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Interactions between the arbuscular mycorrhizal fungus *Glomus intraradices* and the plant growth promoting rhizobacteria *Paenibacillus polymyxa* and *P. macerans* in the mycorrhizosphere of *Cucumis sativus*

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ABSTRACT

Interactions between the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* and bacteria from the genus *Paenibacillus* (*P. macerans* and *P. polymyxa*) were examined in a greenhouse pot experiment with *Cucumis sativus* with and without organic matter amendment (wheat bran). *P. polymyxa* markedly suppressed AM fungus root colonization irrespective of wheat bran amendment, whereas *P. macerans* only suppressed AM fungus root colonization in combination with wheat bran amendment. Dual inoculation with *P. macerans* and *G. intraradices* in combination with wheat bran amendment also caused severe plant growth suppression. Inoculation with *G. intraradices* was associated with increased levels of dehydrogenase activity and available P in the growth substrate suggesting that mycorrhiza formation accelerated the decomposition of organic matter resulting in mobilization of phosphorus. Inoculation with both *Paenibacillus* species increased all measured microbial fatty acid biomarkers in the cucumber rhizosphere, except for the AM fungus biomarker 16:1ω5, which was reduced, though not significantly. Similarly, inoculation with *G. intraradices* increased all measured microbial fatty acid biomarkers in the cucumber rhizosphere, except for the Gram-positive bacteria biomarker 15:0 anteiso, which was overall decreased by *G. intraradices* inoculation. In combination with wheat bran amendment *G. intraradices* inoculation caused a 39% reduction in the amount of 15:0 anteiso in the treatment with *P. polymyxa*, suggesting that *G. intraradices* suppressed *P. polymyxa* in this treatment. In conclusion, plant growth promoting species of *Paenibacillus* may have suppressive effects of AM fungi and plant growth, especially in combination with organic matter amendment. The use of an inert plant growth media in the present study allowed us to study rhizosphere microbial interactions in a relative simple substrate with limited interference from other soil biota. However, the results obtained in the present work mainly show potential interactions and should not be directly extrapolated to a soil situation.

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1. Introduction

Microbial interactions in the mycorrhizosphere are complex both at the physical, metabolic and functional levels (Gryndler, 2000; Johansson et al., 2004; Barea et al., 2005; Artursson et al., 2006; Bending et al., 2006). Although, it is generally accepted that mycorrhizosphere microorganisms play a significant role in crop production, the interactions between arbuscular mycorrhizas and other soil microorgansims are still far from fully understood. Bacteria associated with the mycorrhizosphere have been suggested to be involved in plant growth and establishment of mycorrhizal fungi (Toljander et al., 2006), and species from the genus *Paenibacillus* seem to be associated with mycelium and spores of AM fungi (Mansfeld-Giese et al., 2002; Hildebrandt et al., 2002).

The genus *Paenibacillus* belongs to the group of Gram-positive spore forming bacteria. *Paenibacillus* is frequently recovered from rhizosphere soil (Guemori-Athmani et al., 2000; Von der Weid et al., 2000) and has been found associated with mycelium of both ecto-mycorrhizal fungi (Poole et al., 2001) and AM fungi (Mansfeld-Giese et al., 2002). Two species, *P. polymyxa* and *P. macerans*, show particularly interesting traits in relation to plant growth promotion, such as production of plant hormones (Timmusk et al., 1999), nitrogen fixation (Coelho et al., 2003), phosphorous solubilization

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(Viveganandan and Jauhri, 2000) and soil aggregation formation (Bezzate et al., 2000). Moreover, they have a broad range of enzymatic capabilities (Nielsen and Sorensen, 1997; Budi et al., 2000).

Arbuscular mycorrhizas are known to affect the composition of the microbial community, not only in the rhizosphere, but also to promote specific bacterial populations in the hyphosphere (Mansfeld-Giese et al., 2002). Isolates of *Paenibacillus* have been shown to improve the formation of arbuscular mycorrhizas and suppress a broad range of root pathogens (Budi et al., 1999; Li et al., 2007), suggesting that the disease control feature of arbuscular mycorrhizas may be accomplished in consortia with helper bacteria. Many researchers have studied the biotrophic nature of AM fungi and recently it has been shown that they are able to complete their life cycle in the absence of a host plant when accompanied by an isolate of *Paenibacillus* (Hildebrandt et al., 2002). These results show that AM fungi may be able to grow in the absence of a plant root with an alternative partner, which would radically change the present understanding of the role of AM in soil ecosystems and also open new possibilities for exploiting AM fungi in plant production.

The objective of the present study was to examine the interaction between two plant growth promoting isolates of *Paenibacillus* and the AM fungus *Glomus intraradices* with and without organic matter amendment, and to examine the influence of such microbial inoculations on plant growth, rhizospheric traits and background rhizosphere microorganisms measured as biomarker fatty acids and enzymatic activities.

2. Materials and methods

2.1. Experimental design

A greenhouse pot experiment was carried out with three main factors in a fully factorial design: (1) organic matter amendment in terms of wheat bran (WB) (two levels, without and with); (2) AM (two levels, without and with inoculation with the AM fungus *Glomus intraradices* (Gi)); and (3) *Paenibacillus* (*P.* spp.) (three levels, without inoculation and inoculation with *P. macerans* (Pm) or *P. polymyxa* (Pp)) giving a total of 12 treatments each with four replicates ($n = 48$).

2.2. Biological material and experimental set-up

Two *Cucumis sativus* L. seeds were sown in 100 ml plastic beakers with sterilized quartz sand and vermiculite (2/1; v/v). The different treatments with wheat bran, mycorrhiza and *Paenibacillus* were applied to the growth substrate prior to sowing. Treatments with wheat bran received 0.5 g sterile crude wheat bran (Lyngby Mølle, Denmark) per pot. Full chemical characterization of wheat bran is available from the Danish Food Composition Databank hosted by the Technical University of Denmark (http://www.foodcomp.dk/fcdb-_details.asp?FoodId=0086). In brief, wheat bran has high fibre content (40.2%), which mainly consists of cellulose. The N, P and K content are 2.6, 1.1 and 1.3%, respectively. In treatments with AM fungus each pot received 5 g of a crude soil inoculum with spores, mycelium and root fragments of the AM fungus *Glomus intraradices* (BEG 87) obtained from a dried maize pot culture. A 10 ml filtrate of the soil based mycorrhiza inoculum free from AM fungal structures was added to all treatments and mixed into the substrate to obtain a similar microbial background in all treatments. The two isolates of *Paenibacillus* used in the present experiment (*P. macerans* isolate MB1376 and *P. polymyxa* isolate MB376) were isolated from a cucumber rhizosphere (Mansfeld-Giese et al., 2002). To obtain a batch culture, colonies of each isolate were added to separate Erlenmeyer flasks containing 100 ml of 0.8% tryptic soy broth and pre-cultured for 24 h on a vertical rotary shaker (30 °C and 150 rpm). The batch cultures were harvested by centrifugation (10 min at 2000 rpm) after which the first pellet was re-suspended in 0.9% NaCl, and further washed similarly twice in sterile ddH_2O and re-suspended in a volume of 100 ml ddH_2O. In order to determine the number of colony forming units (cfu) added to the growth substrate, the *Paenibacillus* inocula were plated on tryptic soy agar plates and the cfu were counted after 24 and 48 h incubation at 30 °C in darkness. *P. macerans* MB1376 and *P. polymyxa* MB376 were added to their respective treatments at the concentration of 2.5 and 1.25×10^9 cfu $cm^{-}3$, respectively. Plants were grown under greenhouse conditions with temperatures ranging from 25 ± 3 °C day to 15 ± 3 °C night, a 16/8 h light/dark photoperiod and a relative humidity of 80–90%. Long Ashton nutrient solution with low P (10 mg P $kg^{-}1$) was supplied when watering (every second day).

2.3. Harvest and analyses

Plants were harvested 4 weeks after sowing, and the shoot and root was dried at 65 °C for 48 h and weighed. Before drying, a root subsample (1 g) was cut into 5–10 mm pieces and AM colonization was estimated by the method described by Kormanik and McGraw (1982) except that Trypan Blue was used instead of acid fuchsin. Easily extractable glomalin, operationally measured as easily extractable protein in this study, was determined by the method described by Wright and Upadhyaya (1998). Substrate samples (0.2 g) were mixed with Na citrate 20 mM pH 7.0 and autoclaved at 121 °C for 30 min. The protein in the supernatant was measured by Bradford reaction using BSA, as described by Wright et al. (1996). Dehydrogenase activity was determined following Skujins, 1976, as modified by García et al. (1997). For this, 1 g of substrate sample at 60% field capacity was mixed with 0.2 ml of 0.4% INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazolium chloride) in distilled water for 20 h at 22 °C in darkness. The INTF (iodo-nitrotetrazolium formazan) formed was extracted with 10 ml of methanol, by shaking vigorously for 1 min, filtering through filter paper (Whatman No. 5) and measured spectrophotometrically al 490 nm. β-Glucosidase activity was determined using *p*-nitropenyl-β-D-glucopyranoside (PNG, 0.05 M; Masciandro et al., 1994) as substrate. Two millilitres of 0.1 M maleate buffer (pH 6.5) and 0.5 ml of substrate were added to 0.5 g of substrate and incubated at 37 °C for 90 min. The reaction was stopped with tris-hydroxymethylaminomethane (THAM) according to Tabatabai (1982) and the *p*-nitrophenol formed was determined spectrophotometrically at 398 nm (Tabatabai and Bremner, 1969). Available phosphorus (P) in the growth substrate was determined by the method described by Olsen and Sommers (1982), after extraction with 0.5 M $NaHCO_3$ (pH 8.5). Substrate pH (water) was measured in a substrate/water paste (2/5). Whole cell fatty acids were extracted from the growth media (1.0 g) according to Sasser (1990). To enable quantification of the extracted fatty acid methyl esters (FAME) a known amount of an internal standard (nonadeconoate fatty acid methyl ester 19:0) was added to each sample. Gas chromatography analyses and FAME identification were performed using the software library TSBA41 (Parsley, 1996). The FAMEs 16:1w5 and 15:0 anteiso were employed as biomarkers of *G. intraradices* (Olsson, 1999) and the two *Paenibacillus* strains, respectively. FAME profiles of the two *Paenibacillus* strains (Table 1) were obtained from Mansfeld-Giese et al. (2002). The sum of the following FAMEs were used to estimate the total microbial biomass: 14:0, 15:0, 15:0 anteiso, 15:0 iso, 16:0 iso, 16:1w5, 16:1w7, 17:0, 17:0 anteiso, 17:1w8, 18:1w7, 18:1w9, 18:2w6, 20:4 and 19:0 cyclo. See Ratledge and Wilkinson (1988) for FAME biomarker specificity.

2.4. Statistics

For all the measured variables, ANOVAs were performed to test the effects of wheat bran amendment, AMF and *Paenibacillus*

Table 1
Percent fatty acid composition in pure cultures of *P. macerans* (1376) and *P. polymyxa* (376) used in the present experiment

Fatty acid	*P. macerans* (1376)	*P. polymyxa* (376)
13:0 anteiso	0	0.4
14:0 iso	3.23	1.62
14:0	2.96	0.7
15:1 anteiso 1	0.15	0
Feature 1	0	0.33
15:0 iso	7.15	8.51
15:0 anteiso	**35.58**	**73.8**
15:0	0.87	0.32
16:1 iso H	0	0.35
16:0 iso	17.91	3.66
Feature 3	0	0.86
16:0	16.51	1.04
15:0 2OH	0.57	0
Anteiso 17:1w9	0	1.4
17:0 iso	1.26	0.63
17:0 anteiso	10.64	5.65
17:0 10 methyl	0	0.19
18:0	0.15	0.54

inoculation, and all their possible interactions, using the procedures of Statgraphics *Plus* version 5.1 (Statpoint Inc., Virginia, USA). Data sets not meeting assumptions for ANOVA were transformed as required, but the results are presented in their original scale of measurement. Means were compared by the orthogonal contrast test (Petersen, 1977), using the procedures of JMP® software, version 7.0 (SAS Institute, Cary, NC, USA). Statistical significance was determined at $p < 0.05$.

3. Results

3.1. Plant growth parameters

A significant three-way interaction was found for shoot dry weight (Table 2). In the absence of wheat bran shoot dry weight did not differ between treatments (Fig. 1a). In the presence of wheat bran dual inoculation with *G. intraradices* and *P. macerans* resulted in 46% reduction in shoot dry weight compared to the corresponding treatment without microbial inoculation (Fig. 1a). Combination of wheat bran amendment and single *G. intraradices* inoculation resulted in 84% increase in shoot dry weight as compared to single inoculation with *G. intraradices* without wheat bran (Fig. 1a).

In terms of root dry weight significant interactions were observed for WB × Gi and Gi × *P.* spp. (Table 2). In the absence of wheat bran root dry weight did not differ between treatments (Fig. 1b). In the presence of wheat bran dual inoculation with *G. intraradices* and *P. macerans* caused a 63% reduction in root dry weight compared to the corresponding treatment without microbial inoculation (Fig. 1b).

A significant *P.* spp. × WB interaction was found concerning AM fungus root colonization. Inoculation with *G. intraradices* resulted in 60 and 81% root colonization in treatments without and with wheat bran, respectively. However, these treatment means did not differ significantly (Fig. 2). In treatments without wheat bran inoculation with *P. macerans* had no effect on AM fungus root colonization, whereas inoculation with *P. polymyxa* resulted in 95% reduction in AM fungus root colonization (Fig. 2). In treatments with wheat bran amendment inoculation with *P. macerans* and *P. polymyxa* resulted in 88 and 91% reduction in AM fungus root colonization, respectively (Fig. 2).

3.2. Growth substrate measures

Wheat bran application and *G. intraradices* inoculation both significantly increased the dehydrogenase activity whereas the bacterial inoculants had no effect (Table 2, Fig. 3a). No significant interactions were found between the main factors (Table 2). In the absence of wheat bran the dehydrogenase activity did not differ between treatments (Fig. 3a). In the presence of wheat bran single inoculation with *G. intraradices* resulted in 119% increase in the dehydrogenase activity compared to the corresponding treatment without *G. intraradices* inoculation (Fig. 3a). Dual inoculation with *G. intraradices* and *P. polymyxa* reduced the dehydrogenase activity with 132% compared to the treatment with single inoculation of *G. intraradices* (Fig. 3a).

Concerning glucosidase activity a significant three-way interaction was found for the main factors (Table 2), but due to high variation no significant differences in glucosidase activity were found between treatments either with or without wheat bran (Fig. 3b).

Wheat bran application and *G. intraradices* inoculation both significantly increased the amount of easily extractable protein in the growth media (Fig. 3c, Table 2). The bacterial inoculants had no effect on easily extractable protein. No significant interactions were found between the main factors (Table 2). In the absence of wheat bran the amount of easily extractable protein did not differ

Table 2
Probability (p) values from three-way analyses of variance from the measured parameters with wheat bran (WB), *Glomus intraradices* inoculation (Gi) and *Paenibacillus* inoculations (*P.* spp) as the main factors, and their interactions

	WB	Gi	*P.* spp	WBxGi	WBxPspp	GixPspp	WBxGixPspp
Plant measurements							
Shoot dry weight	***	***	0.118	0.321	***	*	*
Root dry weight	0.546	***	0.351	**	0.052	*	0.223
Root colonization[a]	0.241	–	***	–	***	–	–
Growth media measurements							
Dehydrogenase activity	***	***	0.483	0.086	0.111	0.879	0.685
Glucosidase activity	***	***	0.130	0.542	*	0.080	*
Easily extractable proteins	***	***	0.246	0.586	0.552	0.515	0.292
Phosphorous	***	***	0.169	0.546	0.353	0.233	0.931
pH	***	0.231	0.110	***	*	0.460	*
Total microbial fatty acids	*	**	***	0.991	**	0.360	0.609
16:1w5 (*G. intraradices*)	0.980	**	0.237	0.885	0.997	0.197	0.985
15:0 anteiso (*Paenibacillus*)	***	***	***	0.450	***	0.138	0.448

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
[a] Two-way analyses of variance employed with wheat bran and *Paenibacillus* as main factors.

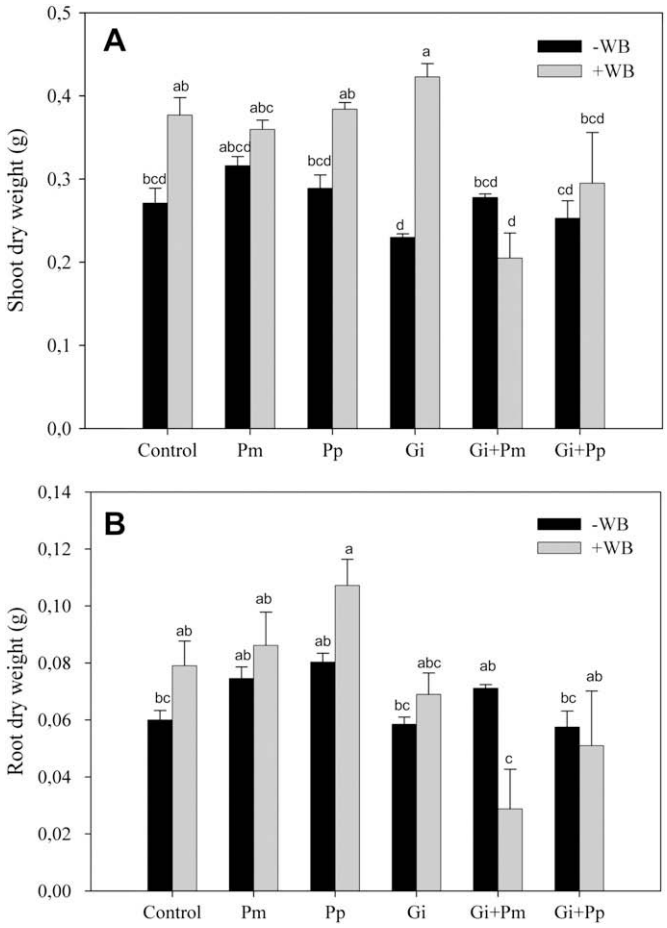

Fig. 1. Cucumber shoot (A) and root (B) dry weight as affected by microbial inoculation with *G. intraradices* (Gi), *P. macerans* (Pm) and *P. polymyxa* (Pp) and organic matter amendment in terms of wheat bran (WB). Different letters indicate significant difference according to the orthogonal contrasts test ($p < 0.05$). Bars denote mean + SE ($n = 4$).

between treatments (Fig. 3c). In the presence of wheat bran dual inoculation with *G. intraradices* and *P. macerans* increased the amount of easily extractable protein with 80% compared to the treatment with *P. macerans* inoculation alone (Fig. 3c).

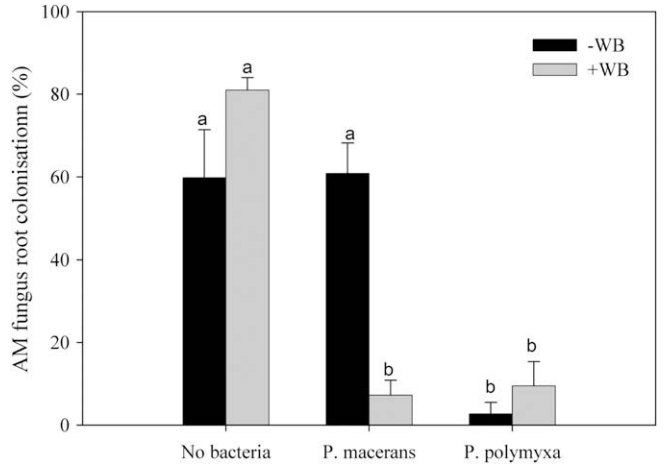

Fig. 2. Root colonization with *G. intraradices* as affected by organic matter amendment in terms of wheat bran (WB) and inoculation with *P. macerans* (Pm) and *P. polymyxa* (Pp). Different letters indicate significant difference according to the orthogonal contrasts test ($p < 0.05$). Bars denote mean + SE ($n = 4$).

Wheat bran application and *G. intraradices* inoculation both increased available P in the growth substrate (Fig. 3d, Table 2). The bacterial inoculants had no effect on available P in the growth substrate. No significant interactions were found between the main factors (Table 2). In the absence of wheat bran the amount of available P did not differ between treatments (Fig. 3d). In the presence of wheat bran dual inoculation with *G. intraradices* and *P. macerans* increased the amount of available P in the substrate with 149% compared to the corresponding treatment with single *P. macerans* inoculation (Fig. 3c).

Concerning pH a significant three-way interaction was found for the main factors (Table 2). In the absence of wheat bran, inoculation with either *P. macerans* or *P. polymyxa* reduced the pH in the substrate by 0.64 and 0.59 pH units, respectively, compared to the treatment without bacterial inoculation (Fig. 3e). In the presence of wheat bran pH in the substrate did not differ between treatments (Fig. 3d).

3.3. Microbial biomarkers

The different groups of biomarker fatty acids specific to Gram-negative bacteria (hydroxylic and cyclic FAMEs), Gram-positive bacteria (branched FAMEs), Actinomycetes (methylated FAMEs), saprotrophic fungi (FAME 18:2w6,9) and protozoa (FAME 20:4) were in general all increased by the microbial inoculations with *G. intraradices* and the two *Paenibacillus* species as well as by the wheat bran amendment (data not presented), so only the fatty acid biomarkers of the biomarker FAMEs of the microbial inoculants and the sum of all microbial fatty acids are presented.

A significant WB × *P.* spp. interaction was found in terms of total microbial fatty acids. Inoculation with *G. intraradices* overall significantly increased the sum of total microbial biomass (Table 2; Fig. 3f)). In the absence of wheat bran inoculation with *P. polymyxa* alone and in combination with *G. intraradices* increased the total amount of microbial fatty acids 250 and 363%, respectively, compared to the control treatment without microbial inoculation (Fig. 3f). In the presence of wheat bran dual inoculation with *P. macerans* and *G. intraradices* increased the total amount of microbial fatty acids with 294% compared to the corresponding treatment without microbial inoculation (Fig. 3f).

The amount of the AM fungus biomarker fatty acid 16:1w5 in the growth substrate overall increased by *G. intraradices* inoculation (Table 2; Fig. 4a.), whereas neither wheat bran amendment nor *Paenibacillus* inoculation had any significant effects on the amount of 16:1w5 in the growth substrate (Table 2; Fig. 4a). Due to high variation the orthogonal contrast test did not reveal any significant difference between any of the treatments (Fig. 4a).

A significant WB × *P.* spp. interaction was found in terms of the biomarker fatty acid 15:0 anteiso and *G. intraradices* inoculation overall significantly reduced the amount of 15:0 anteiso (Table 2; Fig. 4b). In the absence of wheat bran inoculation with *P. polymyxa* alone and in combination with *G. intraradices* increased the amount of 15:0 anteiso by 1300 and 925% compared to the corresponding treatments without inoculation with *P. polymyxa* (Fig. 4b). In the presence of wheat bran single inoculation with *P. macerans* or *P. polymyxa* increased the amount of 15:0 anteiso by 271 and 343%, respectively, compared to the corresponding treatment without microbial inoculation (Fig. 4b). In combination with *G. intraradices*, inoculation with *P. macerans* or *P. polymyxa* did not significantly increase the amount of 15:0 anteiso in the substrate compared to the treatment with single *G. intraradices* inoculation (Fig. 4b). The amount of 15:0 anteiso was reduced by 39% in the treatment with dual inoculation with *P. polymxa* and *G. intraradices*, compared to the corresponding treatment with single inoculation of *P. polymyxa* (Fig. 4b).

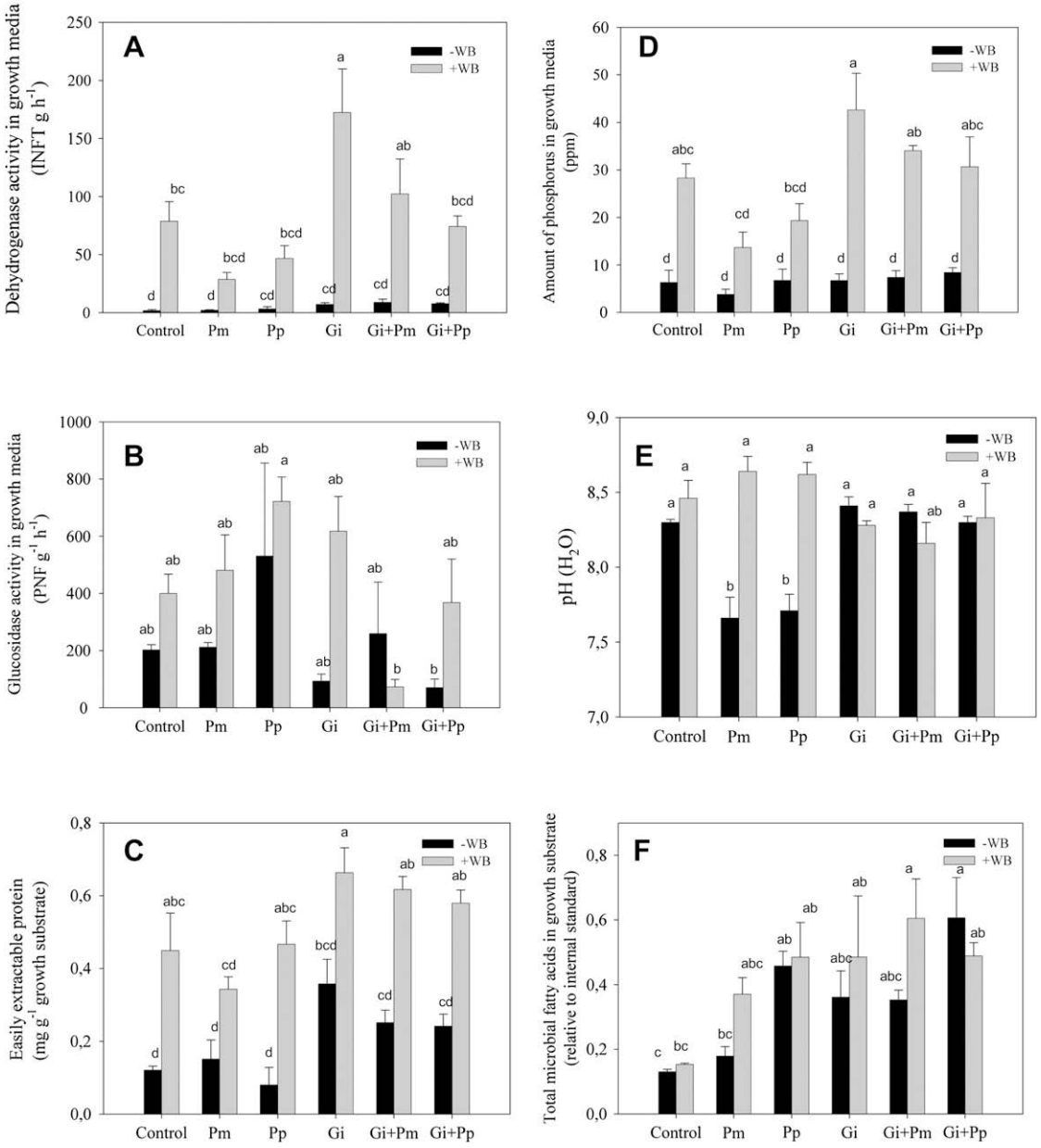

Fig. 3. Dehydrogenase (A) and glucosidase (B) activity, easily extractable protein (C), amount of phosphorous (D), pH (E) and total microbial biomass (F) in the rhizosphere as affected by microbial inoculation with *G. intraradices* (Gi), *P. macerans* (Pm) and *P. polymyxa* (Pp) and organic matter amendment in terms of wheat bran (WB). Different letters indicate significant difference according to the orthogonal contrasts test ($p < 0.05$). Bars denote mean + SE ($n = 4$).

4. Discussion

4.1. Interactions between microbial inoculants

It has previously been shown that *Paenibacillus validus* can support the growth of AM fungi (Hildebrandt et al., 2006), but in the present work we show that two other species from the same genus, *P. macerans* and *P. polymyxa*, have the potential to suppress an AM fungus, *G. intraradices*, and furthermore our results show that this AM fungus may also suppress bacteria from *Paenibacillus*. Similar mutual inhibition has been reported between *G. intraradices* (BEG87) and biocontrol fungi (Green et al., 1999; Ravnskov et al., 2006).

Organic matter, in the form of wheat bran, influenced the effect of the two *Paenibacillus* isolates on *G. intraradices*. The AM fungus root colonization was suppressed by *P. polymyxa* regardless of wheat bran amendment, whereas *P. macerans* only suppressed the root colonization in combination with wheat bran amendment. Li et al. (2008) also reported differential effects of *Paenibacillus* spp. on cucumber mycorrhizas. In the present experiment the differential effect of *P. macerans* and *P. polymyxa* may simply be related to a difference in population density, since the biomass of *P. macerans* increased markedly by wheat bran amendment, whereas *P. polymyxa* did not respond to wheat bran. However, the mode of interaction between *G. intraradices* and the two *Paenibacillus* isolates cannot be clarified from results obtained in the present experiment.

Co-inoculations of plant beneficial microorganisms in plant production systems may improve the efficacy of desired features in relation to biocontrol and plant growth promotion. The possibility of developing microbial consortia with microorganisms from different functional groups such as AM fungi, P solubilizers, N fixers and biocontrol agents seems to be a particularly interesting

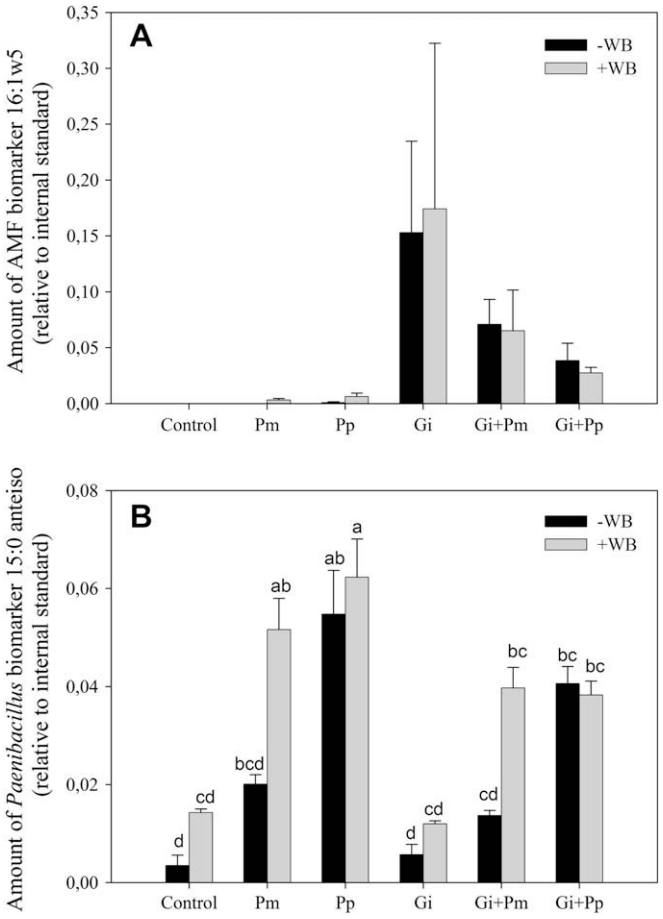

Fig. 4. Amount of AM fungus (A) and *Paenibacillus* (B) biomarker fatty acids in the rhizosphere as affected by microbial inoculation with *G. intraradices* (Gi), *P. macerans* (Pm) and *P. polymyxa* (Pp) and organic matter amendment in terms of wheat bran (WB). Different letters indicate significant difference according to the orthogonal contrasts test ($p < 0.05$). Bars denote mean + SE ($n = 4$).

approach and this area is receiving increased attention (Barea et al., 2002; Vestberg et al., 2004; Roesti et al., 2006; Kohler et al., 2007). Our results emphasize the importance of examining compatibility between different groups of plant beneficial rhizosphere microorganisms.

4.2. Influence of microbial inoculants and organic matter amendment on plant growth

In treatments without organic matter amendment inoculation with *G. intraradices* caused plant growth suppression. Similar plant growth suppression in cucumber mycorrhizas have been reported in several cases (e.g. Ravnskov et al., 2002; Larsen et al., 2003). In contrast, the highest plant growth was found in combination with wheat bran and *G. intraradices*, which may suggest that inoculation with *G. intraradices* accelerated organic matter decomposition, mobilizing nutrients from the organic matter, which has also been reported by Hodge et al. (2001) and Ravnskov et al. (2006).

The plant growth decrease observed in treatments with dual inoculation with *G. intraradices* and *P. macerans* in combination with organic matter amendment was not expected. In this treatment the roots were markedly damaged and showed abnormal growth, caused by an unknown factor released during the decomposition process. The glucosidase activity in this treatment was also reduced compared to the control without microbial inoculation, which may suggest a lower level of organic matter decomposition. Previously wheat bran has been shown to suppress the growth of cucumber (Paulitz and Linderman, 1991).

The inert plant growth media used in the present experiment allowed us to study rhizosphere microbial interactions in a relatively simple substrate with limited interference from other soil biota. However, the results obtained in the present work mainly show potential interactions and should not be directly extrapolated to a soil situation.

4.3. Influence of the microbial inoculants on other rhizosphere microorganisms

It is well documented that AM fungi can affect rhizosphere microorganisms and several reviews on microbial interactions in the mycorrhizosphere are available (Gryndler, 2000; Johansson et al., 2004; Barea et al., 2005; Artursson et al., 2006; Bending et al., 2006; Marschner and Timonen, 2006). Changes in microbial communities (Andrade et al., 1997; Wamberg et al., 2003; Marschner and Baumann, 2003; Ravnskov et al., 2006) and microbial activity (Christensen and Jakobsen, 1993; Vivas et al., 2006; Raiesi and Ghollarata., 2006) due to AM fungus inoculation has also been reported. In the present study inoculation with *G. intraradices* increased the total rhizosphere microbial biomass and activity measured as dehydrogenase activty. A similar increase in dehydrogenase activity has also been shown with other AM fungi (Vivas et al., 2006; Kohler et al., 2007).

Inoculation with the *Paenibacillus* species increased the amount of the fatty acid 15:0 anteiso, which is one of the most abundant fatty acids in the two *Paenibacillus* isolates suggesting that they were both actively growing in the present experiment. A similar approach was successfully used in experiments on the interactions between *G. intraradices* and the biocontrol bacteria *Burkholderia cepacia* (Ravnskov et al., 2002; Larsen et al., 2003; Albertsen et al., 2006), where cyclic fatty acids were employed as biomarkers of *Burkholderia cepacia* showing differential interactions between *G. intraradices* (BEG87) and five *Burkholderia cepacia* isolates.

The decrease in rhizosphere pH after inoculation with the two *Paenibacillus* species in the absence of wheat bran may be the result of acidification caused by respiration from the increased biological activity in these treatments, but could also be related to secretion of organic acids from roots and/or microorganisms. However, possible mechanisms involved in these pH effects are difficult to unravel at this point and further studies are needed.

The amount of easily extractable protein has been suggested as a possible simple way to measure the AM fungal produced glomalin, which is a recalcitrant glycoprotein (Rillig et al., 2001). Our results show that inoculation with *G. intraradices* increased the amount of easily extractable protein in the growth media, whereas the bacterial inoculation had no effect on the easily extractable proteins, supporting the idea of using easily extractable protein as a measure of glomalin.

5. Conclusions

In conclusion, our results add further to the complexity of interactions between AM fungi and other rhizosphere microorganisms showing that *P. macerans* and *P. polymyxa* may not only have mycorrhiza helper features but also suppressive effects on AM fungi and cause plant growth inhibition when dually inoculated with AM fungi. To improve our understanding of the ecology of AM fungi both beneficial and deleterious bacterial associations should be further examined.

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