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# Interactions between a plant growth-promoting rhizobacterium, an AM fungus and a phosphate-solubilising fungus in the rhizosphere of *Lactuca sativa*

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**Abstract**

This study evaluated the interactions between the inoculation with an arbuscular mycorrhizal fungus, *Glomus intraradices* Schenck & Smith, a plant growth-promoting rhizobacterium, *Bacillus subtilis*, and a filamentous soil fungus, *Aspergillus niger*, with respect to their effects on growth of lettuce plants and on indicators of biological soil quality (microbial biomass C, water-soluble C and carbohydrates and dehydrogenase, urease, acid phosphatase and benzoyl argininamide hydrolyzing protease activities). Water-soluble carbohydrates and microbial biomass were increased only in the rhizosphere soil of *G. intraradices*-plants. Rhizosphere soil from all microbial inoculation treatments had significantly higher dehydrogenase activity than the control soil, particularly in the soil inoculated with *B. subtilis* (about 21% higher than control soil). Inoculation with *A. niger* or *B. subtilis* increased significantly the urease, protease and phosphatase activities of the rhizosphere soil of the lettuce plants. The foliar P and K contents increased significantly with the *B. subtilis* or *G. intraradices* inoculation, alone or in combination. The most effective co-inoculation was observed in the combined treatment of inoculation with *G. intraradices* and *B. subtilis*, which synergistically increased plant growth compared with singly inoculated (about 77% greater with respect to the control plants).


*Keywords:* Arbuscular mycorrhizal fungi; Enzyme activity; Microbial biomass C; Plant growth-promoting rhizobacteria; Sustainable agriculture

## 1. Introduction

Co-inoculations of beneficial rhizosphere microorganisms into soils, reducing the inputs of environmentally deleterious agro-chemicals required for optimal plant growth, are gaining increased attention in sustainable agroecosystems (Barea et al., 1997). It is known that microorganisms are activated in the soil–plant interface where a microcosm system, the rhizosphere, develops (Cordier et al., 2000). Carbon fluxes are critical for rhizosphere functioning. Many microbial interactions are responsible for key environmental processes, such as the biogeochemical cycling of nutrients and matter and the maintenance of plant health and soil quality. There are several groups of beneficial rhizosphere microorganisms. The bacteria that provide benefits to the plant either form symbiotic relationships with the plant or are free-living in the soil, but found near or even within the roots. Beneficial free-living soil bacteria are usually referred to as plant growth-promoting rhizobacteria or PGPR (Kloepper et al., 1989), a group that includes the genus Bacillus. PGPR participate in many key ecosystem processes, such as those involved in the biological control

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of plant pathogens (Weller and Thomashow, 1994), solubilisation of nutrients (Rodriguez and Fraga, 1999) and phytohormone synthesis (Gutiérrez-Mañero et al., 2001), and therefore deserve particular attention for sustainable agriculture.

In the case of ubiquitous rhizosphere arbuscular mycorrhizal (AM) fungi, the beneficial effect on plant development including crop plants has been well studied. Inoculation with AM fungi is an effective method of enhancing the ability of the host plants to become established and to cope with stress situations such as nutrient deficiency, drought and soil disturbance (Caravaca et al., 2003a). In fact, several authors have indicated that mycorrhizal fungi may improve the performance of seedlings, by stimulating water uptake (Augé, 2001) or increasing nutrient uptake by the plant, particularly N and P (Jeffries et al., 2003), or by improving soil aggregation in eroded soils (Caravaca et al., 2002). In exchange, mycorrhizal plants provide the fungus with photosynthetic C, which in turn is delivered to the soil via fungal hyphae. As a consequence, mycorrhiza formation can affect the microbial population in the rhizosphere, directly or indirectly, through changes in root exudation patterns, or through fungal exudates. Conversely, soil microorganisms can affect AM formation and function. Particularly, the so-called mycorrhiza helper bacteria are known to stimulate mycelial growth of mycorrhizal fungi or to enhance mycorrhizal formation (Toro et al., 1997).

Another large group of beneficial microorganisms in the rhizosphere are the phosphate-solubilising filamentous soil fungi, such as *Aspergillus niger* (Caravaca et al., 2005). Their effects may be of great interest in soils with scarce assimilable P, such as those in semiarid agroecosystems. Nevertheless, the effectiveness of phosphate solubilisation by microorganisms inoculated directly into the soil under field conditions is unclear because of the possible re-fixation of phosphate ions on their way to the root surface. The microbiologically solubilised phosphate could, however, be taken up by a mycorrhizal mycelium, thereby developing a synergistic microbial interaction (Barea et al., 1997). The combined inoculation of selected rhizosphere microorganisms has been recommended for maximising plant growth and nutrition (Probanza et al., 2001). The study of the antagonic or synergic effects of the different microbial inoculants when co-inoculated is a crucial step in the development of effective host-microorganism combinations. In previous studies, it was shown that the presence of *A. niger* stimulated the growth of ectomycorrhizal shrub species (Caravaca et al., 2005). It has also been reported that dual inoculation with *Glomus intraradices* and *Bacillus subtilis* promoted the establishment of the introduced AM fungus and increased plant biomass and tissue P accumulation (Toro et al., 1997). However, reports on co-inoculation of the AM fungi with both phosphate-solubilising fungi (Aspergillus) and plant growth-promoting rhizobacteria (Bacillus) are uncommon.

The effectiveness of microorganisms as modifiers of soil fertility and facilitators of plant establishment classically has been ascertained by measuring changes in the nutritional and development status of the plant. The use of soil biological markers related to microbial activity, for instance microbial biomass, enzyme activities and labile carbon fractions, has been proposed (Naseby and Lynch, 1997; Caravaca et al., 2003b). This approach provides a comprehensive view of the impact of an inoculant on the functioning of the soil ecosystem. However, there are relatively few studies regarding the use of such parameters as a method of monitoring the effects of microbial inoculation.

The objectives of this study were: (1) to assess the interactions between three groups of microorganisms (AM fungi, PGPR, filamentous soil fungi) with respect to their effects on the promotion of plant growth in a typical crop species (lettuce) under greenhouse conditions, and (2) to determine their combined effects on soil properties considered to be indicators of soil quality, such as labile C fractions (microbial biomass C, water-soluble C and carbohydrates) and enzyme activities (dehydrogenase, urease, protease-BAA and acid phosphatase). The selected species were: *G. intraradices*, representing the AM fungi, *B. subtilis*, representing the PGPR, and *A. niger*, a typical filamentous soil fungus.

## 2. Materials and methods

### 2.1. Soil and plant

An agricultural soil, used to cultivate lettuce was collected near Murcia (SE Spain). The climate is semiarid Mediterranean with an average annual rainfall of 300 mm and a mean annual temperature of 19.2 °C; the potential evapo-transpiration reaches 1000 mm year$^{-1}$. The main characteristics of the agricultural soil used were: pH (1:5) 8.89; electrical conductivity 0.18 dS m$^{-1}$; TOC 1.80%; total N 2.01 g kg$^{-1}$; available P, 70 μg g$^{-1}$; extractable K, 440 μg g$^{-1}$; cationic exchange capacity, 15 cmol kg$^{-1}$.

The plant used in the experiment was lettuce (*Lactuca sativa* L. cv. Cherry). Seeds of lettuce were grown for 15 days in peat substrate under nursery conditions, without any fertilization treatment.