Pedobiologia 55 (2012) 227–232

Contents lists available at SciVerse ScienceDirect

Pedobiologia - International Journal of Soil Biology

journal homepage: www.elsevier.de/pedobi

Differential interaction between two *Glomus intraradices* strains and a phosphate solubilizing bacterium in maize rhizosphere

Laura Fernández Bidondo*, Josefina Bompadre, Mariana Pergola, Vanesa Silvani, Roxana Colombo, Fabiana Bracamonte, Alicia Godeas

Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, 4to piso, Pabellón 2, 1428 Buenos Aires Argentina

ARTICLE INFO

Article history:
Received 18 January 2012
Received in revised form 17 April 2012
Accepted 19 April 2012

Keywords:
Glomus intraradices
Bacillus megaterium
Extraradical-mycelium growth patterns
Microbial interactions

ABSTRACT

Arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) have a positive effect on plant productivity primarily through increasing phosphate availability. In order to study the interaction between AM fungi and PSB, we used *Bacillus megaterium*, a PSB isolated from the sterilized surface of AM germinated spores, and two strains of the AM fungus *Glomus intraradices* with different mycelial architecture. A greenhouse experiment was designed with maize as host plant with the addition of tribasic calcium phosphate. We tested the hypothesis that PSB, intimately linked with AM fungi, could interact differentially with the two AM strains. We concluded that inoculation with the PSB positively affected maize mycorrhization. Insoluble phosphate alone did not influence the AM extraradical mycelium (ERM) length and maize mycorrhization when bacteria were not inoculated. The results provide evidence that the adverse effect on infectivity for some AM strains might be caused by solubilized phosphorus release to the rhizosphere by PSB. Differences related to the mycelium architecture of each AM strain were observed: the density of PSB in rhizosphere soil was significantly higher only with the GA8 strain coinciding with the highest values of maize biomass. The density of bacteria associated with GA8 mycelium could be the result of the transfer of photosynthates through the rhizosphere; this close contact would favor the persistence of the intimate relationship between PSB and AM hyphae. In the bacteria-free treatments, soil adherence was not significantly altered. Although the highest development of ERM occurred with GA5, plants inoculated with GA8 showed the highest values for soil adherence. This may be due to the AM mycelium which modifies bacterial persistence in the rhizosphere and consequently soil adherence. Our results show that for potential applications, some characteristics of the AM strains are key in the selection of the AM fungi–PSB combinations. These include the tolerance to soluble phosphorus, the rate of root colonization, and ERM development that favors the persistence of bacteria in rhizosphere soil.

Introduction

Soil microorganisms form a community that depends on the organic compounds that are provided by root exudates (Lynch and Whipps 1991). Therefore, microbial activity in the rhizosphere is particularly high compared to that in bulk soil (Gryndler 2000). Gerhardson and Clarholm (1986) reported that rhizospheric bacteria usually reach a density twenty times higher than bacteria inhabiting bulk soils. Rhizospheric bacteria produce exopolysaccharides that result in significant increases in soil adherence (Kaci et al. 2005). The combined effect of root hairs and mucilage, either produced by roots or by rhizosphere microorganisms (Watt et al. 1994), can lead to the formation of specific structures called rhizosheaths. These structures have been observed across a wide range of plant species, especially in grasses (Hinsinger et al. 2009).

Exudates of AM fungi are also released into the mycorrhizosphere, and may influence selectively the presence of rhizospheric microorganisms (Marschner and Timonen 2005). The extraradical mycelium (ERM) of AM fungi provides a habitat for soil microorganisms, which is different from that provided by roots. Bacterial colonization and formation of biofilm-like structures on the surface of AM hyphae have been reported (Frey-Klett et al. 2007; Silvani et al. 2008). When bacteria are inoculated into the soil they could remain attached on the hyphae of AMs, decreasing their presence in the rest of the soil (Frey-Klett et al. 1999). This close contact could benefit both soil microorganisms, facilitating metabolic interactions and exchange of nutrients (Artursson et al. 2006).

Plants can directly absorb soil phosphate through high-affinity transporters in roots; however, this direct absorption is limited when phosphorus levels decrease in the soil solution near the roots.

* Corresponding author. Tel.: +54 1145763300.
E-mail address: laurafernandez@conicet.gov.ar (L. Fernández Bidondo).

0031-4056/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.pedobi.2012.04.001

The ERM network grows beyond the depletion zone, absorbing mineral phosphates and translocating them to the AM intraradical structures and then to the root cortex cells. This mechanism is considered the main benefit of the AM symbiosis to the host plant (Smith and Read 1997). Nevertheless, Chiou et al. (2001) suggested that, probably due to the increasing levels of phosphorus in roots, the expression of radical phosphate transporters (MtPT1) in *Medicago truncatula* plants decreased when mycorrhization rates of *Glomus intraradices* and *Glomus versiforme* were augmented.

Several studies have demonstrated a synergistic interaction between AM fungi and phosphate solubilizing bacteria (PSB) (Barea 1997; Kim et al. 1998), with an increase in phosphorus acquisition by the host plant (Toro et al. 1997). The development of the rhizospheric microbial community is involved in plant productivity (Andrade et al. 1997). Therefore, AM fungi and different groups of bacteria, which promote plant growth by different mechanisms, could be considered in the formulation of biofertilizers into the context of sustainable agriculture.

Given this background, the main objective of this study was to analyze the interactions between the PSB *Bacillus megaterium*, isolated from AM propagules, and two strains of *G. intraradices* in the rhizosphere of maize plants. These findings could improve our ability to select beneficial combinations of AM fungi and their associated bacteria.

Considering the number of microorganisms that develop in association with AM structures in soil, our goal was to test the hypothesis that PSB, intimately linked with AM fungi, could synergistically interact with two axenically propagated AM strains.

Materials and methods

Biological material and experimental design

Seeds of maize (*Zea mays*) were surface-sterilized with 70% (v/v) of ethanol solution for 20 min, 20% (v/v) of sodium hypochlorite solution plus Tween 20 (0.1%) for 30 min, rinsed three times with sterile distilled water, and germinated on moist filter paper for 4 days. Maize seedlings uniform in size were transplanted into 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})). Half of the pots were homogeneously amended with 1 g of tribasic calcium phosphate ($Ca_3(PO_4)_2$) per 1000 g of sterile substrate to establish a high insoluble P treatment, while allowing AM symbiosis establishment. Inorganic insoluble phosphates are solubilized by microorganisms due to the release of organic acids (Nautiyal 1999).

Roots of maize seedlings were inoculated at transplanting time separately with one of the *G. intraradices* strains: GA5 (BGIV, http://www.bgiv.com.ar/strains/glomus-intraradices/ga5) or GA8 (BGIV, http://www.bgiv.com.ar/strains/glomus-intraradices/ga8). Previously, differences between the two AM fungal strains in architecture of external mycelium and production of their hyphal structures were observed using monoxenic cultures with transformed carrot roots as the host plant (Silvani 2011). GA5 strain is an extensive and fast colonizer both in soil and *in vitro* conditions. The extraradical mycelium spreads throughout the growth substrate by the development of abundant runner hyphae. In contrast, GA8 strain is characterized by a different mycelial growth pattern. This strain is an intermediate and limited colonizer under both growth conditions; it develops a mycelium network composed of profuse hyphal branches and typical branched absorbing structures (BAS) (Silvani 2011).

Inoculation with AM fungi was carried out by placing 1-cm^3 plugs of 3-month-old *G. intraradices* monoxenic cultures, containing mycorrhizal transformed carrot (*Daucus carota*) roots, approximately 250 spores, and abundant ERM. These cultures were routinely grown in Minimal Medium (MM) (Bécard and Fortin 1988) and incubated in an inverted position at 25 °C in the dark. The non-mycorrhizal control plants were prepared as previously detailed, except that roots were inoculated with 1-cm^3 plugs of MM with non-mycorrhizal transformed carrot roots.

Half of the plants in each AM treatment (*G. intraradices* strain GA5, *G. intraradices* strain GA8 and non-mycorrhizal control) were inoculated with the phosphate solubilizer bacteria (PSB) *B. megaterium* strain SJ5R7 (GenBank accession number JN845569). Two milliliters of concentrated bacterial suspension were added per pot (10^9 cell ml^{-1}) to ensure bacterial survival in the soil in a concentration of at least 10^7 cell ml^{-1}. An association with AM propagules was previously observed for SJ5R7 strain (Silvani et al. 2008). SJ5R7 was initially recovered from the sporosphere of surface-sterilized (15 min in a 5% chloramine-T (Merck) solution) and germinated spores of the AM fungus *Glomus margarita* strain J5 (FCEyN, UBA).

Five pots per treatment were established as follows: the AM fungal treatments (GA5, GA8 and non-mycorrhizal) singly or co-inoculated with the PSB, and supplemented with P or non-treated. Pots were placed in a completely random design, and maize plants were grown with natural light and room temperature under greenhouse conditions. During the assay pots were irrigated with 50 ml of Hewitt (1952) nutritive solution without P every 10 days.

Analysis and harvesting

Fungi

The production of extraradical mycelium of both *G. intraradices* strains was determined as the length of ERM from the soil attached to roots following the methods in Graham et al. (1982). Samples of air-dry roots (48 h in the dark at room temperature) were vigorously shaken to remove the soil. Soil particles were washed into a beaker and dried until constant weight. The ERM length (mm) of GA5 and GA8 were quantified after staining with trypan blue in lactic acid (0.02%). Values were calculated for 1 g of the attached dried soil samples (Brundrett et al. 1994). AM colonization of maize roots was observed using the modified Phillips and Hayman (1970) method: roots were cleared with KOH (10%, w/v, 15 min, 90 °C) and stained with trypan blue in lactic acid (0.02%, 10 min, 90 °C). 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.

Soil

A portion of maize roots was removed at harvest time, then dried and vigorously shaken to remove loose soil, as described previously. The layer of adhering soil was washed by immersion in 90 ml sterile distilled water; the soil suspensions were decanted and dried until constant weight. The amount of adhering soil in maize roots was expressed as grams of dry soil/grams of fresh root (Graham et al. 1982).

Bacteria

From rhizospheric soil suspensions previously obtained serial dilutions (1/10) were performed, and 100 μl were uniformly sowed on Petri dishes containing NBRIP solid medium with $Ca_3(PO_4)_2$ (5 g l^{-1}), and incubated at 29 °C in the dark for a week. The density of inoculated PSB on maize rhizospheric soil was determined by counting colonies surrounded by a halo on the medium surface,