available at www.sciencedirect.com

ScienceDirect

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

Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions

Hanane Hamdali [a,b,c], *Mohamed Hafidi* [c], *Marie Joëlle Virolle* [b], *Yedir Ouhdouch* [a,*]

[a] *Faculté de Sciences Semlalia, Université Cadi Ayyad (UCAM), Laboratoire de Biologie et de Biotechnologie des Microorganismes, Marrakech, Morocco*

[b] *Institut de Génétique et Microbiologie, Université Paris XI, 91405 Orsay, France*

[c] *Faculté de Sciences Semlalia, Université Cadi Ayyad (UCAM), Laboratoire d'Ecologie Végétale, sol et Environnement, Marrakech, Morocco*

ARTICLE INFO

Article history:
Received 16 December 2007
Received in revised form
25 July 2008
Accepted 6 August 2008

Keywords:
Rock phosphate solubilizing actinomycetes
P-deficient soil
Wheat growth
Biocontrol
Damping-off

ABSTRACT

Micromonospora aurantiaca- and *Streptomyces griseus*-related strains isolated from Moroccan phosphate mines (MA^{MPM} and SG^{MPM}) were previously selected for their rock phosphate (RP) solubilizing abilities and their multiple plant growth promoting properties demonstrated in laboratory conditions. In order to assess whether these interesting properties could have a direct effect on plant growth and fitness, seeds of the wheat plant (*Triticum durum* L. cv. Vitron) coated or not with mycelium of these strains and of the reference strain *S. griseus* M1323, were grown in a sterile soil deficient in soluble phosphate supplemented or not with soluble phosphate or with the insoluble RP, under greenhouse conditions. These studies revealed that the presence of the actinomycete strains in the soil supplemented with RP significantly promoted the growth of the wheat plants. MA^{MPM} and SG^{MPM} had the greatest stimulatory effect on plant growth with 50–47% and 80–78% weight increase of shoots and roots, respectively, in comparison with the sterile control. This increase correlated with a significant increase in the N and P content of plant tissues. The MA^{MPM}- and SG^{MPM}-dependent growth promotion in the RP supplemented soil was on average 10–13% lower than that achieved by the soluble phosphate supplement. Furthermore, in a soil infested with *Pythium ultimum*, the mediator of damping-off disease, the coating of wheat seeds with the mycelium of MA^{MPM} strain resulted in a clear protection of the plant. The level of protection achieved by MA^{MPM} was 14% lower than that conferred by the commercial bio-fungicide agent (Mycostop®). This study demonstrated that MA^{MPM} in association with pulverized RP could constitute a novel and non-polluting bio-fertilizer/biocontrol product useful for the development of sustainable agriculture.

1. Introduction

Phosphorus (P) is an important element for plant growth and agricultural yields (Vassilev et al., 2006b). However, many soils throughout the world are deficient in P readily available for plant growth since most of the P present in the soil is in the form of insoluble metal chelates (Vassilev et al., 2006a). Phosphate is thus added to agricultural soil in the form of soluble chemical P

* *Corresponding author at*: Faculté de Sciences Semlalia, Université Cadi Ayyad, Département de Biologie, Laboratoire de Biologie et de Biotechnologie des Microorganismes, Avenue Prince My. Abdellah, BP 23 90, Marrakech 40 000, Morocco. Tel.: +212 2 43 46 49; fax: +212 2 43 74 12.
E-mail address: ouhdouch@ucam.ac.ma (Y. Ouhdouch).

0929-1393/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsoil.2008.08.001

fertilizers. However, only a small fraction (0.1%) of the added P is made available to plants (Scheffer and Schachtschabel, 1989) since most of it is rapidly converted into insoluble forms (Reddy et al., 2002) or washed away into fresh and ground waters, making a regular but unfortunately polluting application necessary (Shigaki et al., 2006). Soil supplementation with soluble chemical P fertilizers is thus a costly and contaminating practice, especially if one considers the highly polluting mode of production of these fertilizers that involves sulphuric acid (Shigaki et al., 2006; Vassilev et al., 2006a). It is now becoming urgent to reduce the environmental impact of agriculture and, for example, to replace the expensive soluble chemical P fertilizers by novel, cheaper, more ecological but nevertheless efficient P fertilizers (Macias et al., 2003).

The natural rock phosphate (RP), used in traditional agriculture, might indeed constitute a valuable alternative source of P fertilizer (Zapata and Zaharah, 2002), if a natural and non-polluting way could be found to promote its solubilization. Several fungi such as *Penicillium* sp. and arbuscular mycorrhizal fungi (Wakelin et al., 2004; Ouahmane et al., 2007) or bacteria such as *Bacillus subtilis*, *Pseudomonas* sp., *Rhizobium* sp. (Rodriguez and Fraga, 1999) and *Mesorhizobium* sp. (Peix et al., 2001) have been previously reported to be phosphate solubilizing microorganisms (PSM). These PSM use different strategies to do so, that include acidification, ion chelation or ion exchange (Rodriguez and Fraga, 1999; Gyaneshwar et al., 2002). Some of these microorganisms such as *Penicillium* sp. (Wakelin et al., 2004), *Enterobacter* sp. and *B. subtilis* (Toro et al., 1997), *Serratia marcescens* EB 67 and *Pseudomonas* sp. CDB 35 (Hameeda et al., 2006) and *Pseudomonas* sp. BR2 (Antoun and Babana, 2006) have been shown to improve plant growth in P-deficient soil supplemented with RP. In addition, some of these PSM can contribute to plant health by producing substances active against some specific plant pathogens (Vassilev et al., 2006b; Wani et al., 2007).

Among these PSM, actinomycetes (Banik and Dey, 1982; Mba, 1997; Hamdali et al., 2008a,b) are of special interest since these filamentous bacteria are often able to colonize plant tissue and to produce spores, a resistant form important for survival in agricultural soil. Furthermore, these Actinobacteria are important natural producers of antibiotics or anti-fungals that could protect the plants against various devastating phytopathogen agents such as *Pythium ultimum*, the cause of damping-off disease in wheat seedlings (Ikeda, 2003; Jain and Jain, 2007). These interesting characteristics of actinomycetes were mainly established under laboratory conditions (Banik and Dey, 1982; Mba, 1997; Hamdali et al., 2008a) but not in soil with the exception of the study of Mba (1994). We previously selected two Actinobacteria from the Moroccan phosphate mines (MPM), *Streptomyces griseus*- and *Micromonospora aurantiaca*-related strains, for their RP-solubilization abilities (Hamdali et al., 2008a) and their multiple plant growth promoting properties demonstrated under laboratory conditions (Hamdali et al., 2008b). However, the ability of these selected two Actinobacteria to improve plant growth in P-deficient soil is not yet well established. The main objective of this study was thus to determine whether these two Actinobacteria were able to improve growth of the wheat plant (*Triticum durum* L.) in a sterile soil deficient in soluble P supplemented or not with RP. In addition, we examined the ability of these Actinobacteria to limit the pathogenic effects of *P. ultimum*.

2. Materials and methods

2.1. Soil sampling and analysis

Soil samples were collected from a non-fertilized field located 10 km south of Marrakesh city, 31°37′10″N, 8°0′29″W, Morocco in October 2006. The climate of the area is semi-arid with an average annual rainfall of 250 mm. According to the FAO-UNESCO (1990) classification, the soil is a calcareous calcisol with a low available phosphorus content. The soil samples were collected from 0 to 30 cm depth after removing 3 cm of the soil surface, sieved (<2 mm) and placed in sterile tightly closed polyethylene bags. The samples were stored at 4 °C for a maximum of 48 h before being used for plant tests.

The soil was autoclaved for 2 h at 121 °C, on 2 consecutive days, to eliminate native microorganisms. After autoclaving, the soil composition was determined according to DIN ISO 19683 (1997). The total carbon (Ct) and nitrogen (Nt) contents of the soil were determined after dry combustion using a CNS elemental analyzer (LECO Corporation, St. Joseph, MI) according to DIN-ISO 13878 (1998), and DIN ISO 15178 (2001), respectively. The molybdenum blue method was used to determine the total and soluble phosphorus content (Olsen and Sommers, 1982). Soil pH was measured potentiometrically in a 1:5 (w/v) aqueous solution. The physicochemical characteristics of the soil were as follows: pH (H_2O) 7.4; clay 19.3%; fine silt 19.7%; coarse silt 4.0%; fine sand 19.1%; coarse sand 37.9%. The total concentrations of Ct, Nt and Pt were 230, 9 and 3 mg per 100 g of soil, respectively, and soluble phosphate only 0.09 mg per 100 g of soil.

The molted rock phosphate used as a supplement is a calcium hydroxyapatite constituted by O: 56.53%; F: 2.42%; Na: 1.81%; Mg: 1.94%; Al: 2.03%; P: 9.37%; S: 0.77%; Sn: 0.12%; Ca: 16.35%; Fe: 0.60% (Hamdali et al., 2008a).

2.2. Actinomycete strains and preparation of the inocula

S. griseus (SG^{MPM})- and *M. aurantiaca* (MA^{MPM})-related strains, isolated from Moroccan phosphate mines were previously selected for their RP-solubilization abilities (Hamdali et al., 2008a). The performance of these selected strains was compared to the reference strain *S. griseus* SG^{M1323} (IPC Paris, France).

Spores of these strains, stored in 20% sterile glycerol at −20 °C, were used to inoculate (at 10^6 spores ml^{-1}) 50 ml cultures of liquid Bennett medium (Jones, 1949) incubated in 250 ml Erlenmeyer flasks for 3 days at 28 °C under constant agitation on a rotary shaker (180 g min^{-1}). The mycelium was centrifuged at 10,000 × *g* for 10 min, washed twice with phosphate buffer saline (PBS; pH 7.2, 10 mM K_2HPO_4–KH_2PO_4, 0.14 M NaCl), fragmented through the needle of a sterile syringe and re-suspended in 10 ml of sterile deionised water. Five milliliters of the mycelial suspension was added to 2.5 g of wet carboxymethylcellulose (CMC, Merck). This paste was then mixed with 50 g of surface sterilized wheat seeds. Each seed was coated by a thin layer of wet CMC containing 10^6