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Promotion of growth of bean (*Phaseolus vulgaris* L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of *Micromonospora endolithica*

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ABSTRACT

The aim of this study was to determine the potential of non-streptomycete actinomycetes (NSA) to solubilize insoluble phosphates in soil and to promote plant growth. Thirty-one NSA were isolated using *Streptomyces* phage and dry heat techniques, from a calcareous soil deficient in available phosphorus (P) in the United Arab Emirates. Nine of these NSA isolates solubilized powdered rock phosphate (PRP) in solid and liquid media. Five isolates were initially selected based on their rhizosphere competence. Among them, an isolate of *Micromonospora endolithica* caused a significant drop in pH in a liquid medium amended with PRP, solubilized considerable amounts of P, produced acid and alkaline phosphatases, as well as a variety of organic acids. In addition, this isolate of *M. endolithica* was chosen from among the five isolates because it also showed exceptional rhizosphere competence and a strong ability to colonize bean (*Phaseolus vulgaris* L.) roots up to a depth of 14 cm. In the greenhouse, the application of *M. endolithica* to soil amended with either single super-phosphate (SP) or PRP significantly promoted the growth of roots and shoots of bean plants compared with those of plants grown in non-inoculated soil amended with SP or PRP. This was also evident in the significant increases in the concentration of available P in the soil and in the levels of N, P, K, S, Mg, Fe and Zn in the roots and those of N, P, K, S, Mg and Fe in the shoots of inoculated plants. The plant growth promotion by *M. endolithica* was most pronounced in the presence of SP as soil amendment compared to PRP. In comparison, a non-phosphate-solubilizing, non-rhizosphere-competent NSA isolate of *M. olivasterospora* failed to increase available soil P, nutrient levels in roots and shoots or to promote plant growth. Plant growth regulators tested do not appear to be involved in the plant growth stimulation observed. Neither *M. endolithica* nor *M. olivasterospora* produced detectable levels of indole-acetic acid, indole-pyruvic acid, gibberellic acid, isopentenyl adenine, isopentenyl adenoside or zeatin *in vitro*. This study is the first published report to demonstrate the potential of phosphate-solubilizing NSA to promote plant growth. In addition, it is also the first published report of rhizosphere-competent actinomycetes capable of solubilizing SP or PRP, in soils.

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1. Introduction

The role of phosphate-solubilizing microorganisms (PSM) in phosphate solubilization in soil has been attributed mainly to their abilities to reduce the pH of the surroundings by the production of organic acids (Sperber, 1958; Kim et al., 1998; Chen et al., 2006), production of acid and alkaline phosphatases (Rodriguez and Fraga, 1999) and to H^+ protonation (Illmer and Schinner, 1995). These organic acids can either dissolve phosphates as a result of anion exchange or can chelate Ca, Fe or Al ions associated with the phosphates (Gyaneshwar et al., 2002).

Various kinds of bacteria (Rodriguez and Fraga, 1999; Harris et al., 2006; Perez et al., 2007) and fungi (Whitelaw, 2000; Wakelin et al., 2007) have been isolated and characterized for their ability to solubilize unavailable reduced phosphorus (P) to available forms. Such transformations increase P availability and promote plant growth (Rodriguez and Fraga, 1999; Whitelaw, 2000; Rudresh et al., 2005; Harris et al., 2006).

Although a few studies have demonstrated the ability of streptomycete actinomycetes (SA) to solubilize insoluble forms of phosphates (Taha et al., 1969; Banik and Dey, 1982; Molla et al., 1984; Mba, 1994a), there appears to be no record in the literature on the enumeration and isolation of phosphate-solubilizing, non-streptomycete actinomycetes (NSA) or rhizosphere-competent, phosphate-solubilizing actinomycetes and evaluation of their abilities to improve the availability of P to plants. Only three reports dealing with phosphate-solubilizing NSA exist (Sperber, 1958; Mba, 1997; Chen et al., 2006). These isolates were only tested *in vitro* for their abilities to solubilize phosphates and no effort was made in soil to study their effects on nutrient uptake or plant growth. As a part of a large scale study on the potential of NSA as plant growth promoters and biocontrol agents of soil-borne fungal plant pathogens (El-Tarabily and Sivasithamparam, 2006), our studies was also extended to investigate the potential of NSA to solubilize P in relation to their ability to enhance plant growth.

It has been shown that rhizosphere competence confers on plant growth promoting rhizobacteria (PGPR) the potential to be effective in a site naturally positioned on and/or in root tissues to directly promote growth of the inoculated plants (Benizri et al., 2001). It is therefore logical to expect that strains of PSM selected as inoculants, should be capable of rapidly colonizing the roots. None of the studies on phosphate-solubilizing SA or NSA to date have involved selection and use of rhizosphere-competent strains as inoculants.

Many calcareous soils such as those of the United Arab Emirates (UAE) are characterized by the high content of exchangeable calcium and alkaline pH which negatively affects nutrient availability thus influencing soil fertility (Satchell, 1978). When soluble forms of inorganic P fertilizers are applied to such soils, they are rapidly converted to insoluble non-available forms (Satchell, 1978). Accordingly, the main objectives of the current study were to (i) isolate and identify phosphate-solubilizing NSA; (ii) examine the abilities of these isolates to solubilize powdered rock phosphate (PRP) *in vitro* and to produce organic acids as well as acid and alkaline phosphatases; and (iii) determine the potential of the most promising isolate to be rhizosphere-competent, to improve plant nutrient levels, to produce plant growth regulators (PGRs) and to enhance the growth of bean (*Phaseolus vulgaris* L.) plants in a calcareous soil amended with or without single super-phosphate (SP) or PRP.

2. Materials and methods

2.1. Enumeration and isolation of NSA

Brownish-yellow calcareous loamy sandy soil deficient in available P was collected from Al-Ain city, 140 km east of Abu-Dhabi, UAE. The soil characteristics were: pH of 8.6 (in 0.01 M $CaCl_2$); electrical conductivity 1.51 dSm^{-1}; organic carbon 0.17%; the following nutrients are expressed in mg $kg\ soil^{-1}$; total and available P 128 and 9.8, respectively; bicarbonate extractable K 217; NO_3^-–N 14; NH_4^+–N 1; SO_4 101 and Fe 116.

Seven free-draining pots (Smith and Nephew, Vic., Australia) (5 cm diameter) were filled with 1 kg of air-dried soil. The soil was amended with 100 g PRP $kg\ soil^{-1}$ (30% P) (Tianjin Crown Champion International Co., Limited, Tianjin, China) and the soils were saturated to field capacity. Soils were incubated at 30 ± 2 °C for 4 weeks in order to enhance the populations and activities of PSM. Two methods were used to isolate NSA. These were (i) the use of polyvalent *Streptomyces* phage (Kurtböke et al., 1992) and (ii) the dry heat technique (Nonomura and Ohara, 1969). The aims of these two methods were to reduce the dominance of SA and to facilitate the recovery of NSA.

For the phage technique, four polyvalent *Streptomyces* phages (El-Tarabily, 2006) were used. The stock phage suspension was prepared by combining high-titre phage suspensions ($\times 10^{12}$ plaque forming units ml^{-1}) of each phage, and the stock suspension was then used to treat 10 g of soil suspensions (eight replicates) in dilution tubes. Actinomycete colonies were isolated using the spread plate technique over humic acid vitamin agar medium (HVA) (Hayakawa and Nonomura, 1987). HVA plates were dried in a laminar flow cabinet for 20 min and incubated at 28 °C in the dark for 2 weeks. Plates without phage treatments were used as control.

For the dry heat technique, 10 g of soil (eight replicates) were heated at 120 °C for 1 h. One gram of the heat-treated soil was added to 10 ml of sterile distilled water in a test tube and vortex-mixed for 5 min and further dilutions (10^{-2}, 10^{-3} and 10^{-4}) were prepared in sterile distilled water. The control treatments consisted of 1 g of unheated soils. Actinomycete colonies were isolated using the spread plate technique over arginine vitamin agar (AVA) (Nonomura and Ohara, 1969). AVA plates were dried in a laminar flow cabinet for 20 min and were incubated at 28° in the dark for 3 weeks to encourage the growth of NSA.

Cooled (45 °C) sterile HVA and AVA were amended with cycloheximide (50 $\mu g\ ml^{-1}$) (Sigma Chemical Company, St. Louis, MO, USA) and nystatin (50 $\mu g\ ml^{-1}$) (Sigma) immediately prior to pouring plates. SA and NSA colonies were counted from each medium and were expressed as $\log_{10}$ colony forming units (cfu) g dry $soil^{-1}$. Differentiation between SA and NSA colonies was based on morphological criteria and according to the presence or absence of aerial mycelium, distribution (aerial/substrate) and form of any spores present and stability or fragmentation of substrate mycelium (Cross, 1989). All NSA isolates were purified on oatmeal agar plates