Soil Biology & Biochemistry 90 (2015) 1–9

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus *Rhizophagus irregularis* DAOM 197198

Salma Taktek [a], Martin Trépanier [b], Paola Magallon Servin [a], Marc St-Arnaud [c], Yves Piché [d], J.-André Fortin [d], Hani Antoun [a, *]

[a] Centre de recherche en innovation sur les végétaux and Département des sols et de génie agroalimentaire, Université Laval, Québec, QC, Canada G1V 0A6
[b] Premier Tech, 1 avenue Premier, Campus Premier Tech Rivière-du-Loup, QC, Canada G5R 6C1
[c] Institut de recherche en biologie végétale, Université de Montréal, Jardin botanique de Montréal, Montréal, QC, Canada H1X 2B2
[d] Centre d'étude de la forêt and Département des sciences du bois et de la forêt, Faculté de foresterie, de géographie et de géomatique, Université Laval, Québec, QC, Canada G1V 0A6

ARTICLE INFO

Article history:
Received 5 January 2015
Received in revised form
1 July 2015
Accepted 20 July 2015
Available online 3 August 2015

Keywords:
AMF extraradical hyphae
Burkholderia
Glomus irregulare
Phosphate rock solubilization
Rhizobacteria
Hyphosphere

ABSTRACT

A simple method is described for trapping phosphate solubilizing bacteria (PSB) strongly attached to the hyphae of the arbuscular mycorrhizal fungus (AMF) *Rhizophagus irregularis* (*Ri*). Bacteria were isolated from the hyphosphere of mycorrhizal leek plants growing on Turface previously inoculated with soil suspensions, obtained from the mycorrhizosphere of mycorrhizal plants growing in agricultural settings or maple forests in Quebec, Canada. Among the best PSB strongly attached to the hyphae of *Ri*, 26 isolates belonged to *Burkholderia* spp. and one was identified as *Rhizobium miluonense*. Four hyphobacteria exhibiting high potential of inorganic and organic P mobilization were further compared with four equivalent mycorrhizobacteria directly isolated from mycorrhizospheric soils sampled. In general, hyphobacteria were superior in mobilizing P from hydroxyapatite and from a low reactivity igneous phosphate rock from Quebec. Release of gluconic acid or the product of its oxidation 2-ketogluconic acid, are the main mechanisms involved in P solubilization. In a two compartments Petri plate system, *Ri* extraradical hyphal exudates, supported PSB growth and activity. In the absence of PSB *Ri* showed a negligible P solubilization activity. In the presence of PSB a substantial increase in P mobilization was observed, and the superiority of hyphobacterial activity was also observed under this system. Our results suggest that in developing a bioinoculant based on selected PSB, their interaction with AMF hyphae should not be overlooked.

1. Introduction

The dynamic processes that characterize relationships between plants and microbial communities are complex. Soil microorganisms have an important influence on soil fertility and plant growth (Andrade et al., 1997; Miransari, 2011).

Phosphorus (P) is a major essential macronutrient limiting crop yields and is required by plants in relatively large amounts. Most soils are frequently deficient in soluble orthophosphate (Pi), the P form directly available to plants (Richardson, 2001). Therefore, low Pi availability limits plant growth and agricultural productivity worldwide and to support and maintain crop production, P must be provided to plants as soluble chemical fertilizers. However, applied P is rapidly fixed in soil and it is estimated that only 10–20% of chemical P fertilizers are used by plants the year of application (Richardson, 2001). Phosphate rock (PR), the cheapest P-fertilizer, was recognized as a valuable alternative for sustainable agriculture (Vassilev et al., 2001; Reddy et al., 2002). Unfortunately, plants respond to fertilization with PR in an erratic way, and generally yields obtained with PR are lower than those produced with soluble phosphate fertilizers (Khasawneh and Doll, 1978).

In most natural terrestrial plant ecosystems, P is obtained from the soil with the help of symbiotic mycorrhizal fungi (Smith and Read, 1997). The arbuscular mycorrhizal fungi (AMF), forming the oldest and most widespread type of mycorrhizal association, are key components of the soil microbial community, influencing water and nutrients uptake (phosphorus, nitrogen and various micronutrients), and reducing the incidence of plant diseases to their

* Corresponding author. Tel.: +1 418 6562131x3650; fax: +1 418 6563515.
E-mail address: hani.antoun.1@ulaval.ca (H. Antoun).

http://dx.doi.org/10.1016/j.soilbio.2015.07.016
0038-0717/

host (St-Arnaud and Vujanovic, 2007; Bonfante and Anca, 2009). In addition, AMF can affect the diversity and structure of bacterial communities in the rhizosphere (Toljander et al., 2006). The soil zone influenced by both the mycorrhizal roots and the AMF hyphae extending into the soil from the colonized roots has been called the mycorrhizosphere (Linderman, 1988) and is formed of two principal components: the rhizosphere which is directly influenced by roots, and the hyphosphere, which refers only to the soil zone surrounding individual fungal hyphae (Andrade et al., 1997, 1998). The hyphae of AMF provide an increased area for interactions with other soil microorganisms, especially bacteria which may in turn synergistically interact with AMF and thereby promote plant growth (Johansson et al., 2004). AMF can only absorb phosphate ions from soil solutions but are unable to extract P by themselves from PR (Antunes et al., 2007). However, when associated with some bacteria (Villegas and Fortin, 2001) or fungi (Kucey, 1987), AMF can obtain P from PR and translocate it to their host plants.

Phosphate solubilizing bacteria (PSB) are considered to play an important role in P mobilization. They increase soil fertility through the solubilization of inorganic phosphates by releasing organic acids, and the mineralization of organic phosphates by producing phosphatases and phytases (Dobbelaere et al., 2003). PSB were already proposed as a viable solution to resolve the problem of precipitation of soluble superphosphate fertilizers (Omar, 1998; Narula et al., 2000; Whitelaw, 2000; Khan et al., 2007; Hameeda et al., 2008). Mycorrhizae can affect both the numbers and the composition of bacterial communities in the rhizosphere and the hyphosphere (Meyer and Linderman, 1986; Linderman, 1988; Paulitz and Linderman, 1991; Offre et al., 2007). In fact, qualitative and quantitative differences in bacterial communities between bulk soil and hyphosphere suggest preferential associations between some bacterial taxa and fungal hosts (Andrade et al., 1997, 1998). Interaction between AMF and PSB suggests mutual beneficial effects. The nutritional dynamics of bacteria and mycorrhizal fungi may be ecologically important. In fact, fungal exudates and other molecules could be used by bacteria as nutrients (Bonfante and Anca, 2009). On the other hand, hyphospheric bacteria are involved in the production of growth factors (affecting both plants and AMF), the reduction of stress and the inhibition of antagonists and competitors (Frey-Klett et al., 2007).

In order to establish efficient tandems between AMF and soil bacteria capable of extracting P from PR, we hypothesized that this beneficial interaction would be more effective with PSB strongly attached to hyphae, suggesting a close association with AMF hyphae, rather than PSB freely growing in the mycorrhizosphere.

The aim of this work is to isolate PSB closely attached to hyphae of the commercially used AMF *Rhizophagus irregularis* (*Ri*) DAOM 197198, hereafter called hyphobacteria. To reach this aim, a novel approach is described. We also present the first conclusive evidence that hyphospheric PSB display the ability to colonize AMF extraradical mycelium on a minimal growth medium and mobilize phosphates more efficiently than mycorrhizospheric bacteria, as a result of the PSB-*Ri* interaction.

2. Materials and methods

2.1. Soil sampling

In order to isolate PSB, thirteen soil samples were collected at a depth of 10–30 cm, from different sites near Quebec City, Canada and analyzed (Table S1). From each site, three subsamples were collected from adjacent plants and well mixed to form a composite sample. All tools used in soil sampling were surface disinfected using 70% ethanol and soils were placed in sterile Whirl-Pak® sample bags, transported on ice to the laboratory, stored at 4 °C and processed within a week. Each sample was homogenized in sterile saline (0.85% NaCl, w:v), serially diluted and used as described below.

2.2. Chemical composition and reactivity of Quebec phosphate rock

Igneous PR came from a phosphate rock open-pit mine located at Lac à Paul in the Saguenay–Lac-Saint-Jean region of Quebec, Canada and owned by Arianne Phosphate Inc., a Canadian mining exploration company. The chemical composition of the PR is presented in Table S2. The total P content of the PR used was 146 mg g^{-1} and its solubility was 3.4% in 2% formic acid and 5% in 2% citric acid, indicating a low reactivity.

2.3. Isolation of PSB strongly attached to hyphae or directly from the mycorrhizosphere

Leek (*Allium ampeloprasum* L., Norseco Inc.) seeds were planted in 200-cells seedling flats containing pasteurized and moistened Agro-mix substrate (Fafard, Saint-Bonaventure, Québec, Canada), homogeneously mixed with MYKE® PRO PS3, a commercial powdered inoculum containing spores of the AMF *R. irregularis* (*Ri*) DAOM 197198 (previously *Glomus irregulare*), to give a final concentration of 1200 spores L^{-1} of substrate. MYKE® PRO PS3 was kindly supplied by Premier Tech, Rivière-du-Loup, Québec, Canada, and was produced under aseptic conditions in a bioreactor. Plants were grown for 4 weeks in a greenhouse set to 14 h diurnal temperature of 22 °C and 18 °C at night. After 30 days, seedlings were transplanted into 15 cm diameter pots filled with Turface (Athletics™, Profile Products, Buffalo Grove, IL). This substrate, formed by calcined montmorillonite clay particles, allowed easily the separation and collection of AMF hyphae. Pots were placed in the greenhouse, watered daily and supplied twice a week for 15 d with a water soluble 20–2–20 (N–P–K) fertilizer diluted to a final concentration of 100 mg N L^{-1}, with 150 mg N L^{-1} for an additional 30 d, and finally with 200 mg N L^{-1} until the end of the experiment. After two-month, time required for the establishment of abundant extraradical AMF hyphae, each pot containing one mycorrhizal plant was inoculated with 10 mL of a diluted soil suspension in sterile saline, containing bacteria (approximately 10^6 CFU mL^{-1}) prepared from each of the 13 soil samples. Uninoculated control plants received 10 mL of sterile saline. The experiment was organized in a completely randomized block design with 14 treatments (13 soil samples and an uninoculated control) and 4 blocks. Additional set of pots were prepared as described above with or without inoculation with *Ri* DAOM 197198 and receiving diluted soil suspension or sterile saline. These pots were used to monitor leek plant colonization by *Ri* and the presence of PSB on hyphae. For the duration of the experiment no root colonization by AMF was observed in all treatments not inoculated with *Ri* DAOM 197198, and receiving soil suspension or saline. Plants were harvested two months after inoculation with the soil bacteria. Our observations indicated that this time was required for a good colonization of hyphae by PSB. At harvest, the percentage of root colonization by *Ri* ranged from 18 to 43%. From each pot, a 5 g subsample of Turface plus mycorrhizal roots was suspended in 50 mL of sterile dechlorinated tap water. Each suspension was vortexed three times for 5 min. Floating AMF hyphae were recovered by filtrating the supernatant using a synthetic nylon with a mesh size of 50 μm (Dynamic Aqua Supply Ltd, Surrey B.C., Canada) and then resuspended in 6 mL of sterile saline. The hyphae were washed three times with sterile water (2 min per wash) to remove loosely attached bacteria, and then ground using a Kontes Pellet Pestle (Fisher Scientific) to isolate the PSB strongly attached to AMF hyphae and endophytes. Ground hyphae were resuspended in 1 mL of sterile saline solution