

Impact of antifungals producing rhizobacteria on the performance of *Vigna radiata* in the presence of arbuscular mycorrhizal fungi

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Abstract Plant growth-promoting rhizobacteria (PGPR) that produce antifungal metabolites are potential threats for the arbuscular mycorrhizal (AM) fungi known for their beneficial symbiosis with plants that is crucially important for low-input sustainable agriculture. To address this issue, we used a compartmented container system where test plants, *Vigna radiata*, could only reach a separate nutrient-rich compartment indirectly via the hyphae of AM fungi associated with their roots. In this system, where plants depended on nutrient uptake via AM symbiosis, we explored the impact of various PGPR. Plants were inoculated with or without a consortium of four species of AM fungi (*Glomus coronatum*, *Glomus etunicatum*, *Glomus constrictum*, and *Glomus intraradices*), and one or more of the following PGPR strains: phenazine producing (P^+) and phenazine-less mutant (P^-), diacetylphloroglucinol (DAPG) producing (G^+) and DAPG-less mutant (G^-)

strains of *Pseudomonas fluorescens*, and an unknown antifungal metabolite-producing *Alcaligenes faecalis* strain, SLHRE425 (D). PGPR exerted only a small if any effect on the performance of AM symbiosis. G^+ enhanced AM root colonization and had positive effects on shoot growth and nitrogen content when added alone, but not in combination with P^+ . D negatively influenced AM root colonization, but did not affect nutrient acquisition. Principal component analysis of all treatments indicated correlation between root weight, shoot weight, and nutrient uptake by AM fungus. The results indicate that antifungal metabolites producing PGPR do not necessarily interfere with AM symbiosis and may even promote it thus carefully chosen combinations of such bioinoculants could lead to better plant growth.

Keywords Antifungal-producing bacteria · PGPR · Mycorrhiza helper bacteria · AMF

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Introduction

The rhizosphere is a dynamic environment harboring a large and diverse community of prokaryotic and eukaryotic microbes that interact with each other and with the plant root. One member of this community can affect the growth and physiology of the others, as well as the physical and chemical properties of the soil. The sum of these interactions influences not only plant mineral nutrient acquisition but also the tolerance of plants to adverse chemical soil conditions and susceptibility to diseases (Rainey 1999). In particular, one group of bacteria—collectively called plant growth-promoting rhizobacteria (PGPR)—can stimulate plant growth through direct or indirect interactions with roots (Kloepper 1993). Antagonistic PGPR suppress plant pathogens, through competition for carbon and nitrogen as

nutrients or signals, competition for iron, preemptive competitive (niche) pathogen exclusion from favored sites of infection courts, and induction of systemic resistance or direct pathogen inhibition by antimicrobial compounds (antibiotics, hydrogen cyanide, etc.). Some PGPR can also directly stimulate plant growth by increasing the availability of mineral nutrients or the levels of phytohormones (Thomashow and Weller 1995; Whipps 2001; Haas and Keel 2003; Dwivedi and Johri 2003; Haas and Défago 2005; Raaijmakers et al. 2006; Weller et al. 2007; Rezzonico et al. 2007). Strains of *Pseudomonas fluorescens* that produce the polyketide antibiotic 2,4-diacetylphloroglucinol (DAPG) are some of the most effective PGPR controlling root and seedling diseases. For example, *P. fluorescens* CHA0 suppresses black root rot of tobacco, crown and root rot of tomato, *Pythium* damping off of cucumber, and take all of wheat (Keel et al. 1992; Schnider-Keel et al. 1995; Landa et al. 2002). In some soils, DAPG producers also play a key role in the natural suppression of take-all disease of wheat, known as take-all decline (Raaijmakers and Weller 1998; Raaijmakers et al. 1999; Haas and Défago 2005). Phenazine-1-carboxylic acid producing *P. fluorescens* 2–79 also suppresses take all of wheat by inhibiting *Gaeumannomyces graminis* var. *tritici* (Bull et al. 1991). Besides these bacteria, a group of fungi beneficial to plants, the arbuscular mycorrhizal (AM) fungi in the phylum Glomeromycota, form symbiotic associations with the roots of most terrestrial plants (Schussler et al. 2001; Toljander et al. 2006). The extraradical hyphae of the AM fungi explore the soil by an extended network, thereby increasing the nutrient absorption surface area of their host plant (Rhodes and Gerdemann 1975). Conversely, they also act as conduits for the transport of energy-rich assimilates from the plants to the soil microflora that may enhance unlocking of secluded mineral nutrients (Mäder et al. 2000; Hodge 2001; Oehl et al. 2003; Johnson et al. 2002; Toljander et al. 2006) and water acquisition (Ebel et al. 1996). It has been suggested that bacteria in the mycorrhizosphere influence the growth and establishment of AM fungi by stimulating spore germination (Xavier and Germida 2003) and root colonization (Budi et al. 1999). Moreover, it has been shown that these interactions are facilitated by the close proximity of bacteria to the fungus, but little is known about the influence of such associations in plants when different antifungal-producing bacteria interact with AM fungi. In addition, it has been shown that PGPR can invade and colonize the exterior and interior of AM fungal spores (Levy et al. 2003).

Any application of antagonistic antifungal-producing PGPR such as *Pseudomonas* spp. as biocontrol agents in the field requires particular attention because of the possibility that it may antagonize not only fungal phyto-

pathogens but also the beneficial AM fungi (Barea et al. 1998; Vázquez et al. 2000; Gaur et al. 2004). Nevertheless, combinations of AM fungi and PGPR suitable as biocontrol agents and biofertilizers enhancing both plant health and growth may be found (Akköprü and Demir 2005). A mixed inoculum of pseudomonads and AM fungi has been reported to promote tomato growth due to improved P acquisition (Gamalero et al. 2004). Enhanced knowledge regarding the beneficial interactions of plants with AM fungi and PGPR is of special importance for low-input sustainable agriculture that relies on biological processes and resources, rather than on the use of agrochemicals for maintaining soil fertility and plant health (Smith and Read 1997; Artursson et al. 2006).

The aim of the present work was to investigate if the symbiotic performance of green gram, *Vigna radiata* (L.) R. Wilczek, with AM fungi is affected by bacteria that produce antifungal compounds, namely the phenazine-producing *P. fluorescens* 2–79, the DAPG producing *P. fluorescens* CHA0rif and their respective mutants deficient in the bacterium of these antifungals, and the *Alcaligenes faecalis* SLHRE 425 that was newly isolated from the wheat rhizosphere and produces an unknown antifungal compound. Phenazines are broad-spectrum antifungal compounds that are active against phytopathogenic fungi and other bacteria (Boronin and Thomashow 1998), whereas DAPG is a broad-spectrum antibiotic that exhibits antifungal, antibacterial, antihelminthic, and phytotoxic activities (Haas and Défago 2005). Both groups of compounds have proved to be effective in suppressing *Gaeumannomyces* var. *tritici*, the cause of take-all disease of wheat (Schnider-Keel et al. 1995).

We have used a compartmented container system (Wyss et al. 1991) consisting of a root-free nutrient-rich middle compartment separated by a fine screen (30 mm mesh) from nutrient deficient side root compartments. Since the large compartment is accessible to the hyphae only, a large mycorrhiza dependency of the plants is expected, and thus, the impact of the PGPR on the AM symbiosis performance, if any, should be much more pronounced and more easily recognizable than in a simple pot system. While it is certainly also possible for nutrients to pass through the mesh walls into the plant compartments, experience has shown that such a transfer is generally negligible, in particular in the case of phosphorus, as compared to the more efficient transport of nutrients by means of the fungal hyphae. One factor underlying this phenomenon is the method by which the plants were watered. They are watered in the side compartments only, and hence, the mass flow of water is directed inward toward the central compartment, minimizing the outward transport of dissolved nutrients by mass flow. This allowed us to

specifically study the influence of PGPR on AM functionality in terms of plant nutrient acquisition, root colonization, and soil microbial population structure in a laboratory setting (Mäder et al. 2000).

Materials and methods

Container system

The compartmented container system (Wyss et al. 1991) used in this study is illustrated in Fig. 1. The side compartments containing the test plants were inoculated or not with AM fungi (root hyphal compartment, RHC), while the middle compartment was accessible only to the fungal hyphae and not the plant roots (hyphal compartment, HC) as it was separated from the RHC by a nylon screen (30 mm mesh; Zurcher Beuteluchfabrik, Ruschlikon, Switzerland) that could be penetrated by the AM hyphae but not by the roots. The RHC on both sides were further subdivided into five subcompartments, which allowed sampling of individual plants without disturbing neighboring ones.

Microbial inoculants

The bioinoculants used are listed in Table 1.

Microbial wash

All plants received a microbial wash containing an indigenous microbial population to maintain the natural microbial community in addition to the added inoculants.

The microbial wash was prepared from 60 g of indigenous wheat soil and, additionally, to ascertain the supply of compatible rhizobia, from 60 g of soil taken from pots in which green gram inoculated with its rhizobia had been grown. Each of these soil samples was stirred with 600 ml sterile water until homogeneous soil suspensions were obtained; the suspensions were then filtered through Whatmann No. 1 paper holding back all AM fungal propagules. This soil filtrate, the microbial wash, was added at a ratio of filtrate from 1 g of soil per 1 kg of soil substrate.

Culture conditions and soil inoculation

Bacterial cultures were grown overnight in King's B medium (20 g Proteose peptone (Fluka, Switzerland), 1 g K_2HPO_4 , 0.4 g $MgSO_4$, 8 ml glycerol in 1,000 ml distilled water) at 28°C on a rotary shaker (200 rpm). Cells were harvested by centrifugation for 10 min at 6,000×g and resuspended in 0.85% NaCl. Following two washes with 0.85% NaCl, the cells were resuspended in sterile distilled water. The optical density of the suspension was measured in a spectrophotometer (Shimadzu, Japan) to quantify cell number (an OD_{600} of 0.125 corresponded to 10^8 cells/ml). A volume of 1.25 ml (0.4 OD_{600}) of bacterial suspension was inoculated per 100 g of soil in each of the ten side compartments.

Mycorrhizal inoculum consisted of air-dried soil containing all four AM fungi (Table 1) in equal amounts (infective propagules) in the form of AM fungal hyphae, spores, and AM-colonized roots of *Tagetes* sp. in a mixture of equal volumes of quartz sand and Lecadan clay (Wyss et al 1991). A nutrient poor subsoil from Therwil, Switzerland

Fig. 1 The container system consisting of a central nutrient-rich, hyphal compartment (HC) plus a series of root hyphal compartment (RHC) at both sides in which the test plants; *V. radiata* (L.) R. Wilczek, were grown in a nutrient deficient substrate. The compartments were separated by a 30-mm nylon mesh, allowing the passage of AM hyphae, but preventing the passage of roots. TCP tricalcium phosphate

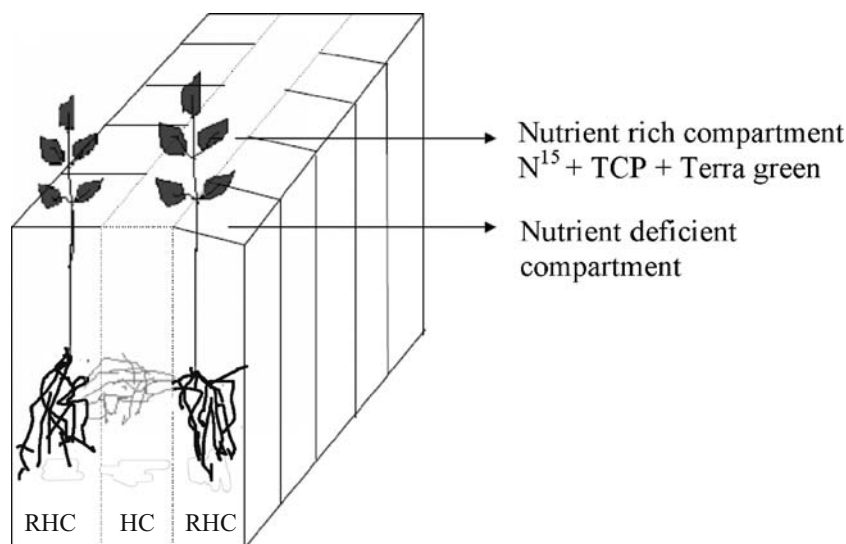


Table 1 The list of bioinoculants used in this study

Inoculants used	Properties	Abbreviations used
<i>Pseudomonas fluorescens</i> 2–79 ^a	Phenazine producer	P ⁺
<i>Pseudomonas fluorescens</i> 2–79 D::lacZ ^a	Phenazine negative mutant	P [−]
<i>Pseudomonas fluorescens</i> CHA0rif ^a	DAPG producer	G ⁺
<i>Pseudomonas fluorescens</i> CHA630 ^a	DAPG negative mutant	G [−]
<i>Alcaligenes faecalis</i>	Unknown antifungal producer, select isolate	D
Consortium of all bacteria	P ⁺ P [−] G ⁺ G [−] D	B
<i>Glomus coronatum</i> ^b , <i>G. etunicatum</i> ^b , <i>G. constrictum</i> ^c , and <i>G. intraradices</i> ^c	Mycorrhizal consortium ^d	M

^a Antibiotic selection characteristics for these strains were as follows: 2–79 and CHA630, 25 µg/ml of kanamycin and 40 µg/ml of ampicillin; 2–79 D::lac Z and CHA0rif, 100 µg/ml of rifampicin and 40 µg/ml of ampicillin

^b From TERI, New Delhi

^c From the Botanical Institute, Basel

^d The four different AM fungi were mixed in equal amounts

was homogenized by sieving (2 mm). This soil, washed quartz sand (Type K 30, size 0.2–0.5 mm, Trafor AG, Basel, Switzerland), and Terragreen were autoclaved separately at 121°C overnight. All ten RHC on both sides of the compartmented units were filled with 100 g sand/soil mixture (1:1, w/w). In the HC, the middle compartment, a mixture of 500 g containing Terragreen/sand/soil (1:1:1, w/w), supplemented with 20 g ¹⁵N manure + hay (Langmeier et al. 1998) and tricalcium phosphate (100 mg), was prepared and placed in the middle compartment. The compartment systems were saturated with (sterile) water by soaking them from the bottom, until the soil appeared wet at the top surface.

Green gram, *V. radiata* (L.) R. Wilczek, seeds were soaked with water on a wet tissue paper for 24 h to germinate. Four or five germinated seeds were planted into the side chambers, with 6 g of mycorrhizal inoculum and 1.25 ml of bacterial suspension. Nonmycorrhizal controls received only the bacterial suspensions whereas mycorrhizal controls received only the mycorrhizal inoculum. In addition, 1 ml aliquots of a microbial wash (refer as above) were applied to all compartments, to provide an equal general bacterial background population.

Nonmycorrhizal and mycorrhizal green gram plants were grown in the RHC. Each experiment consisted of eight treatments, with four replicates per treatment.

In each treatment, plants were fertilized six times per week with 3 ml of one fifth of modified Hoagland solution without phosphorus by irrigation in the RHC. Full-strength modified Hoagland solution (Hoagland and Arnon 1950) was applied once per treatment to the saturation level in the middle chamber. The modified Hoagland's solution consisted of *macroelements* (per ml) 0.5 g KNO₃, 0.11 g Ca(NO₃)₂·4H₂O, 0.13 g KH₂PO₄,

0.49 g MgSO₄·7H₂O, and 0.2 g Sequestren (Fe-EDTA) and *microelements* (per ml) 0.3 mg MnSO₄·1H₂O, ZnSO₄·7H₂O, 0.04 mg CuSO₄·5H₂O, 1 mg Na₂B₄O₇·10H₂O, and 0.05 mg (NH₄)₆Mo₇O₂.

Plants were grown in a green house from mid-May to mid-August (13 weeks), at a temperature between 18°C and 25°C. Supplementary light was used to extend the day length to 14 h. The position of growth containers in the glasshouse was rerandomized at 3 days intervals.

Sampling strategy

Total roots from two plants of each of four replicates were cut and shaken gently to remove adhered soil particles. The roots were weighed, and the shoots were dried in an oven at 80°C for 2 days. Roots (0.5 g) were crushed in 10 ml of water and serially diluted up to 10^{−6} for plating. The remaining root fraction was washed with tap water, pat-dried with tissue paper, weighed, and cut into 1 cm pieces and kept for mycorrhizal root staining in 0.1% KOH overnight. Plants were harvested 13 weeks after planting when they were at fruiting stage.

Mycorrhizal root staining

The root staining method that was modified by Phillips and Hayman (1970) was used. A weighed fresh root sample from each treatment was stained with trypan blue in lactophenol according to Phillips and Hayman (1970). The percentage of root length colonization by AM fungi was determined microscopically using the gridline intersection method of Giovannetti and Mosse (1980).

Nutrient analysis

The uptake of nutrients, mainly phosphorus and nitrogen, is greatly aided by mutualistic associations with AM fungi. These fungi extend long hyphal threads outside the roots transporting phosphorus and other nutrients into the plant roots. In order to follow the uptake, we supplied ^{15}N in the nutrient-rich middle compartment as well as phosphorus-rich terra green and tricalcium phosphorus such that the fungal hyphae could extend from the side compartment into the middle compartment and thus provide nutrients to the roots of the plants growing in the side compartments.

Analysis of phosphorus content

Dry shoot matter was milled, digested, and analyzed for P content using the method of Watanabe and Olsen (1965) and Onishi et al. (1975).

Analysis of nitrogen and ^{15}N content

Shoots were ground to a fine powder using a ball mill, and total N content was measured using a Leco CHN analyzer. ^{15}N was determined by mass spectrophotometry using the ANCA-SL/GSL at the Analytical Service of Europa Scientific Ltd., Crewe, UK. ^{15}N enrichments of green gram shoots were calculated by subtracting the natural ^{15}N abundance control (0.366%).

Statistical analysis

There were four replicates per treatment with two plants per container system, giving a total of 32 assemblies, in a randomized block. Treatments were analyzed by one-way analysis of variance. Differences were considered as statistically significant at $P < 0.05$. Total performance of the green gram plants in response to different treatments was analyzed with principal component analysis (PCA) with the use of the statistical package Plus 6.1 software (Insightful Corp., USA). The PCA plot with correlation scaling was used to analyze the relationship between the different treatments and the different plant attributes. In this analysis, the treatments are defined as the objects and the different plant attributes as the descriptors (represented by the vectors) of the multivariate analysis. Since descriptors were measured in different units, they were standardized before the analysis. The PCA is presented as a two-dimensional correlation plot (Fig. 7).

Results

Effect of mycorrhiza on the *Pseudomonas* population

The mycorrhizal control, M, had a significantly higher population than *P. fluorescens* 2–79 D::lacZ-treated mycorrhizal plants (MP⁻). There was no significant difference in the total pseudomonad population for other treatments. However, all mycorrhiza (M)-treated bacterial plants: SLHRE425 (D), *P. fluorescens* 2–79 (P⁺), *P. fluorescens* CHA0 (G⁺), *P. fluorescens* CHA0rif (G⁻), and P⁺G⁺ had lower colony forming units than nonmycorrhizal bacterial control treatments, B (Fig. 2). The nonmycorrhizal control, B, had maximum counts ($2.21 \pm 0.21 \times 10^8$). The lowest counts were observed for MD ($2.93 \times 10^7 \pm 2.03$ cells/g of soil; Fig. 2).

Effect of treatments on plant growth

The root fresh weight was significantly highest in MG⁺ plants, compared to MG⁻ plants as well as compared to the rest of the treatments and the nonmycorrhizal control (Fig. 3a). The values of root dry weight were not significantly different for the remaining treatments.

MG⁺ plants had a significantly higher shoot dry weight than the MP⁺, MD, and the MP⁺G⁺ consortium plants. The shoot dry weights of MP⁺ and MP⁺G⁺ were significantly

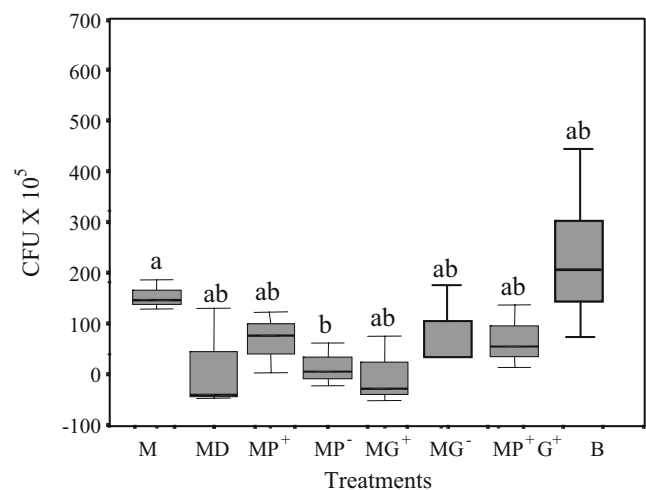
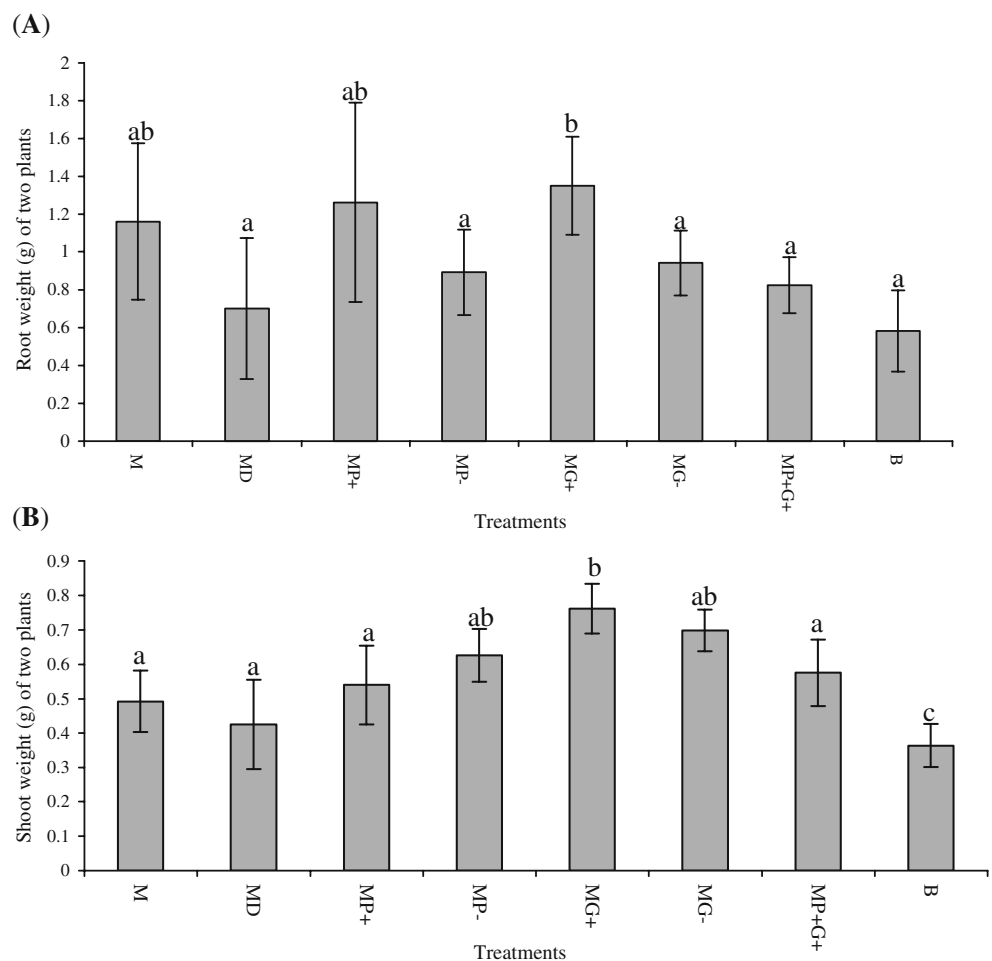


Fig. 2 The effect of mycorrhiza on the development of various antifungal-producing PGPR populations in the green gram (*V. radiata*) rhizosphere established after a plant growth period of 13 weeks in the root hyphal compartment. M plants with mycorrhiza only, D (SLHRE425; unknown antifungal producer), P⁺ (2–79; Phz⁺), P⁻ (2–79 D::lacZ; Phz⁻), G⁺ (CHA0rif; DAPG⁺), G⁻ (CHA630; DAPG⁻), B (all bacteria). Box plots indicate median values (lines in boxes), 75th and 25th percentiles (limits of the box). Upper and lower horizontal bars indicate 10th and 90th percentiles, respectively. CFU colony forming units. Different lowercase letters above boxes indicate a significant difference between treatments ($P < 0.05$, $n = 4$)

Fig. 3 The effect of different antibiotic metabolite-producing bacteria on the growth of mycorrhizal green gram plants grown for 13 weeks in root hyphal compartment. Root fresh weight (a) and shoot dry weight (b) of two plants per container system. M plants with mycorrhiza only, D (SLHRE425; unknown antifungal producer), P^+ (2-79; Phz⁺), P^- (2-79 D::lacZ; Phz⁻), G^+ (CHA0rif; DAPG⁺), G^- (CHA630; DAPG⁻), B (all bacteria). The error bars represent standard errors of means of root and shoot weight, respectively ($n=4$). Different lowercase letters above the bars indicate significant differences between the treatments ($P<0.05$)



higher than that of the nonmycorrhizal control, B, although it was not significantly different from M. There was no significant difference in shoot dry weight of green gram plants versus the other treatments (Fig. 3b).

Effect on mycorrhizal colonization

MG^+ had a highly significant positive influence on mycorrhizal colonization (average of 67.75%; Fig. 4) as compared to MG^- and other treatments. The vesicles and arbuscules were also observed in higher numbers (data not shown). In contrast, MG^- and MD gave significantly lower percentages of mycorrhizal colonization of 34.5% and 28.41%, respectively. MP^+ also resulted in significantly lower percentage of mycorrhizal colonization (40.75%) compared to MG^+ but there was no significant difference to the mycorrhizal control, M (44.32%). Interestingly, MP^+G^+ consortium inoculated plants had a reduced mycorrhization as compared to the individually inoculated MP^+ and MG^+ plants. MD had significantly lower mycorrhizal colonization (28.42%).

Effect on nutrient content (nitrogen and phosphorus) in plant shoots

MG^+ and MP^+ plants had a significantly higher P content than MD. The consortium MP^+G^+ induced a significantly decreased P content compared to four of the individually inoculated mycorrhizal plants (Fig. 5). The nonmycorrhizal control (B) had significantly lower P content compared to all mycorrhizal plants with the exception of the MP^+G^+ plants (Fig. 5).

MG^+ had a significantly higher N content than MP^+ (Fig. 6a) although it was not significantly different from MG^- and MP^- . MP^+G^+ consortium had significantly lower N content than MG^+ . MG^+ and MG^- had significantly higher N contents compared to all other treatments except to MP^- . Nonmycorrhizal control and mycorrhizal controls were nonsignificantly different from each other. MD had a significantly lower N content than MP^+ and MG^+ (Fig. 6a).

MG^+ had a significantly higher N^{15} content than MP^+ , MD, and mycorrhizal control M. MP^+G^+ consortium had a significantly lower N content than MG^+ . Moreover, the

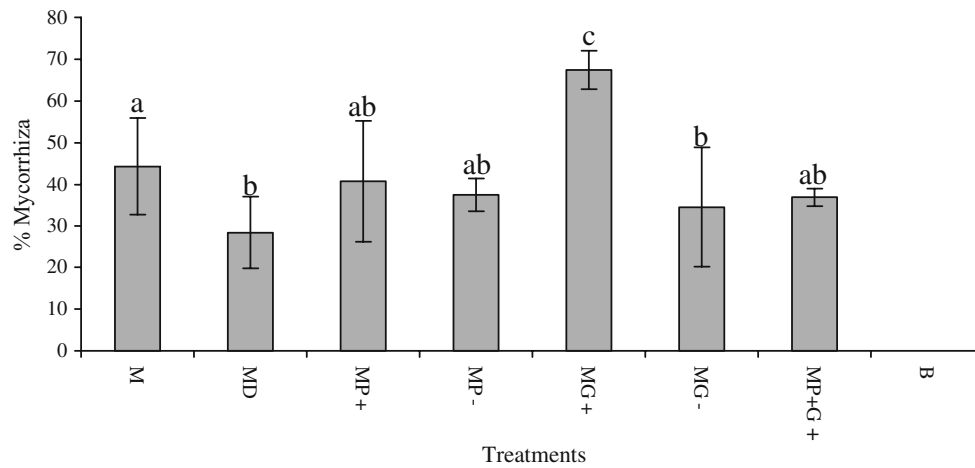


Fig. 4 The percent mycorrhizal colonization of green gram roots grown for 13 weeks in a root hyphal compartment. *M* plants with mycorrhiza only, *D* (SLHRE425; unknown antifungal producer), *P*⁺ (2–79; Phz⁺), *P*⁻ (2–79 *D*::*lacZ*; Phz⁻), *G*⁺ (CHA0rif; DAPG⁺), *G*⁻

(CHA630; DAPG⁻), *B* all bacteria. Error bars represent standard errors of means of percent mycorrhiza, respectively ($n=4$). Different lowercase letters above the bars indicate significant differences between the treatments ($P<0.05$)

nonmycorrhizal control *B* had a significantly lower N¹⁵ content than rest of the treatments except mycorrhizal control *M* and *MD* (Fig. 6b).

Principal component analysis

In order to determine the effects of various treatments and the correlation of different functional parameters with each other, principal component analysis with correlation scaling was performed and is shown in Fig. 7 as a two-dimensional plot (Pielou 1984; Garland and Mill 1991; Legendre and Legendre 1998). The first component or principal axis accounts for 69.4% whereas the second component or axis

is 18.61%. The vectors representing ¹⁵N content, N content, and shoot weight were oriented in the same direction and were significantly positively correlated. The vectors for three other factors, P content, root weight, and % mycorrhization were positively correlated with each other. Moreover, MP⁺ treatment was positively associated with these factors. MG⁺ treatment was associated positively with all factors. Indeed, the orthogonal projection of an object (treatment) on to a descriptor (vector representing different plant attributes) allows an assessment of the correlation between the object and the descriptor. Thus *P. fluorescens* CHA0rif (*G*⁺) act as mycorrhiza enhancing bacteria. It seems that DAPG had a beneficial impact affording

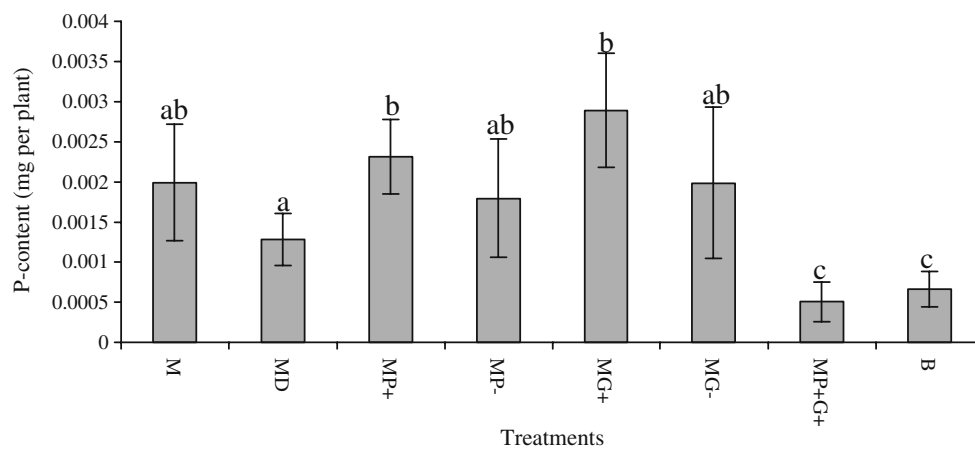
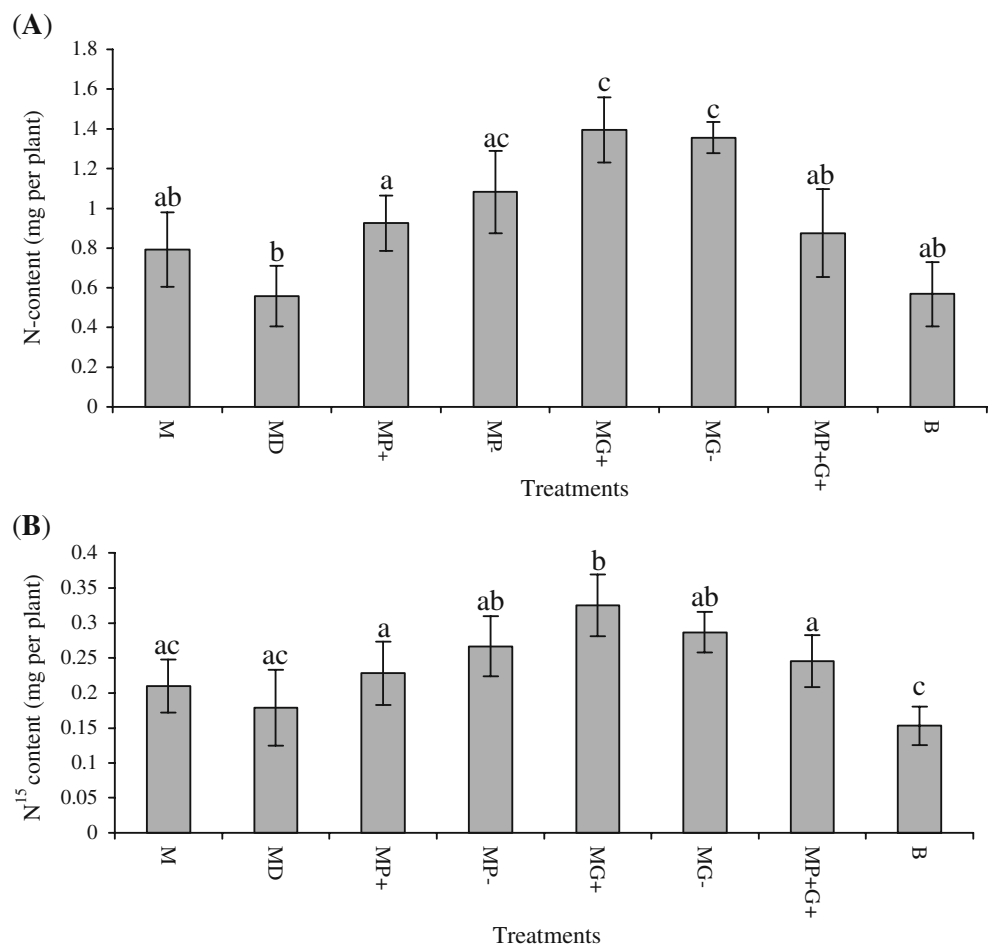


Fig. 5 The effect of different antibiotic metabolite-producing bacteria on the phosphorus content of shoots of green gram plants grown for 13 weeks in root hyphal compartments. *M* plants with mycorrhiza only, *D* (SLHRE425; unknown antifungal producer), *P*⁺ (2–79; Phz⁺), *P*⁻ (2–79 *D*::*lacZ*; Phz⁻), *G*⁺ (CHA0rif; DAPG⁺), *G*⁻ (CHA630;

DAPG⁻), *B* all bacteria. The error bars represent standard errors of means of P content, respectively ($n=4$). Different lowercase letters above the bars indicate significant differences between the treatments ($P<0.05$)

Fig. 6 The effect of different antibiotic metabolite-producing bacteria on the N content of shoots of green gram plants grown for 13 weeks in root hyphal compartments **a** N content and **b** ^{15}N content. The error bars represent standard errors of means of N content, respectively ($n=4$). *M* plants with mycorrhiza only, *D* (SLHRE425; unknown antifungal producer), *P*⁺ (2-79; Phz⁺), *P*⁻ (2-79 D::lacZ; Phz⁻), *G*⁺ (CHA0rif; DAPG⁺), *G*⁻ (CHA630; DAPG⁻), *B* all bacteria. The error bars represent standard errors of means of N content, respectively ($n=4$). Different lowercase letters above the bars indicate significant differences between the treatments ($P<0.05$)



increased plant weight, mycorrhization, and nutrient content. Similarly, phenazine production does not have a deleterious effect on mycorrhizal fungi and benefited plant growth. In contrast, the MD and MP⁺G⁺ treatments had the least effect upon % mycorrhization, root weight, P content, N content, and shoot weight.

Discussion

The comparative study of antifungal metabolite-producing bacteria suggests that mycorrhizal–rhizobacterial interactions could bring about subtle changes in the mycorrhizosphere. In the present work, total pseudomonad count was higher in nonmycorrhizal control treatment suggesting a suppressive impact of the AM symbiosis on the bacterial population. Various reports have indicated an influence of AM symbiosis on the microbial community structure in the rhizosphere (Meyer and Linderman 1986a, b; Schreiner et al. 1997; Ravnskov et al. 1999; Linderman 2000). Paulitz and Linderman (1989) found a decrease in numbers of *P. fluorescens* in the rhizosphere of cucumber in the presence of *Glomus intraradices* and *Glomus etunicatum*.

Plants were most benefited when they were treated with both PGPR and mycorrhiza. This was best visualized through PCA that indicates the degree to which plant attributes were affected by various treatments (Fig. 7). There were positive correlations within the treatments for mycorrhizal colonization, P content, and root weight. N content, ^{15}N content, and shoot weight were also positively correlated with each other. This again indicates that nutrient uptake increases concomitantly with better mycorrhizal colonization.

AM colonization was greatly affected by a change in the microbial population. “Mycorrhiza-helper bacteria” (MHB) have been described for the ectomycorrhizal symbiosis (Garbaye 1994) and only a few examples of MHB have been reported for the AM symbiosis (Toro et al. 1997; Singh and Kapoor 1998). The mechanisms by which these bacteria stimulate AM colonization are still poorly understood, but certain bacterial activities, such as the production of metabolites increasing root cell permeability and hormone synthesis, are expected to be involved (Barea et al. 1998). Meyer and Linderman (1986a, b) have previously emphasized that pseudomonads in the rhizosphere are a diverse group of bacteria and their interactions with plants

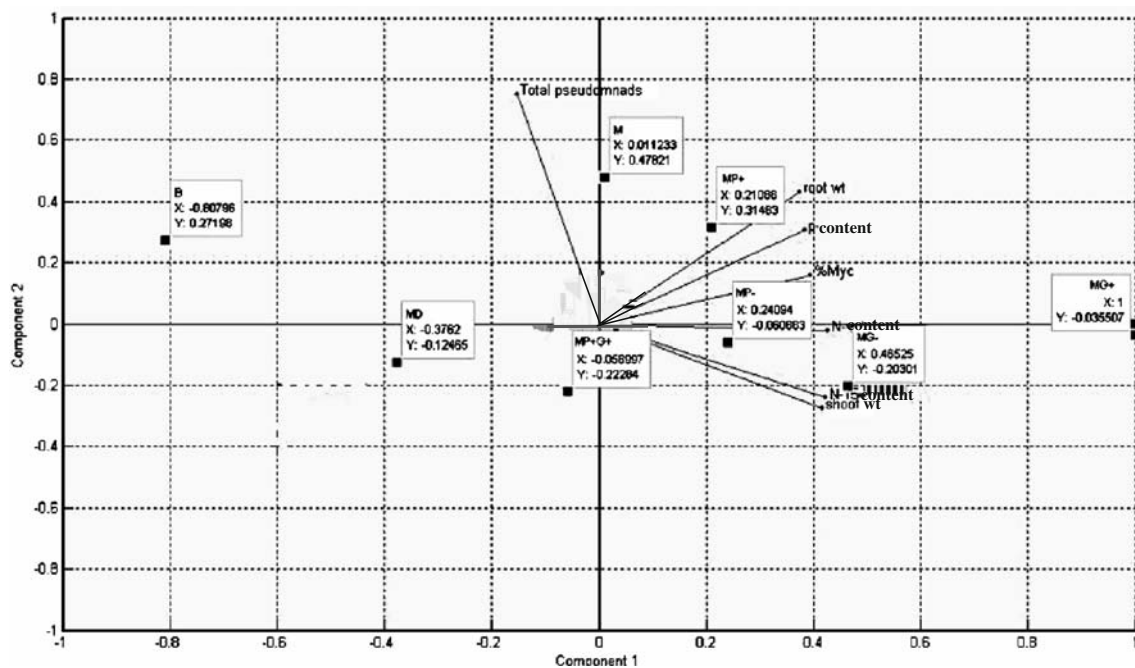


Fig. 7 Principal component analysis (correlation scaling) of all of the samples. It was performed using Matlab 7.0.1. The plant data were normalized before the analysis. Descriptors (arrows) indicate the shoot weight, ^{15}N content, % mycorrhization, P content, N content, root weight, and total pseudomonad count and objects are the treatments.

M plants with mycorrhiza only, *D* (SLHRE425; unknown antifungal producer), P^+ (2–79; Phz⁺), P^- (2–79 D::lacZ; Phz⁻), G^+ (CHA0rif; DAPG⁺), G^- (CHA630; DAPG⁻), *B* average value of all bacterial single controls, *PC1* principal component axis 1, *PC2* principal component axis 2

and mycorrhizal fungi will differ between strains, e.g., Bowen and Theodorou (1979) observed a fluorescent pseudomonad markedly suppressing the development of several ectomycorrhizal fungal species in *Pinus* roots. Mycorrhizal root colonization was significantly stimulated by G^+ which has also been previously found using various other bacteria (Azcón-Aguillar and Barea 1995; Barea et al. 1998; Vosátka and Gryndler 1999; Duponnois and Plenclhett 2003). DAPG production appears to have a positive influence on mycorrhization as the DAPG negative mutant G^- induced a significantly decreased AM colonization. G^+ has been reported to act as MHB (Garbaye 1994; Frey-Klett et al. 1997) exerting a positive effect on mycorrhizal associations. P^+ brought about a significant decrease in AM colonization compared to MG^+ but a nonsignificant one compared to the mycorrhizal control (*M*) indicating that its influence was noninhibitory. The mutant (P^-), however, induced a significantly reduced AM colonization. Also, *MD* had a negative effect on AM colonization. Interestingly, the consortium MP^+G^+ induced a decreased colonization. DAPG producer, G^+ , alone stimulated mycorrhizal colonization but had no such impact when inoculated with the phenazine producer, P^+ , indicating a suppressive effect of the latter one. This indicates that antifungal-producing PGPR can behave as MHB but if there is an excessive antifungal pressure in the rhizosphere, a negative influence on the mycorrhization might occur.

All pseudomonad isolates singly inoculated had a positive impact on P content of the mycorrhizal plants compared to inoculation as consortium as well as to the singly inoculated *A. faecalis* treatment. The beneficial effect of AM fungi on plants for nutrient acquisition has been well documented (Duponnois and Plenclhett 2003; Govindarajulu et al. 2005) and recently, it has been suggested that in AM symbiosis, plants receive all their phosphorus via fungal symbionts (Harrison 2005). AM fungi and phosphorous solubilizing rhizobacteria could also have a synergistic effect on plant growth as reported previously (Linderman 1988; Barea and Jeffries 1995). Increased plant P content has also been ascribed to a release of plant hormones by rhizobacteria (Azcón et al. 1976), continuous release of PO_4^- ions by phosphate-solubilizing pseudomonads, and subsequent uptake by AM fungi (Toro et al. 1997). Since *A. faecalis* (*D*) is a nonphosphorus solubilizer, there was no such synergistic effect on P content. It is intriguing to note that the P content in MP^+G^+ consortium-treated plants was much smaller than in the other treatments of the mycorrhizal plants, a phenomenon that could be due the impact of the bacterial consortium in the mycorrhizosphere. It further indicates that for application, the right selection of bioinoculants is essential.

Higher shoot nitrogen contents in the G^+ -treated mycorrhizal green gram plants, compared to the nonmycorrhizal and mycorrhizal controls, demonstrate the close relation-

ship between AM colonization and N content. This increased N content in the MG⁺ plants might be due to the access of the plants to the nutrient-rich middle compartment via their associated AM fungi. However, unlike in the case of the plant P content, no such an increase in N content was found in the other treatments of the mycorrhizal plants. This could be due to the soil filtrate, which was added to ensure the presence of a native bacterial population that also included the appropriate rhizobia. This was done to provide the natural rhizospheric conditions to the plants. Rhizobia, by way of nitrogen fixation in the nodule symbiosis, are known to provide nitrogen to the plants and this capability is enhanced when plants are colonized by AM fungi (Ianson and Linderman 1993; Barea et al. 2002). It seems logical therefore that all mycorrhizal and nonmycorrhizal treatments did not have much difference in nitrogen uptake due to direct nitrogen fixation in the nodules. MG⁺ might have facilitated nitrogen uptake because of the enhanced root mycorrhization.

In summary, the CHA0rif (G⁺) treatment had a stimulatory effect on mycorrhizal root colonization probably due to the DAPG production. *P. fluorescens* 2–79 (P⁺) did not exert antifungal action against the different AM fungi used in this study. The consortium of both these bacteria induced a decreased AM colonization and plant P content compared to their single treatments. Hence, it indicates that even if the PGPR strains improve plant growth individually, they may not necessarily do so when other strains are present. This demonstrates the complex relationships between microorganisms and, as a consequence, the difficulties in preparing an efficient bacterial consortium for biofertilization products. On the basis of these observations and considering the importance of both rhizobacteria and rhizofungi, the release of microbial bioinoculants that produce antifungal metabolites deserves a more detailed analysis to determine possible interference with the performance of AM fungi.

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