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ORIGINAL PAPER

Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant

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Abstract Bacterial strains from mycorrhizal roots (three belonging to *Comamonadaceae* and one to *Oxalobacteraceae*) and from non-mycorrhizal roots (two belonging to *Comamonadaceae*) of *Medicago truncatula* and two reference strains (*Collimonas fungivorans* Ter331 and *Pseudomonas fluorescens* C7R12) were tested for their effect on the in vitro saprophytic growth of *Glomus mosseae* BEG12 and on its colonization of *M. truncatula* roots. Only the Oxalobacteraceae strain, isolated from barrel medic mycorrhizal roots, and the reference strain *P. fluorescens* C7R12 promoted both the saprophytic growth and root colonization of *G. mosseae* BEG12, indicating that they acted as mycorrhiza helper bacteria. Greatest effects were achieved by *P. fluorescens* C7R12 and its influence on the saprophytic growth of *G. mosseae* was compared to that on *Gigaspora rosea* BEG9 to determine if the bacterial stimulation was fungal specific. This fungal specificity, together with plant specificity, was finally evaluated by comparing bacterial effects on arbuscular mycorrhizal symbiosis when each of the fungal species was inoculated to two different plant species (*M. truncatula* and *Lycopersicon esculentum*). The results obtained showed that promotion of saprophytic growth by *P. fluorescens* C7R12 was expressed in vitro towards *G. mosseae* but not towards *G. rosea*. Bacterial promotion of mycorhization was also expressed towards *G. mosseae*, but not *G. rosea*, in roots of *M. truncatula* and *L. esculentum*. Taken together, results indicated that enhancement of arbuscular mycorrhiza development was only induced by a limited number of bacteria, promotion by the most efficient bacterial strain being fungal and not plant specific.

Keywords Arbuscular mycorrhizas · *Comamonadaceae* · *Oxalobacteraceae* · *Pseudomonas fluorescens* C7R12 · Mycorrhiza helper bacteria

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Introduction

Arbuscular mycorrhizas (AM) are symbiotic associations which occur between the large majority of land plant families (Wang and Qiu 2006) and obligate biotrophic AM fungal species belonging to the Glomeromycota phylum (Schüßler et al. 2001). The AM association represents an ancient symbiosis with fossil evidence dating back to 400 millions years (Remy et al. 1994) and it has been proposed that AM have contributed to the colonization of land by early plants (Pyrozinski and Malloch 1975; Redecker et al. 2000). AM have a central position in terrestrial nutrient cycling processes and are of particular interest because of their positive effects on plant growth and health and protection against biotic and abiotic stresses (Smith and Read 1997; Cordier et al. 1998; Barea et al. 2002; Berta et al. 2005).

Even if AM associations are generally considered to be non-specific (Sanders 2002), reciprocal interactions based

on feedback mechanisms between plants and AM fungi have been proposed (Bever et al. 2002) based on observations of differential responses of plant species to individual isolates and species of AM fungi (Streitwolf-Engel et al. 1997) which lead to variations in ecosystem plant composition and productivity according to AM fungal diversity and identity (van der Heijden et al. 1998; Klironomos et al. 2000). In return, plant species, even closely related, can differentially affect the AM fungal community associated with mycorrhizal roots (Vandenkoornhuyse et al. 2003; Pivato et al. 2007).

The long co-evolution of plants and AM fungi is expected not to have occurred independently from the associated bacterial flora. Indeed, from an evolutionary point of view, one may expect that these bacteria would at least not be deleterious or even be beneficial to mycorrhiza development as has been shown for a fluorescent pseudomonad representative of populations preferentially associated with ectomycorrhizas (Frey et al. 1997). Bacterial strains, isolated from sporocarps or spores of *Glomus* species, were also shown to stimulate fungal spore germination and arbuscular mycorrhiza formation (Budi et al. 1999; Xavier and Germida 2003). Moreover, hyphal elongation and branching of *Gigaspora margarita* were shown to be higher when harboring the bacteria *Candidatus* Glomeribacter gigasporarum, indicating that this bacterial species enhanced the presymbiotic growth of the AM fungus (Lumini et al. 2007). Free-living and endo-symbiotic bacteria promoting the establishment of mycorrhiza by increasing fungal contact with and colonization of host roots have been called "mycorrhiza helper bacteria" (MHB) (Garbaye 1994). The corresponding promoting effects and the conditions of their expression have been recently reviewed by Frey-Klett et al. (2007).

Studies on bacteria associated with AM fungi and on their effects on the AM symbiosis have mostly been conducted in bioassays involving a given plant species, isolate of AM fungi and group or even strain of bacteria. In this context, we have undertaken research to identify in an untargeted way (1) bacterial populations preferentially associated with mycorrhizal roots harboring indigenous AM fungi and inversely (2) the impact of these bacterial populations on the AM symbiosis. The first step in this research was based on studies performed with *Medicago truncatula*, as a model plant, cultivated in a fallow soil from the Mediterranean area (Mas d'Imbert, France) and corresponding to the diversification zone of annual medics (Offre et al. 2007). By growing a mutant of *M. truncatula* impaired in its ability to establish AM in this soil and comparing with the corresponding wild-type genotype, the genetic structure of bacterial communities associated with mycorrhizal and non-mycorrhizal roots, assessed by automated-ribosomal interspace analysis (A-RISA) from DNA directly extracted from root tissues, was shown to differ significantly. Cloning and sequencing of partial 16S rDNA and 16S-23S intergenic spacer (IGS) sequences allowed us to identify microbial groups preferentially associated with AM as belonging to the *Burkholderiales,* more specifically to the *Oxalobacteraceae,* and to a lesser extent to the *Comamonadaceae* (Offre et al. 2007). The diversity of the populations belonging to these families was further characterized by analysing partial 16S rDNA-IGS sequences obtained directly from mycorrhizal and non-mycorrhizal roots or from bacteria isolated from these roots. These analyses confirmed the higher abundance of *Oxalobacteraceae* associated with mycorrhizal roots and led to the isolation of bacteria representative of the diversity of the two families (Offre et al. 2008).

The aims of the present study were to test if bacterial isolates, belonging to families preferentially associated with mycorrhizal roots (Offre et al. 2007), promote mycorrhization and to determine whether effects by a selected MHB are fungal and/or plant specific. More generally, bacterial effects on AM were assessed by evaluating the impact of the three partners—bacteria, AM fungi, and plant—on the interactions. The strategy followed consisted first in comparing the effect of (1) bacterial strains from mycorrhizal roots (one belonging to *Oxalobacteraceae* and three to *Comamonadaceae*) and non-mycorrhizal roots (two belonging to *Comamonadaceae*) and of (2) two reference strains (one belonging to *Oxalobacteraceae* and one to *Pseudomonadaceae*) on the presymbiotic growth of an AM fungal species (*Glomus mosseae*) to test their possible activity as MHB. The promoting activity of the most efficient bacterial strain (*Pseudomonas fluorescens* C7R12) was then compared on another AM fungal species (*Gigaspora rosea). Finally, the influence of both AM fungal and plant species on the promoting effect of P. fluorescens C7R12 was tested by comparing mycorrhiza development in two plant species (M. truncatula and Lycopersicon esculentum) inoculated with either G. mosseae or G. rosea, in the presence or not of the bacterium.*

Materials and methods

Bacterial strains and growth conditions

Bacterial strains used in this study (Table 1) included: six bacterial strains selected from a previous study and two reference strains. The six selected bacterial strains belonged to *Burkholderiales* (β-*Proteobacteria*), and more specifically to *Comamonadaceae* (five strains) and to *Oxalobacteraceae* (one strain). They were chosen for their association with mycorrhizal (J5) and non-mycorrhizal (TRV25) roots of *M. truncatula*, as established in a previous study (Offre et al. 2008). The reference strains were *Collimonas fungivorans*

Table 1 Bacterial strains used in this study

Bacterial strain	Family	Origin	Reference
J5A3	*Comamonadaceae*	*Medicago truncatula* J5	Offre et al. 2008
J5B1	*Comamonadaceae*	*M. truncatula* J5	Offre et al. 2008
J5B5	*Comamonadaceae*	*M. truncatula* J5	Offre et al. 2008
T25A2	*Comamonadaceae*	*M. truncatula* TRV25	Offre et al. 2008
T25D3	*Comamonadaceae*	*M. truncatula* TRV25	Offre et al. 2008
J5B4	*Oxalobacteraceae*	*M. truncatula* J5	Offre et al. 2008
Collimonas fungivorans Ter331	*Oxalobacteraceae*	*Ammophila arenaria*	de Boer et al. 2004
Pseudomonas fluorescens C7R12	*Pseudomonadaceae*	*Linum usitatissimum*	Eparvier et al. 1991

Ter331 (β-*Proteobacteria*, *Oxalobacteraceae*), a chitinolytic bacterial strain isolated from slightly acid dune soils on the Wadden Island Terschelling (de Boer et al. 2004), and *P. fluorescens* C7R12 (γ-*Proteobacteria*, *Pseudomonadaceae*), isolated from the Châteaurenard soil naturally suppressive to fusarium wilts (Lemanceau et al. 1988). Both reference strains were previously shown to be potential biocontrol agents (Lemanceau and Alabouvette 1991; Kamilova et al. 2007). All strains were grown on 0.1×tryptic soy agar (TSA) (Forbes et al. 1998) for 5 days at 25°C and stored at −80°C in tryptic soy broth (TSB) (Forbes et al. 1998) containing 20% glycerol.

AM fungi and spore disinfection

The two arbuscular mycorrhizal (AM) fungal strains used in this study were *G. mosseae* (Nicol and Gerdemann) Gerd. & Trappe BEG12 and *G. rosea* Gerd. & Trappe BEG9. *G. mosseae* BEG12 originated from a pot culture of strawberry at East Malling Research Station in Kent (Mosse 1956) and *G. rosea* BEG9 was originally isolated from soybean rhizosphere soil in North Florida (Nicolson and Schenck 1979).

Sporocarps of *G. mosseae* BEG12 and spores of *G. rosea* BEG9 were obtained from Agrauxine-Biorize (Dijon, France). Spores of *G. mosseae* BEG12 were collected with a pipette from sporocarps that were carefully opened with fine point tweezers under a stereomicroscope Leica MZ16 (Leica Microsystems, Wetzlar, Germany). Spores of both AM fungal species were surface-sterilized by very gently shaking them in a 2% chloramine T solution twice for 10 min each and a 200 μg ml^{-1} streptomycin and 100 μg ml^{-1} gentamycin solution four times for 1 min each, as described by Bécard and Fortin (1988). The same sterilization process was applied again 1 week later. Spores were kept in sterile demineralized water at 4°C.

In vitro spore germination and hyphal growth

The effect of the eight bacterial strains on spore germination and hyphal growth of *G. mosseae* BEG12 was assessed in Petri dishes as follows. One AM fungal spore was placed at each vertex of an octagon (i.e. eight spores per Petri dish) at the surface of 1% Bactoagar (Fluka, Buchs, Switzerland). An aliquot (1 ml) of a bacterial suspension was mixed into the medium to obtain a final concentration of 10^7 colony forming unit (CFU) ml^{-1}. Each treatment (BEG12 in the absence or in the presence of one of the bacterial strains) was replicated in 15 plates.

After 7 days' incubation at 25°C in the dark, percentage spore germination was determined using a stereomicroscope Leica MZ16 (Leica Microsystems), connected with a camera Leica DFC320 (Leica Microsystems). The length of hyphae per germinated spore was measured from images obtained using the software Leica Application Suite v. 2.4 (Leica Microsystems).

The bacterial strain shown to have the strongest promoting effect on spore germination and hyphal growth of *G. mosseae* BEG12 was further tested on *G. rosea* BEG9 under the same experimental conditions as described above.

Treatments and plant growth conditions

M. truncatula Gaertn. cv Jemalong line J5 and *Lycopersicon esculentum* Mill. cv Guadalete plants were cultivated in a mixture of 0.3–0.7 mm coarse grade quartz sand (Punto Elle, Turin, Italy) previously sterilized at 200°C for 3 h and distributed into 600-ml containers (650 g per container). Before sowing, seeds of *M. truncatula* were scarified and surface sterilized by gently shaking them in 98% sulphuric acid for 2 min, 95% ethanol for 5 min, and 3.5% sodium hypochlorite solution for 10 min, and finally rinsed in sterile distilled water six times for 5 min. Tomato seeds (Solanum c.s.a., Lodi, Italy) were surface sterilized by gently shaking them in a 5% sodium hypochlorite solution for 3 min and were rinsed six times for 5 min and four times for 20 min in sterile distilled water. Surface sterilized *M. truncatula* and tomato seeds were germinated at 25°C for 2 days on 0.7% Bactoagar (Fluka, Buchs, Switzerland) and 3 days on moist sterile filter paper, respectively. One pregerminated seed of either plant was sown in each container. Plants were cultivated in a growth chamber (16/8 h light/dark photoperiod, 24/20°C light/dark thermoperiod, 150 μE m^{-2} s^{-1} light irradiance at pot height, 60% relative humidity) and were

watered to saturation three times per week with a modified Long Ashton nutrient solution containing 32 μM phosphate (Trotta et al. 1996) and 10 mM nitrate.

Microbial inoculations were performed after sowing by (1) applying 25 ml bacterial suspension at the surface of each treated container to obtain a density of 10^7 CFU per gram of dry sand and (2) placing 200 fungal spores of AM fungi in contact with the root system of each pregerminated seed. Five containers were cultivated per experimental condition: non-inoculated (control), inoculated with a bacterial and an AM fungal strain, either separately or in combination.

Two successive experiments were performed. In the first one, the effect of each of the eight bacterial strains on barrel medic root colonization by *G. mosseae* BEG12 was assessed 12 weeks after sowing. In the second, the effects of the most efficient bacterial strain was assessed, 7 weeks after sowing, on (1) the colonization of barrel medic and tomato roots by *G. mosseae* BEG12 or *G. rosea* BEG9, and (2) the growth of these two plant species.

Root colonization by AM fungi

Root colonization by AM fungi was assessed on five different plants per treatment. Thirty root pieces (1 cm long each) were sampled per root system. These pieces were cleared for 30 min at 60°C in 10% KOH, stained with 1% methyl blue in lactic acid and finally were mounted on slides. The percentages of root fragments colonized by the fungus (F%) and the intensity of colonization of the root cortex (M%) in all fragments, and of arbuscular abundance in the mycorrhizal root cortex (A%) were evaluated microscopically using the notation scale described by Trouvelot et al. (1986) and the Mycocalc software freely available at http://www.dijon.inra.fr/Mychintec/Mycocalc-prg/download.html. M% and A% were determined by calculating weighted indices based on scoring frequencies of root fragments in classes ranging from 0 to 5 according to the root area colonized by the fungus and from 0 to 3 according to the amount of arbuscules, respectively.

Plant and root growth

Growth of plants was assessed by plant fresh weight and that of roots by quantifying total length, surface area and volume, as described by Gamalero et al. (2004). To evaluate root growth parameters, whole root systems were placed in a transparent water container and scanned using a dedicated Epson Twain Pro 2.10 (Régent Instruments, Quebec, Canada) scanner, equipped with a special lighting system for root measurement. Digitized root images were analyzed by WinRhizo Pro v. 2002c (Régent Instruments, Quebec, Canada) and morphometric parameters evaluated.

Statistical analysis

All data were submitted to analysis of variance followed by Fisher's least significant difference test ($P \leq 0.1$) using Statview® software (SAS Institute, Cary, NC, USA). Parameters of AM fungal spore germination and root colonization were submitted to angular transformation prior to statistical analysis.

Results

Effects of bacteria on AM spore germination and hyphal growth in vitro

Germination rate of spores and hyphal length of *G. mosseae* BEG12 in the absence of bacteria were low (1.41% and 5.93 mm, respectively) (Table 2). These values were increased by all the bacterial strains tested except for J5A3 and Ter331 which even decreased germ tube length, significantly for Ter331. Among all the strains tested, C7R12 promoted most the spore germination rate and germ tube length. When the effect of *P. fluorescens* C7R12 was tested on the presymbiotic growth of *G. rosea* BEG9, as compared to that of *G. mosseae* BEG12 (Table 3), the promoting effect of C7R12 on spore germination and germ tube length was observed for *G. mosseae* BEG12 but only for the spore germination for *G. rosea* BEG9.

Effects of bacteria on root colonization by AM fungi

Most of the eight bacterial strains tested did not affect the frequency (F%) and intensity (M%) of AM colonization and the abundance of arbuscules (A%) in roots of *M. truncatula* (Table 4). However, C7R12 promoted the

Table 2 Effect of *Comamonadaceae* strains J5A3, J5B1, J5B5, T25A2, T25D3 and *Oxalobacteraceae* strain J5B4, reference strains *Collimonas fungivorans* Ter331 and *Pseudomonas fluorescens* C7R12 on spore germination and hyphal growth of *Glomus mosseae* BEG12

Treatment	Germination (%)	Hyphal length (mm)
BEG12	1.41 a	5.93 b
BEG12+J5A3	2.16 a	3.31 ab
BEG12+J5B1	28.28 cd	12.53 e
BEG12+J5B5	12.93 b	10.46 d
BEG12+T25A2	38.85 de	12.07 e
BEG12+T25D3	24.39 c	7.66 c
BEG12+J5B4	29.96 cd	12.96 e
BEG12+Ter331	2.51 a	2.64 a
BEG12+C7R12	44.12 e	17.62 f

Observations were made after 7 days of incubation. Means with the same letter are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

Table 3 Effect of *Pseudomonas fluorescens* C7R12 on spore germination percentages and hyphal length of *Glomus mosseae* BEG12 and *Gigaspora rosea* BEG9

Treatment	Germination (%)	Hyphal length (mm)
BEG12	1.04 a	1.57 a
BEG12+C7R12	35.71 b	19.35 b
BEG9	3.94 A	72.43 A
BEG9+C7R12	12.74 B	69.23 A

Observations were made after 7 days of incubation. Means with the same lowercase (BEG12 treatments) or capital (BEG9 treatments) letter are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

mycorrhization of *M. truncatula* by *G. mosseae* BEG12 for all parameters and J5B4 only promoted the abundance of arbuscules. In contrast, J5B5 depressed both AM colonization and the abundance of arbuscules.

The influence of *P. fluorescens* C7R12 on F%, M%, and A% of roots of barrel medic (Fig. 1) and tomato (Fig. 2) inoculated with *G. mosseae* BEG12 or *G. rosea* BEG9 was further tested. In plants inoculated with *G. mosseae* BEG12, C7R12 increased significantly M% in barrel medic (Fig. 1) and all parameters in tomato (Fig. 2). In plants inoculated with *G. rosea* BEG9, *P. fluorescens* C7R12 significantly depressed all mycorrhization parameters in barrel medic (Fig. 1) and did not have any effect in tomato (Fig. 2).

Compared effects of *G. mosseae* BEG12 and *G. rosea* BEG9, inoculated separately or in combination with *P. fluorescens* C7R12, on shoot growth of barrel medic and tomato

The effect of *G. mosseae* BEG12 and *P. fluorescens* C7R12, inoculated separately or in combination, on plant growth of barrel medic and tomato plants is shown in Fig. 3a and that of *G. rosea* and *P. fluorescens* C7R12 in Fig. 3b. *G. mosseae* BEG12 and *P. fluorescens* C7R12 promoted barrel medic growth when inoculated together but not when inoculated separately (Fig. 3a). In contrast, both microorganisms promoted tomato growth when inoculated separately, with a significant higher promotion for *P. fluorescens* C7R12 than for *G. mosseae* BEG12, but their co-inoculation did not bring any additional beneficial effect (Fig. 3a). *G. rosea* BEG9 and *P. fluorescens* C7R12, inoculated separately or in combination (Fig. 3b), did not promote growth of barrel medic, but had growth promoting effects on tomato plants similar to *G. mosseae* BEG12 (Fig. 3b).

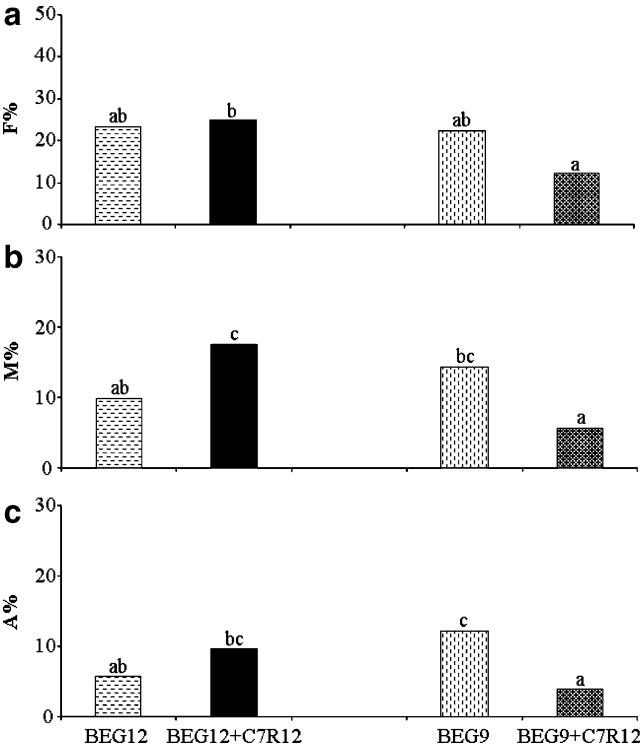

Fig. 1 Effect of *Pseudomonas fluorescens* C7R12 on frequency F% (**a**) and intensity M% (**b**) of arbuscular mycorrhizal colonization, and frequency of arbuscules A% (**c**) in *Medicago truncatula* roots inoculated with *Glomus mosseae* BEG12 or *Gigaspora rosea* BEG9. Observations were made 7 weeks after sowing. *Bars* with the same *letter* are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

Table 4 Effect of *Comamonadaceae* strains J5A3, J5B1, J5B5, T25A2, T25D3 and *Oxalobacteraceae* strain J5B4, reference strains *Collimonas fungivorans* Ter331 and *Pseudomonas fluorescens* C7R12 on frequency (F%) and intensity (M%) of arbuscular mycorrhizal colonization, and frequency of arbuscules (A%) in *Medicago truncatula* roots inoculated with *Glomus mosseae* BEG12

Treatment	F%	M%	A%
BEG12	51.33 ab	40.97 b	12.12 b
BEG12+J5A3	41.33 ab	33.94 ab	11.50 ab
BEG12+J5B1	58.00 abc	39.47 ab	7.67 ab
BEG12+J5B5	33.84 a	22.76 a	7.55 a
BEG12+T25A2	50.00 ab	37.63 ab	7.31 ab
BEG12+T25D3	60.67 b	41.31 bc	8.14 b
BEG12+J5B4	71.33 b	58.57 bc	22.45 c
BEG12+Ter331	51.33 ab	36.88 ab	5.46 ab
BEG12+C7R12	82.00 c	67.05 c	22.30 c

Observations were made 12 weeks after sowing. Means with the same letter are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

Compared effects of *G. mosseae* BEG12 and *G. rosea* BEG9, inoculated separately or in combination with *P. fluorescens* C7R12, on root growth of barrel medic and tomato

The effects of *G. mosseae* BEG12 and *G. rosea* BEG9, when inoculated separately or in combination with *P. fluorescens* C7R12, on root growth are shown in Figs. 4 and 5. *P. fluorescens* C7R12 did not modify root growth of barrel

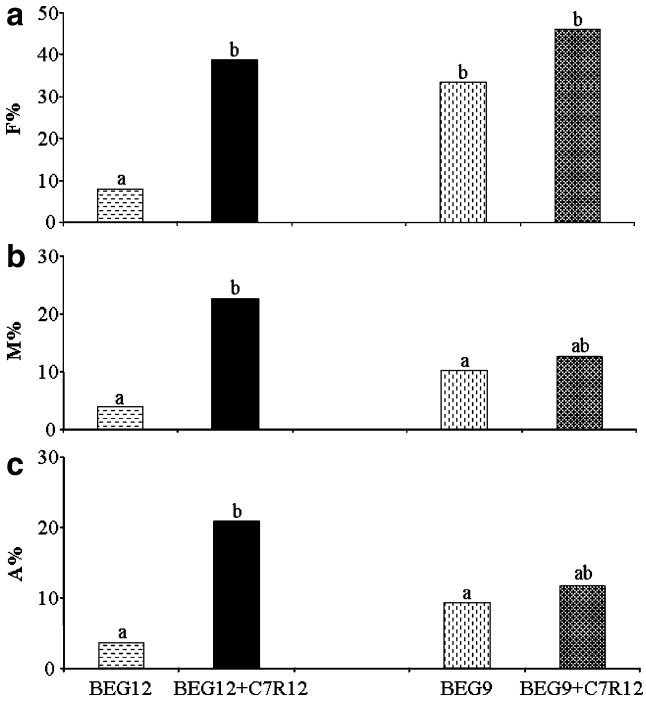

Fig. 2 Effect of *Pseudomonas fluorescens* C7R12 on frequency F% (**a**) and intensity M% (**b**) of arbuscular mycorrhizal colonization, and frequency of arbuscules A% (**c**) in *Lycopersicon esculentum* roots inoculated with *Glomus mosseae* BEG12 or *Gigaspora rosea* BEG9. Observations were made 7 weeks after sowing. *Bars* with the same *letter* are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

medic plants but promoted all parameters in tomato plants (Figs. 4 and 5). *G. mosseae* BEG12 increased both barrel medic root surface area and volume (Fig. 4) and even in the presence of *P. fluorescens* C7R12. Total root length of barrel medic was only promoted when plants were co-inoculated with *G. mosseae* BEG12 and *P. fluorescens* C7R12. *G. mosseae* BEG12 promoted the three root parameters in tomato plants, this promotion being however significantly lower than that achieved by *P. fluorescens* C7R12. Combined inoculation of these two microorganisms did not bring any additional positive effect.

G. rosea BEG9 did not modify significantly barrel medic root growth when inoculated separately or in combination with *P. fluorescens* C7R12 (Fig. 5). *G. rosea* BEG9 increased significantly all parameters in tomato plants, this promotion being however significantly lower than that achieved by *P. fluorescens* C7R12. Co-inoculation of *G. rosea* BEG9 and *P. fluorescens* C7R12 did not bring any further significant increase compared to *G. rosea* BEG9.

Discussion

The first part of this study was aimed at assessing if, as hypothesized previously (Offre et al. 2007), bacteria preferentially associated with mycorrhizal roots were at least not deleterious or even beneficial towards AM fungi and the symbiosis. Bacterial strains, isolated from mycorrhizal and non-mycorrhizal roots of *M. truncatula* (Offre et al. 2008), were tested for their effect on AM fungal growth in vitro and on root colonization, in comparison with two reference strains. To our knowledge, this is the first study to assess if indigenous bacterial populations preferentially associated with AM can promote mycorrhization.

As indicated by Garbaye (1994) and Frey-Klett et al. (2007) in their reviews, mycorrhiza promotion by MHB is at least partly ascribed to the stimulation of the presymbiotic fungal growth which leads to an increase in root–fungus contacts and root colonization. The effect of the eight bacterial strains on spore germination and hyphal growth was tested using *G. mosseae* as a model AM fungal species since it was previously shown to be abundant in the roots of *M. truncatula* from which the six tested bacterial strains were isolated (Pivato et al. 2007; Offre et al. 2008). As previously described with other strains of *G. mosseae* (Giovannetti et al. 2003), a low presymbiotic growth was recorded in vitro for *G. mosseae* BEG12 in the absence of

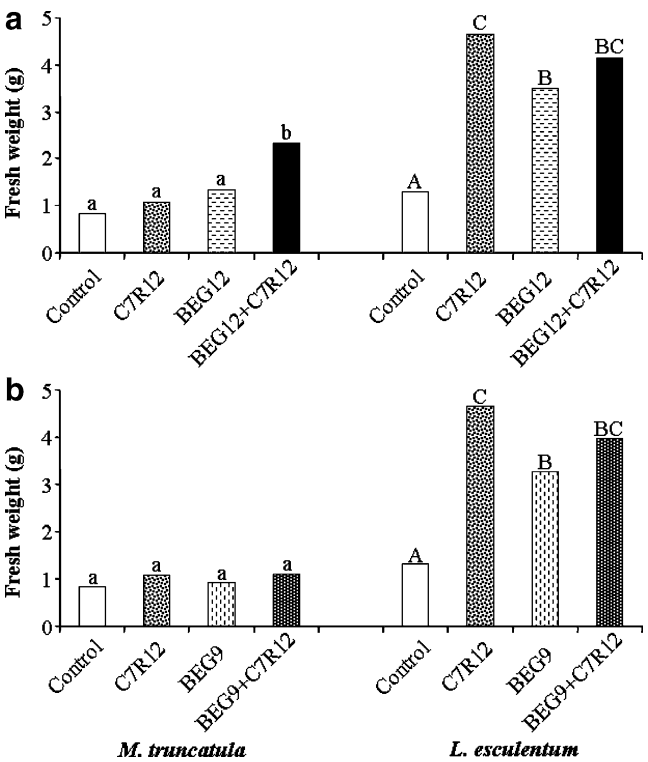

Fig. 3 Compared effects of *Glomus mosseae* BEG12 (**a**) or *Gigaspora rosea* BEG9 (**b**), inoculated separately or in combination with *Pseudomonas fluorescens* C7R12, on fresh weight of *Medicago truncatula* and *Lycopersicon esculentum*. Observations were made 7 weeks after sowing. *Bars* with the same *lowercase* (*M. truncatula*) or *capital* (*L. esculentum*) *letter* are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

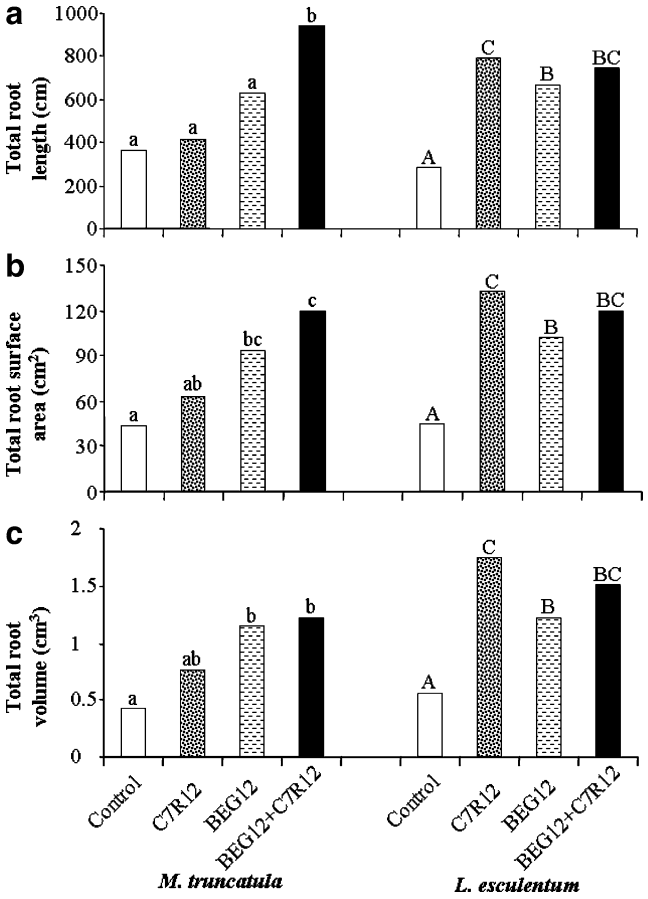

Fig. 4 Compared effects of *Glomus mosseae* BEG12, inoculated separately or in combination with *Pseudomonas fluorescens* C7R12, on total root lenght (**a**), area (**b**) and volume (**c**) of *Medicago truncatula* and *Lycopersicon esculentum*. Observations were made 7 weeks after sowing. *Bars* with the same *lowercase* (*M. truncatula*) or *capital* (*L. esculentum*) *letter* are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

bacteria, whereas all bacterial strains promoted spore germination and hyphal growth, except for J5A3 and Ter331 which depressed hyphal growth. These growth-promoting effects of most of the tested bacterial isolates are in agreement with previous reports (Mosse 1962; Azcón-Aguilar et al. 1986; Azcón 1989).

When all the eight bacterial strains were further tested for effect on root colonization of barrel medic by *G. mosseae* BEG12, only J5B4 and C7R12 promoted root colonization parameters (A% for J5B4 and F%, M%, and A% for C7R12). The bacterial strain J5B4 was the only *Oxalobacteraceae* strain tested in the present study, due to the very low number of isolates available from mycorrhizal barrel medic roots belonging to this family (Offre et al. 2008). The promotion of both fungal growth and mycorrhization by the only *Oxalobacteraceae* strain isolated from mycorrhizal roots of barrel medic would rather be in favor of our initial hypothesis, even if additional *Oxalobacteraceae* strains preferentially associated with mycorrhizal roots should be tested before being able to draw any definitive conclusion.

The only strain tested which had a deleterious effect on AM fungal growth in vitro was the strain *C. fungivorans* Ter331 isolated by de Boer et al. (1998, 2004) from Marram grass and not from mycorrhizal roots of barrel medic. This observation is consistent with the fact that the *C. fungivorans* Ter331 was shown to express mycophagy through its chitinolytic ability and to be a biocontrol agent against fungal soil-borne pathogens (Kamilova et al. 2007). Despite this deleterious effect recorded in vitro, *C. fungivorans* Ter331 did not suppress significantly any of the mycorrhization parameters. This discrepancy could be related to the short turnover time for hyphae and arbuscules of AM (Staddon et al. 2003) allowing *Collimonas* Ter331 (de Boer et al. 2004) to feed on AM fungal structures without depressing the level of mycorrhization. In contrast

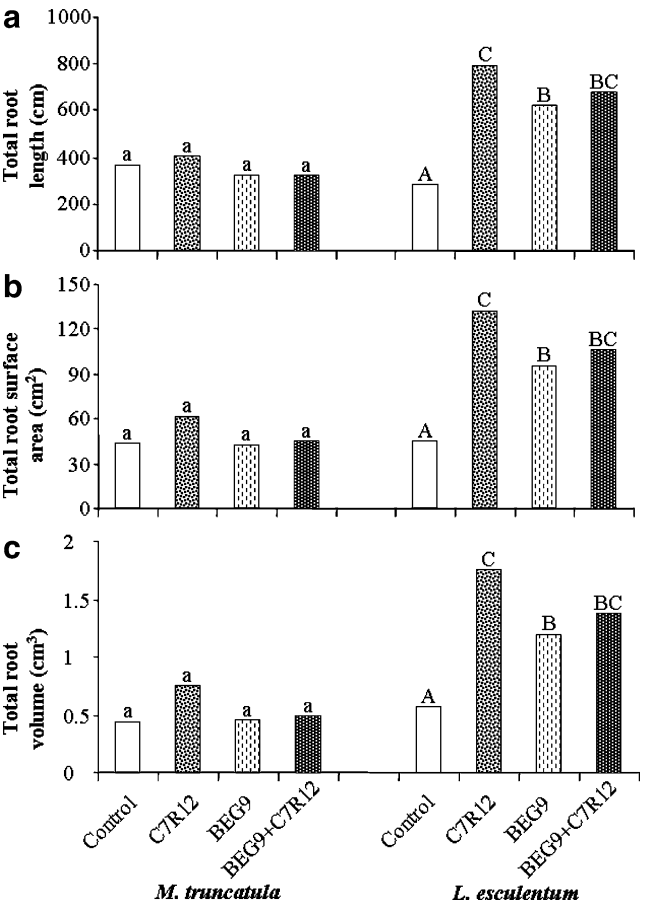

Fig. 5 Compared effects of *Gigaspora rosea* BEG9, inoculated separately or in combination with *Pseudomonas fluorescens* C7R12, on total root lenght (**a**), area (**b**) and volume (**c**) of *Medicago truncatula* and *Lycopersicon esculentum*. Observations were made 7 weeks after sowing. *Bars* with the same *lowercase* (*M. truncatula*) or *capital* (L. esculentum) *letter* are not significantly different ($P \leq 0.1$) according to Fisher's least significant difference test

with *C. fungivorans* Ter331, the MHB strain J5B4 was shown to be not chitinolytic (Uroz, personal communication) which may account for the difference in behavior between these two *Oxalobacteraceae* strains.

None of the *Comamonadaceae* strains tested was identified as being MHB, two of them were even deleterious, one on the in vitro growth of *G. mosseae* BEG12 (strain J5A3) and the other on the mycorrhization of barrel medic by *G. mosseae* BEG12 (strain J5B5). In this context, it is worthwhile recalling that, in contrast to the *Oxalobacteraceae*, the culturable approach developed by Offre et al. (2008) did not allow us to confirm the high frequency of *Comamonadaceae* in mycorrhizal roots previously shown following a non-culturable approach (Offre et al. 2007). In summary, the only MHB strain isolated from *M. truncatula* belongs to the *Oxalobacteraceae*, a family which was previously shown to be significantly more represented in mycorrhizal than in non-mycorrhizal roots (Offre et al. 2007, 2008). In contrast, none of the strains belonging to the *Comamonadaceae*, a family which is well-represented both in mycorrhizal and non-mycorrhizal roots (Offre et al. 2008), promoted mycorrhization whether they were isolated from mycorrhizal or non-mycorrhizal roots. Taken together, these data are in favor of our initial hypothesis according to which bacteria preferentially associated with mycorrhizal roots would promote mycorrhization, even if additional strains should be tested before being able to further conclude.

Among all the bacterial strains tested, the reference strain *P. fluorescens* C7R12 gave the greatest positive effect on *G. mosseae* BEG12 presymbiotic growth and colonization of *M. truncatula* roots. Interestingly the corresponding wild-type *P. fluorescens* strain C7 was selected among a large collection of fluorescent pseudomonads for its ability to suppress fusarium-wilt in non-gnotobiotic conditions (Lemanceau and Alabouvette 1991), where the host plant was probably mycorrhizal. This may be relevant to its positive effect on symbiosis with indigenous AM fungi. This hypothesis is supported by the observations made with the bacterium *Paenibacillus* sp. B2 which was shown to suppress a soil-borne fungal pathogen, but to promote AM fungal root colonization (Budi et al. 1999; Selim et al. 2005). Frey-Klett et al. (2007) suggested that MHB could have evolved selective mechanisms of interaction with their microbial surroundings, having neutral or positive effects on their host mycorrhizal association but negative effects on the root pathogens that might threaten their very habitat. The differential activities toward pathogenic and symbiotic fungi found in *P. fluorescens* C7R12 and in *Paenibacillus* sp. B2 lend support to this hypothesis.

The second part of this study consisted in testing whether AM promotion by *P. fluorescens* C7R12 was fungal and plant specific. Despite the importance of such specificity from both an ecological and an applied point of view, hardly any information is available in the literature. For this, a second AM fungus (*G. rosea*, order *Diversisporales*), phylogenetically distant from *G. mosseae* (order *Glomerales*) (Schüßler et al. 2001; Walker and Schüßler 2004), and a second plant species (*L. esculentum*), taxonomically distant from *M. truncatula*, were chosen. Moreover, these two plant species are known to respond in a different way to *G. mosseae* and *G. rosea*; with corresponding differences being related to the level of colonization, nutrient uptake and growth, and plant gene expression (Monzon and Azcón 1996; Burleigh et al. 2002; Sanchez et al. 2004; Smith et al. 2004).

Data on the effect of *P. fluorescens* C7R12 on the saprophytic growth of the two AM fungi in absence of the host plant clearly showed bacterial promotion to be fungal specific. Indeed *P. fluorescens* C7R12 increased 34 times the level of spore germination of *G. mosseae* BEG12 and only three times that of *G. rosea* BEG9, and increased 12 times the hyphal growth of *G. mosseae* BEG12 whereas no significant effect was detected on *G. rosea* BEG9. In vivo experiments with the two plant species confirmed the positive effect of *P. fluorescens* C7R12 towards *G. mosseae* BEG12 with promotion of fungal colonization by the bacterial strain both in barrel medic (M%) and in tomato (F%, M%, A%) roots. The fungal-specific effect of the bacterial strain was also confirmed by no significant (tomato) or even a depressing (barrel medic) effect of *P. fluorescens* C7R12 on mycorhization with *G. rosea* BEG9. The bacterial promotion of mycorrhization of *M. truncatula* by *G. mosseae* BEG12 led to an increased plant growth, whereas each microorganism had no effect separately. The lack of effect of *G. mosseae* BEG12 on barrel medic growth, already reported by Monzon and Azcón (1996), could be ascribed in our experimental conditions to the low level of mycorrhization. The additive effect of *P. fluorescens* C7R12 and *G. mosseae* BEG12 on plant growth may have resulted from a significant increase in root length and surface by the microbial combination compared to that in presence of each microorganism separately. In contrast, both *G. mosseae* BEG12 and *P. fluorescens* C7R12 inoculated separately significantly promoted tomato plant growth, in such way that no additional effect was obtained by their combination. Similar types of observation were made on root growth for which no additional effects were gained by the microbial combination. Promotion of tomato growth by *G. mosseae* BEG12 is in agreement with Gamalero et al. (2002). Based on these observations, the promotion by *P. fluorescens* C7R12 of mycorhization appears to be fungal rather than plant specific. Further studies are required to test this conclusion with other MHB and to analyze possible bacterial and fungal traits mediating such specificity.

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