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Beneficial effects of indigenous Cd-tolerant and Cd-sensitive *Glomus mosseae* associated with a Cd-adapted strain of *Brevibacillus* sp. in improving plant tolerance to Cd contamination

A. Vivas [a], A. Vörös [b], B. Biró [b], J.M. Barea [a], J.M. Ruiz-Lozano [a], R. Azcón [a,*]

[a] *Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain*
[b] *Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, Herman O.út 15., Budapest H-1022, Hungary*

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Abstract

In this study, we investigated the basic interactions between plant, rhizosphere microorganisms and Cd pollution. The effectiveness of the inoculation of an indigenous Cd-adapted arbuscular mycorrhizal (AM) strain of *Glomus mosseae* and/or a Cd-adapted bacterium (*Brevibacillus* sp.), both isolated from Cd-contaminated soil, was assayed in *Trifolium* plants growing in Cd-polluted soil. The behavior of the Cd-adapted *G. mosseae* strain was compared with that of a collection *G. mosseae* isolate (BEG 119). Results showed the functional compatibility of autochthonous microorganisms, which resulted in a biomass increase of 545% (shoot) and 456% (roots) compared to non-mycorrhizal plants. Single AM fungi (autochthonous or reference strains) and bacterial inoculation also proved highly effective in increasing plant biomass and nutrition. *G. mosseae* (BEG 119, reference strain) was less efficient than *Brevibacillus* sp. in increasing shoot growth. Co-inoculation with both microorganisms increased to the highest extent root biomass and symbiotic structures (nodules and AM colonization), and this may be responsible for the beneficial effect found. While plant acquisition of N and P were consistently enhanced by the application of the Cd-adapted autochthonous *Brevibacillus* sp. plus AM fungus, the Cd uptake by *Trifolium* plants decreased in dual AM fungus–bacterium treatments. Thus, the combined microbial inoculation conferred tolerance to Cd by increasing nutrient status and rooting development, and by decreasing Cd availability and uptake by the plant. The indigenous bacterial isolate was able to growth at increasing Cd concentrations (from 25 to 100 $\mu g\, g^{-1}$ Cd) while the growth of a reference, non-Cd-adapted, bacterial strain fell to zero in medium having 25 $\mu g\, g^{-1}$ Cd, indicating the tolerance to Cd of the indigenous bacterium. The beneficial effect of inoculated microorganisms on symbiotic and nutritional plant values is relevant for the increased growth and nutrition of plants growing in Cd-contaminated soils. The inoculation of suitable symbiotic and saprophytic rhizosphere microorganisms isolated from Cd-polluted soils plays an important role in the development and metal tolerance by plants and in soil bioremediation.

Keywords: Arbuscular mycorrhiza (AM); *Glomus mosseae*; *Brevibacillus* sp.; Cd tolerance; microbial interactions; native microorganisms

1. Introduction

Symbiosis between mycorrhizal endophytes and plants has extraordinary importance because of its impact on revegetation processes and on adverse

* Corresponding author. Tel.: +34-958-181600; fax: +34-958-12-96-00.
E-mail address: rosario.azcon@eez.csic.es (R. Azcón).

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environments (Barea and Jeffries, 1995; Barea et al., 2002). The arbuscular mycorrhizal (AM) symbiosis not only increases nutrient acquisition by the plant but also resistance to biotic and abiotic stresses (Bethlenfalvay and Linderman, 1992). In fact, many plant nutritional, biochemical, physiological and morphological responses are attributable to this association (Smith and Read, 1997).

Important ecological problems have arisen due to exploitation of natural resources causing degradation of natural ecosystems and polluted areas which results in unfavorable conditions for plant growth due to nutrient imbalances and depletion of microbial population (García et al., 1999). High levels of Cd negatively affected soil biomass (García et al., 1999; Chander and Brookes, 1991a,b) and had a negative effect on dehydrogenase activity (endocellular enzyme), which was similar to the Cd effect on the microbial biomass (Moreno-Ortego et al., 1999). Nevertheless, in polluted areas, plants are more dependent on microbial activity and the microorganisms are able to enhance their metabolic activity to combat stress (Moreno-Ortego et al., 1999; Killham and Firestone, 1983). Depending on the severity of the environmental damage the diversity and activity of the soil biota are increased to a certain threshold value preceding the final loss of function.

Microbial inoculations in polluted soils can help the plant through toxin decomposition as well as promoting plant growth (Puppi et al., 1994). Thus, microbial inoculation using strains adapted to the high Cd concentrations can restore the biomass values, since the stimulation of indigenous soil microbial activity is essential to enhance natural detoxification of contaminated environments (Vörös et al., 1998). Approaches that are still in the early stages of testing include inoculation of the soil/plant system with microorganisms having specific biotransforming abilities or that enhance phytoaccumulation.

Based on the chemical properties of Cd, the possibility of conversion to a less toxic form is likely to be very low. But AM mycelium has a high metal sorption capacity relative to other microorganisms (Joner et al., 2000). The adsorption was maximal in a metal tolerant *Glomus mosseae* isolate that adsorbed up to 0.5 μg Cd mg^{-1} which was three times the binding capacity of non-tolerant fungi (Joner et al., 2000). But contrasting results have been reported for this subject since some authors found no differences between sensitive or resistant strains of *G. mosseae* in highly Cd-contaminated soils (Gildon and Tinker, 1983; Weissenhorn et al., 1995).

Fungal isolates within one species vary in symbiotic effectiveness (Van der Heijden and Kuyper, 2001; Monzón and Azcón, 1996) depending on the compatibility among the fungus, soil microorganisms and plant in the rhizosphere niche. In fact, the rhizosphere has specific features such as pH, lower concentration of ions and higher content of low molecular weight organics, which are affected by species diversity and activity of the soil biota.

Studies have reported that different isolates of AM fungi showed high variability in their tolerance to heavy metals (Leyval et al., 1991; Weissenhorn et al., 1993; Bartolomé-Esteban and Schenck, 1994). Thus, the objective of this study was to compare the effectiveness of an autochthonous *G. mosseae* strain isolated from a Cd-polluted soil versus a non-autochthonous *G. mosseae* strain (isolate BEG 119) not adapted to Cd, in terms of plant growth, nutrition, heavy metal uptake and symbiotic development under Cd toxicity. In addition, we tested the interactions between each *G. mosseae* isolate and an autochthonous Cd-adapted bacterium.

2. Materials and methods

2.1. Experimental design

The experiment consisted of two treatments for each mycorrhizal inoculum. AM autochthonous and *G. mosseae* (BEG 119) from our laboratory collection, were assayed singly or in co-inoculation with *Brevibacillus* sp. (autochthonous strain). Non-mycorrhizal controls inoculated with bacterium, or non-treated, were also used.

All treatments were replicated five times with a total of 30 pots and placed in a randomized complete block design.

2.2. Soil microorganisms and plant growing conditions

A calcareous loamy soil from Granada province (Spain) was sieved (2 mm), diluted with quartz–sand

(<1 mm) (4:1 soil:sand (v/v)) and sterilized by steaming (100 °C for 1 h for 3 days). The soil had a pH of 7.2 (water); 1.63% organic matter, nutrient concentrations (mg kg^{-1}): N, 2.1; P, 1.7 ($NaHCO_3$-extractable P); K, 80. The soil contained 58% sand, 19% clay and 23% silt.

After sterilization, the soil/sand mixture was supplemented with 30 μg g^{-1} of Cd by adding an adequate amount of an aqueous solution of $CdSO_4$. After 2 weeks of soil incubation (for metal stabilization) the available Cd was determined, according to the Lakane and Erviö (1971) methodology, to be 13 μg g^{-1} Cd.

The soil samples for microbial inocula production were taken from Cd-treated long-term field experiment (10 year-old) in Nagyhörcsök (Hungary) (Kádár, 1995). The Cd-adapted AMF and bacterial populations were isolated from this soil and were cultivated for inocula production.

The indigenous mycorrhizal inoculum isolated from the Cd-polluted soil (Nagyhorcsock, Hungary) was identified as a *G. mosseae* strain (determined morphologically). It was bulked in an open-pot culture of red clover and consisted of soil, spores, mycelia and infected root fragments with overall colonization of 70%. Ten grams of inoculum were added to appropriate pots at sowing time just below the clover seeds.

A *G. mosseae* strain (isolate BEG 119) from our collection was used as reference AM inoculum. It was also bulked in an open-pot culture of clover. The inoculum consisted of 10 g of soil, spores, mycelia and infected roots fragments with 80% colonization. It was added to the appropriate pots at sowing time just below the clover seeds.

A bacterial strain, later identified as a *Brevibacillus* sp., was isolated from the Cd-contaminated soil at Nagyhörcsök (Hungary). It was the most abundant bacterial type in such soil. When appropriate, pots were inoculated with 1 ml of bacterial culture (10^8 cfu ml^{-1}) grown in nutrient broth medium for 24–48 h at 28 °C of temperature.

A suspension of *Rhizobium leguminosarum* bv. *trifolii* was added to each pot (1 ml, 10^8 cfu per pot). It was prepared following standard procedure (Azcón, 1993).

Following incubation, the Cd-amended soil/sand mixture (30 μg g^{-1} of Cd as $CdSO_4$) was added to the pots. One-third of the pots was inoculated with the *G. mosseae* (Cd-adapted inoculum) selected from the original contaminated soil, and another third with *G. mosseae* reference strain (BEG 119). Non-mycorrhizal pots received the same amount of autoclaved inoculum and a filtrate (2 ml) of AM inoculum to add the microbial population free of AM propagules. Plants of *Trifolium* L. *repens* were grown for 3 months in the 100 cm^3 pots in a greenhouse under controlled climatic conditions (18–24 °C, with a 18/6 light/dark period and 50% relative humidity). Throughout the experiment, the plants were fertilized with 10 ml of Hewitt's (Hewitt, 1952) nutrient solution lacking N and P.

2.3. Measurements

At harvest, the dry biomass of roots and shoots, nutrient and metal concentrations and symbiotic development (mycorrhizal infection and nodulation) were determined.

Shoot concentrations (mg g^{-1}) of N (micro-Kjeldahl) and P, as well as of Pb, Cd, Ni and Zn (μg g^{-1}) were also determined from three different measurements made on a pooled sample containing the five replicates per treatment after wet digestion of the air-dried plant samples with $HNO_3 + H_2O_2$ by inductively coupled plasma-atomic emission spectrometry (ICP-AES), as described by Takács et al. (2001).

The percentage of mycorrhizal root length was estimated by microscopic observation of fungal colonization after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (v/v), according to Phillips and Hayman (1970). Mycorrhizal colonization was quantified using the grid-line intersect method (Giovannetti and Mosse, 1980). Nodule numbers were estimated by direct observation using a binocular microscope. Five replicates were examined per treatment.

2.4. Molecular identification of the bacterial isolate

Bacterial identification was carried out by 16S rDNA cloning and sequencing as described by Vivas et al. (in press). Database searches for 16S rDNA sequence similarity unambiguously identified the Cd-tolerant bacterium as a member of the genus *Brevibacillus*.

2.5. Bacterial growth under increasing Cd levels in the medium

Growth of the Cd-tolerant bacteria under increasing Cd levels was assayed in comparison to a reference *Brevibacillus* strain from our collection. Both bacterial strains were cultivated at 28 °C in nutrient broth supplemented with 0, 25, 50, 75 or 100 μg g^{-1} Cd as $CdSO_4$. The number of viable cells was estimated at 1 h intervals from 0 to 16 h.

2.6. Statistics

The results (except concentration and content of mineral elements in shoot) were statistically evaluated by factorial analysis of variance with bacterial treatment, mycorrhizal treatment and bacterial treatment–mycorrhizal treatment interaction as sources of variation. Percentage values were arcsine-transformed before statistical analysis.

3. Results

The results of factorial ANOVA are given in Table 1. Mycorrhizal colonization and bacterial (*Brevibacillus* sp.) inoculation were critical for plant growth in highly Cd-polluted soil. Nevertheless, responses of *Trifolium* to the autochthonous and reference *G. mosseae* strains were different. The native AM inoculum clearly caused the highest beneficial effect on shoot and root growth particularly when associated with the indigenous bacterium although the interaction between AM and bacterial inoculation was not significant (Fig. 1, Table 1).

Table 1
Significance of the main treatment effects and their interactions based on factorial ANOVA

	F-values		
	AM treatment	Bacterial treatment	Mycorrhiza* bacteria
Shoot dry weight	213.0***	310.0***	3.4 ns
Root dry weight	84.7***	40.70***	1.8 ns
Number of nodules	16.43**	7.05*	12.3**

ns: not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

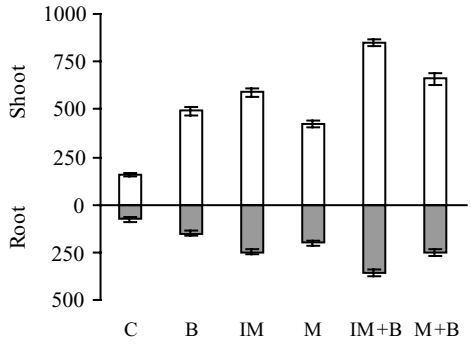

Fig. 1. Shoot and root dry weights (mg per plant) of *Trifolium* plants cultivated in soil amended with 30 μg g^{-1} Cd. Treatments are C (Control), (B) *Brevibacillus* sp., (IM) mycorrhizal inoculation with indigenous or (M) collection *G. mosseae*. Vertical bars represent standard errors.

Brevibacillus sp. increased shoot and root growth similarly to the non-native *G. mosseae* (BEG 119) (Fig. 1). Plants colonized by the reference *G. mosseae* (isolate BEG 119) when associated with *Brevibacillus* sp. had equal shoot and root growth to the single mycorrhizal plants colonized by the indigenous *G. mosseae* (Fig. 1). These growth responses were closely related to P nutrition and total root colonization (Table 2, Fig. 2).

Similar percentage of AM colonization and similar bacterial enhancement of mycorrhizal infection were recorded in mycorrhizal plants regardless of the fungal strain involved. The association of autochthonous microorganisms increased total mycorrhizal root length to a greatest extent than any other treatment (Fig. 2).

The number of nodules formed on roots of *Trifolium* was strongly increased by mycorrhizal colonization. This effect was particularly marked in dually-inoculated plants, with the indigenous *G. mosseae* plus *Brevibacillus* sp. being the best treatment for enhancing this value (Fig. 3, Table 1).

The effect of *G. mosseae* (isolate BEG 119) on plant P content was lower than that of *Brevibacillus* sp. and co-inoculation with both microorganisms did not increase this value. The interaction of the indigenous microorganisms (*G. mosseae* and *Brevibacillus* sp.) was highly effective in enhancing phosphorus content. Dual inoculation with native inocula increased plant P content by 610% over control (Table 2).

Table 2
Cd, P, N, Fe Mn, Zn, Ni and Pb concentration or content in *Trifolium* plants grown in soil artificially contaminated with 30 μg g^{-1} Cd

Microbial treatments	Cd (μg g^{-1})	P (mg g^{-1})	N (mg g^{-1})	Fe (μg g^{-1})	Mn (μg g^{-1})	Zn (μg g^{-1})	Ni (μg g^{-1})	Pb (μg g^{-1})
Concentrations								
C	11.58	1.215	19.460	0.166	0.172	0.0634	0.0441	0.0409
B	11.17	1.080	10.310	0.389	0.185	0.0623	0.0586	0.0580
IM	12.98	0.977	17.580	0.241	0.095	0.0540	0.0557	0.0418
M	11.87	1.046	31.310	0.485	0.202	0.0551	0.0657	0.0657
IM + B	2.79	1.365	27.860	0.262	0.125	0.0535	0.0323	0.0382
M + B	2.44	0.828	24.400	0.400	0.230	0.0468	0.0860	0.0768
	Cd (μg)	P (mg)	N (mg)	Fe (μg)	Mn (μg)	Zn (μg)	Ni (μg)	Pb (μg)
Content								
C	1.807	0.190	3.036	0.026	0.027	0.0100	0.0070	0.0060
B	5.475	0.529	5.051	0.191	0.091	0.0310	0.0290	0.0280
IM	7.659	0.577	10.370	0.142	0.056	0.0320	0.0330	0.0250
M	5.021	0.442	13.245	0.205	0.085	0.0230	0.0280	0.0280
IM + B	2.368	1.160	23.681	0.223	0.106	0.0450	0.0270	0.0320
M + B	1.610	0.547	16.104	0.264	0.152	0.0310	0.0570	0.0510

Values given were the means of three determinations from a pooled sample including the five replications per treatment. Treatments are C (Control), (B) *Brevibacillus* sp., (IM) mycorrhizal inoculation with indigenous or (M) from collection *G. mosseae*.

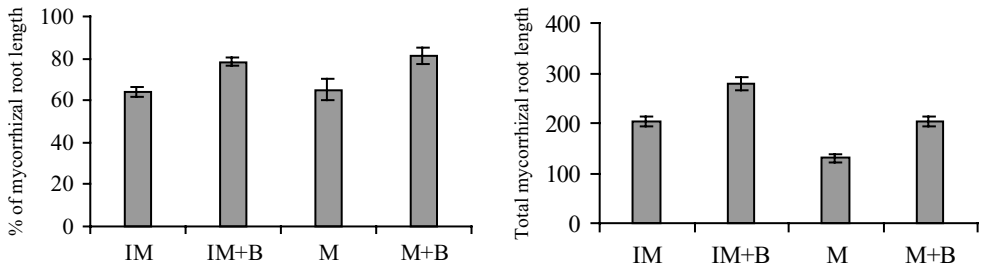

Fig. 2. Effect of *Brevibacillus* sp. (B) and mycorrhizal inoculation of *G. mosseae* [indigenous (IM) or from collection (M)] on mycorrhizal colonization evaluated as percentage and as total mycorrhizal root length (cm) in soil artificially contaminated with 30 μg g^{-1} Cd. Vertical bars represent standard errors.

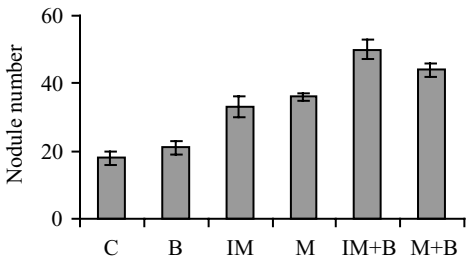

Fig. 3. Nodule number in *Trifolium* plants cultivated in soil amended with 30 μg g^{-1} Cd. Treatments are as in Fig. 1. Vertical bars represent standard errors.

The effectiveness of the indigenous mycorrhizal fungus in increasing plant growth and N–P content was greatest when it was dually inoculated with *Brevibacillus* sp. (Fig. 1, Table 2).

Nitrogen uptake was increased by mycorrhizal colonization, particularly when both indigenous microorganisms were co-inoculated, which increased N content by 780% over control (Table 2).

Different effects of *Brevibacillus* sp. on N nutrition were observed depending on the origin of the associated *G. mosseae*. No stimulating effect of the bacterium plus *G. mosseae* (BEG 119) on plant N content was found, while the interaction was positive when the autochthonous *G. mosseae* was involved (Table 2).

In general, the effect of the indigenous *Brevibacillus* sp. was more apparent in plants colonized by the indigenous AM fungus, when shoot and root biomass as well as N, P, and Zn content were increased (Table 2). Not only plant growth and nutrition responses but also symbiotic values were increased to a greater extent by co-inoculation with indigenous microorganisms. In contrast, metals such as Ni and Pb were particularly increased in the dual *G. mosseae* (BEG 119) plus *Brevibacillus* sp.-inoculated plants.

The most important result of this study is that plant Cd uptake was clearly reduced in dually-inoculated plants as compared to single-inoculated plants. This is an interesting microbial effect for plant growing in Cd-polluted soils. Additionally, to test if the autochthonous bacterial strain isolated from Cd-polluted soil had a particular ability to grow under high Cd concentrations this bacterium was grown under increasing Cd levels (from 25 to 100 $\mu g\,g^{-1}$) and compared with a non-indigenous bacterium used as a reference strain (both belonging to the same genus). This reference bacterium was only used under "in vitro" conditions for this comparative study but not in the microcosm experiment. The results show that the

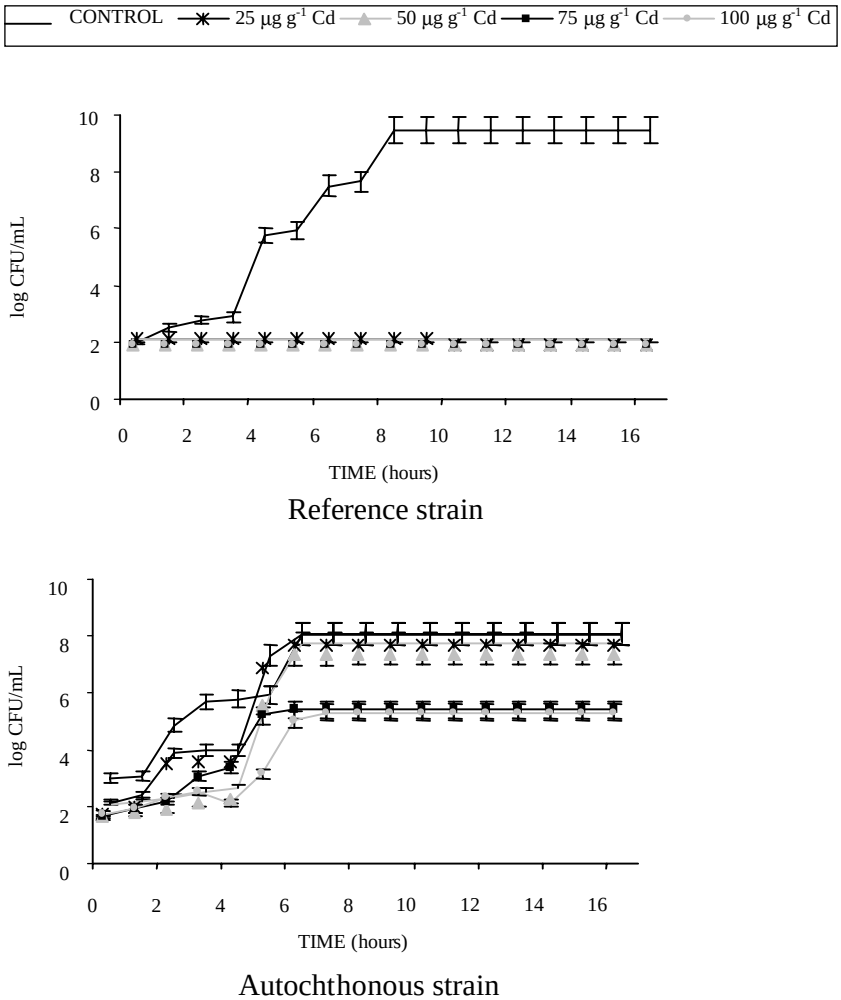

Fig. 4. Viable cells (log cfu ml^{-1}) of reference and autochthonous *Brevibacillus* strains at different time intervals in nutrient broth medium supplemented with increasing Cd levels. Vertical bars represent standard errors.

growth of the autochthonous and the reference strains of *Brevibacillus* sp. in nutrient broth at increasing Cd concentrations was different (Fig. 4). The reference bacterium was found to be intolerant to this metal while the native isolate reached 10^7 cfu after 6 h at the highest Cd concentration in the growing medium (from 75 to 100 $\mu g\, g^{-1}$).

4. Discussion

Mycorrhization by the autochthonous *G. mosseae* was more effective in terms of enhancing shoot and root growth and nutrient uptake than the reference AM strain of *G. mosseae* (BEG 119). The dual inoculation with AM fungi and the native *Brevibacillus* sp. maximized mycorrhizal efficiency in all cases. As a consequence, inoculation with both indigenous microorganisms lead to the best plant nutrition in this Cd-polluted soil. Several mechanisms can be involved in such bacterial effect (Puppi et al., 1994).

The ability of *Brevibacillus* sp. to produce auxin-indole derivatives (3.62 $mg\, l^{-1}$ as measured in axenic liquid culture medium) may cause such stimulating effects. In a previous microcosm experiment using the same soil and microorganisms but a lower Cd contamination (15 $\mu g\, g^{-1}$ Cd applied), the IAA determined in the rhizosphere of single *Brevibacillus*-inoculated soil was 162 $mg\, kg^{-1}$. This value increased to 287 and 225 $mg\, kg^{-1}$ when autochthonous *G. mosseae* or BEG 119, respectively were inoculated with the bacterium. The bacterial stimulating effect may in part be explained by such a mechanism.

Linderman (1992) and Azcón (1987, 1989, 1993) described the stimulation of mycorrhizal root colonization by IAA-producing bacteria since growth promoting compounds were able to stimulate plant susceptibility to AM infection and growth of extraradical AM mycelium. The amount of IAA in the rhizosphere of *Brevibacillus* sp.-inoculated plants may be related to the effect of this bacterium, not only in the stimulation of root growth, total mycorrhizal colonization and consequently extraradical mycelium production, but also of plant growth and nodule formation as can be observed in this study.

As our results show, AM fungi have a wide environmental distribution but the symbiotic effectiveness changes depending on the fungal origin. While the percentage mycorrhizal colonization by the indigenous and the reference AM inocula was similar (64 and 65%, respectively), the total mycorrhizal root length differed to a greater extent than colonization according to the origin of AM isolates.

Weissenhorn and Leyval (1995) isolated a Cd-tolerant AM fungus which was compared with a Cd-sensitive reference strain and determined that only the tolerant AM strain was able to colonize plants with up to 5 $mg\, l^{-1}$ Cd and this Cd level suppressed infection by the sensitive fungal strain. However, even in AM colonized plants the negative effect of Cd was not alleviated. Koomen et al. (1987) and Díaz and Honrubia (1995) reported that indigenous isolates are not necessarily highly effective in terms of plant growth responses in the soil of their origin. In agreement, Shetty et al. (1994, 1995) and Weissenhorn et al. (1995) found no differences between sensitive and resistant AM strains of *G. mosseae* on maize grown in highly contaminated soils and Weissenhorn and Leyval (1995) reported similar results in sand culture medium amended with a range of Cd concentrations. Also, Gildon and Tinker (1983) found that the resistant AM fungal strain and the sensitive Cd strain have similar effects on plant P uptake. In contrast, under the experimental conditions used here, AM colonization by indigenous or reference AM fungi was not suppressed by an available Cd level of 13 $\mu g\, g^{-1}$, and both *G. mosseae* strains were effective in promoting growth and nutrition of the host plant. Nevertheless, the *G. mosseae* isolate adapted to the soil conditions was able to stimulate plant growth to a higher extent than the non-indigenous isolate of *G. mosseae* (BEG 119).

No information on the influence of indigenous bacteria associated with native AM fungi on plant Cd tolerance has been previously reported. The most relevant effect of the bacterial co-inoculation with *G. mosseae* (indigenous or reference strains) was the strong decrease in Cd concentration in the host plants. Hence, dual inoculations acted as Cd excluders since the host plants refused metal transfer to the shoot biomass. These results provide evidence that the ability of plants to grow in polluted soil was related to the associated microorganism. Mechanisms involved in the observed effect are not totally determined and need further study. However, some explanations can be offered. It has been suggested that fast-growing

bacteria could be efficiently used for the removal of Cd from a medium (Bååth, 1989). Previously, in an "in vitro" experiment with 10 $\mu g\, g^{-1}$ of Cd, we determined that *Brevibacillus* sp. was able to accumulate 73% of Cd from the culture solution (unpublished results). The amount of Cd accumulated was the same after 1 and 3 h. This bacterial ability may explain the decreasing Cd content in dual mycorrhizal plus bacterium treated plants. The results demonstrate the potential use of these microbial inocula for *Trifolium* growth in Cd-polluted soil. However, in the present study this bacterial activity was evidenced only in mycorrhizal rhizospheric soil. To explain that, it should be considered that plants associated to AM fungi can change bacterial populations (Medina et al., 2003) and redox conditions in the rhizosphere (Bååth and Arnebrant, 1994) and this will cause different Cd mobility and concentration in soil (Speir et al., 1999).

Not only the bacterial inoculation, but also the AM inoculation, and particularly the dual association, increased the concentration of most of the nutrients and metals in *Trifolium*, with the exception of Cd, which was present in the growing medium in a supraoptimal level (polluted conditions). Weissenhorn and Leyval (1995) reported that, independently of the Cd content in the substrate, Cd concentration in the shoots of plants was decreased by mycorrhizal colonization. Joner et al. (2000) reported that AM mycelium has a high metal sorption capacity. It has been postulated that AM fungi restrict metal transport to host tissues by metal binding or sequestration (Leyval et al., 1997). The external mycelium is able to produce glycoprotein, with binding properties, and glomalin. As well, changes in storage and/or internal transportation have been proposed by Turnau et al. (1993) and Boddington and Dodd (1999). In the present study, both mycorrhizal mechanisms seem to be stimulated by the bacterium. The bacterial isolate of *Brevibacillus* sp. also acted as a mycorrhiza-helping organism (Garbaye, 1994), improving the mycorrhizal functionally. If AM mycelium has a high metal sorption capacity (Joner et al., 2000), any factor (such as the bacterium) increasing AM mycelium could decrease Cd availability for the plant.

On the other hand, the phytotoxicity caused by Cd may be alleviated due to the effect of increased absorption of major nutrients such as N and P (Sieverding, 1991). In fact, in the treatments having a greater N and P uptake, Cd content was reduced, and consequently enhanced plant growth was observed.

In conclusion, dual inoculation of native microorganisms seems to be a strategy which can be recommended for promoting plant growth under Cd-polluted conditions. Thus, isolation of efficient metal-adapted microorganisms may be an interesting biotechnological tool for inoculation purposes in contaminated soils (Dodd and Thomson, 1994). The functioning of beneficial microbial interactions are crucial from an ecological and practical point of view in the case of Cd-polluted soil.

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