d,e]Replicate 1, 2 and 3, respectively.
[f]Not identified.

microorganisms, *P. corrugata* strain 1, *P. fluorescens* subgroup G strains 3 and 5, *P. marginalis* and *P. putida* subgroup B strain 1 increased the percentage of emerged *P. aphanidermatum* inoculated seedlings.

Pseudomonas marginalis was the only microorganism out of the 40 tested that significantly reduced damping-off incidence caused by both pathogens with 62.5 and 87.5% of emerged seedlings when inoculated with *P. ultimum* and *P. aphanidermatum,* respectively. None of the fungi tested significantly increased the percentage of emerged seedlings.

Discussion

In this study, a two-step procedure involving *in vitro* and *in vivo* assays was used to select antagonist microorganisms with the ability to control *Pythium* damping-off of tomato seeds (Okamoto et al., 2000; Dal Bello et al., 2003). In order to have a reasonable number of microorganisms for *in vivo* tests, the 237 microorganisms isolated from the different greenhouse tomato growing media were tested for their ability to inhibit

Table 2. Effect of antagonistic microorganisms on the emergence of tomato seedlings and the decay of tomato seeds inoculated with *Pythium ultimum* in rockwool

Microorganisms	Emerged seedlings (%)		Decayed seeds (%)	
Infected control	0	a	100	e
Non-infected control	100	d	0	a
Acremonium potronii	0	a	87.5	de
Acrodontium griseum	0	a	75	cde
Enterobacter cloacae	0	a	50	abcde
Fungus 2-8-8	12.5	ab	62.5	bcde
Fungus 5-12-6	0	a	75	cde
Fungus 8-13-1	0	a	62.5	bcde
Fusarium oxysporum strain 1	0	a	75	cde
Fusarium oxysporum strain 2	25	abc	37.5	abcd
Fusarium tumidum	0	a	75	cde
Penicillium brevicompactum	0	a	62.5	bcde
Penicillium griseofulvum	0	a	62.5	bcde
Penicillium janthinellum strain 1	0	a	75	cde
Penicillium janthinellum strain 2	0	a	62.5	bcde
Penicillium janthinellum strain 3	0	a	62.5	bcde
Penicillium janthinellum strain 4	0	a	50	abcde
Penicillium janthinellum strain 5	0	a	50	abcde
Penicillium restrictum	0	a	100	e
Penicillium simplicissimum strain 1	12.5	ab	50	abcde
Penicillium simplicissimum strain 2	0	a	87.5	de
Penicillium solitum	12.5	ab	62.5	bcde
Pseudomonas corrugata strain 1	37.5	abc	37.5	abcd
Pseudomonas corrugata strain 2	50	abcd	50	abcde
Pseudomonas corrugata strain 3	62.5	bcd	12.5	ab
Pseudomonas fluorescens subgroup F	62.5	bcd	25	abc
Pseudomonas fluorescens subgroup G strain 1	62.5	bcd	12.5	ab
Pseudomonas fluorescens subgroup G strain 2	75	cd	12.5	ab
Pseudomonas fluorescens subgroup G strain 3	25	abc	37.5	abcd
Pseudomonas fluorescens subgroup G strain 4	75	cd	12.5	ab
Pseudomonas fluorescens subgroup G strain 5	50	abcd	25	abc
Pseudomonas fluorescens subgroup G strain 6	12.5	ab	62.5	bcde
Pseudomonas marginalis	62.5	bcd	25	abc
Pseudomonas putida subgroup B strain 1	50	abcd	12.5	ab
Pseudomonas putida subgroup B strain 2	25	abc	50	abcde
Pseudomonas resinovorans	50	abcd	37.5	abcd

Table 2. (Continued)

Microorganisms	Emerged seedlings (%)		Decayed seeds (%)	
Pseudomonas syringae strain 1	62.5	bcd	12.5	ab
Pseudomonas syringae strain 2	25	abc	75	cde
Pseudomonas syringae strain 3	50	abcd	50	abcde
Pseudomonas viridiflava	62.5	bcd	37.5	abcd
Trichoderma atroviride	0	a	87.5	de
Trichoderma longibrachiatum	25	abc	25	abc

In each column, means followed by the same letter are not significantly different ($P > 0.05$) according to the Fisher protected LSD test ($n = 8$).

the *in vitro* mycelial growth of both pathogens, *P. ultimum* and *P. aphanidermatum*. Of those, 40 microorganisms were shown to inhibit the growth of both *P. ultimum* and *P. aphanidermatum* presumably by producing antifungal compounds. These microorganisms were identified as *Acremonium potronii, Acrodontium griseum, Enterobacter cloacae, Fusarium oxysporum, F. tumidum, Penicillium brevicompactum, P. griseofulvum, P. janthinellum, P. restrictum, P. simplicissimum, P. solitum, Pseudomonas corrugata, P. fluorescens* subgroups F and G, *P. marginalis, P. putida* subgroup B, *P. resinovorans, P. syringae, P. viridiflava, Trichoderma atroviride* and *T. longibrachiatum*. Three fungi were not further identified. These microorganisms were then tested *in vivo* for their ability to reduce damping-off caused by both pathogens. Among these microorganisms, *P. corrugata* strains 1 and 3, *P. fluorescens* subgroups F and G strains 1, 2, 3, 4 and 5, *P. marginalis*, *P. putida* subgroup B strain 1, *P. syringae* strain 1 and *P. viridiflava* reduced damping-off caused by *P. ultimum* or *P. aphanidermatum*. These microorganisms were previously reported for their ability to reduce the development of various pathogens mainly in soil.

Pseudomonas putida is a bacterium frequently isolated from soil which received a lot of attention for its capacity to degrade complex aromatic molecules (Koshelava et al., 2000; Sultana et al., 2001). In this study, two strains of *P. putida* subgroup B were isolated and showed *in vitro* inhibition of both pathogens. However, only *P. putida* strain 1 reduced damping-off of tomato caused by *P. aphanidermatum*. Previous works have reported the biocontrol ability of this bacterium against *Pythium* damping-off of sugarbeet (Shah-Smith and Burns, 1996) and safflower (Liang et al., 1996), durum wheat tan spot caused by *Pyrenophora tritici-repentis* (da Luz et al., 1998), potato silver scurf caused by

Table 3. Effect of antagonistic microorganisms on the emergence of tomato seedlings and the decay of tomato seeds inoculated with *Pythium aphanidermatum* in rockwool

Microorganisms	Emerged seedlings (%)		Decayed seeds (%)	
Infected control	0	a	100	d
Non-infected control	100	d	0	a
Acremonium potronii	0	a	62.5	abcd
Acrodontium griseum	0	a	50	abcd
Enterobacter cloacae	25	ab	50	abcd
Fungus 2-8-8	0	a	62.5	abcd
Fungus 5-12-6	25	ab	62.5	abcd
Fungus 8-13-1	0	a	87.5	cd
Fusarium oxysporum strain 1	0	a	62.5	abcd
Fusarium oxysporum strain 2	0	a	25	abc
Fusarium tumidum	0	a	50	abcd
Penicillium brevicompactum	0	a	100	d
Penicillium griseofulvum	0	a	100	d
Penicillium janthinellum strain 1	0	a	75	bcd
Penicillium janthinellum strain 2	0	a	75	bcd
Penicillium janthinellum strain 3	0	a	75	bcd
Penicillium janthinellum strain 4	37.5	abc	25	abc
Penicillium janthinellum strain 5	12.5	ab	37.5	abcd
Penicillium restrictum	0	a	87.5	cd
Penicillium simplicissimum strain 1	0	a	37.5	abcd
Penicillium simplicissimum strain 2	0	a	75	bcd
Penicillium solitum	25	ab	62.5	abcd
Pseudomonas corrugata strain 1	62.5	bcd	12.5	ab
Pseudomonas corrugata strain 2	25	ab	37.5	abcd
Pseudomonas corrugata strain 3	50	abcd	12.5	ab
Pseudomonas fluorescens subgroup F	25	ab	50	abcd
Pseudomonas fluorescens subgroup G strain 1	12.5	ab	62.5	abcd
Pseudomonas fluorescens subgroup G strain 2	12.5	ab	37.5	abcd
Pseudomonas fluorescens subgroup G strain 3	62.5	bcd	12.5	ab
Pseudomonas fluorescens subgroup G strain 4	12.5	ab	50	abcd
Pseudomonas fluorescens subgroup G strain 5	62.5	bcd	37.5	abcd
Pseudomonas fluorescens subgroup G strain 6	12.5	ab	75	bcd
Pseudomonas marginalis	87.5	cd	0	a
Pseudomonas putida subgroup B strain 1	62.5	bcd	25	abc
Pseudomonas putida subgroup B strain 2	12.5	ab	62.5	abcd
Pseudomonas resinovorans	50	abcd	25	abc

Table 3. (Continued)

Microorganisms	Emerged seedlings (%)		Decayed seeds (%)	
Pseudomonas syringae strain 1	0	a	75	bcd
Pseudomonas syringae strain 2	12.5	ab	75	bcd
Pseudomonas syringae strain 3	25	ab	0	a
Pseudomonas viridiflava	25	ab	25	abc
Trichoderma atroviride	0	a	87.5	cd
Trichoderma longibrachiatum	0	a	100	d

In each column, means followed by the same letter are not significantly different ($P > 0.05$) according to the Fisher protected LSD test ($n = 8$).

Helminthosporium solani (Elson et al., 1997; Martinez et al., 2002) and potato soft rot caused by *Erwinia carotovora* (Colyer and Mount, 1984). *P. marginalis* isolated from a mixture of peat and compost strongly reduced the incidence of damping-off caused by both pathogens. To our knowledge, no study has previously reported the antagonistic activity of *P. marginalis* against Oomycetes, but such an activity was reported against *Colletotrichum graminicola* on sorghum (Michereff et al., 1994).

Pseudomonas corrugata was reported to display antagonism against various plant pathogens in different substrates including soil (Pandey et al., 2001), peat moss (Georgakopoulos et al., 2002) and rockwool (Rankin and Paulitz, 1994). Antagonism against *P. ultimum* in maize (Pandey et al., 2001), cucumber and sugar beet (Georgakopoulos et al., 2002), *P. aphanidermatum* in cucumber (Rankin and Paulitz, 1994) and *Gaeumannomyces graminis* var. *tritici* in wheat (Ryder and Rovira, 1993) has been observed. While *P. syringae* was previously reported for its biocontrol activity against apple blue (*Penicillium expansum*) and gray (*Botrytis cinerea*) mold (Zhou et al., 2001, 2002) and against the foodborne pathogen *Escherichia coli* (Janisiewicz et al., 1999) on apple. In the present study, *P. corrugata* and *P. syringae* reduced significantly damping-off of tomato caused by *P. ultimum*. However, their potential use as biocontrol agents of tomato pathogens must be viewed with caution. Indeed, *P. corrugata* and *P. syringae* pv. tomato are well-known tomato pathogens causing pith necrosis and bacterial speck, respectively (Richard and Boivin, 1994).

Pseudomonas fluorescens has been extensively studied for its ability to stimulate the growth of plants (Howie and Echandi, 1983; Kloepper et al., 1988; Kurek and Jaroszuk-Ściseł, 2003) and to reduce the development of various plant pathogens (Paulitz et al., 1992;

Ramamoorthy et al., 2002a). Numerous studies have demonstrated the potential of different strains of *P. fluorescens* as biocontrol agents of different pathogens including *Fusarium sambucinum* (Schisler et al., 2000), *Pythium* sp. group G (Bardin et al., 2003) and *P. ultimum* (Carisse et al., 2003). The ability of isolates of *P. fluorescens* subgroups C and E to reduce cucumber root rot caused by *P. aphanidermatum* has also been reported (Paulitz et al., 1992). Of particular interest, studies have reported the biocontrol potential of specific strains of *P. fluorescens* against *Fusarium* wilt of tomato grown in soil (Larkin and Fravel, 1998), *P. aphanidermatum* damping-off of tomato grown in soil (Ramamoorthy et al., 2002b) and *P. ultimum* damping-off of tomato seedlings grown in Petri dish bioassays (Hultberg et al., 2000). Our results showed the efficiency of different strains of *P. fluorescens* of the subgroups F and G to reduce the incidence of tomato damping-off caused by either *P. ultimum* or *P. aphanidermatum* in rockwool.

Biocontrol agents exert disease suppression by different modes of action including competition, direct parasitism, antibiosis and induction of plant resistance mechanism (Whipps, 2001). None of the mechanisms are necessarily mutually exclusive and frequently several modes of action are exhibited by a single biocontrol agent. The mechanism by which *P. fluorescens* exerts its antagonism against *Pythium* sp. has been extensively studied and appears to involve the production of a variety of antibiotic compounds (Howell and Stipanovic, 1980; Sharifi-Tehrani et al., 1998; Thrane et al., 2000), competition (Mohamed and Caunter, 1995; Ellis et al., 1999) and induced resistance (Benhamou et al., 1996; Ramamoorthy et al., 2002a). *P. putida*, *P. corrugata* and *P. marginalis* were also reported to exert an antagonistic activity through the production of antimicrobial compounds (de Freitas et al., 1991; Harris et al., 1997), competition (Paulitz et al., 1992; Fukui et al., 1994) or induction of plant resistance mechanism (Chen et al., 1998; Ongena et al., 2000, 2002;). In this study, the presence of an inhibition zone occurring between the pathogens and *P. fluorescens*, *P. putida*, *P. corrugata* and *P. marginalis* in dual culture on agar plate suggests that the production of antibiotic compounds is involved in the antagonism observed *in vivo*. However, other modes of action such as competition and induced resistance can not be ruled out.

It is interesting to note that certain microorganisms allowed to reduce the incidence of seed decay caused by either *P. ultimum* or *P. aphanidermatum* but did not allow to decrease the incidence of damping-off. For instance, *F. oxysporum* strain 2 reduced seed decay incidence caused by both pathogens but failed to reduce the incidence of damping-off. Such a phenomenon which was also observed with

T. longibrachiatum, P. janthinellum and *P. resinovorans* suggests that some microorganisms protected the seed from decay but failed to protect the epicotyl leading to emergence.

In this study, a two-step procedure including *in vitro* screening has allowed to identify several microorganisms with the ability to reduce damping-off caused by *P. aphanidermatum* or *P. ultimum. In vitro* screening is often used as a first filter to identify potential biocontrol agents (Okamoto et al., 2000; Dal Bello et al., 2003). However microorgansims showing no antagonistic activity *in vitro* may display a biocontrol ability *in vivo* (Knudsen et al., 1997). For this reason, the 197 microorganisms showing no antagonistic activity *in vitro* will be tested in future work for their ability to control damping-off in rockwool.

Very few works have reported on successful biocontrol of *Pythium* in greenhouse hydroponic production systems. The results presented in this study have led to the identification of several microorganisms displaying the ability to control tomato damping-off caused by either *P. ultimum* or *P. aphanidermatum* in rockwool. These findings may open the way for new avenues of research in the biological control of *Pythium* diseases in hydroponic systems.

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