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Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil

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ABSTRACT

Both arbuscular mycorrhizal (AM) fungi and phosphate solubilizing bacteria (PSB) are involved in phosphorus (P) mobilization and turnover but the influence of their interaction on organic P mineralization in the root free soil (hyphosphere) have been little studied. We investigated the interactive effects of an AM fungus (*Rhizophagus irregularis*, RI) and/or PSB (*Pseudomonas alcaligenes*, PA) on phytate mineralization and subsequent transfer to the host plant (*Medicago sativa*) using a two-compartment microcosm with a central 30 μm nylon mesh barrier. The root growth compartment containing 5 mg inorganic P (Pi, KH_2PO_4) kg^{-1} soil was inoculated with RI or uninoculated and the AM fungal hyphal soil containing 75 mg organic P (Po, Na-phytate) plus 0 or 5 mg Pi kg^{-1} soil was inoculated with PA or uninoculated. Sole inoculation with RI increased shoot P content compared with the uninoculated treatment and dual inoculation with both RI and PA did not increase shoot P compared with sole RI inoculation. Sole PA inoculation significantly increased microbial biomass P (MBP). Compared with sole PA inoculation soil MBP increased under zero-Pi addition but decreased under 5 mg Pi kg^{-1} soil addition in the dual inoculation RI/PA treatment. The uninoculated microcosms had the lowest acid phosphatase activity and the highest phytate-P remaining in the soil. Inoculation with PA led to higher acid phosphatase activity and lower phytate-P than did RI. Dual RI/PA inoculation had the highest acid phosphatase activity and the lowest phytate-P remaining in the soil. Addition of 5 mg Pi kg^{-1} soil to the hyphal compartment decreased phytate-P remaining in the RI and/or PA treatments. The phytate-P remaining in the soil was negatively correlated with soil acid phosphatase activity or MBP in the presence of RI but there was no correlation between shoot P and soil phytate-P. In conclusion, our results indicate that the mineralization of soil phytate was promoted by the interaction between the AM fungus and its hyphosphere PSB.

1. Introduction

Soil microorganisms are key drivers in soil functional processes including organic matter decomposition, turnover and release of nutrients, particularly nitrogen (N) or phosphorus (P) for subsequent plant capture (Van der Heijden et al., 2008). Among the wide diversity of soil microorganisms arbuscular mycorrhizal (AM) fungi and phosphorus solubilizing bacteria (PSB) are two key beneficial functional groups that are directly involved in P turnover and subsequent plant P acquisition. AM fungi are able to release protons for mobilization of insoluble soil phosphates and to extend their extensive hyphae from the P depletion zone to explore a greater soil volume for inorganic P sources (Smith and Smith, 2011). However, accounting for 40% of the culturable bacterial population and widely distributed in soil (Kucey, 1983; Jorquera et al., 2008), PSB can increase soil available P through the release of organic acids and phosphatases, which enhance the mineralization of organic P sources (Rodriguez and Fraga, 1999).

Interactions between AM fungi and PSB in influencing plant P uptake have been studied since the late 1990's. Previous studies have shown positive effects of dual inoculation with AM fungi and

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PSB on increasing PSB numbers on root surfaces (Andrade et al., 1998), mycorrhizal colonization rates (Singh and Kapoor, 1998; Sabannavar and Lakshman, 2009), and AM fungal hyphal length and phosphatase activity (Kim et al., 1998; Vázquez et al., 2000; Kohler et al., 2007). Most studies have been conducted using pot culture in which the rhizosphere has been inoculated with both microbial types simultaneously in the presence of plant roots (Andrade et al., 1998; Singh and Kapoor, 1998; Sabannavar and Lakshman, 2009). The results obtained therefore do not necessarily reflect direct interactions between AM fungi and PSB as their confounding interactions with plant roots cannot be ruled out. The transcriptome of *Glomus intraradices* (DAOM 197198) has revealed that this AM fungus (AMF) may have a low capability of utilizing phytate because it lacks phytase protein (Tisserant et al., 2012). In addition, AM fungi also affect the growth of soil fungi or bacteria directly (Larsen et al., 1998; Mansfeld-Giese et al., 2002) or indirectly via their AM spore-associated bacteria (Larsen et al., 2003; Johansson et al., 2004; Bharadwaj et al., 2012). Furthermore, soil bacteria interact with AM fungi through their ability to colonize the surfaces of AM hyphae and spores (Artursson and Jansson, 2003; Toljander et al., 2006; Scheublin et al., 2010), thus affecting AM colonization and hyphal growth (Frey-Klett et al., 2007; Leigh et al., 2011) and nutrient release from organic material for AM hyphae capture (Hodge et al., 2001; Leigh et al., 2009; Herman et al., 2012). These results imply that some bacteria in the hyphosphere (the volume of soil influenced by the AM fungal hyphae) may play a similar role to AM in organic P turnover and capture for the host plant. However, at present only few experimental evidences have been obtained to demonstrate such a similarity (Ravnskov et al., 2002; Medina et al., 2007).

Understanding the interactions between AM fungi and their associated bacteria may give insight into manipulating specific microorganisms to enhance P availability in soils (Richardson and Simpson, 2011). Phosphorus availability in soils is determined by the competition between chemical and biological sinks (Attiwill and Adams, 1993). Plants and soil microbial biomass are two major biological sinks: P held in microbial biomass acts as a labile pool which prevents fixation and becomes available for plant capture during microbial turnover. Plants must, however, compete with other soil microorganisms for available P (Schmidt et al., 1997; Rousk et al., 2007; Marschner et al., 2011) and this is regulated by the ratio of soil organic C and available P (Marschner, 2008). In general, microbes contribute to plant P uptake when the soil C:P ratio is <200 but compete with plants for P at C:P >300 (Stevenson, 1986). However, while it has been established that AM fungi can enhance P capture for their associated host plants (Smith and Smith, 2011; Karasawa and Takebe, 2012), it is unknown to what extent or how AM fungi compete with other microbes in the hyphosphere soil for P or the consequence of such P competition for plant P nutrition. In this study we tested the following hypotheses. Firstly, AM fungi and PSB can facilitate the utilization of organic P by plants as PSB will convert organic P to available P, which the extraradical mycelia of AM fungi will acquire and then transfer to the host plant. Secondly, competition between these two microbial groups may also occur but will depend on the soil C:P ratio and if PSB have converted insoluble phosphate into microbial biomass, P will then become unavailable to the fungi but such competition is likely to be minimal when the soil C:P ratio is low (i.e. <200).

2. Materials and methods

2.1. Biological materials and soil

The host plant used was *Medicago sativa* cv. Aohan which had a relatively small biomass at the seedling stage and it was therefore easy to observe the effects of the AM fungi–bacteria interaction on the host plant. The AM fungal strain was *Rhizophagus irregularis* BEG 141 (RI, formerly *G. intraradices*, kindly provided by Professor Vivienne Gianinazzi-Pearson, INRA, France), a widely studied strain that has often been used as a model AM fungus. The bacterial strain used was *Pseudomonas alcaligenes* M20 (PA) which was isolated from the rice rhizosphere in rural areas of Beijing, China for its lecithin-hydrolyzing ability. Then PA also has been demonstrated to be effective in mineralizing and utilizing phytin as sole P source (refer to Fig. S1).

A moderately acid (pH_{water} 6.4; water:soil = 5:1) soil (a brown earth according to the USDA classification system) from Tai'an, Shandong province, China was used as the growth medium. This soil contained 5.19 g organic matter kg^{-1}, 7.2 mg mineral N (NO_3^- and NH_4^+) kg^{-1}, 4.9 mg Olsen-P kg^{-1}, and 117.3 mg NH_4Cl-exchangeable K kg^{-1}. After collection in May 2011 the soil was air dried, sieved (2 mm) and the following chemicals were added to one kg soil: 200 mg N (NH_4NO_3), 200 mg K (K_2SO_4), 50 mg Mg ($MgSO_4 \cdot 7H_2O$), 5 mg Zn ($ZnSO_4 \cdot 7H_2O$), 5 mg Mn ($MnSO_4 \cdot H_2O$), and 2 mg Cu ($CuSO_4 \cdot 5H_2O$). The mixed soil was then sterilized with 10 kGy ^{60}Co γ-radiation at the Beijing Radiation Application Research Center to kill indigenous microorganisms prior to transfer to the microcosms (see Fig. S2).

2.2. Plant growth conditions

Two-compartment microcosms were constructed to meet the experimental requirements (Fig. S2). Each microcosm comprised a 10 × 15 × 3.5 cm (l × *h* × *w*) root compartment and a 10 × 15 × 5 cm hyphae-only compartment. The two compartments were separated from each other with 30 μm nylon mesh (Anping Wire Mesh Industrial Ltd, Hebei, China). In the hyphae only compartment, a buffer section of 2 cm width from the mesh window was set up to minimize the influence of roots on the hyphal compartment soil. Thus, the soils in the three sections are referred to as root soil, buffer soil and hyphal soil (see Fig. S2). In the root soil compartment, 18 surface sterilized *M. sativa* seeds (10% hydrogen peroxide for 10 min, and then 70% ethanol for 3 min) were planted and thinned to 5 seedlings two days after germination.

15 g dry weight of AM (RI, BEG 141) inoculum propagated on maize roots consisting of spores, mycelium, fine root segments and soil, was placed on the top 5 cm soil depth to the +RI root compartment while 15 g autoclaved inoculum with 10 mL filtered washings of this AM inoculum was added to the −RI (AM control) root compartment. The filter was used to remove AM propagules but maintain similar bacterial communities (Hodge et al., 2001). The PA inocula were cultured in liquid LB medium (Sambrook et al., 1989) for 2 d at 180 rpm at 37 °C and then centrifuged at 6000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended and then diluted to 10^9 CFUs mL^{-1} using sterilized 0.85% (w/v) NaCl solution. After 30 d of plant growth, 10 mL of this bacterial suspension was added to the hyphal soil in the +PA treatment and 10 mL autoclaved bacterial suspension was added to the −PA treatment (bacterial control).

2.3. Experimental set-up

The experiment considered three factors: (1) two Pi levels as 0 or 5 mg Pi (KH_2PO_4) kg^{-1} soil (P0 or P5) in the hyphal soil, (2) two *R. irregularis* (RI) levels, with or without RI in the root soil, and (3) two *P. alcaligenes* (PA) levels, with or without PA in the hyphal soil. Thus, a total of 8 treatments as the following four treatments for each KH_2PO_4 kg^{-1} dry weight (DW) soil level in the hyphal soil: (1) no inoculation (control), (2) mono inoculation with *R. irregularis* (RI), (3) mono inoculation with *P. alcaligenes* (PA), (4) dual