Comparative Effect of *Pseudomonas*, Strain F 113 [Biocontrol Agent (Antifungal)] and its Isogenic Mutant, Strain F113G22 [Impaired Biocontrol Ability] on Spore Germination and Mycelial Growth of *Glomus mosseae* under Monoxenic Conditions

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Summary.- A study was carried out under monoxenic conditions to ascertain whether or not the biocontrol (antifungal) agent, *Pseudomonas* F113, affects the development of the mycorrhizal fungus *Glomus mosseae*, in comparison with its isogenic mutant F113 G22, which lacks such antifungal ability. Each bacterial strain was co-cultured with surface-sterilized spores of *Glomus mosseae* in water-agar at 25° C during 28 days. Despite there being no significant effects on spore germination, the hyphal development was significantly stimulated by both bacteria. Therefore, it can be concluded that the antifungal effect of *Pseudomonas* F113 was not exerted on a representative beneficial mycorrhizal fungus.

Keywords: Pseudomonas sp., Glomus mosseae, *spore germination, hyphal development*

Introduction

Many studies have shown interactions between arbuscular mycorrhizal (AM) fungi and other soil microorganisms (2). Despite it is well accepted that these interactions can affect the optimal functioning of mycorrhizal symbiosis, the management of these rhizosphere activities and the underlying responsible mechanisms are not completely understood. Particularly, soil microorganisms, included fungi (6, 7) and some rhizosphere bacteria (1, 10, 12), have been described to influence germination and growth of AM fungi *in vitro*. Besides, it has also been stated that AM fungi spore germination can be suppressed in some non-sterile soils by antagonistic microbiota (14).

Bacteria living in the rhizosphere may have beneficial effects on plant growth by providing available nutrients and growth factors, or by producing antibiotics and siderophores, which antagonize phytopathogenic fungi and bacteria.

There is a considerable experimental support for the idea that the so called plant growth promoting rhizobacteria (PGPR) may be used as bio-fertilizers or biological control agents to increase plant productivity (8, 9). In addition, beneficial bacteria are being genetically modified to improve the expression of their relevant traits such as that of biocontrol ability. However, the introduction of bacterial strains, either wildtype or genetically modified, in plant rhizosphere, as it is presently being investigated, must be throughly controlled because it could cause some disturbance in rhizosphere biology/ecology. A number of studies are aimed at this concern. The main objective of the present study aims to ascertain whether or not the antifungal agent *Pseudomonas* F113 affects the development of the AM fungi *Glomus mosseae in vitro*, in comparison with its isogenic mutant, which lacks such an ability.

Materials and Methods

Both the biocontrol agent *Pseudomonas* strain F113 (antifungal) and its isogenic mutant strain F113G22 (impaired biocontrol ability) were grown at 28° C for 24 hr on LB (triptone, 10 g/L; yeast extract, 5 g/L; Na Cl, 5 g/L; Difco Agar, 15 g/L) medium and then resuspended in sterile water. The cultures were adjusted to an optical density of 0.4 (λ= 650 nm) corresponding to 10^8 for the F113 strain and 10^7 for the F113G22 colony forming units (cfu). Serial dilutions (10^{-1} to 10^{-5}) were prepared and 50 µL of each dilution inoculated in Petri dishes (9 cm diameter) containing water-agar (0.8 %, Bacto-Difco) buffered with MES [2-(N-Morpholino) ethanesulfonic acid] (10 mM) (6). The final pH after sterilization at 120° C for 20 minutes, was 7.0. *Glomus mosseae* resting spores freshly excised from sporocarps, were surface sterilized in 2 % w/v chloramine T plus 200 µg/mL of streptomycin and one drop of Tween 80 (11). This solution was applied for 20 minutes and the

spores were then washed five times in sterile desionized water prior to transferring them, with sterile capillary pipettes, to Petri dishes. Five surface sterilized spores, located on the vertices of an imaginary pentagon of about 3.5 cm per side, were grown per plate. Five replicates per dilution and bacterial culture were prepared. The Petri dishes were incubated at 25° C in the dark and plates were sealed with parafilm to reduce dehidration and contamination risks.

Germination rate and hyphal development were periodically examined under a light microscope. Mycelial growth was estimated by using a gridline (0.9 mm side) intersect method.

Data of hyphal length after 28 days of co-culture were processed by Student's t test ($P < 0.01$).

Results

Spore germination was hardly affected by either of the two bacteria (*Pseudomonas* F113 or its mutant G22) tested (Figure 1).

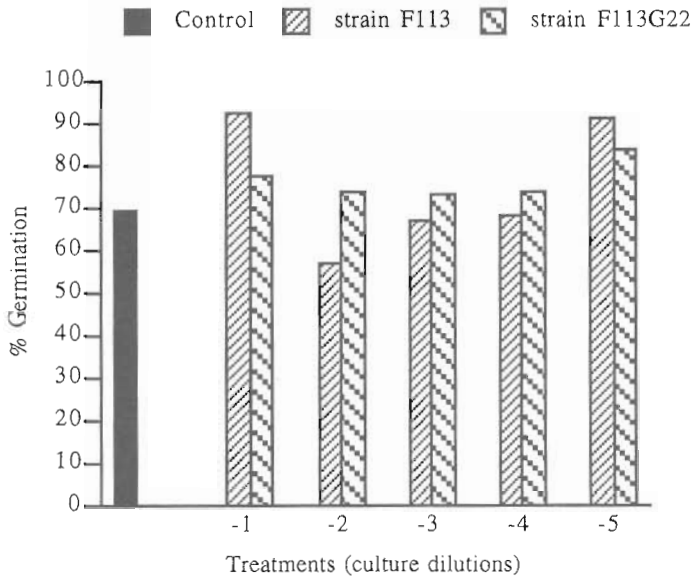

Figure 1. Effect of *Pseudomonas* sp. (F113 and F113G22) on the germination of *G. mosseae* spores, as affected by the density of the bacterial inoculum (10^{-1} to 10^{-5}) after 28 days of co-culture under monoxenic conditions.

Despite the lack of any significant effects on spore germintion, the growth of the hyphae was significantly stimulated by the two *Pseudomonas* sp. (Figure 2).

Discussion

The stimulation of AM fungi mycelial growth *in vitro* by other rhizosphere microorganisms found in the reported study corfirms previous results (1, 7, 10, 12).

Both strains assayed showed stimulatory effects on the development of the mycelium of *G. mosseae*, but they did no exert any effect on the germination rate. This mycelial growth stimulation was greater at the lower density of the inoculum tested. The mechanisms involved may be the production of stimulatory compounds, like aminoacids, vitamins, hormones, etc (3), which can be released to the growing medium (3, 4).

Recently, some experiments carried out with *Gigaspora margarita* and different strains of *Rhizobium leguminosarum* and *Pseudomonas fluorescens* showed physical interactions between the bacteria and the AM fungus, which could be involved in the synergistic or antagonistic effects caused by soil bacteria on the mycelial growth of the AM fungus (5).

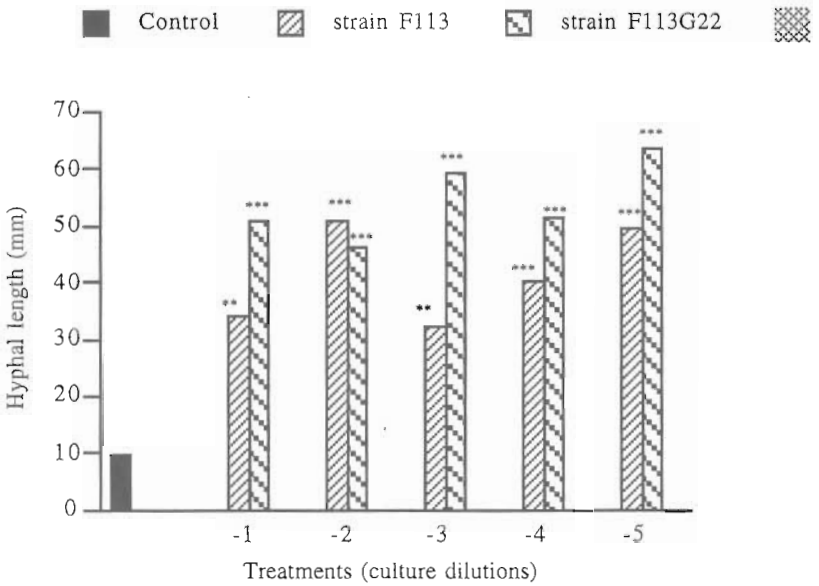

Figure 2. Effect of *Pseudomonas* sp. (F113 and F113G22) on growth of the hyphae of *G. mosseae*, as affected by the density of the bacterial inoculum (10^{-1} to 10^{-5}), after 28 days of co-culture under monoxenic conditions. ** Indicates effects significantly different from control at $P< 0.01$ and *** at $P< 0.001$.

All in all, the most noteworthy effect was that the antifungal activity of *Pseudomonas* F113 was not exerted on *G. mosseae*, a representative mycorrhizal fungus, as already described for other antifungal biological agents like *Trichoderma* sp. (7), or *Gliocladium* (13). Furthermore, the antifungal *Pseudomonas* exerted a rather high stimulatory effect on mycelial development from *G. mosseae* spores.

Obviously, the results, corresponding to a study carried out under the particular set of conditions typical of monoxenic experiments, should be seen with some caution. Microcosm and field testing trials might follow to try to ascertain the actual meaning of the conclusions reached in this study. A confirmation under more realistic conditions of the lack of any inhibitory activity from a biocontrol *Pseudomonas* against a beneficial fungal symbiont, while it antagonizes pathogenic fungi would be relevant both in rhizosphere ecology and in term of target biotechnological approaches concerning management of the rhizosphere environment to improve plant growth and health.

Acknowledgments: The *Pseudomonas* strains were provided by Prof. O'Gara, Microbiology Dept., University College of Cork (Ireland), in the network of scientific relationship sponsored by the BIOTECH EU. Programme, IMPACT Project (BIO 2-CT 93-0053).

References

1. AZCON, R. (1987). Germination and hyphal growth of *Glomus mosseae in vitro*: effects of rhizosphere bacteria and cell-free culture media. *Soil Biol. Biochem.* 4, 417-419.
2. AZCON-AGUILAR, C. & BAREA, J. M. (1992). Interactions between mycorrhizal fungi and other rhizosphere microorganisms. In: *Mycorrhizal Functioning: An Integrative Plant-Fungal Process* (Ed. by M. J. Allen), pp. 163-198. CRC Press, New York.
3. AZCON-AGUILAR, C., DIAZ-RODRIGUEZ, R. M. & BAREA, J. M. (1986). Effect of soil microorganisms on spore germination of the vesicular arbuscular mycorrhizal fungus *Glomus mosseae*. *Trans. Br. Mycol. Soc.* **86**, 337-340.
4. BECARD, G. & PICHE, Y. (1989). Fungal growth stimulation by CO_2 and root exudates in vesicular-arbuscular mycorrhizal symbiosis. *Appl. Environm. Microbiol.* **55**, 2320-2325.

5. BIANCIOTTO, V., PEROTTO, S., LANFRANCO, L. & BONFANTE, P. (1994). The role of arbuscular mycorrhizal fungi in the impact project: cellular interactions between arbuscular mycorrhizae and plant growth promoting soil bacteria. BIOTECH Sectorial Meeting on Microbial Ecology and Biosafety. Cork (Irland), September, 27-29. Abstracts, pag. 62.
6. CALVET, C. (1989). Micorrizas vesículo-arbusculares en cultivos intensivos sobre sustratos orgánicos: interacciones con hongos saprofitos y patógenos de la rizosfera. Ph D. Thesis, Universidad de Granada, Spain.
7. CALVET, C., BAREA, J. M. & PERA, J. (1992). *In vitro* interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* and some saprophytic fungi isolated from organic substrates. *Soil Biol. Biochem.* **24**, 757-780.
8. DAVISON, J. (1988). Plant beneficial bacteria. *Biotechnology* **6**, 282-286.
9. KLOEPPER, J. W. (1992). Plant growth-promoting rhizobacteria as biological control agents. In: *Soil Microbial Ecology. Applications in Agriculture, Forestry and Environmental Management* (Ed. by F. Blaine), pp. 255-274. Marcel Dekker, New York.
10. LINDERMAN, R. G. & PAULITZ, T. C. (1990). Mycorrhizal-rhizobacterial interactions. In: *Biological Control of Soil-borne Plant Phathogens* (Ed. by D. Hornby), pp. 261-283. Wallington, CAB International.
11. MOSSE, B. (1962). The establishment of vesicular-arbuscular mycorrhiza under aseptic conditions. *J. Gen. Microbiol.* **27**, 509-520.
12. MUGNIER, J. & MOSSE, B. (1987). Spore germination and ability of a vesicular arbuscular mycorrhizal fungus *Glomus mosseae*. *Trans. Br. Mycol. Soc.* **88**, 411-413.
13. PAULITZ, T. C. & LINDERMAN, R. G. (1991). Lack of antagonism between the biocontrol agent *Gliocladium virens* and vesicular arbuscular mycorrhizal fungi. *New Phytol.* **117**, 303-308.
14. WILSON, G. W. T., DANIELS, B. A., HETRICK & GERSCHEFSKE KITT, D. (1989). Suppression of vesicular-arbuscular mycorrhizal fungus spore germination by nonsterile soil. *Can. J. Bot.* **67**, 18-23.