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Pre-symbiotic and symbiotic interactions between *Glomus intraradices* and two *Paenibacillus* species isolated from AM propagules. *In vitro* and *in vivo* assays with soybean (AG043RG) as plant host

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ABSTRACT

Two indole-producing *Paenibacillus* species, known to be associated with propagules of arbuscular mycorrhizal (AM) fungi, were examined for their mycorrhization helper bacteria activity at pre-symbiotic and symbiotic stages of the AM association. The effects were tested under *in vitro* and *in vivo* conditions using an axenically propagated strain of the AM fungus *Glomus intraradices* and *Glycine max* (soybean) as the plant host. The rates of spore germination and re-growth of intraradical mycelium were not affected by inoculation with *Paenibacillus* strains in spite of the variation of indole production measured in the bacterial supernatants. However, a significant promotion in pre-symbiotic mycelium development occurred after inoculation of both bacteria under *in vitro* conditions. The *Paenibacillus rhizosphaerae* strain TGX5E significantly increased the extraradical mycelium network, the rates of sporulation, and root colonization in the *in vitro* symbiotic association. These results were also observed in the rhizosphere of soybean plants grown under greenhouse conditions, when *P. rhizosphaerae* was co-inoculated with *G. intraradices*. However, soybean dry biomass production was not associated with the increased development and infectivity values of *G. intraradices*. *Paenibacillus favisporus* strain TG1R2 caused suppression of the parameters evaluated for *G. intraradices* during *in vitro* symbiotic stages, but not under *in vivo* conditions. The extraradical mycelium network produced and the colonization of soybean roots by *G. intraradices* were promoted compared to the control treatments. In addition, dual inoculation had a promoting effect on soybean biomass production. In summary, species of *Paenibacillus* associated with AM fungus structures in the soil, may have a promoting effect on short term pre-symbiotic mycelium development, and little impact on AM propagule germination. These findings could explain the associations found between some bacterial strains and AM fungus propagules.

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1. Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous components of most soil ecosystems. They are obligate symbionts that need a host plant to complete their life cycle. Among the propagules of AM fungi are spores that germinate under favorable conditions developing an extraradical mycelium (ERM) network. However, this mycelium stops growing within approximately 20 days post germination in the absence of a host root (Giovannetti, 2000). Intraradical mycelium (IRM) developed within roots is also a source of inoculum for some AM fungi (Silvani et al., 2008).

Among physiological changes that occur in plants after AM root colonization is the alteration of the composition of root exudates (Azaizeh et al., 1995). Changes in the rhizosphere of mycorrhizal plants modify the community of microorganisms, resulting in the mycorrhizosphere effect (Linderman, 1988). As well, exudates from AM fungi can modify soil pH (Bago and Azcón-Aguilar, 1997) and soil structure (Rillig et al., 2002). Intraradical and extraradical AM hyphae create a unique habitat for soil microorganisms; in turn, some of these organisms are beneficial for the establishment of AM symbiosis and host plant growth (Marschner and Timonen, 2005).

Diverse bacterial taxonomic groups live in association with AM structures in different stages of their life cycle and in a wide variety

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of soil environments (Bonfante and Anca, 2009). Various bacteria form biofilms on spores and mycelia and may occur within the cytoplasm of both spores and mycelia (Bianciotto et al., 1996).

Usually, bacteria have been detected using either classical (Xavier and Germida, 2003) or molecular (Roesti et al., 2005) methods. Bacteria from IRM inside roots of several host plants have been isolated (Silvani et al., 2008). Frequently, bacteria from the genus *Paenibacillus* have been isolated in relation to AM structures in the mycorrhizosphere (Budi et al., 1999; Mansfeld-Giese et al., 2002), and several rhizospheric species within this genus have the ability to promote plant growth and development (Maes and Baeyen, 2003).

Ecological relationships established between bacteria and AM fungi in the mycorrhizosphere could be determined by its trophic character (Bonfante and Anca, 2009). Abdel-Fatah and Mohamedin (2000) observed an enhancement of chitinolytic activity in the rhizosphere when sorghum plants were co-inoculated with the AM fungus *Glomus intraradices* and *Streptomyces coelicolor*. Roesti et al. (2005) showed that most of the bacteria found in association with spore walls of the AM fungi, *Glomus geosporum* and *Glomus constrictum*, were able to degrade biopolymers such as cellulose and chitin. These authors suggested that the increase in germination of *Glomus* spores could be related to the degradation of external layers of spore walls by soil bacteria. Also, toxic compounds that inhibit germination of AM propagules and mycelial growth could be reduced by these bacteria (Roesti et al., 2005).

Bacteria have also been shown to enhance AM fungal growth. For example, Hildebrandt et al. (2002) reported that *Paenibacillus validus* under *in vitro* conditions supported mycelial growth of *G. intraradices* up to the stage of new spore formation in the absence of a host root. This was determined to be due to the production of sugars such as raffinose (Hildebrandt et al., 2006). Close contact between bacteria and AM fungi could facilitate these metabolic interactions and nutrient exchange (Artursson et al., 2006).

Some bacterial strains, including some *Paenibacillus* species, are able to promote the development of the AM symbiosis. These bacteria are considered to be mycorrhization helper bacteria (MHB) (Garbaye, 1994). Stimulation of AM propagule germination, hyphal growth, and root colonization was observed in the presence of MHB (Mayo et al., 1986; Xavier and Germida, 2003) even when their soluble exudates were added (Vósatka and Gryndler, 1999).

Some MHB are also plant growth-promoting rhizobacteria (PGPR). They produce hormone-type substances such as auxins, which regulate plant development and stimulate differentiation and growth of plant tissues (Tate, 1995). In addition, increased levels of auxins in mycorrhizal plants have been observed (Meixner et al., 2005), suggesting that these hormones could be signals of the AM colonization process (Ludwig-Müller and Güther, 2007).

In this study, the MHB ability of two indole-producing strains of *Paenibacillus* isolated from AM propagules was tested on pre-symbiotic and symbiotic development of the AM fungus *G. intraradices* under an axenic system with transformed soybean roots. Also, an assay under greenhouse conditions was performed using plants with the same soybean genotype as the transformed roots. We hypothesized that these bacteria that are intimately linked with AM fungi would have a positive effect on the development of the symbiosis both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Biological material

Two indole-producing *Paenibacillus* species were used: *Paenibacillus rhizosphaerae* and *Paenibacillus favisporus*. An association with AM propagules was previously observed for both species (Silvani et al., 2008). *P. rhizosphaerae* isolate TGX5E (GenBank accession number GU830879) was recovered from the endophytic environment of *Solanum lycopersicum* roots colonized by the AM fungus *Glomus fasciculatum* strain GX5 originating from the *Chaco Serrano* Woodland of Córdoba province in Argentina (Banco de Glomeromycota In vitro (BGIV) http://www.bgiv.com.ar/strains/glomus-fasciculatum/gx5). The *P. favisporus* isolate TG1R2 (GenBank accession number GU937424) was obtained from surface-sterilized (15 min in a 5% chloramine-T (Merck) solution) germinated sporocarps of *Glomus mosseae* strain G1 originating from native grassland of the *Pampeana* phytogeographic region, from Buenos Aires province in Argentina (collection of FCEyN, University of Buenos Aires, Argentina).

The identity of these bacterial species was confirmed: bacterial DNA was extracted using a genomic DNA purification kit (Geneclean II Kit) according to the instructions. 16S rDNA genes from isolates were PCR amplified with bacterial universal primers, fD1 and rD1 (Weisburg et al., 1991). PCR conditions consisted of an initial denaturing cycle (94 °C, 5 min), 30 amplification cycles (94 °C, 30 s; 55 °C, 45 s; 72 °C, 1 min), and a final elongation cycle (72 °C, 10 min). PCR products were ligated into the vector pCR® 2.1 (Invitrogen) following manufacturer's instructions, and transformed into competent *Escherichia coli* DH5α cells. Finally, 16S rRNA genes were restricted in two small fragments by using EcoRI and subcloned into the vector pUC18 (Invitrogen). The 16S rRNA gene sequences obtained were compiled using the BioEdit Sequence Alignment Editor 7.0 software, and compared with sequences from the GenBank database. DNA similarity was performed using NCBI BLAST server (http://www.ncbi.nlm.nih.gov).

A monoxenic culture of *G. intraradices* strain GA5 (GenBank accession number GU140042) was established to obtain viable, pure and homogeneous AM fungal spores and mycelia. This is a typical *in vitro* fast-growing *G. intraradices* strain (BGIV, http://www.bgiv.com.ar/strains/glomus-intraradices/ga5). The monoxenic cultures were established with transformed carrot (*Daucus carota*) roots grown in Minimal Medium (MM) (Bécard and Fortin, 1988) and incubated in an inverted position at 25 °C in the dark. A two-compartment Petri plates with the distal root-free side lacking sucrose was used for enhancing spore production as described by Douds (2002). This co-culture system is routinely used for AM propagation to obtain a large amount of AM propagules within a short time. After 3 months of dual culture, the spores in the distal compartment and the colonized roots from the proximal compartment were recovered by solubilization of MM in 10 mM sodium citrate buffer (pH 6.0) (Doner and Bécard, 1991).

2.2. Indole acetic acid (IAA) detection

Bacterial isolates were grown in liquid BDN medium supplemented with tryptophan (100 μg ml^{-1}) for 24 h at 30 °C (Fuentes-Ramírez et al., 1993). Bacterial cells were removed by centrifugation (6.000 rpm, 10 min) and IAA production was detected in the supernatants with Salkowski chromogenic reagent (strong mineral acid plus oxidant) (Ehmann, 1977). The absorbance of supernatants for IAA production was measured at 530 nm, and compared with a standard curve of commercial IAA (Indole-3-acetic acid, Merck).

2.3. In vitro assay

Pre-symbiotic development and symbiotic establishment of *G. intraradices* GA5 in response to directly released and diffusible substances produced by the *Paenibacillus* isolates TG1R2 and TGX5E were evaluated under *in vitro* conditions, using transformed soybean (*Glycine max* AG043RG NIDERA) roots (TSR). In a previous study of the symbiotic association between this AM fungus and

soybean (Fernández et al., 2009), we found comparable results with the TSRs *in vitro* system and whole plants *in vivo* conditions using the same plant genotype.

The bacterial strains were grown in liquid BDN medium supplemented with tryptophan as previously described (Fuentes-Ramírez et al., 1993), and centrifuged (10.000 rpm, 10 min) to obtain cell-free bacterial medium. This supernatant (diffusible substances) was filter-sterilized (Millipore 0.2 μm pore size) and the pellets (bacterial cell suspension) were re-suspended with 10 mM SO_4Mg to a final concentration of 10^9 cells ml^{-1}.

Pre-symbiotic parameters: In both assays utilizing cell suspensions and diffusible substances, groups of 30 spores or 10 colonized root fragments (1 cm length) previously removed from the monoxenic culture of *G. intraradices* on transformed carrot roots (and designated as IRM), were transferred to Petri plates with 0.35% w/v Gel-Gro® (ICN Biochemicals, Aurora, OH, USA) plus 0.03% w/v $MgSO_4$ (pH 6). Each type of inoculum was homogeneously mixed with 100 μl of cell suspensions or sterilized supernatants. The same amount of $MgSO_4$ solution or sterile medium was added to the control treatments. Plates were sealed and incubated in the dark at 25 °C for ten days. All treatments (spores/IRM with or without each bacterial strain) were replicated in five Petri plates. The effect of bacterial strains on spore germination (%), and the number of root fragments that showed AM hyphal re-growth (%), were assessed under a stereobinocular microscope (Nikon SMZ645) as described by Silvani et al. (2008). The ERM length (mm) was measured using the method proposed by Brundrett et al. (1994) after staining with 0.02% trypan blue in lactic acid. Measurements were made with a binocular microscope (Nikon OPTIPHOT-T2) at 100× magnification.

Symbiotic parameters: A 1 cm^3 plug of inoculum from 3-month-old *G. intraradices* monoxenic culture, containing colonized roots (30% of frequency and 50% of intensity), approximately 250 spores and abundant ERM, was placed in proximity to fresh TSR explants (Fernández et al., 2009) into Petri plates with 10 ml of MXB with 42 mg/l KH_2PO_4. 100 μl of cell suspensions or sterilized supernatants were added and plates were sealed and incubated in the dark at 25 °C for forty days. Control treatments were performed in the same way as in the pre-symbiotic assay. Each treatment (*G. intraradices* co-cultured with TSR in presence or absence of bacterial strains) was replicated in five Petri plates. The effect of bacterial strains on length of *G. intraradices* ERM was measured using the grid intersects method (Marsh, 1971). In addition, the number of newly formed spores was assessed by counting 10 cells of 1 cm^2 for each replicate. The establishment of AM symbiosis was also checked for presence or absence of mycorrhizal structures. To accomplish this, soybean roots were removed, cleared in KOH (10% w/v, 15 min, 90 °C) and stained with 0.02% trypan blue in lactic acid for 10 min at 90 °C (modified from Phillips and Hayman, 1970). Intraradical colonization was quantified by examination of 50 randomly selected root pieces, in groups of ten, and the frequency (%*F*) of mycorrhizal colonization was calculated as the percentage of root segments containing hyphae, arbuscules or vesicles (Declerck et al., 2004). All measurements were made with a Nikon binocular microscope at 100× magnification.

2.4. In vivo assay

Soybean seeds of the same genotype of TSR were used to evaluate the effects of rhizobacteria and the AM symbiosis established with *G. intraradices* under greenhouse conditions.

Seeds were surface-sterilized with 70% v/v of ethanol solution for 15 min followed by 20% v/v of sodium hypochlorite solution plus Tween 20 (0.1%) for 20 min, rinsed three times with sterile distilled water, and then germinated on moist filter paper for 48 h. Soybean seedlings were grown in pots with 500 g of an autoclaved (100 °C for 1 h, three consecutive days), mixture of 1:1:1 perlite, vermiculite and soil pH 7.1; total C 12.08 and N 1.1 (g kg^{-1}); P 34.2 mg kg^{-1}; K 0.9, Ca 7.5, Mg 1.7 and Na 0.2 (cmol kg^{-1}) Pots were placed in a greenhouse with natural light and room temperature. Each pot was watered to saturation twice a week and irrigated with 50 ml of Hewitt (1952) nutritive solution every fifteen days.

Roots of soybean seedlings were inoculated by placing a 1-cm^3 plug of *G. intraradices* monoxenic culture containing colonized roots, ERM and spores. The non-mycorrhizal control plants were prepared in the same way, except that roots were inoculated with 1-cm^3 plug of MM with non-mycorrhized transformed carrot roots. Both rhizobacteria were grown as previously described and 2 ml of bacterial suspension (10^9 cells ml^{-1}, to ensure a high bacterial density) were applied together with *G. intraradices* inoculum.

Five pots per treatment were established for soybean control plants (non-inoculated), soybean plants inoculated with each bacterial strain and *G. intraradices*, either separately and co-inoculated.

Soybean roots were carefully separated from pots and stained for microscopic examination of AM colonization at weeks 3, 4 and 6 as previously described.

At the final harvest date, fresh and dry weight (DW) of shoots and roots from each pot were recorded after drying at 80 °C. Microbial inoculation effect (MIE) on soybean biomass was calculated according to Bagyaraj (1992): MIE = (DW treated plant-DW untreated plant/DW treated plant) × 100.

Also the ERM length of *G. intraradices* from soil attached to soybean roots was determined according to Graham et al. (1982) by vigorously shaking the air-dry roots for 48 h in the dark at room temperature, and removing the loose soil. The adhering soil particles were washed into a beaker and dried until constant weight. The length of *G. intraradices* ERM was quantified from 1 g of the dried soil samples.

2.5. Statistical analysis

The experiments were arranged in a completely randomized design with equal replications in each treatment. All data were subjected to analysis of variance (one-way ANOVA) with the inoculated bacterial strain as sole variation source. When significant differences among treatments were detected ($P < 0.05$) *post hoc* comparisons among mean values in each treatment were made using the least significant difference (LSD) test (Clewer and Scarisbrick, 2001). Statistical procedures were carried out with the software package STATISTICA 6.0 for Windows XP.

3. Results

Both *Paenibacillus* strains isolated were indole-positive; but in the presence of 100 μg ml^{-1} of tryptophan the supernatants of *P. favisporus* contained a significantly higher concentration of IAA (70 μM) than those of *P. rhizosphaerae* (13 μM) after 24 h growth. Despite the fact that these two bacterial strains belong to the same genus, their IAA production potential was quite different and they caused a variable effect on AM fungi in both the *in vivo* and *in vitro* assays.

3.1. In vitro bacterial effects

There were significant differences ($p < 0.05$) among treatments for all the *in vitro* variables analyzed, with the exception of the percent of root fragments with AM hyphal re-growth in the exudates assay ($p > 0.05$). The percentage of *G. intraradices* germinated spores after exposure to the *P. rhizosphaerae* cell

suspension or its exudates was not affected under *in vitro* conditions. However there was a significant decrease with cell suspensions but not with diffusible substances released by *P. favisporus* (Fig. 1A).

The pre-symbiotic mycelial growth from *G. intraradices* germinated spores increased with *P. rhizosphaerae* in all treatments. There was also a significant enhancement of hyphal development from spores in response to the exudates of *P. favisporus* (Fig. 1B).

The cell suspension of *P. rhizosphaerae* led to a significant increase in rates of re-growth of intraradical fungal structures from root fragments and the hyphal length developed by *G. intraradices*, but exudates did not cause any noticeable effects (Fig. 1C, D).

Finally, the re-growth rate of *G. intraradices* IRM was not affected either by exposure to the cell suspension or exudates of *P. favisporus*, (Fig. 1C), but the development of hyphal length increased significantly after hyphal outgrowth from root fragments (Fig. 1D).

A significant increase in ERM development of *G. intraradices* after forty days of *in vitro* co-culture with TSRs was observed when cell suspension and exudates of *P. rhizosphaerae* were present; whereas the opposite trend was found for the ERM length when *P. favisporus* was added. This effect was not present when bacterial exudates were added (Fig. 2A).

The number of spores newly formed by *G. intraradices* grown in co-culture with TSRs in absence of bacteria was approximately ten. This value was almost doubled by cell suspension and exudates of *P. rhizosphaerae*, and diminished by half in *P. favisporus* treatments (Fig. 2B).

Successful establishment of *in vitro* AM symbiosis between *G. intraradices* and TSRs occurred with 30% of colonization frequency. There was a significant stimulation on colonization of TSRs by the AM fungus in all treatments with *P. rhizosphaerae*, and a significant decline produced by *P. favisporus* (Fig. 2C).

3.2. In vivo bacterial effects

Significant differences ($p \leq 0.05$) between treatments were observed for all *in vivo* variables analyzed. A significant effect on soybean mycorrhization by *G. intraradices* was observed when inoculated in combination with bacteria. The frequency of mycorrhizal colonization increased significantly with time in plants inoculated with AM fungus and both *Paenibacillus* strains (Fig. 3A).

The influence of bacteria on ERM production by *G. intraradices* in the soil strongly attached to soybean roots was estimated at the end of the assay period. The ERM of *G. intraradices* was significantly increased when *P. favisporus* and *P. rhizosphaerae* were inoculated in mycorrhized soybean plants (Fig. 3B).

The effect of both *Paenibacillus* species on soybean plant growth, separately and co-inoculated with *G. intraradices*, was evaluated. Inoculation of *G. intraradices* promoted soybean growth with an increase up to 17.32% with respect to non-mycorrhizal control plants (Fig. 3C). The highest dry biomass was found in soybean plants treated with *P. favisporus*, when this species was inoculated separately (MIE 10.6%) or in combination with the AM fungus (MIE 18.18%) (Fig. 3C).

The values of DW increased when soybean plants were co-inoculated with *P. rhizosphaerae* and *G. intraradices*, nevertheless a decline of this measurement was observed when only bacteria were present. When it occurred, plants were smaller than control treatments, and a negative value of the MIE was obtained (Fig. 3C).

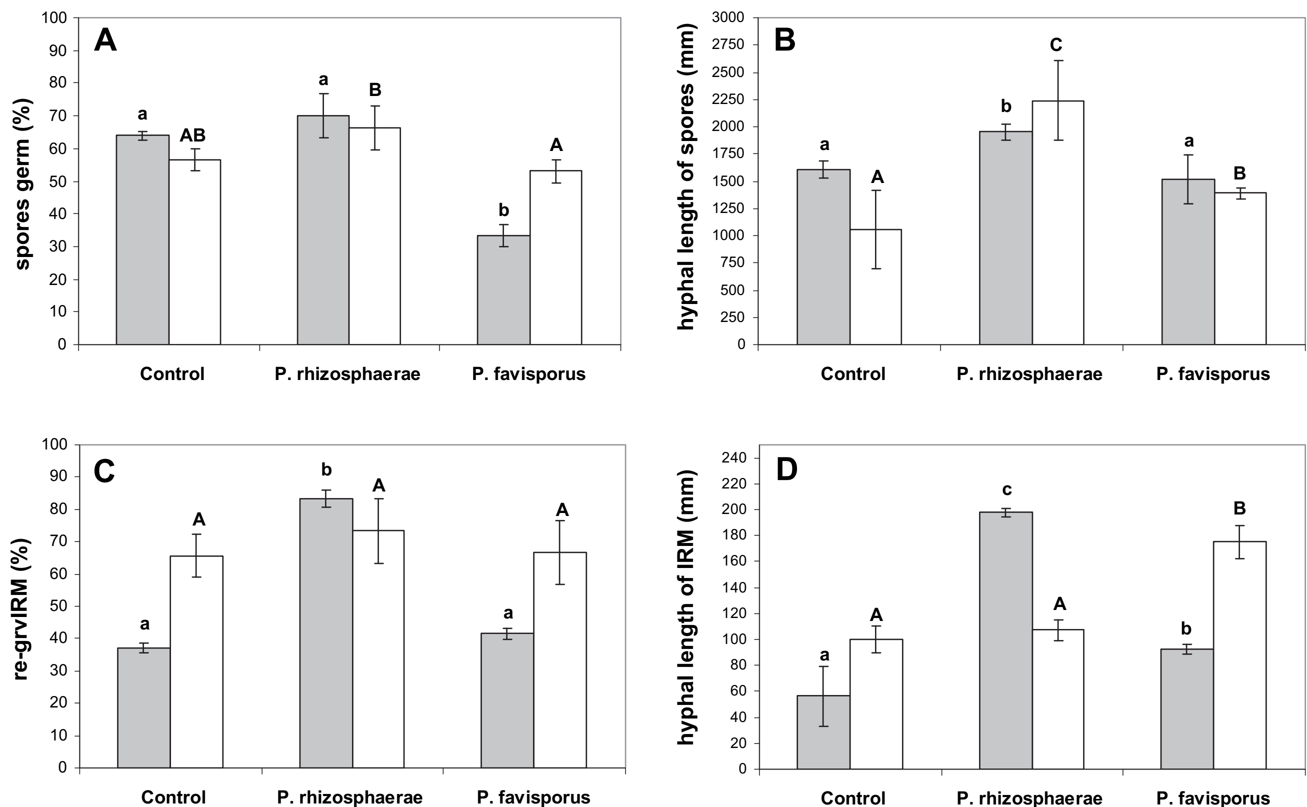

Fig. 1. Effect of cell suspensions (grey columns, small letters) and diffusible substances (white columns, capital letters) of *P. rhizosphaerae* TGX5E and *P. favisporus* TG1R2 on *G. intraradices* GA5: spore germination (%) (A) and pre-symbiotic hyphal length of germinating spores (mm) (B), and root fragments with AM hyphal re-growth (%) (C) and pre-symbiotic hyphal length of root fragments (mm) (D). Values are the means of five observations (±standard error) after ten days of co-culture. Bars with different letters are significantly different (LSD test, $p < 0.05$).

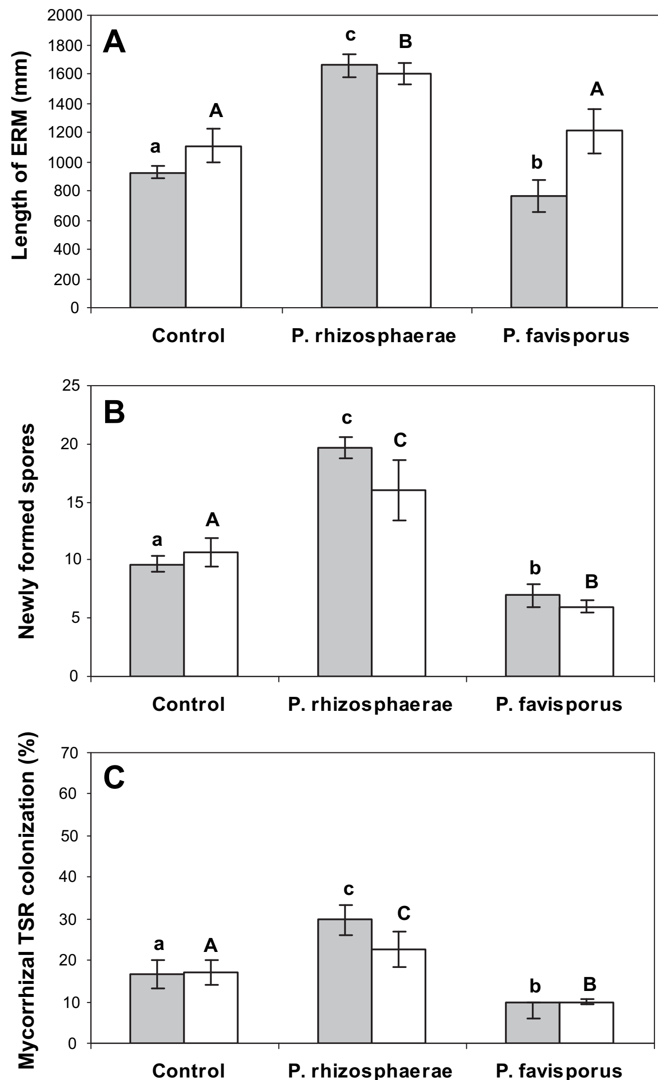

Fig. 2. Effect of cell suspensions (grey columns, small letters) and diffusible substances (white columns, capital letters) of *P. rhizosphaerae* TGX5E and *P. favisporus* TG1R2 on ERM length of *G. intraradices* GA5 (A), the number of newly formed spores (B) and the frequency of mycorrhizal colonization (C). Values are the means of five observations (±standard error) after forty days of co-culture. Bars with different letters are significantly different (LSD test, $p < 0.05$).

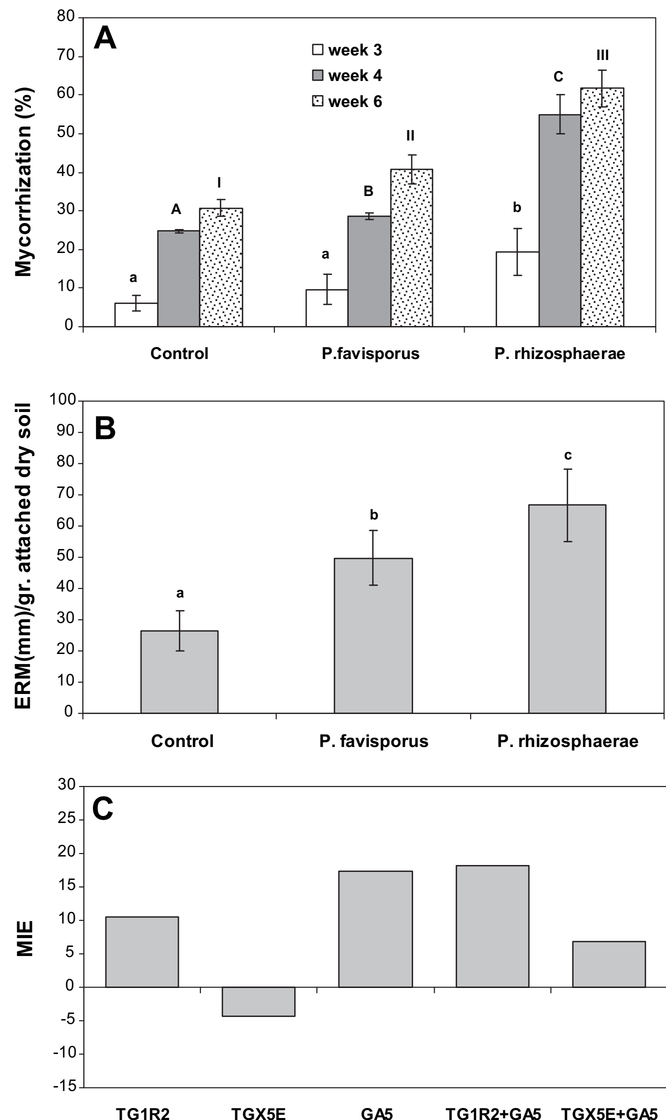

Fig. 3. Effect of *P. favisporus* TG1R2 and *P. rhizosphaerae* TGX5E on frequency colonization of soybean roots by *G. intraradices* GA5 (A) at week 3 (white columns, small letters), week 4 (grey columns, capital letters) and week 6 (dotted columns, Greek numbers). ERM length of *G. intraradices* GA5 produced per gram of dry soil (B) and MIE of bacteria and AM fungi on soybean biomass (C). Figures A and B, values represent the means of five observations (±standard error). Bars with different letters are significantly different (LSD test, $p < 0.05$).

4. Discussion

In this study, two *Paenibacillus* strains were isolated from AM propagules: *P. favisporus* was obtained from surface-sterilized and germinated sporocarps of *G. mosseae*, and *P. rhizosphaerae* was obtained from the intraradical mycelium of *G. fasciculatum* in *S. lycopersicum* roots. Mansfeld-Giese et al. (2002) isolated *Paenibacillus* bacteria associated with external mycelium of *G. intraradices*, and Hildebrandt et al. (2002) found that *P. validus* endospores associated with *G. intraradices* spores survived surface sterilization methods. Budi et al. (1999) identified a bacterial isolate as *Paenibacillus* sp. from the mycorrhizosphere of *Sorghum bicolor* inoculated with *G. mosseae*. This *Paenibacillus* strain produced stimulatory effects on AM fungal root colonization, spore germination, and hyphal growth of *G. mosseae* (Budi et al., 1999). These findings suggest that *Paenibacillus* species are widely distributed in the mycorrhizosphere and could have a positive effect on the development of the *in vivo* and *in vitro* AM symbiosis.

Several mechanisms have been proposed to explain this effect (1) changes in the receptivity of the root, (2) changes in the root-fungus recognition, (3) stimulation of fungal growth, (4) modification of the chemistry of the rhizospheric soil and (5) germination of the fungal propagules (Johansson et al., 2004; Bending et al., 2006).

The effects of the *Paenibacillus* strains tested in this study varied depending on pre-symbiotic and post-symbiotic *G. intraradices* development and root source (TSR vs. intact plant). The use of a single soybean genotype (AG043RG, NIDERA) in *in vitro* and *in vivo* assays allowed meaningful comparison of results (Fortin et al., 2002)

In the *in vitro* assay, inoculation with the endophytic strain *P. rhizosphaerae* TGX5E, associated with the intraradical mycelium, did not influence germination of *G. intraradices* spores but affected the development of both intra and extraradical mycelium by

promoting IRM re-growth and ERM length produced by both types of AM propagules. Similarly, a significant increase in the ERM network and an increase in sporulation were observed during symbiotic association with TSR. These results were correlated with the highest values of TSR colonization observed with *P. rhizosphaerae*. On the other hand, the length of the *G. intraradices* ERM in the rhizosphere of soybean plants showed the highest values when *P. rhizosphaerae* was co-inoculated. This bacterial strain had a promoting effect on *G. intraradices in vitro* infectivity, and led to an increase in the frequency of soybean colonized roots under *in vivo* conditions. Nevertheless, the high rate of infectivity was not correlated with an improvement of soybean dry biomass production.

In the *in vitro* pre-symbiotic assay, the rhizospheric strain *P. favisporus* TG1R2 did not affect germination of *G. intraradices* propagules. Nevertheless, during the symbiotic stage with TSR, the effects were clearly negative for all the variables measured. On the other hand, *P. favisporus* did not decrease the *in vivo* parameters. The length of ERM in soybean plants inoculated with this bacterium was promoted compared to control plants. Also, there was an increase in colonization of soybean roots by *G. intraradices* when plants were co-inoculated with *P. favisporus*. This species had a promoting effect on soybean biomass production when inoculated alone and a synergistic effect when co-inoculated with *G. intraradices*. We hypothesized that the highest values of IAA production of this bacterial strain could be responsible for the negative effects observed *in vitro*, where there was a closer proximity of the AM propagules to the bacterial supernatants in the limited space of Petri plates compared with the extensive growth of soybean roots in pots. Even if some cell-free bacterial exudates containing plant hormones can promote AM development and infection (Azcón et al., 1978), indole-producing bacteria could also have negative effects on AM fungi under certain conditions. Gryndler et al. (1998) found that a concentration of 30 μM of IAA suppressed for *Glomus fistulosum* hyphal growth.

Spore germination and re-growth of IRM were not affected by inoculation with *Paenibacillus* strains and the different IAA production in the bacterial supernatants. On the other hand, the clear promotion of in vitro pre-symbiotic mycelium network developed from spores and IRM caused by inoculation of both bacteria could explain the intimate association found between these bacterial strains and AM propagules. These results could indicate that the interaction between *G. intraradices* and *Paenibacillus* strains could not only be related to the production of indole compounds by the bacteria. Souchie et al. (2007) found that a similar value of IAA produced by some bacterial isolates showed different effects on AM hyphal growth. They hypothesized that other substances might also be responsible for growth promotion. As discussed by Johansson et al. (2004), rhizobacterial hormone-linked positive and negative modulation of mycorrhization is not a universal phenomenon.

In contrast, Azcón (1989) and Medina et al. (2003) suggested that the enhancement of mycelial growth and mycorrhization when bacteria were present resulted in a plant growth promotion response. However, additional combinations between AM fungi and indole-producing strains should be tested before firm conclusions can be drawn on the mode of interaction between these microorganisms.

The degree of specificity/selectivity of microbial associates could be related to the ability of some bacteria to behave as AM helper bacteria as defined by Garbaye (1994). Some exudates and/or differences in surface morphology of fungal propagules could be the possible reasons for the interaction observed (Artursson et al., 2006). The intra and extraradical AM fungal structures are different ecological niches for specific bacterial populations adapted to their environment. Therefore, the specificity of bacteria associated with AM fungi in different fungal developmental stages should be further examined to resolve the mechanisms involved in bacterial–mycorrhiza interactions.

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