Mycorrhiza (2012) 22:437–447
DOI 10.1007/s00572-011-0418-7

ORIGINAL PAPER

Interactions among *Glomus irregulare*, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions

Dharam Parkash Bharadwaj · Sadhna Alström · Per-Olof Lundquist

Received: 8 April 2011 / Accepted: 17 October 2011 / Published online: 15 November 2011

Abstract Arbuscular mycorrhizal (AM) fungi interact with bacteria (AM fungi-associated bacteria, AMB) in the mycorrhizosphere. We previously identified a set of AMB that enhance AM fungal colonization, plant growth, and inhibit pathogens. Here, we used transformed carrot root cultures in a two-compartment plate system for further in vitro studies on interactions taking place among *Glomus irregulare* (syn. *Glomus intraradices*), AMB, and plant pathogens. We found that exudates of *G. irregulare* stimulated growth of all ten AMB isolates tested in multi-well plates. AMB growth stimulation was observed also during co-cultivation of three of these AMB with *G. irregulare* in the hyphal compartment. In addition, co-cultivation stimulated growth of *G. irregulare* hyphae and spore production, as well as *G. irregulare* root colonization. GC/MS analysis in a preliminary screening of metabolites revealed differences in concentrations of several identified but also unidentified compounds in *G. irregulare* hyphal exudates. Exudates in presence of three different AMB isolates co-cultivated with *G. irregulare* contained several additional compounds that differed in amount compared with *G. irregulare* alone. The results indicate that *G. irregulare* exudates contain carbohydrates, amino acids, and unidentified compounds that could serve as a substrate to stimulate AMB growth. With regard to effects on plant pathogens, growth inhibition of *Rhizoctonia solani*, *Verticillium dahliae*, and *Pectobacterium carotovorum* ssp. *carotovorum* was evident in the presence of the AMB isolates tested together with the *G. irregulare* exudates. These in vitro studies suggest that *G. irregulare* and AMB stimulate growth of each other and that they together seem to provide an additive effect against growth of both fungal and bacterial pathogens.

Keywords Arbuscular mycorrhizal fungi · Exudate analysis · *Glomus irregulare* · Interactions · Potato pathogens · Spore-associated bacteria

D. P. Bharadwaj · S. Alström
Department of Mycology and Plant Pathology, Uppsala BioCenter, Swedish University of Agricultural Sciences, Box 7026, SE-750 07 Uppsala, Sweden

P.-O. Lundquist (✉)
Department of Plant Biology and Forest Genetics, Uppsala BioCenter, Swedish University of Agricultural Sciences, Box 7080, SE-750 07 Uppsala, Sweden
e-mail: Per-Olof.Lundquist@slu.se

Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous soil inhabitants and constitute an integral component of terrestrial ecosystems by forming obligate symbiotic associations with plant root systems (Smith and Read 1997). The AM fungi interact with bacterial communities in the rhizosphere and on their own hyphal network, commonly referred to as the "mycorrhizosphere" (Rambelli 1973; Meyer and Linderman 1986a; Barea et al. 2005; Linderman 2008; Pivato et al. 2008). Members of bacterial families like Pseudomonadaceae, Paenibacillaceae, and Oxalobacteriaceae have been identified to occur in these AM-associated communities (Bharadwaj et al. 2008a; Offre et al. 2008; Nazir et al. 2010). Some of these bacteria can directly influence plant physiology while others may create a more indirect synergism in the mycorrhizosphere to support plant growth (Barea 1997; Bharadwaj et al. 2008b). The indirect synergism may be due to improved nutrient acquisition with the bacteria, thus acting as plant growth-promoting bacteria (PGPB; Barea et al. 2002). On the other hand, some bacteria have been reported to stimulate spore germination and hyphal growth rate and enhance the extent of

AM fungal colonisation. These are called mycorrhiza helper bacteria (MHB) (Mayo et al. 1986; Meyer and Linderman 1986b; Carpenter-Boggs et al. 1995; Barea et al. 2002; Frey-Klett et al. 2007; Bharadwaj et al. 2008b; Nazir et al. 2010). They are mostly fungal-specific but can also be host plant-specific (Garbaye 1994; Bharadwaj et al. 2008a; Pivato et al. 2009). Production of volatile compounds that can positively influence germination of AM spores, provision of nitrogen through nitrogen fixation, solubilization of soil phosphate sources, detoxification of the fungal microhabitat, change of pH and level of siderophores are some of the proposed mechanisms underlying stimulation of mycorrhization. An additional mechanism that has been investigated is their ability to produce cell wall-degrading enzymes that may soften the root cell walls and make it easier for the AM fungi to penetrate the roots (Mosse 1962; Garbaye 1994; Gryndler et al. 2000; Bharadwaj et al. 2008a, 2008b; Nazir et al. 2010).

Certain MHB bacteria have been demonstrated to exert strong antagonistic ability against fungal pathogens in the mycorrhizosphere. A *Paenibacillus* bacterium isolated from the mycorrhizosphere of *Sorghum bicolor* when grown in the presence of *Glomus mosseae* was shown to inhibit different fungal pathogens, as well as stimulate AM colonization (Budi et al. 1999). Li et al. (2007) also reported that a *Paenibacillus* strain isolated from the mycorrhizosphere of cucumber plants or the hyphosphere of *Glomus intraradices* reduced damping-off in cucumber caused by *Pythium aphanidermatum*. Bacteria belonging to the genera *Pseudomonas*, *Stenotrophomonas*, and *Bacillus* isolated from surface-decontaminated spores of *G. mosseae* and *G. intraradices* have been found to inhibit the growth of fungi and bacteria pathogenic to potato (Bharadwaj et al. 2008a, 2008b). Thus, some bacteria associated with AM fungi are increasingly interesting as an unexploited novel resource for sustainability in agriculture and horticulture.

AM fungi have also been shown to be involved in the reduction of harmful effects of several plant pathogens (Azcón-Aguilar and Barea 1996; Singh et al. 2000; Whipps 2004). Fungal pathogens belonging to the genera *Phytophthora* (Mark and Cassells 1996; Norman et al. 1996; Trotta et al. 1996; Cordier et al. 1998), *Fusarium* (Zambolim and Schenck 1983), *Aphanomyces* (Rosendahl 1985), *Sclerotium* (Krishna and Bagyaraj 1983), and *Rhizoctonia* (Berta et al. 2005) have been reported to be controlled by AM fungi. Several mechanisms have been suggested to be involved in the reduction of plant pathogens in mycorrhized plants. These include competition, improved plant nutrient status, changes in root morphology, changes in microbial flora in the rhizosphere, and induced resistance in plants (Azcón-Aguilar and Barea 1996; Whipps 2004). It has also been found that the chemical composition of root exudates changes as a result of mycorrhizal establishment (Lioussanne et al. 2008). Exudates of mycorrhizal roots have been found to affect germination, chemotactic response, growth and sporulation of pathogenic fungi (Meyer and Linderman 1986a; Norman and Hooker 2000; Lioussanne et al. 2008), and thus mycorrhizal exudates may play an important role in the reduction of plant pathogens by AM fungi.

The majority of plant–microbe interactions in the soil rely on the release of various compounds from roots that can affect the growth and activity of microbes (Hartmann et al. 2009). Root exudates of mycorrhizal plants also contain hyphal exudates and deposition of AM fungal mycelial products that could serve as substrates for bacterial growth and impose a direct influence on bacterial communities in the mycorrhizosphere. Filion et al. (1999) suggested that mycelial exudates released by *G. intraradices* could have both antagonistic and stimulatory effects on fungi and bacteria depending on the organism. Toljander et al. (2007) found that mycelial exudates of *G. intraradices* increased bacterial growth and vitality but also changed bacterial community composition. The effects of interactions between AM fungi and bacteria associated with AM fungi (AMB, AM fungi-spore associated bacteria; Bharadwaj et al. 2008a) on production of exudates and growth of AM fungi and AMB, as well as their interaction effects on fungal and bacterial pathogens, have not received much attention. In our previous studies, we found that the composition of an AMB community depended on the type of interacting AM fungal species and plant species (Bharadwaj et al. 2008a). Some of the AMB we studied strongly inhibited the growth of fungal and bacterial pathogens and affected plant growth and AM fungal colonization in potato (Bharadwaj et al. 2008a, 2008b). They were also shown to produce an array of different hydrolytic enzymes as well as indole acetic acid (IAA) and siderophores depending upon the isolate (Bharadwaj et al. 2008b).

The present study examined the interactions occurring among AM fungi, bacteria, and plant pathogens under in vitro conditions. The aims were to determine: (1) the effect of *Glomus irregulare* (syn. *G. intraradices*) exudates on the growth of selected AMB, (2) the effect of AMB on the growth of *G. irregulare*, and (3) interaction effects of AMB and *G. irregulare* on some fungal and bacterial pathogens. We also made an attempt to investigate differences and identities of compounds released as a result of interaction in the *G. irregulare* hyphosphere in the absence or presence of added AMB.

Materials and methods

Microorganisms and culturing conditions

A total of ten previously isolated and characterized AMB isolates (FWC14, FWC94, FWC101, LWC2, and LYC39 (*Stenotrophomonas maltophilia*), FWC16 (*Pseudomonas*

putida biotype B), FWC30 (*P. fluorescens* biotype F), FWC42 (*Bacillus subtilis*), FWC70 (*P. putida* biotype A), and FWC110 (*Arthrobacter ilicis*) (Bharadwaj et al. 2008a, 2008b) were used. They were selected on the basis of their multifunctional characteristics, including antagonistic activity against four different potato pathogens (Bharadwaj et al. 2008a, 2008b). The isolates were cultured on diluted tryptic soy broth agar (TSA10, per liter 10 g tryptic soy broth, 15 g agar, Difco Ltd) for 24 h at 22°C and suspended in sterile phosphate buffer saline solution (PBS; 0.14 M NaCl, 0.003 M KCl, and 0.01 M phosphate buffer in 1,000 mL, pH 7.4) so as to obtain 10^7 colony forming units (cfu)/mL in the inocula used in different experiments.

The AM fungus *Glomus irregulare* Blaszk Wubert, Renker and Busco (Stockinger et al. 2009; syn. *G. intraradices* MUCL 41833) and root organ culture of Ri T-DNA transformed (T-) roots of carrot (*Daucus carota* L.) without AM fungus was obtained from the Glomeromycota in vitro collection (GINCO) (http://www.mbla.ucl.ac.be/ginco-bel). The roots with and without *G. intraradices* were multiplied on modified minimal (MM^+) medium (Filion et al. 1999) in the dark at 25°C. The MM^+ medium, pH 5.5, contained (milligrams per liter) $MgSO_4{\cdot}7H_2O$ 731; KNO_3 80; $Ca(NO_3)_2{\cdot}4H_2O$ 288; KCl 65; KH_2PO_4 4.8; Na-FeEDTA 8; KI 0.75; $MnCl_2{\cdot}4H_2O$ 6; $ZnSO_4{\cdot}7H_2O$ 2.65; H_3BO_3 1.5; $CuSO_4{\cdot}5H_2O$ 0.13; $Na_2MoO_4{\cdot}2H_2O$ 0.0024; glycine 3; thiamine 0.1; pyridoxine 0.1; nicotinic acid 0.5; and myoinositol 50, and 10 g/L sucrose. The medium was solidified using 0.4% (*w*/*v*) gellan gum (Phytagel™ Sigma Chemicals, St. Louis, MO, USA).

The fungal pathogens *Rhizoctonia solani* Kühn and *Verticillium dahliae* Kleb., the causal organisms of black scurf/stem canker and wilt in potato, respectively, were obtained from our own culture collection. Their pathogenicity was confirmed in potato before starting the experiments. They were routinely multiplied on potato dextrose agar (PDA, Oxoid Ltd). A small circular disc (5 mm) of freshly grown culture was inoculated aseptically to the potato dextrose broth (PDB) at 20°C to obtain stationary broth cultures. After 1 week, the mycelial mat was cut into a homogeneous suspension in PBS solution using a sterile scalpel before use in different experiments. The bacterial pathogen *Pectobacterium carotovorum* ssp. *carotovorum*, the causal organism of soft rot in potato, was also obtained from our own culture collection and its pathogenicity was confirmed before the start of the experiments (Bharadwaj et al. 2008b). *P. carotovorum* ssp. *carotovorum* was multiplied on King's medium B agar (KBA, King et al. 1954), and a suspension was prepared in PBS solution in the same manner as described above for AMB.

Two-compartment Petri plate system

A two-compartment Petri plate system was set up as previously described (St-Arnaud et al. 1996; Filion et al. 1999; Toljander et al. 2007). In brief, the plate (90× 15 mm^2) consisted of a root and a hyphal compartment. The root compartment contained the sterile MM^+ medium with all its ingredients. A slope was created in the hyphal compartment using MM^+ medium lacking sucrose, EDTA, and vitamins, referred to here as MM^-. The *G. irregulare*-mycorrhized or the non-mycorrhized T roots (control) were inoculated aseptically in the root compartment and incubated at 26°C for 8 weeks in the dark. Plates were placed at an angle of 45º to allow *G. intraradices* mycelium to grow over the slope of MM^- in the hyphal compartment (Fig. 1). Only *G. irregulare* mycelium was allowed to grow into the hyphal compartment, and roots were cut manually when needed. The liquid present in contact with hyphae in the non-gellan gum area in the hyphal compartment was considered *G. irregulare* exudate in MM^- leachate (*G. irregulare* exudate) and was collected aseptically and stored at −18°C until use in several experiments. About 80 μL exudates was collected per plate from at least 60 plates in total and pooled and used in all experiments described below. Plates with T carrot roots only were maintained as controls and the liquid present in the non-gellan gum area in the hyphal compartment was considered MM^- gellan gum leachate in the absence of *G. intraradices* (MM^- leachate).

Effect of *G. irregulare* exudate on the growth of AMB

Using multiwell plate system

Multiwell plates (24 wells/plate) were used to study the growth of AMB in the presence of *G. irregulare* exudate at one time point. For this purpose, exudate was collected aseptically and carefully from the hyphal compartment 8 weeks after inoculation of mycorrhizal roots into the root compartment (Fig. 1). Ten microliters of each AMB suspension were inoculated to a mixture of 50 μL *G. irregulare* exudate in MM^- broth+50 μL MM^- broth and 100 μL KB broth. The corresponding controls consisted of a mixture of 50 μL of MM^- leachate+50 μL MM^- broth and 100 μL KB broth. The KB broth was added to prevent nutrient starvation conditions, if any, during AMB growth. Four replicates per treatment combination were prepared. Plates were incubated while shaking at 150 rpm at 22°C for 48 h. Growth of each AMB was recorded by measuring absorbance at 560 nm wavelength at different time intervals up to 48 h.

Using two-compartment plate system

The effect of co-cultivation of *G. irregulare* and AMB on the growth of AMB was also examined for the four isolates FWC30, FWC42, FWC70, and LWC2. This was done in

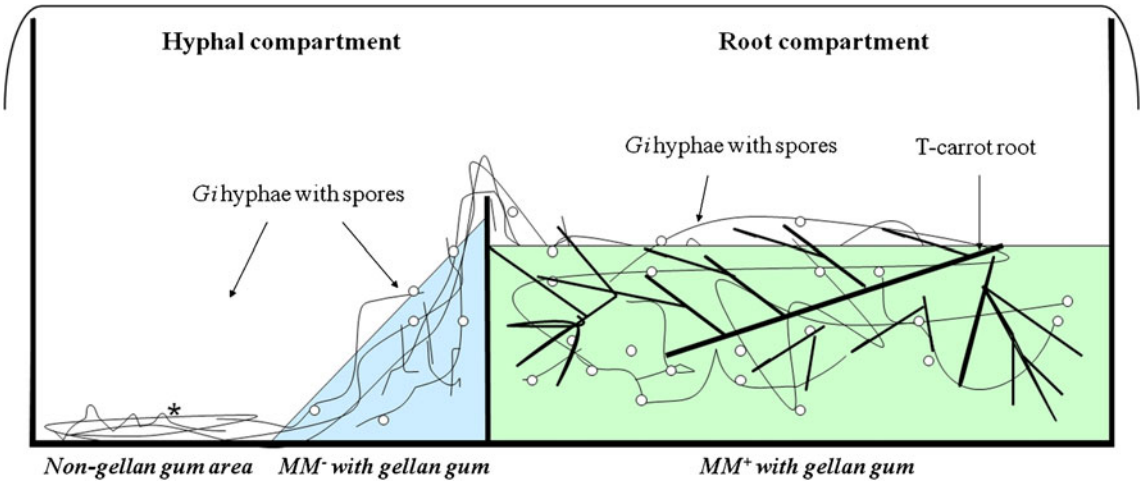

Fig. 1 Illustration of the two-compartment plate system used in the present study. Root compartment: transformed (T) carrot root mycorrhized with *Glomus irregulare* (syn *G. intraradices*) on modified minimal medium (MM^+, Filion et al. 1999) in gellan gum. Hyphal compartment: extraradical *G. irregulare* hyphae grow across a slope of MM^- medium (MM^+ lacking vitamins, EDTA, and sucrose) in gellan gum into a non-gellan gum area that was initially empty. MM^- leachate or *G. irregulare* exudates in MM^- leachate were collected in the non-gellan gum area (*asterisk*)

two-compartment plates prepared as above (Fig. 1) to determine the growth of AMB in the presence of continuous growth of *G. irregulare* hyphae, i.e., at different time points. The AMB isolates were selected based on their strong antagonistic activity against potato pathogens in our previous studies (Bharadwaj et al. 2008a, 2008b). Control plates were prepared accordingly but without AMB. After 8 weeks of incubation, 2 mL of MM^- broth and 100 μL of each AMB suspension were added to the hyphal compartment. The growth of AMB was estimated in two replicates of each after 8 and 24 h using the classical dilution plate counting method. For this, the serial dilutions were spread on TSA 10 in duplicate plates, incubated for 48 h and colony-forming units per milliliter was estimated. All the steps were carried out in aseptic conditions. The pH in the hyphal compartment was recorded using a pH electrode (Mettler Toledo AB, Stockholm) at each time-point and for each combination.

Effect of AMB on *G. irregulare*-colonized carrot roots and *G. irregulare* spore production

The effect of actively growing AMB in the hyphal compartment on the growth of *G. irregulare*-mycorrhized T roots and on spore production in the hyphal compartment was also studied (Fig. 1). One hundred microliters of each AMB suspension plus 2 mL of TSB 10 (per liter 10 g tryptic soy broth, Difco Ltd) were inoculated in the hyphal compartment of 8-week-old *G. irregulare* cultures and then incubated for 2 weeks at 22°C. Five replicates were prepared for each combination. The plates were sealed with parafilm to retain any water-soluble bacterial volatile metabolites produced. Total number and length of newly formed *G. irregulare*-mycorrhized T roots were assessed in relation to marked positions in the plate and recorded as measures of the impact of AMB on the growth of mycorrhizal root. The effect on spore production in the hyphal compartment was evaluated for the three isolates, FWC30, FWC70, and LWC2. The spores were counted manually under a light microscope. The plates without AMB were used as controls for comparison.

Analysis of exudates by gas chromatography/mass spectroscopy

The exudates from the experiments using the two-compartment plate system described above were used to screen for differences in compounds produced during the in vitro growth of *G. irregulare* in the presence and absence of the AMB isolates FWC30, FWC70, and LWC2. In this initial study, we analyzed the following samples using a metabolomic approach: (1) sterile MM^- broth (control); (2) MM^- leachate collected from the hyphal compartment from plates with non-mycorrhized T roots in the root compartment (control); (3) *G. irregulare* exudate collected from the hyphal compartment; (4) leachate from the hyphal compartment where AMB was co-cultivated with *G. irregulare*; and (5) sterile suspension of gellan gum (control). The samples were collected from five replicates for each treatment and filtered through micropore filters (pore size 0.20 μm, Pall Corporation, Gelman Laboratory, Michigan). All the above samples were subjected to extraction for metabolites, treated with trimethylsilyl (TMS) and analyzed using a metabolomics protocol adapted for qualitative

detection of organic acids, amino acids, fatty acids, amines, mono- and disaccharides, and sterols among other compounds, using a Pegasus III time-of-flight mass spectrometer GC/TOFMS at the metabolomic facility at Umeå Plant Science Centre, Umeå University, Sweden. The data obtained was processed as described by Gullberg et al. (2004) and Jonsson et al. (2005).

Effect of AMB on growth of pathogens in *G. irregulare* exudate

The interactive effects of *G. irregulare* with the four AMB isolates, FWC30, FWC42, FWC70, and LWC2 on the growth of potato pathogens were studied in sterile 24-multiwell plates. The bacterial suspensions were prepared by culturing them in half-strength KB broth and shaking at 150 rpm at 22°C for 24 h. Ten microliters of each AMB suspension were inoculated to a mixture containing 50 μL *G. irregulare* exudates+50 μL MM⁻ broth+100 μL half-strength PDB in case of fungal pathogens. Equal portions (1 mm^2) of the actively growing mycelium from freshly cultured *R. solani* or *V. dahliae* on PDA were transferred separately to each well. Four replicates per treatment were prepared. After 1 week of incubation at 20°C, the effect on the pathogen was measured in terms of mycelial dry weight after drying the mycelial mat at 60°C for 24 h. The growth of the fungal pathogens was compared with their growth in two different controls with either PDB only or *G. irregulare* exudate only.

In order to further investigate the effect of combined inoculation of AM fungal exudates and AMB on the growth of the bacterial pathogen *P. carotovorum* ssp. *carotovorum*, 10 μL of each AMB suspension were inoculated into a mixture of 50 μL *G. irregulare* exudate+50 μL MM⁻ broth+100 μL half-strength KB broth, followed by 10 μL of *P. carotovorum* ssp. *carotovorum* suspension in 24-well plates. The controls were prepared with 100 μL MM⁻ broth and without *G. irregulare* exudate. The plates were then incubated at 20°C for 48 h, with three replicates per treatment. Using dilution plate counting, the growth of *P. carotovorum* ssp. *carotovorum* and AMB were assessed. Serial dilutions were thus spread on KBA or TSA10 plates and incubated at 22°C for 48 h. KBA was used to distinguish the fluorescent colonies of FWC30 and FWC70 from non-fluorescent *P. carotovorum* ssp. *carotovorum* colonies, and TSA10 was used to distinguish the morphologically different colonies of FWC42, LWC2, and *P. carotovorum* ssp. *carotovorum*. All data were transformed to colony-forming units per milliliter.

Statistical analysis

The effect of inoculation on total root length, number of T carrot roots, and mycelial dry weight of *R. solani* and *V. dahliae* was analyzed using ANOVA and Tukey's pairwise comparisons ($P<0.05$) in Minitab statistical software, Release 14 (Minitab Inc. 2003, State College, Pennsylvania).

Results

Effect of *G. irregulare* exudate on the growth of AMB

The growth of all AMB isolates studied was found to be improved in the presence of *G. irregulare* exudates. No apparent negative effect of *G. irregulare* exudate was found on the growth of any of the bacterial isolates in multiwell plates. The growth pattern of all ten AMB isolates showed that the log phase was extended in the presence of *G. irregulare* exudate compared with that in their absence. Data for three isolates (FWC30, FWC70, and LWC2) are shown in Fig. 2. The growth pattern of isolate FWC42 was not scored because its mucoid morphology made it impossible to distinguish individual colonies in a satisfactory way. The growth patterns of the remaining six AMB were similar to those shown in Fig. 2. There was a tendency for continued increased growth of these AMB isolates during co-cultivation of AMB with *G. irregulare* in the hyphal compartment compared with growth in the absence of *G. irregulare* both after 8 and 24 h (Table 1). No change in pH was observed during bacterial growth in the presence or absence of *G. irregulare*.

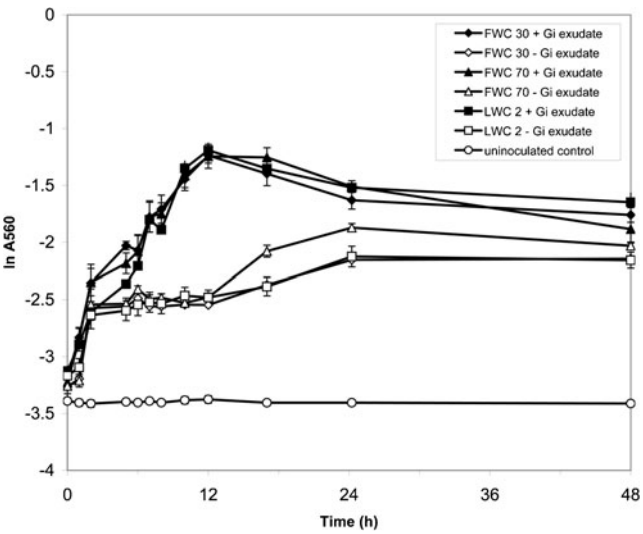

Fig. 2 Growth of some arbuscular mycorrhizal fungal spore-associated bacteria (AMB; data shown for the three isolates FWC30, FWC70, and LWC2) in the presence and absence of *Glomus irregulare* (syn *G. intraradices*) exudates in MM⁻ leachate. Patterns were similar for the remaining seven AMB isolates. *Error bars* represent standard errors of means of bacterial growth ($n=4$)

Table 1 Effect of *Glomus irregulare* (syn *G. intraradices*) on AMB growth during co-cultivation in the compartment plate system at 8 and 24 h of incubation (growth compared as the total number of colony-forming units (cfu) formed)

AMB isolate[a]	Cfu×10^7/mL after 8 h		Cfu×10^7/mL after 24 h	
	−*G. irregulare*	+*G. irregulare*	−*G. irregulare*	+*G. irregulare*
FWC30	18±1 a	40±2 b	153±4 a	212±3 b
FWC70	27±2 a	47±3 b	180±4 a	268±3 b
LWC2	21±4 a	33±2 a	169±4 a	228±3 b

Data is presented as means±standard error (n=2 for each time point). Different letters indicate significant differences in response to the *G. intraradices* alone (P< 0.05)

[a] AMB: bacteria associated with the arbuscular mycorrhiza spores. The data for AMB FWC42 was not included because the mucoid growth habit of this isolate made it impossible to score individual colonies

Effect of AMB on *G. irregulare*-colonized carrot roots and *G. irregulare* spore production

None of the AMB showed an inhibitory effect on root growth. The presence of actively growing FWC70 in the hyphal compartment resulted in a significant (P<0.05, n= 5) increase in the growth of *G. irregulare* mycorrhized T roots in the root compartment when evaluated as increase in the total number of newly formed roots (+133%) and length of roots (+129%) compared with the untreated controls. No significant effects of the other nine AMB tested were observed on the growth of T roots, although a positive tendency was observed in the presence of FWC94, FWC101, and FWC110.

In addition, spore formation by *G. irregulare* in the hyphal compartment increased by 55%, 80%, and 15%, respectively, in the presence of the three isolates FWC30, FWC70, and LWC2 compared with that in the AMB-free control (P<0.05, n=5).

Analysis of exudate by gas chromatography/mass spectroscopy

In this preliminary screening of changes in the content of a range of compounds using gas chromatography/mass spectroscopy (GC/MS), the compounds detected were asparagine, β-D-methylglucopyranoside, cadaverine, citric acid, fructose, glucose, glutamine, hypoxanthine, inositol, leucine, pinitol, proline, pyroglutamic acid (a putative derivative artefact from glutamate), raffinose, succinic acid, sucrose, trehalose, tryptophan, tyrosine, uric acid, and several unidentified TMS derivatives.

Further comparisons focused only on compounds and TMS derivatives that were at least 30-fold higher or lower in amount between treatments. This level was chosen subjectively to reduce risk for over-interpretation of the results. Based on this, the levels of fructose and four unidentified TMS derivates were found to increase in *G. irregulare* exudate in MM^- leachate in the hyphal compartment (Fig. 1) compared with that in MM^- leachate without *G. irregulare*, while the levels of lactose, lysine, and three unidentified TMS derivates decreased. Uric acid and two unidentified TMS derivatives that were present in MM^- leachate without *G. irregulare* disappeared in the presence of *G. irregulare*. In the presence of the three AMB isolates, FWC30, FWC70, and LWC2 with *G. irregulare*, the levels of fructose and two of the four TMS derivatives were found to decrease, while the levels of lactose, lysine, uric acid, and four of the five unidentified compounds were found to increase. In the presence of at least one AMB isolate, tryptophan, tyrosine, and 13 TMS derivatives increased.

On the other hand, the amounts of some compounds were found to decrease. Six compounds (fructose, sucrose, β-D-methylglucopyranoside, asparagine, pinitol, raffinose) decreased in the presence of all three isolates. In the presence of at least one AMB isolate, citric acid, trehalose, tyrosine, and five unidentified TMS derivatives decreased.

Effect of AMB on growth of pathogens in *G. irregulare* exudate

Actively growing AMB in the presence of the *G. irregulare* exudate inhibited growth of the fungal pathogens. Growth of both *R. solani* and *V. dahliae* in PDB medium with *G. irregulare* exudate and AMB was significantly reduced, by 15% and 55%, respectively, compared with their growth in PDB only (Table 2). Furthermore, compared with growth in *G. irregulare* exudate as substrate, the presence of AMB in the nutrient-rich PDB still resulted in lower growth. For *R. solani*, the increase in mycelium biomass was reduced by 46%, 81%, 46%, and 73% in the presence of FWC30, FWC42, FWC70, and LWC2, respectively, compared with biomass in *G. irregulare* exudate alone. For *V. dahliae*, the corresponding figures were 33%, 59%, 33%, and 44%, respectively (Table 2). The fungal biomass of *R. solani* in *G. irregulare* exudate was reduced by 62% compared with in PDB alone, but there was no difference in fungal biomass of *V. dahliae* in *G. irregulare* exudate and in PDB.

The growth of the bacterial pathogen *P. carotovorum* ssp. *carotovorum* (measured as colony-forming units per milliliter) was also reduced in the presence of *G. irregulare* exudate alone. The numbers of viable cells of *P. carotovorum* ssp. *carotovorum* were further reduced by two (FWC30 and FWC70) of the three AMB isolates in the presence of *G. irregulare* exudate compared with the

Table 2 Effect of AMB isolates actively growing in *Glomus irregulare* (syn *G. intraradices*) exudates in MM⁻ leachate on growth (mycelial dry weight) of *Rhizoctonia solani* and *Verticillium dahliae* pathogenic to potato

Treatments	*R. solani* (μg/well)	*V. dahliae* (μg/well)
MM⁻ leachate[a]	200±41 bc	600±58 ab
G. irregulare	325±111 b	675±48 a
PDB	850±104 a	700±71 a
PDB+*G. irregulare*+FWC30[b]	175±32 bc	450±64 b
PDB+*G. irregulare*+FWC42	62±12 c	275±48 b
PDB+*G. irregulare*+FWC70	175±25 bc	450±29 b
PDB+*G. irregulare*+LWC2	88±12 c	375±48 b

Data is presented as means±standard error (n=4). Different letters indicate significant differences between means (P< 0.05)

PDB half-strength of potato dextrose broth

[a] See Fig. 1 and text for description

[b] AMB—see Table 1 for details

numbers in *G. irregulare* exudate alone or in KB alone (Table 3). The third isolate (LWC2) reduced the growth of *P. carotovorum* ssp. *carotovorum* only when compared with that in KB alone. The environment in the growth plate was favorable for growth of *P. carotovorum* ssp. *carotovorum* and of four AMB isolates (Table 3), but the mucoid growth habit of FWC42 made it impossible to score individual colonies for this strain on these two media.

Discussion

In the present study, the effect of *G. irregulare* exudate on the growth of selected AMB isolates and vice versa, and the combined effects of *G. irregulare* exudate and AMB on fungal and bacterial plant pathogens, were investigated under in vitro conditions. Most of the AMB isolates tested in this study belong to the phylum γ-Proteobacteria (Bharadwaj et al. 2008a). In a previous study, one of these selected AMB isolates; FWC70 (*P. putida*), was shown to stimulate root colonisation both by *G. mosseae* in γ-sterilized field soil and by a native AMF community in the field (Bharadwaj et al. 2008b). This AMB also stimulated primary and lateral root development, root and shoot length, and number of leaves per plant in potato in gnotobiotic condition, in addition to antagonism to the potato pathogens *P. carotovorum* ssp. *carotovorum*, *Phytophthora infestans*, *R. solani*, and *V. dahliae* (Bharadwaj et al. 2008b). The other AMBs in the same study also stimulated some of these traits depending on the isolate (Bharadwaj et al. 2008b).

When these AMB, including FWC30, FWC70, and LWC2, were inoculated into *G. irregulare* exudate in the present study, the growth of all ten isolates was significantly stimulated for up to 12 h. A similar pattern of stimulation was observed for the three isolates when co-cultivated with *G. irregulare* in the hyphal compartment up to 24 h. No change in pH was observed during bacterial growth in the presence or absence of *G. irregulare*. Overall, these results indicate that *G. irregulare* secrete compounds that make the environment favorable for supporting the growth of bacteria, including those belonging to the γ-Proteobacteria. Synergistic interactions between γ-Proteobacteria and AM fungi, resulting in increased AM colonization and nutrient uptake in plants, have been reported (Arthurson et al. 2006). Filion et al. (1999) and Toljander et al. (2007) also reported that *G. irregulare* exudate increases bacterial growth, particularly in terms of the frequency of occurrence of the γ-Proteobacteria.

The AMB isolate FWC70 significantly increased the total number and length of *G. irregulare*-inoculated carrot roots and also enhanced production of spores by *G. irregulare* under in vitro conditions. In our previous

Table 3 Effect of *Glomus irregulare* (earlier *G. intraradices*) exudates in MM⁻ leachate and four selected actively growing AMB isolates (see Table 1 for details) on the growth (colony forming units, cfu) of *Pectobacterium carotovorum* ssp. *carotovorum* (*P. carotovorum* ssp. *carotovorum*) pathogenic to potato

Treatments	*P. carotovorum* ssp. *carotovorum* (cfu×10^7 per mL)	AMB (cfu×10^7 per mL)
KB+*P. carotovorum* ssp. *carotovorum*	87±2.7 a	0 a
KB+*P. carotovorum* ssp. *carotovorum*+*G. irregulare*	49±1.5 b	0 a
KB+*P. carotovorum* ssp. *carotovorum*+*G. irregulare*+FWC30	39±1.9 c	67±2.3 b
KB+*P. carotovorum* ssp. *carotovorum*+*G. irregulare*+FWC70	18±2.0 d	79±1.7 c
KB+*P. carotovorum* ssp. *carotovorum*+*G. irregulare*+LWC2	45±1.8 bc	51±3.2 d

Data is presented as means±standard error (n=3). Different letters indicate significant differences between means (P< 0.05)

KB half-strength Kings B broth

The data for AMB FWC42 was not included because the mucoid growth habit of this isolate made it impossible to score individual colonies.

study, the same isolate was found to enhance AM fungal colonization of potato roots both in greenhouse and outdoor experiments (Bharadwaj et al. 2008b). Root growth of the AM host plant potato and radicle length of the AM non-host oilseed rape were beneficially affected by FWC70 (Bharadwaj et al. 2008b). This AMB was identified as *P. putida* (Bharadwaj et al. 2008a), a species that has previously been reported to enhance mycorrhizal colonization in subterranean clover (Meyer and Linderman 1986b). The possible explanation behind this stimulation might be its ability to produce compounds such as siderophores and IAA (Bharadwaj et al. 2008b), which could contribute to promote carrot root growth. Furthermore, in response to inoculation of FWC70 in the hyphal compartment containing *G. irregulare*, increased production of *G. irregulare* spores was observed. Although not quantified here, the amount and branching of hyphae per plate also appeared to visually increase. A direct effect of compounds produced by AMB on the production of hyphae and spores cannot be excluded, but it is also possible that compounds from the AMB, such as auxins and siderophores, could have been transported through *G. irregulare* hyphae to the root, leading to increased root growth and as a consequence a stimulation of extraradical *G. irregulare* growth. Plant growth affecting bacteria are also known to produce volatile compounds such as hydrogen cyanide (HCN), ethylene (C_2H_4), and ammonia (NH_3) (Alström 1987). Recently, Bernier et al. (2011) suggested that NH_3 may be a "universal" signal in long-range interactions between physically separated bacteria. The role of ammonia and ethylene in AMB–AM fungi–host plant interactions remains vastly unexplored. Although production of HCN by the studied AMBs was not detected previously (Bharadwaj et al. 2008b), the possibility cannot be excluded that unidentified volatile metabolites produced by AMB during their growth could be involved in stimulation of root growth, irrespective of the presence of *G. irregulare* mycelium.

First indications of possible metabolic interactions occurring between AM fungi and AMB come from our results from the GC/MS analysis. Some differences in the composition and amounts of compounds in hyphal exudates between *G. irregulare* alone and in presence of AMB were observed when compared with the amounts detected in uninoculated MM^- leachate. The major compounds detected were carbohydrates, organic acids, amino compounds, and several unidentified compounds and/or unidentified TMS derivatives formed during the derivatization process. The analyses suggest that fructose, sucrose, β-D-methylglucopyranoside, asparagine, pinitol, raffinose, citric acid, trehalose, glutamine, and tyrosine, and other unidentified compounds supported bacterial growth. Increased growth of pseudomonads in the presence of AM fungal exudates has also been reported previously (Toljander et al. 2007), particularly in mycorrhizosphere soils (Andrade et al. 1997; Mansfeld-Giese et al. 2002).

Compounds such as tryptophan (precursor in IAA biosynthesis), lactose, lysine, uric acid, and some unidentified compounds were found in higher amounts when AMB were co-cultivated with *G. irregulare*, but were markedly absent from *G. irregulare* exudates. These findings seem to indicate that these compounds are involved in, or result from, the interactions among the AMB, AM fungi, and the host plant, as exemplified by the stimulation by AMB of root growth, hyphal growth, spore production, and root colonization discussed above. A more detailed analysis of the identity and location of biosynthesis of the compounds found in the hyphal exudates should give insight into their possible functional role in the interactions among AMB, *G. irregulare*, and the host roots. Although the *G. irregulare* exudates were collected from the root-free hyphal compartment, it is possible that the presence of roots in the root compartment affected the composition of exudates through an action of their volatiles, e.g., CO_2. It is well known that CO_2 produced by plant roots can affect hyphal growth and spore germination (e.g., Becard and Piche 1989), but other volatiles produced by carrot roots such as terpenes (Rosenfeld et al. 2004) can also be present and could have an effect on AM fungal growth and/or physiology. To what extent such volatiles were produced in the mycorrhized and non-mycorrhized roots in the compartment system used in the present study, and the magnitude of their effect on *G. irregulare* exudation, needs further study.

Some of the compounds found, e.g., trehalose, are associated with extra-radical mycelium of AM fungi (Bago et al. 2003) and may therefore have been produced by *G. irregulare*. The presence of low-molecular weight sugars and organic acids in *G. irregulare* exudates has previously been reported (Toljander et al. 2007). Elevated levels of certain amino acids (e.g., glutamic acid, aspartic acid, asparagine) during the most active stages of overall root mycorrhization in *Medicago truncatula* have also been demonstrated (Schliemann et al. 2008). Pinitol is a carbohydrate known to participate in osmotic adjustment during stress in plants (Sheveleva and Bohnert 1998; Ruiz-Lozano 2003). It is possible that the pinitol, inositol, fructose, and glucose detected here in *G. irregulare* exudates moved from the root compartment to the hyphal compartment via *G. irregulare* mycelium, since these compounds were detected in the MM^+ medium in the root compartment. In MM^- leachate without *G. irregulare,* there was no mycelial connection between the two compartments. In the root compartment where gellan gum was used as a solidifier, sucrose (fructose+glucose) and inositol were present in the MM^+ medium. Gellan gum is known to contain the glucopyranoside and glucose, and thus it is

possible that these compounds originated from solubilization of the gellan gum.

Mycelial exudates of *G. irregulare* have been demonstrated to affect bacterial and fungal plant pathogens in a few studies (Filion et al. 1999; Ravnskov et al. 1999). With this in mind, we investigated the interaction between *G. irregulare* exudates and four selected AMB on two fungal pathogens (*V. dahliae* and *R. solani*) and one bacterial (*P. carotovorum* ssp. *carotovorum*) plant pathogen. The AMB isolates were two *Pseudomonas* isolates (FWC30, FWC70), one *Stenotrophomonas* isolate (LWC2), and one *Bacillus* isolate (FWC42), selected on the basis of their strong in vitro antagonistic ability against four potato pathogens (Bharadwaj et al. 2008b). In the present study, we specifically investigated whether *G. irregulare* and AMB could have an interacting effect on the growth of the pathogens, and we found combined effects on inhibition of plant pathogens. The results showed that the growth of *R. solani*, but not of *V. dahliae*, was lower in the presence of *G. irregulare* exudates alone compared with the growth on PDA. Growth of both fungal pathogens was strongly inhibited in the presence of the AMB isolates together with *G. irregulare* exudates. A possible explanation for this difference in behavior between the two fungal pathogens may lie in their differing growth rates and strong competition for nutrients by AMB to which the more slow-growing *V. dahliae* could be less sensitive. For *P. carotovorum* ssp. *carotovorum,* a combined effect of *G. intraradices* exudate and AMB in inhibiting growth of the bacterial pathogen was also observed. Several mechanisms could be involved in this inhibition including siderophore-mediated competition for nutrients and production of *P. carotovorum* ssp. *carotovorum* growth inhibitors.

The AMB isolates FWC30, FWC42, FWC70, and LWC2 produce extracellular enzymes and bioactive compounds, e.g., protease(s), chitinase(s), and siderophores (Bharadwaj et al. 2008b). These AMB seem to be competitive due to production of these compounds, but several mechanisms are possible for the antagonism to pathogens. Competition for nutrients could be one important mechanism against all three pathogens tested here. The role of the *G. irregulare* exudates could be to stimulate AMB growth, but also to produce unidentified metabolites that could have antibacterial and antifungal effects. Further analysis of the compounds found in hyphal exudates and biological tests would unravel the mechanisms involved in antagonism.

To summarize, the beneficial role of AM fungi both in terms of promoting plant growth and antagonism to plant pathogens may partly be due to beneficial traits of bacterial associates such as AMB. Our earlier studies (Bharadwaj et al. 2008a, 2008b) in combination with the present in vitro experiment-based study provides further evidence that *G. irregulare* interacts positively with AMB and vice versa. This interaction seems to take place partly through the hyphal exudates of the AM fungus containing several metabolites produced during their interaction, independent of the presence of living roots. None of the AMB tested seemed to have a deleterious effect on plant growth. We anticipate that combining *G. irregulare* with AMB can provide an additive or synergistic effect against a broad spectrum of plant pathogens. To our knowledge, this is the first attempt to examine interactions taking place between AM fungi and AMB at the metabolite level and their interactions with pathogens. Further experimentation combined with analysis using molecular techniques will help reveal the mechanisms underlying interactions between AMB and *G. irregulare* and the plant host in natural environments.

Acknowledgments We thank Krister Lundgren, Umeå Plant Science Centre, Umeå University, for excellent metabolite analysis. This work was financially supported by the Strategic Programme on Interactions between Micro-organisms and Plants (IMOP) at the Swedish University of Agricultural Sciences (SLU), Uppsala, the Carl Tryggers Foundation for Scientific Research, Stockholm, Stiftelsen Lantbruksforskning (SLF), Stockholm, and the Swedish Board of Agriculture (SJV), Norrköping.

References

Alström S (1987) Influence of root-zone inhabiting bacteria on growth of plants and soil-borne fungal pathogens. Ph. D. thesis. 14, Swedish Univ of Agricultural Sciences, Uppsala, Sweden

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ (1997) Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71–79

Arthurson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens. An overview of the mechanisms involved. Mycorrhiza 6:457–464

Bago B, Pfeffer PE, Abubaker J, Jun J, Allen JW, Brouillette J, Douds DD, Lammers PJ, Shachar-Hill Y (2003) Carbon export from arbuscular mycorrhizal roots involves the translocation of carbohydrate as well as lipid. Plant Physiol 131:1496–1507

Barea JM (1997) Mycorrhiza–bacteria interactions on plant growth promotion. In: Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S (eds) Plant growth promoting Rhizobacteria. OECD Press, Paris, France, pp 150–158

Barea JM, Azcon R, Azcon-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81:343–351

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Becard G, Piche Y (1989) Fungal growth stimulation by CO_2 and root exudates in vesicular–arbuscular mycorrhizal symbiosis. Appl Environ Microbiol 55:2320–2325

Bernier SP, Letoffe S, Delepierre M, Ghigo J-M (2011) Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. Mol Microbiol 81:705–716

Berta G, Sampo S, Gamalero E, Massa N, Lemanceau P (2005) Suppression of Rhizoctonia root-rot of tomato by *Glomus mosseae* BEG12 and *Pseudomonas fluorescens* A6RI is associated with their effect on the pathogen growth and on the root morphogenesis. Eur J Plant Pathol 111:279–288

Bharadwaj DP, Lundquist P-O, Persson P, Alström S (2008a) Evidence for specificity of cultivable bacteria associated with arbuscular mycorrhizal fungal spores. FEMS Microbiol Ecol 65:310–322

Bharadwaj DP, Lundquist P-O, Alström S (2008b) Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. Soil Biol Biochem 40:2494–2501

Budi SW, Van Tuinen D, Martinotti G, Gianinazzi S (1999) Isolation from *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soil-borne fungal pathogens. Appl Environ Microbiol 65:5148–5150

Carpenter-Boggs L, Loynachan TE, Stahl PD (1995) Spore germination of *Gigaspora margarita* stimulated by volatiles of soil-isolated actinomycetes. Soil Biol Biochem 27:1445–1451

Cordier C, Pozo MJ, Barea JM, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defense responses associated with localised and systemic resistance to *Phytophthora parasitica* in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11:1017–1028

Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere micro-organisms. New Phytol 141:525–533

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Gullberg J, Jonsson P, Nordstrom A, Sjostrom M, Moritz T (2004) Design of experiments: an efficient strategy to identify factors influencing extraction and derivatization of *Arabidopsis thaliana* samples in metabolomic studies with gas chromatography/mass spectrometry. Anal Biochem 331:283–295

Gryndler M, Hrselova H, Striteska D (2000) Effect of soil bacteria on hyphal growth of the arbuscular mycorrhizal fungus *Glomus claroideum*. Folia Microbiol 45:545–551

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257

Jonsson P, Johansson A, Gullberg J, Trygg J, Jiye A, Grung B, Marklund S, Sjöström M, Antti H, Moritz T (2005) High through-put data analysis for detecting and identifying differences between samples in GC/MS-based metabolomic analyses. Anal Chem 77:5635–5642

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 44:301–307

Krishna KR, Bagyaraj DJ (1983) Interaction between a *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Can J Bot 61:2349–2351

Li B, Ravnskov S, Xie G, Larsen J (2007) Biocontrol of *Pythium* damping-off in cucumber by arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus*. Biocontrol 52:863–875

Linderman RG (2008) The mycorrhizosphere phenomenon. In: Feldman F, Kapulnik Y, Barr J (eds) Mycorrhiza works. Deutsche Phytomedizinische Gesellschaft, Braunschweig, pp 341–355. ISBN 978-3-941261-01-3

Lioussanne L, Jolicoeur M, St-Arnaud M (2008) Mycorrhizal colonization with *Glomus intraradices* and development stage of transformed tomato roots significantly modify the chemotactic response of zoospores of the pathogen *Phytophthora nicotianae*. Soil Biol Biochem 40:2217–2224

Mansfeld-Giese K, Larsen J, Bødker L (2002) Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microbiol Ecol 41:133–140

Mark GL, Cassells AC (1996) Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). Plant Soil 185:233–238

Mayo K, Davis RE, Motta J (1986) Stimulation of germination of spores of *Glomus versiforme* by spore-associated bacteria. Mycologia 78:426–431

Meyer JR, Linderman RG (1986a) Selective influence on populations of rhizosphere or rhizosplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol Biochem 18:191–196

Meyer JR, Linderman RG (1986b) Response of subterranean clover to dual-inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida*. Soil Biol Biochem 8:185–190

Mosse B (1962) The establishment of vesicular–arbuscular mycorrhiza under aseptic conditions. J Gen Microbiol 27:509–520

Nazir R, Warmink JA, Boersma H, Van Elsas JD (2010) Mechanisms that promote bacterial fitness in fungal-affected soil microhabitats. FEMS Microbiol Ecol 71:169–185

Norman JR, Atkinson D, Hooker JE (1996) Arbuscular mycorrhizal fungal-induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. Plant Soil 185:191–198

Norman JR, Hooker JE (2000) Sporulation of *Phytophthora fragariae* shows greater stimulation by exudates of nonmycorrhizal than by mycorrhizal strawberry roots. Mycol Res 104:1069–1073

Offre P, Pivato B, Mazurier S, Siblot S, Berta G, Lemanceau P, Mougel C (2008) Microdiversity of Burkholderiales associated with mycorrhized and non-mycorrhized roots of *Medicago truncatula*. FEMS Microbiol Ecol 65:180–192

Pivato B, Gamalero E, Lemanceau P, Berta G (2008) Colonization of adventitious roots of *Medicago truncatula* by *Pseudomonas fluorescens* C7R12 as affected by arbuscular mycorrhiza. FEMS Microbiol Lett 289:173–180

Pivato B, Offre P, Marchelli S, Barbonaglia B, Mougel C, Lemanceau P, Berta G (2009) Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. Mycorrhiza 19:81–90

Rambelli A (1973) The rhizosphere of mycorrhizae. In: Marks GL, Koslowski TT (eds) Ectomycorrhizae: their ecology and physiology. Academic, New York, pp 299–343

Ravnskov S, Nybroe O, Jakobsen I (1999) Influence of an arbuscular mycorrhizal fungus on *Pseudomonas fluorescens* DF57 in rhizosphere and hyphosphere soil. New Phytol 142:113–122

Rosendahl S (1985) Interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* and *Aphanomyces eutieches* root rot of peas. Phytopathologische Zeitschrift 114:31–41

Rosenfeld HJ, Vogt G, Aaby K, Olsen E (2004) Interaction of Terpenes with Sweet Taste in Carrots (*Daucus carota* L.). In: McCreight JD and Ryder EJ (eds), Acta Hort. 637, pp 377–386, Proc. XXVI IHC—Advances in Vegetable Breeding

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317

Schliemann W, Ammer C, Strack D (2008) Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. Phytochemistry 69:112–146

Sheveleva E, Bohnert HJ (1998) Plant stress adaptations—making metabolism move. Curr Opin Plant Biol 1:267–274

Singh R, Adholeya A, Mukerji KG (2000) Mycorrhiza in control of soil-borne pathogens. In: Mukerji KG, Chamalo BP, Singh J

(eds) Mycorrhizal biology. Kluwer Academic Plenum Publishers, New York, pp 173–196

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, London

St-Arnaud M, Hamel C, Vimard B, Caron M, Fortin JA (1996) Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an in vitro system in absence of host roots. Mycol Res 100:328–332

Stockinger H, Walker C, Schüβler A (2009) *Glomus intraradices* DAOM 197198, a model fungus in arbuscular mycorrhiza research is not *Glomus intraradices*. New Phytol 183:1176–1181

Toljander J, Lindahl B, Paul L, Elfstrand M, Finlay R (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304

Trotta A, Varese GC, Gnavi E, Fusconi A, Sampo S, Berta G (1996) Interactions between the soilborne root pathogen *Phytophthora nicotianae* var *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. Plant Soil 185:199–209

Whipps JM (2004) Prospects and limitations for mycorrhiza in biocontrol of root pathogens. Can J Bot 82:1198–1227

Zambolim L, Schenck NC (1983) Reduction of the effects of pathogenic root rot infecting fungi on soybean by the mycorrhizal fungus *Glomus mosseae*. Phytopathol 73:1402–1405