Influence of *Pythium oligandrum* on the bacterial communities that colonize the nutrient solutions and the rhizosphere of tomato plants

J. Vallance, F. Déniel, G. Barbier, L. Guerin-Dubrana, N. Benhamou, and P. Rey

Abstract: The influence exerted by the biocontrol oomycete *Pythium oligandrum* on the bacterial populations proliferating in the rhizosphere of tomato plants grown in a hydroponic system and in the circulating solutions is studied in the present experiment. Quantitative PCR and single-strand conformation polymorphism were used to investigate the genetic structure and dynamics of the bacterial communities colonizing the root systems and the various circulating solutions. Quantitative PCR assays showed that bacteria heavily colonized the rhizosphere of tomato plants with, however, no significant density changes throughout the cultural season (April–September). Single strand conformation polymorphism fingerprints revealed the occurrence of transient perturbations in the rhizospheric indigenous bacterial communities following *P. oligandrum* introduction in the root system of plants. This effect was, however, transient and did not persist until the end of the cropping season. Interestingly, the genetic structure of the bacterial microflora colonizing either the roots or the nutrient solutions evolved throughout the cropping season. This temporal evolution occurred whatever the presence and persistence of *P. oligandrum* in the rhizosphere. Evidence is also provided that bacterial microflora that colonize the root system are different from the ones colonizing the circulating solutions. The relationships between these 2 microflora (at the root and solution levels) are discussed.

Key words: bacterial communities, biological control, soilless culture, recirculating solutions, single-strand conformation polymorphism, rhizosphere.

Résumé : L'influence exercée par un oomycète utilisé en bio-contrôle *Pythium oligandrum* sur les populations bactériennes qui prolifèrent dans la rhizosphère des plants de tomates cultivés en système hydroponique et sur les solutions circulantes est étudiée dans l'expérience présente. Une PCR quantitative et le polymorphisme de conformation simple brin (SSCP) ont été utilisés pour examiner la structure génétique et la dynamique des communautés bactériennes qui colonisent les systèmes racinaires et les différentes solutions circulantes. Les tests en PCR quantitative ont montré que les bactéries colonisaient fortement la rhizosphère des plants de tomates, sans changements de densité significatifs au cours de la saison de culture (avril à septembre). Les empreintes en SSCP ont révélé l'existence de perturbations transitoires dans les communautés bactériennes endogènes de la rhizosphère à la suite de l'introduction de *P. oligandrum* dans le système racinaire des plants. Cet effet était cependant transitoire et ne persistait pas jusqu'à la fin de la saison de culture. Fait intéressant, la structure génétique de la microflore bactérienne qui colonise les racines ou les solutions évoluait pendant toute la saison de culture. Cette évolution temporelle se produisait peu importe la présence et la persistence de *P. oligandrum* dans la rhizosphère. Une preuve est aussi apportée que la microflore bactérienne qui colonise le système racinaire diffère de celle des solutions circulantes. Les relations entre ces 2 microflores (racines et solutions) sont discutées.

Mots-clés : communautés bactériennes, contrôle biologique, culture hors-sol, solutions circulantes, polymorphisme de conformation simple brin, rhizosphère.

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J. Vallance. Université Européenne de Bretagne / Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESMISAB, 29280 Plouzané, France; Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France; INRA, ISVV, UMR1065 SAVE, F-33140 Villenave d'Ornon, France.
F. Déniel and G. Barbier. Université Européenne de Bretagne / Université de Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESMISAB, 29280 Plouzané, France.
L. Guerin-Dubrana and P. Rey. Université de Bordeaux, ISVV, UMR1065 Santé et Agroécologie du Vignoble (SAVE), Bordeaux Sciences Agro, F-33140 Villenave d'Ornon, France; INRA, ISVV, UMR1065 SAVE, F-33140 Villenave d'Ornon, France.
N. Benhamou. Centre de Recherche en Horticulture, Pavillon Envirotron, 2480, Boulevard Hochelaga, Université Laval, Québec, QC G1V 0A6, Canada.

Corresponding author: P. Rey (e-mail: prey@bordeaux.inra.fr).

Introduction

Research over the past years has convincingly shown that a rich and complex microflora colonizes 3 ecological niches in soilless greenhouse systems, i.e., the rhizosphere, the substrate, and the circulating solutions (Vallance et al. 2011). In an attempt to characterize this abundant microflora, most efforts have been concentrated on the identification of the microbial populations in both the circulating solutions and the rhizosphere. In the literature, previous reports have provided that huge populations of aerobic heterotrophic bacteria (10^5–10^6 colony-forming units (cfu)·mL^{-1}) proliferate in the circulating solutions as soon as 20 h after tomato planting (Berkelmann et al. 1994) and might reach a peak of 10^{10} cfu·g^{-1} of root fresh mass in the vicinity of young tomato roots (Waechter-Kristensen et al. 1994). Other studies have provided complementary information about the bacterial distribution, highlighting that, whatever the cultural system used, bacterial and fungal populations colonize preferentially the rhizosphere (Koohakan et al. 2004). It is worth mentioning, however, that among the nonspecific bacterial genera, aerobic bacteria, including fluorescent pseudomonads, develop abundantly in the rhizosphere, as well as in the nutrient solutions (Berkelmann et al. 1994; Koohakan et al. 2004; Déniel et al. 2004). This finding suggests that growth of fluorescent pseudomonads (more than 40% of the total bacterial population) may be activated by specific parameters including temperature, nitrogen content, and oxygen concentration of the nutrient solutions.

Although part of the microflora may be pathogenic as reported by several authors (Rey et al. 1996, 1997, 1998, 2001; Stanghellini and Rasmussen 1994), evidence from a number of studies indicates that a large number of microorganisms occurring in the microflora are beneficial due to their ability to suppress diseases by efficiently controlling pathogenic populations (Berger et al. 1996; Chen et al. 1998; Minuto et al. 2007; Postma et al. 2000, 2005; Postma 2004). However, disease suppression mediated by beneficial microorganisms is highly variable, mainly because of the random colonization of the rhizosphere by effective biocontrol agents. To solve this problem, inoculation of plants grown in soilless cultures with specific biocontrol microorganisms was carried out. Le Floch et al. (2003) were the first to report a significant increase in tomato yield upon introduction of the mycoparasite *Pythium oligandrum* in the rhizosphere. Several authors have convincingly shown how *P. oligandrum* exerts antimicrobial activity (Benhamou et al. 1999; Foley and Deacon 1986; Rey et al. 2005) and induces growth promotion and disease resistance on plants (Benhamou et al. 1997; Le Floch et al. 2003, 2005; Rey et al. 2008). However, very little information is available on key questions including the diversity and population dynamics of the microorganisms colonizing the roots and the nutrient solutions in the presence of a biocontrol agent, e.g., *P. oligandrum*. Vallance et al. (2009) reported that the introduction of *P. oligandrum* in soilless tomato cultures resulted in a decrease of *Pythium dissotocum* populations, a well-known minor pathogenic oomycete. But, to our knowledge, no data are available for substantiating the concept that amendment of the rhizosphere and (or) influents with fungal or oomycete biocontrol agents may have an impact on the diversity and growth of the bacterial communities. Therefore, a serious drawback has been the lack of information on important parameters including the influence that may exert the introduction of *P. oligandrum* on the naturally occurring bacterial microflora.

In that context, the present study was undertaken to gain a deeper insight into the influence exerted by *P. oligandrum* on the bacterial populations proliferating not only in the rhizosphere of tomato plants grown in a hydroponic system but also in the circulating solutions. Our objectives were (*i*) to assess the bacterial status and dynamics in the tomato rhizosphere and the circulating solutions and (*ii*) to evaluate whether or not the introduction and persistence of *P. oligandrum* in the rhizosphere affects the bacterial microflora. Quantitative PCR was used to evaluate bacterial density throughout the cultural season (from April to September), and single-strand conformation polymorphism (SSCP) was used to determine the bacterial communities structures and dynamics. One of the main advantages of SSCP is that it can be used to detect rapid changes in microbial communities in the absence of prior knowledge about their composition. This method also avoids the biases introduced by culture-based methods. Additionally, these techniques were recently used to study the diversity and dynamics of bacterial communities in slow filters used to disinfect nutrient solutions in hydroponic systems; these filters were either amended or not amended with suppressive bacteria (Renault et al. 2012).

Materials and methods

Plant material

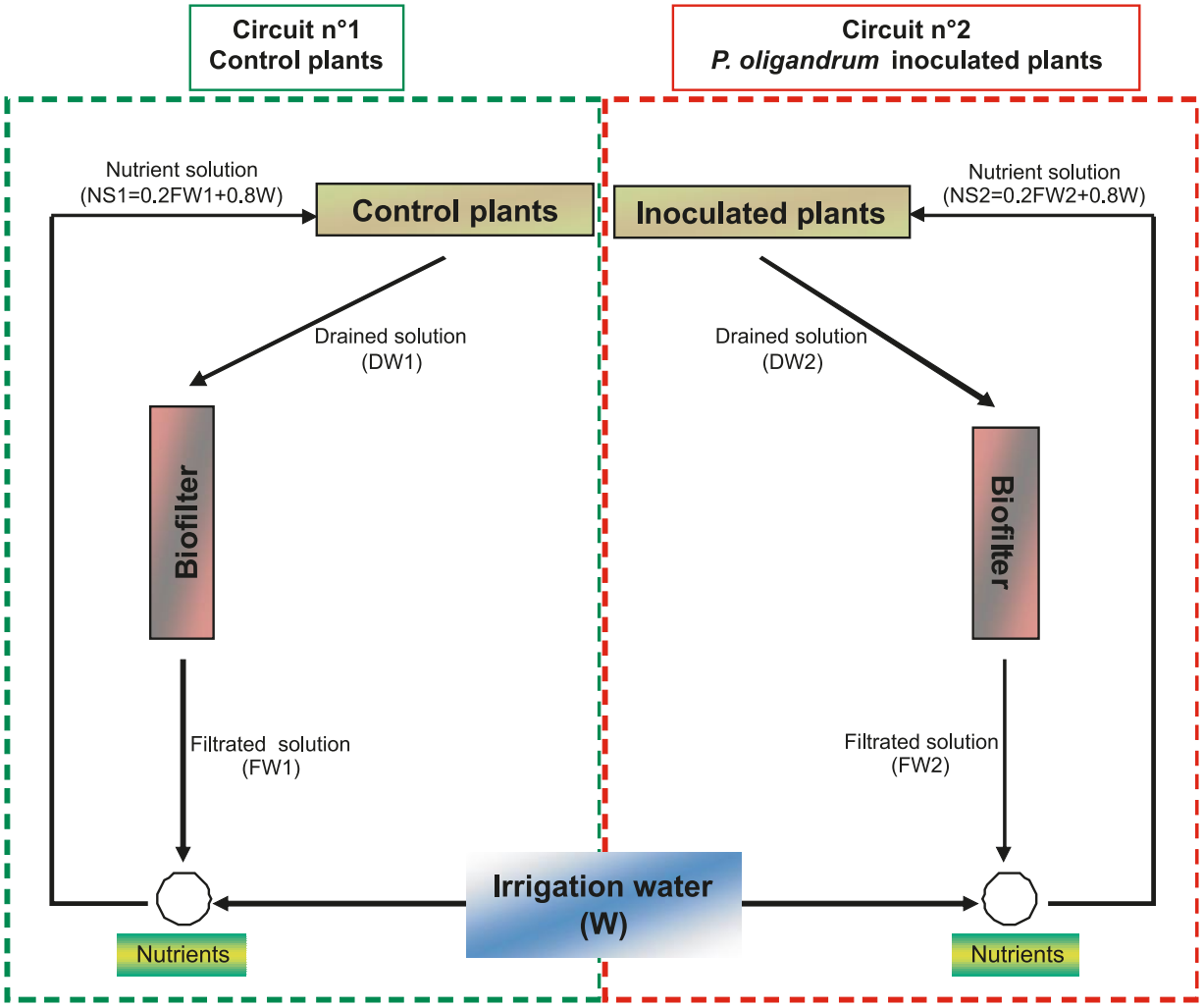
Seeds of tomato (*Lycopersicon esculentum* Mill. cv Durinta) (Western Seed, France) were sown in rockwool cubes prior to being transferred to coconut fiber slabs (4 plants per slab) in 2 experimental soilless greenhouses at CATE precincts (St Pol de Léon, Brittany, France). Each slab was wrapped in a plastic bag to isolate it from the others. A standard nutrient solution (Yara, France) was delivered to each plant through a capillary system set at the crown. The temperature in the greenhouses was regularly monitored and was between 18 °C (±2 °C, night) and 21 °C (±2 °C, day). The pH was regularly monitored and was between 5.5 and 6.2. The culture conditions were similar in the 2 greenhouses, except that the temperature difference between night and day was about 2 °C greater in greenhouse 2 than in greenhouse 1.

In each greenhouse, 3 rows of plants (136 plants) were used as controls, and 2 other rows (136 plants) were inoculated with strains CBS 530.74, CBS 109981, and LMSA 1.01.631 of *P. oligandrum* (Souchotèque de Bretagne, ESMISAB, Plouzané, France). Nutrient solutions were recycled using 2 identical but separated biofiltration systems, one for control plants and the other for *P. oligandrum* inoculated plants (Fig. 1).

Fungal cultures and growth conditions

The strains of *P. oligandrum* Drechsler (strains CBS 530.74, CBS 109981, and LMSA 1.01.631) used in this study were obtained from the souchothèque de Bretagne (ESMISAB, Plouzané, France). They were grown on potato dextrose agar (PDA) at 24 °C in the dark and were regularly subcultured.

Fig. 1. Schematic representation of the irrigation circuits (1, control; 2, *Pythium oligandrum* inoculated) in the greenhouses under study.



Pythium oligandrum inoculation and root colonization

Plants were inoculated twice at a 1-month interval with a mixture of the 3 *P. oligandrum* strains. Fungal homogenates, containing a mixture of oospores and mycelium, were prepared as previously described by Vallance et al. (2009). Briefly, equal volumes of fungal inoculum from each of the 3 strains were mixed and then diluted in distilled water to the required final concentration of oospores. Aliquots of 100 mL of mixed oospore suspensions at a rate of 2.22×10^5 for the first inoculation and 3.38×10^5 oospores·mL^{-1} for the second inoculation were deposited in the vicinity of the collar and the roots of each tomato plant. Two weeks after the second inoculation (end of April), *P. oligandrum* was detected on about 90% of the root systems and persisted at around 50% until the end of the cropping season (Vallance et al. 2009).

Sampling and DNA extraction

Root samples were collected twice per month throughout the cropping season (March–October). Two coconut fiber slabs (samples) per experimental condition (inoculated and control plants) and per greenhouse were randomly selected and opened for root collection. Circulating solution samples were collected every 4 weeks throughout the cultural season (7 months). Circulating solutions included (*i*) water irrigating the greenhouses (W), (*ii*) solutions drained from roots (before biofiltration) (DW1 and DW2), (*iii*) solutions disinfected by biofiltration (FW1 and FW2), and (*iv*) nutrient solutions reinjected on roots (NS1 and NS2) (Fig. 1). DNA was extracted from 200 mg of fresh root tissue with the Fast DNA Spin Kit (MP Biomedicals) and from 200 mL of each of the 4 circulating solutions (filtrated on 0.2 µm cellulose acetate membranes; Sartorius) using the Fast DNA Spin Kit for soil (MP Biomedicals) according to the manufacturer's instructions.

Supplementary DNA extractions were performed from pure cultures of *Pseudomonas putida* to gain standards for running the qPCR analyses. The bacterial standard was obtained from a pure culture of *P. putida* (isolate LMSA 3.06.047; souchotèque de Bretagne, ESMISAB, France). Bacteria were grown for 24 h in a nutrient broth medium (BN, AES). After centrifugation at 10 000 rpm for 10 min, 200 µL of sterile distilled water were added to the bacterial pellet for DNA extraction.

Regarding the tomato genomic DNA, the standard for DNA quantification was obtained from roots of young plants cultured in sterile conditions. Seeds of tomato (Prisca, F1 hybrids, S&G Syngenta Seeds) were disinfected successively in 70% (*v*/*v*) ethanol and 2% (*v*/*v*) sodium hypochlorite for

Table 1. Primers and fluorogenic probes used for quantitative PCR (qPCR) and single-strand conformation polymorphism (SSCP).

Application	Gene	Name	Sequence 5′–3′	Amplicon size
qPCR	Tomato LAT52[a]	up_Lat1	AGACCACGAGAACGATATTTGC	92 bp
		lo_Lat2	TTCTTGCCTTTTCATATCCAGACA	
		Lp	**6-FAM**-CTCTTTGCAGTCCTCCCTTGGGCT-**BHQ1**	
	Bacteria rRNA 16S[b]	W102	CGGTGAATACGTTCYCGG	123 bp
		W105	GGWTACCTTGTTACGACTT	
		W101	**6-FAM**-CTTGTACACACCGCCCGTC-**TAMRA**	
SSCP	V3 region rRNA 16S	W49	ACGGTCCAGACTCCTACGGG	200 bp
		W104	**6-FAM-**TTACCGCGGCTGCTGGCAC	

[a]Yang et al. 2005.
[b]Suzuki et al. 2000.

7 min; and then thoroughly rinsed in sterile distilled water prior to being deposited in tubes containing 0.6% (*w*/*v*) agarose. Seeded tubes were then incubated in a plant growth chamber (Strader, France) set at 20 °C (±2 °C) with a photoperiod of 16 h of light and 8 h of dark (neon light 58 W, Mazda Brillant incandia 830 and Blanc 840). After 30 days of culturing, roots were collected in sterile conditions and processed for DNA extraction.

Quantitative PCR

A MiniOpticon thermocycler (Bio-Rad) was used for qPCR analyses. The reaction volume was 15 µL and consisted of 7.5 µL of 2× Quantitect Probe Mix (Qiagen) and 0.4 µmol·L^{-1} of each primer and probe (Table 1). The cycling parameters were pre-incubations at 50 °C for 2 min and at 95 °C for 15 min followed by 40 cycles at 95 °C for 15 s and 56 °C for 1 min. Fluorescence was monitored during every PCR cycle after the extension step.

The standard curves were generated by plotting the threshold cycle of a 10-fold dilution series of known amounts of genomic DNA (bacteria and tomato DNA) versus the logarithm of the concentration. Thereafter, a regression line was drawn to determine the concentration of the target DNA in the sample tested from the value of its threshold cycle. Opticon Monitor software (version 3.1.32; Bio-Rad Laboratories, Inc.) was used for data analysis. To normalize the data for the root samples, the amount of tomato DNA in each sample was quantified as reported by Le Floch et al. (2007) using specific primers and a probe for the tomato gene *Lat52* (Yang et al. 2005).

Differences in bacterial root colonization between greenhouses 1 and 2 and between control and *P. oligandrum* inoculated plants throughout the cultural season were assessed by a Wilcoxson signed rank test (Dagnelie 1975) in which the means of the 2 repetitions per date and per greenhouse or treatment were used as paired data.

SSCP analyses of the bacterial communities in both the tomato rhizosphere and the circulating solutions

Primers used for SSCP analyses of the bacterial communities are presented in Table 1. DNA was amplified by PCR in a PTC-100 thermocycler (MJ Research, Inc.) with a reaction mixture (50 µL final volume) consisting of 1 µL of DNA template, 0.2 mmol·L^{-1} each dNTP, 2 ng·µL^{-1} of each primer, Pfu Turbo buffer 1×, and 0.05 units of Pfu Turbo DNA polymerase (Stratagene, the Netherlands). The cycling parameters were 95 °C for 2 min followed by 25 cycles at 95 °C for 30 s, 61 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. The PCR products were visualized by 1% Tris–borate–EDTA (TBE) agarose gel electrophoresis prior to SSCP analysis.

SSCP analyses were performed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) with a 47 cm long capillary. One microlitre of a PCR product was mixed with 18.8 µL of formamide Hi-Di (Applied Biosystems) and 0.2 µL of standard internal DNA molecular mass marker Genescan 400 HD ROX (Applied Biosystems). The sample mixture was denatured at 95 °C for 5 min and immediately cooled on ice prior to be loaded onto the instrument. The nondenaturing polymer consisted of 5% POP conformational analysis polymer (Applied Biosystems), 10% glycerol, and EDTA buffer (10×) and water (Applied Biosystems). Migration time was set to 30 min, voltage to 12 kV, and temperature to 32 °C.

Samples were co-migrated with the fluorescent size standard (GeneScan-400 ROX) to allow comparison of migration profiles between samples. Patterns were aligned and studied by principal components analysis (PCA) with Statbox Pro (version 6.6; Grimmersoft, Paris, France).

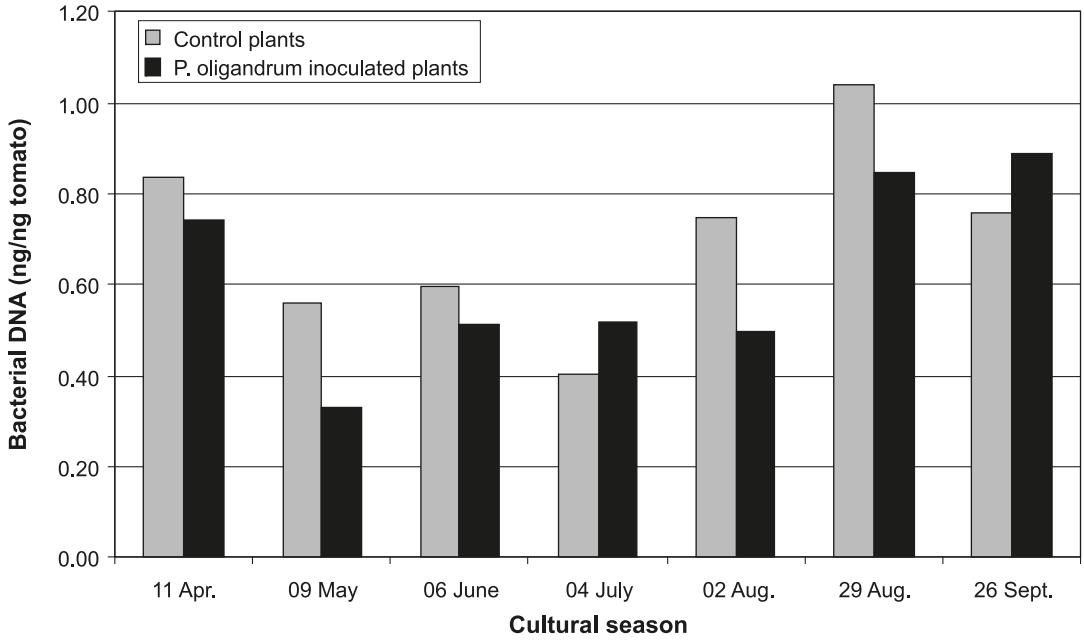
Results

Bacterial density in the tomato rhizosphere

Because experiments were conducted under the same cultural conditions in 2 independent greenhouses, a Wilcoxson signed rank test (Dagnelie 1975) was performed to evaluate whether or not the compartment had an effect on the bacterial colonization of the tomato plant root system. No statistical difference was observed between the 2 greenhouses over the whole cultural season for both control and *P. oligandrum* inoculated tomato plants as estimated by the U_{observed}, which was superior to $U_{\text{Wilcoxson table}}$ for $N = 7$ at $\alpha = 0.05$. Therefore, samples collected in the 2 greenhouses can be used as repeats to compare the bacterial colonization in the rhizosphere of control and *P. oligandrum* inoculated plants.

The amounts of bacterial DNA (nanograms) per unit of tomato DNA (nanograms) ranged from 0.4 to 1 (Fig. 2). For control and *P. oligandrum* inoculated plants, bacterial densities remained stable over the cropping season as assessed by the signed rank test (U_{observed} was superior to $U_{\text{Wilcoxson table}}$ for $N = 14$ at $\alpha = 0.05$).

Fig. 2. Bacterial root colonization assessed by real-time PCR. DNA quantities correspond to amounts of bacterial DNA (ng) per unit of tomato DNA (ng). Median values are represented on the graph as a nonparametric test was used (paired samples) to analyze the 4 samples collected per date and per treatment (the samples collected in the 2 greenhouses were used as repetitions). No significant differences in bacterial DNA quantities were observed over time and between control plants and *Pythium oligandrum* inoculated plants as assessed by the Wilcoxson signed rank test at $\alpha = 0.05$ (Dagnelie 1975).



SSCP analyses of bacterial communities

SSCP analyses of the genetic structure of the bacterial communities in both the rhizosphere and the circulating solutions were based on the detection of the V3 region of the 16S rRNA encoding gene.

Bacterial communities in the tomato rhizosphere

SSCP analyses were performed on root samples taken at an 8-week interval. A total of 31 SSCP profiles were obtained from the tomato root samples throughout the cropping season. By considering the number of peaks and the relative height of the baseline, these SSCP profiles revealed complex bacterial communities (Fig. 3). Sample distribution on the principal plan generated by the PCA is illustrated in Fig. 4. Evidence is provided from the first PCA axis (F1), which explains 38% of the total bacterial variability, that an evolution of the genetic structure of the bacterial communities occurred in the rhizosphere throughout the cropping season. Indeed, samples collected during the first 3 months (April–June) were distributed along the positive *x* axis, whereas those collected thereafter were correlated to the negative *x* axis of the first PCA axis.

Examination of the second PCA axis (F2), which explains 26% of the total bacterial variability, indicated that root samples could be distinguished depending on the presence or the absence of *P. oligandrum*. Support to this concept came from the observation that root samples from *P. oligandrum* inoculated plants were positively correlated on F2, whereas root samples from noninoculated control plants were mainly distributed on the negative *y* axis. Such a difference between inoculated and control plants was more acute at the beginning of the cropping season as samples collected from April to June were more scattered on F2 than samples collected in August and September. Note that *P. oligandrum* was detected on about 50% of the root samples at the end of the cropping season (Vallance et al. 2009).

Root samples were collected at 2 locations for each time point. Interestingly, such duplicates were closely associated one to each other on the graph (points labelled a and b in Fig. 4), thus indicating a great homogeneity in the distribution of the bacterial populations in the rhizosphere.

Bacterial communities colonizing the 4 circulating solutions

Samples collected from the circulating solutions during the cultural season led to 21 SSCP profiles. According to the number of peaks and the relative height of the baseline, it is clear that complex bacterial communities are specifically associated with each type of solution (Fig. 3).

Sample distribution on the principal plan generated by the PCA is illustrated in Fig. 5. Analysis of the first PCA axis (F1, 41% of the total variability) revealed that the bacterial communities from the 4 types of solutions could be distinguished according to their distribution along the axes. In that context, bacterial communities, either drained from roots or recycled, were positively correlated on F1, whereas bacterial communities from the irrigation water and the nutrient solutions were preferentially distributed along the negative *x* axis. Our data indicate also that the bacterial communities do not significantly differ in the circuits irrigating control or *P. oligandrum* inoculated plants as evidenced by the close association of samples from control and inoculated plants on the plan (Fig. 5).

Fig. 3. Example of single-strand conformation polymorphism fingerprints of the bacterial communities colonizing the roots (green), the drainage water (blue), and the nutrient solution (red) in September (end of the cropping season). For each graph, the *x* axis is the frequency of reading (number of scans) and the *y* axis is the fluorescence.

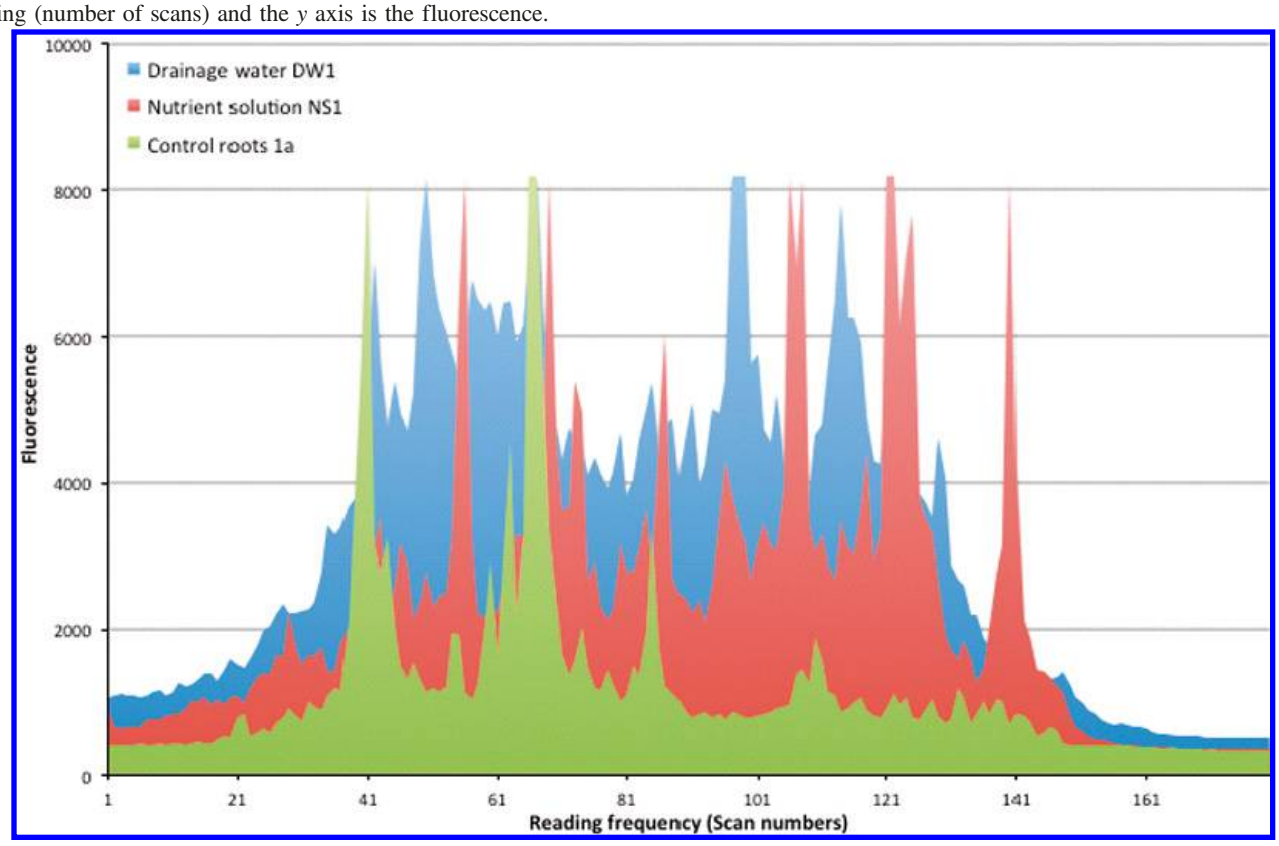


Fig. 4. Principal components analysis of the bacterial communities colonizing the rhizosphere. For each sample, the sampling date is a different color (11 April 2006 (blue), 6 June 2006 (green), 2 August 2006 (black), 26 September 2006 (orange)), the number 1 or 2 refers to the greenhouse, and the letter a or b indicates the repetition. Control plants are represented on the graph by a triangle, and the *Pythium oligandrum* inoculated plants are represented by a square and bold font.

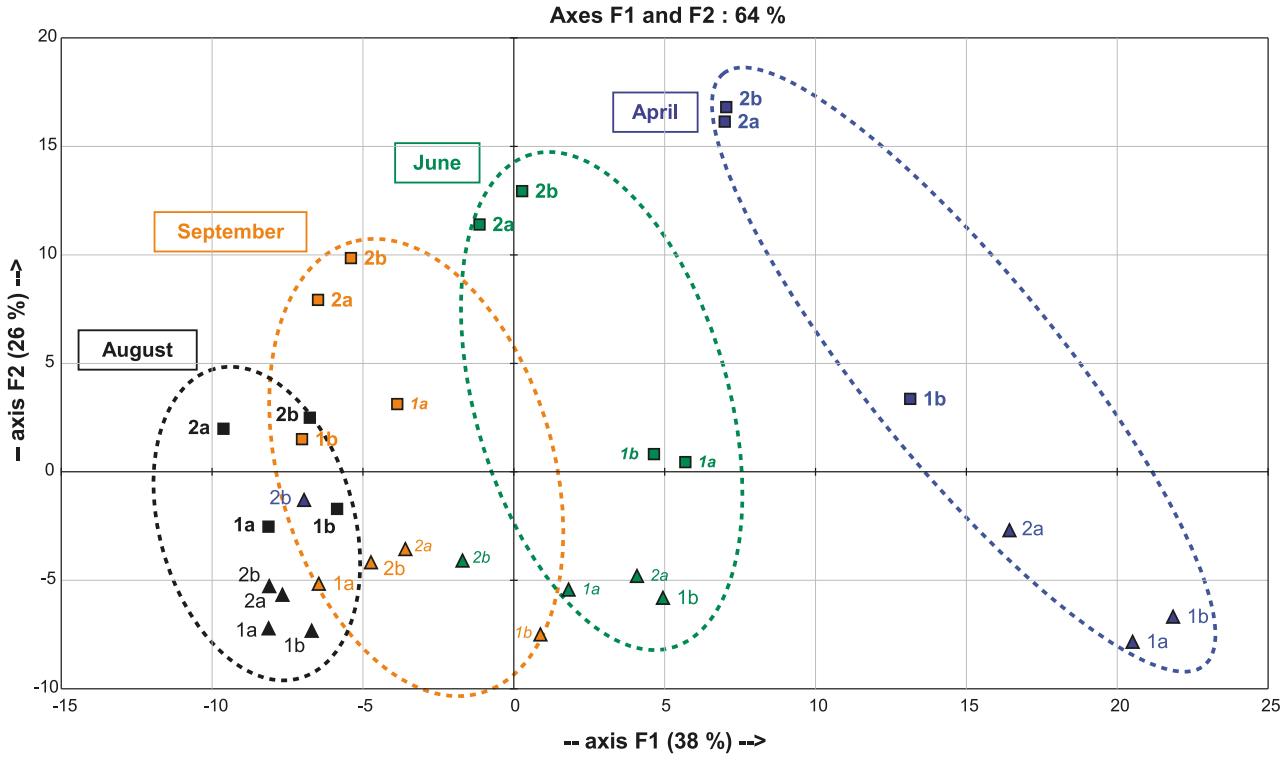
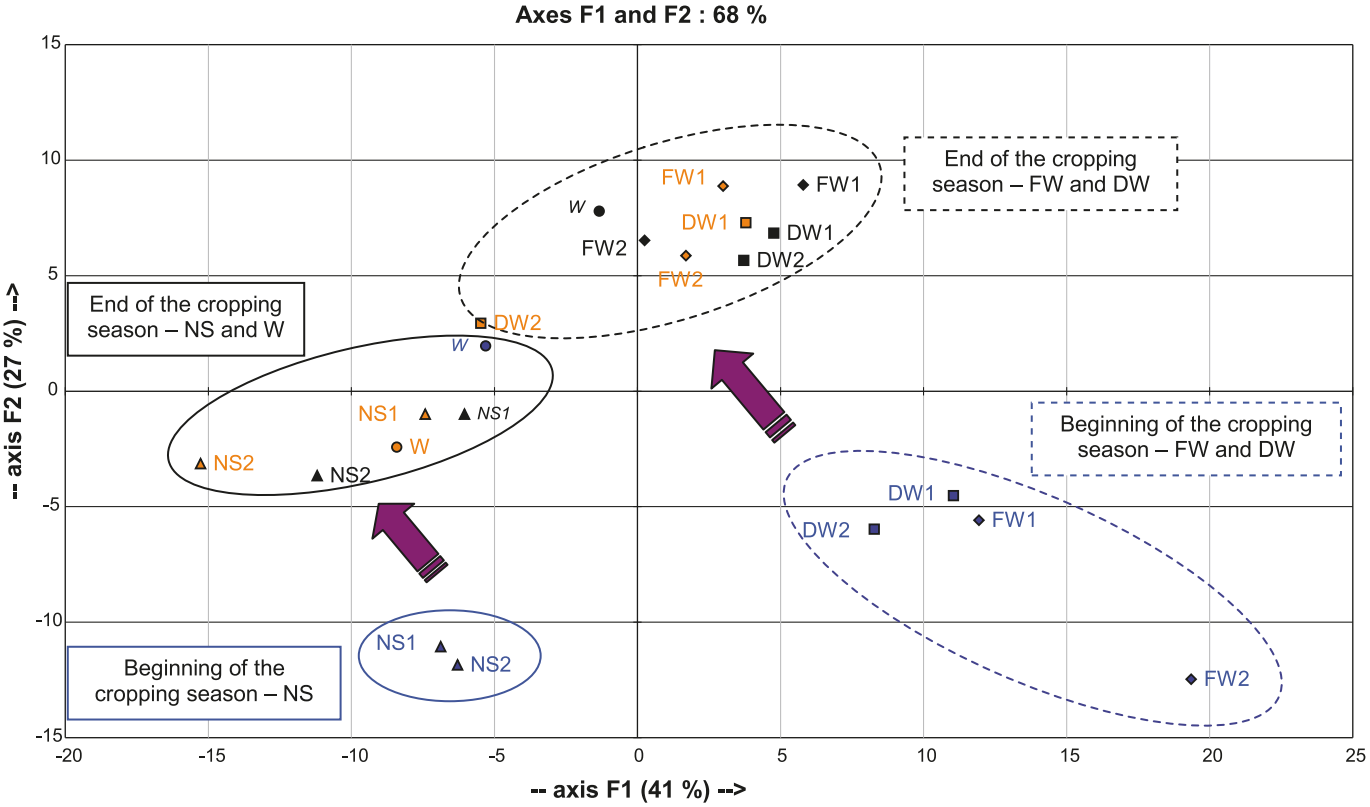


Fig. 5. Principal components analysis of the bacterial communities colonizing the circulating solutions. For each sample, the sampling date is a different color (11 April 2006 (blue), 2 August 2006 (black), 26 September 2006 (orange)), the letters indicate the kind of effluents (W, irrigation water; FW, recycled water; NS, nutrient solution; DW, drained water), and the last number refers to the circuit (1, circuit irrigating the control plants; 2, circuit irrigating the *Pythium oligandrum* inoculated plants).



Observation of the F2 axis (27% of the total variability) revealed that the circulating solutions could be distinguished according to their sampling date. Samples collected in April (beginning of the cropping season) were correlated negatively on F2, whereas those from August and September (end of the cropping season) were predominantly distributed along the positive *y* axis.

Bacterial communities colonizing the solutions or the rhizosphere

Whatever the sampling date, as similar tendencies were obtained from the data generated from root and solution samples, PCA of samples collected only in August is illustrated in Fig. 6.

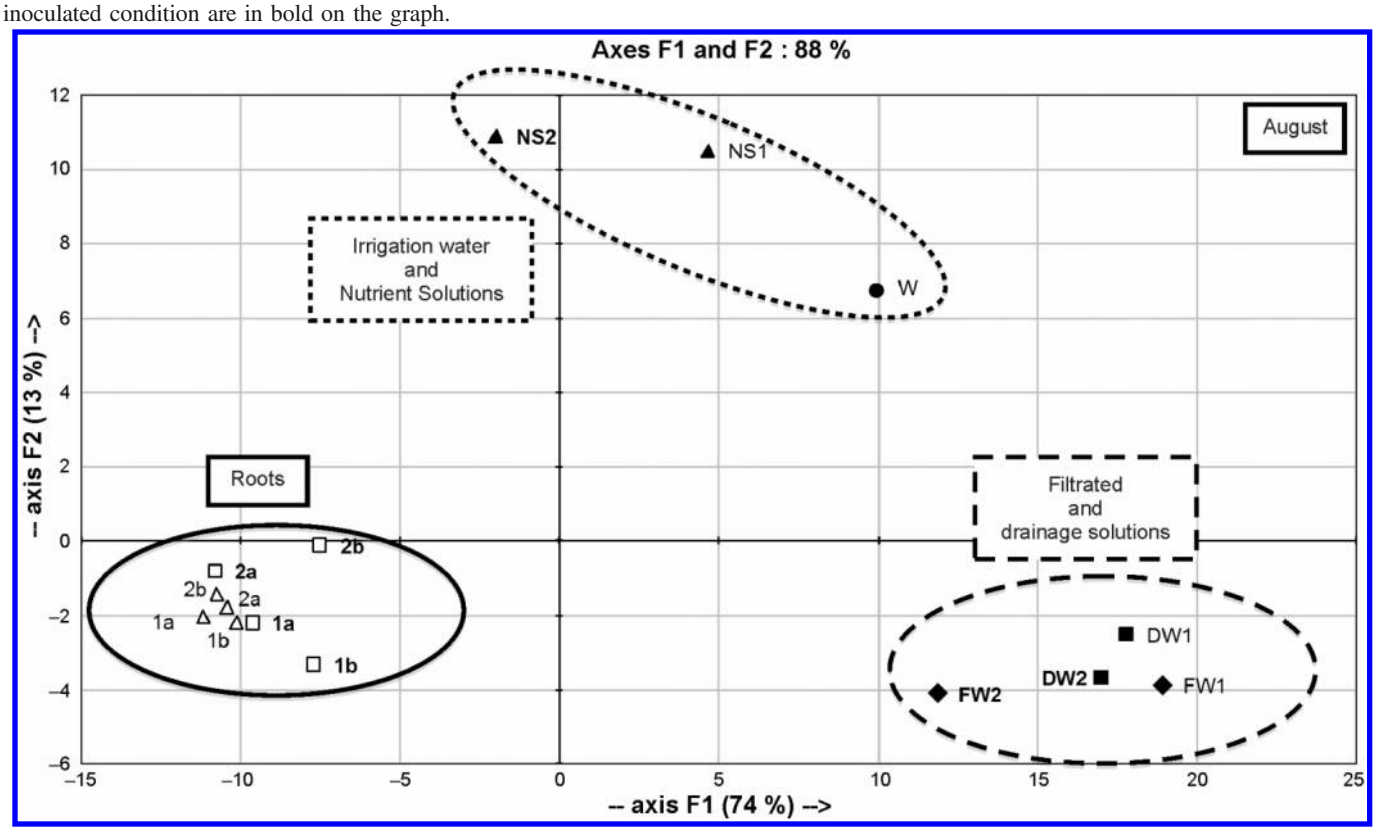
Bacterial communities colonizing the rhizosphere and the circulating solutions are different. Indeed, our results allowed delineating 3 types of bacterial community structures: the first one is associated with the root samples, the second one is associated with the irrigation water and the nutrient solutions, and the last one is associated with the drained and biofiltrated waters (Fig. 6). In spite of such differences, each type of bacterial community structure evolved similarly between the beginning (April) and the end (September) of the cropping season (data not shown). Additionally, the presence of *P. oligandrum* did not influence the 3 types of bacterial community structure as evidenced by the fact that samples belonging to both experimental settings were not separated on the plans generated by the PCA (data not shown).

Discussion

Since the initial description of *P. oligandrum* as a potential biocontrol agent (Drechsler 1943), an increasing number of reports have demonstrated that this oomycete displays remarkable antagonistic activities against a range of microorganisms colonizing the rhizosphere of cultivated plants (Benhamou et al. 1999; Rey et al. 2008). However, and in spite of increasing interest in using *P. oligandrum* as an alternative strategy for controlling root diseases, information is seldom available on the effect that this microorganism may exert on the bacterial microflora. This is especially crucial in the case of soilless cultures in which the abundant microflora plays a key role in the plant growth and development (Vallance et al. 2011).

Based on the use of qPCR, the results clearly indicate that the indigenous bacterial microflora is not significantly affected by the presence of *P. oligandrum*. Our results confirm earlier microbiological observations describing an absence of quantitative evolution in the overall number of bacteria developing around the root system of hydroponically grown tomato plants in a 6-month trial (Khalil and Alsanius 2001). They highlight too that the introduction of a biocontrol agent, e.g., *P. oligandrum*, in soilless growing systems does not necessary result in significant perturbations of the bacterial microflora colonizing the rhizosphere. Our results are also in line with the work of Koohakan et al. (2004) that disclosed that the population of aerobic bacteria colonizing the rhizosphere of 4 types of soilless tomato growing systems started

Fig. 6. Principal components analysis of the bacterial communities colonizing the rhizosphere and the circulating solutions in August. For root samples, the numbers 1 and 2 refer to the greenhouse, and the letters a and b indicate the repetition. Control plants are represented on the graph by a triangle, and the *Pythium oligandrum* inoculated plants, by a square and bold font. For circulating solution samples, the letters indicate the kind of solutions (W, irrigation water; FW, recycled water; NS, nutrient solution; DW, drained water) and the numbers indicate the circuit (1, circuit irrigating the control plants; 2, circuit irrigating the *P. oligandrum* inoculated plants). Solution samples collected in the inoculated condition are in bold on the graph.



to become stable at 10^{10} cfu·g^{-1} by 10 weeks after the beginning of the culture in all the types of systems they studied. Calvo-Bado et al. (2006) reported that no substantial changes in microbial communities were expected to take place in systems when nutrient inputs from the rhizosphere and nutrient solutions were steady.

If bacterial densities over the root system did not vary significantly throughout the cropping season, SSCP fingerprints revealed that transient perturbations in the indigenous bacterial communities occurred after *P. oligandrum* inoculation in the rhizosphere. To our knowledge, this is the first report describing such a bacterial shift resulting from the establishment of an oomycete biocontrol agent in the rhizosphere. *Pythium oligandrum* is well known for its remarkable antagonistic properties against various fungi and oomycetes. Indeed, features of mycoparasitism, antibiosis, and (or) competition for nutrients and space have been abundantly documented (Benhamou et al. 1999; Picard et al. 2000; Rey et al. 2008). By contrast, the possibility that it may exert an antagonistic effect against bacteria has not yet been examined, except for the study of Hase et al. (2006) that attempted to determine the ability of *P. oligandrum* at controlling tomato wilt caused by *Ralstonia solanacearum*. Based on their observations, the authors concluded that *P. oligandrum* mediated plant protection against the bacterial pathogen was achieved through a stimulation of the plant defense strategy rather than by a direct antagonistic process. Thus, it is still unclear whether or not *P. oligandrum* exerts a potential antibacterial effect. According to Bradshaw-Smith et al. (1991), *P. oligandrum* has the ability to secrete volatile antibiotic compounds that seem to be active against some pathogenic fungi, i.e., *Phoma medicaginis* and *Mycosphaerella pinodes*. However, these compounds have not been yet isolated, and further biochemical investigations are needed to determine the ability of *P. oligandrum* to produce molecules with bactericidal activity.

This shift in the structures of the bacterial communities observed when *P. oligandrum* colonize the rhizosphere was found to be transient as it gradually decreased at the end of the cropping season. This observation agrees with recent reports showing that the introduction of a pathogenic oomycete in the rhizosphere of plants grown in soilless cultures induced transient or even negligible effects on the indigenous bacterial and fungal communities (Calvo-Bado et al. 2006). The same trend was observed by Vallance et al. (2009) when they studied the evolution of the indigenous fungal communities after root inoculation by *P. oligandrum*. Similar observations have also been reported following the introduction of other biocontrol fungal agents in the soil, including the nonpathogenic *Fusarium oxysporum*, strain 47 (Fo47) (Edel-Hermann et al. 2009), and *Trichoderma atroviride*, strain SC1 (Savazzini et al. 2009). In both cases, evidence was provided that implementation of the biocontrol agents in different soil environments did not significantly modify the

indigenous microbial communities, even 1 year after the introduction of the antagonistic fungi. Finally, our observations are also in line with the conclusions raised by Scherwinski et al. (2008) showing that the introduction of 3 bacterial antagonists into the rhizosphere of naturally *Rhizoctonia* infested lettuce fields had nearly no impact on nontarget bacteria and endophytic fungi, except for minor or transient effects. In light of these observations, one may reasonably assume that, in soilless cultures, well-established microbial populations are resilient to potential disturbance caused by the introduction of new microorganisms in their environment.

One important facet of our results was the finding that the bacterial community structure in the rhizosphere changed gradually over time, but independently of the establishment of *P. oligandrum*. Interestingly, marked structural differences were detected at the bacterial microflora level throughout the cropping season (from April to September) whether *P. oligandrum* was present or not. Although the mechanisms involved in the evolution of the bacterial genetic structure are not yet elucidated, the possibility that organic compounds released from the roots may have promoted growth and development of the microbial populations in the rhizosphere has to be taken into consideration, as suggested by Waechter-Kristensen et al. (1997). Support to the hypothesis that nutrient parameters are key mediators of active microbial growth in the rhizosphere is further substantiated by the study of Maloney et al. (1997) in which changes within the culturable rhizobacterial populations of lettuce and tomato were found to correlate with plant growth stage, carbon availability, and nitrogen concentration.

Our results show that the shift over the cultural season in the bacterial communities colonizing the rhizosphere is also observed in the circulating solutions. To our knowledge, this is the first study reporting a comparison at different times of the fingerprints obtained from the bacterial communities colonizing the circulating solutions in a tomato hydroponic system. Our observations also revealed that the SSCP profiles from the nutrient solutions were similar to those of the irrigation waters. This result is expected as nutrient solutions are generated from 20% of filtered solution and 80% of irrigation water. By contrast, the similarity between the fingerprints of the drained and the filtrated solutions was more surprising as slow filtration is known to eradicate a great part of the microflora. Déniel et al. (2004, 2006) showed that even though slow filtration eliminates about 67% to 99% of bacteria, 10^3 to 10^4 bacteria·mL^{-1} can still be detected in the effluents flowing out the filtering columns. Using SSCP, denaturing gradient gel electrophoresis, and (or) Biolog analyses, several reports highlight that differences in the composition of the circulating bacterial communities occur before and after the biofiltration process (Postma et al. 1999; van Os and Postma 2000; van Os et al. 2004; Renault 2007). In the present case, the modifications in the bacterial communities induced by the filtration process are likely lower in comparison with the differences observed with the other samples of solution, i.e., drained and (or) filtrated solutions versus irrigation water and (or) nutrient solution.

When samples from roots and effluents are compared, differences in the genetic structure of the bacterial microflora colonizing the rhizosphere and the circulating solutions were detected. Based on these results, 3 kinds of bacterial community structures can be proposed: (*i*) roots, (*ii*) irrigation water and nutrients solutions resupplying roots, and (*iii*) drained and (or) filtrated solutions. Analysis of the SSCP fingerprints clearly shows that specific microflora colonize the root system and the circulating solutions and that their structures evolve throughout the cropping season. It can be assumed that these changes over time in the bacterial microflora are correlated with the colonization of the rhizosphere by new bacterial species, whereas at the same time, others tended to disappear.

A relationship between microflora from either roots or solutions likely exists. Indeed, as the solutions irrigate the root systems, one can presume that part of their bacterial microflora is also able to colonize the roots; as a consequence, the rhizospheric microbial shifts could be partly induced by the shifts observed in the circulating solutions. Further studies have to be performed, for instance, 16S sequencing of the bacterial communities, to characterize the bacterial species that specifically colonize the 2 ecological niches: the roots and the irrigating solutions.

Finally, at least 4 main points can be drawn from this study. (*i*) *Pythium oligandrum* colonizes and persists in the rhizosphere even if a complex and dense indigenous bacterial microflora had previously invaded this ecosystem. It is a key point to get a successful plant protection from a biocontrol agent. (*ii*) *Pythium oligandrum* did it without necessary inducing shifts in the structure of the indigenous bacterial communities. Only a transient shift was observed after *P. oligandrum* inoculation of roots. Consequently, it can be stated that *P. oligandrum* does not affect durably the indigenous bacterial communities even if the oomycete colonizes the rhizosphere over the cropping season. (*iii*) Our observations confirm that the resilience of a well-established microflora allows it to stand the introduction of a biocontrol agent. (*iv*) It was shown also that independent of the biocontrol agent presence, the indigenous bacterial communities of the roots and the solutions as well changed throughout the cultural season. However, this evolution in the bacterial community structures, likely due to bacterial species changes in the indigenous communities, does not negatively affect the *P. oligandrum* persistence in the rhizosphere.

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