Scientia Horticulturae 164 (2013) 270–276

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Effect of *Pseudomonas luteola* on mobilization of phosphorus and growth of young apple trees (Ligol)—Pot experiment

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ARTICLE INFO

Article history:
Received 23 March 2011
Received in revised form 19 August 2013
Accepted 11 September 2013

Keywords:
Pseudomonas
Phosphate solubilizing microorganisms
Siderophores
IAA
Apple trees

ABSTRACT

The use of phosphate solubilizing bacteria as inoculants may increase the concentration of plant-available phosphorus in soil. Among soil microorganisms, bacteria from the genus *Pseudomonas* have received considerable attention as plant growth promoters. A phosphate solubilizing bacterium isolated from non-rhizosphere soil collected in Central Poland, was identified as *Pseudomonas luteola* BN0834 on the basis of biochemical methods and 16SrDNA sequence analysis. *P. luteola* strain BN08-34 was tested for: solubilization of inorganic and organic compounds of phosphorus (calcium phosphate, zinc phosphate, hydroxyapatite and calcium phytate); indole acetic acid (IAA), siderophore production and biosurfactant production; and the ability to grow on a medium without nitrogen added. Young apple trees, cultivar Ligol (rootstock M26), were grown in a pot-house for 14 weeks in pots filled with nonsterilized soil, classified as a sandy loam. The *P. luteola* BN0834 was introduced into the soil in a number equal to the number of native phosphate solubilizing microorganisms (PSM) (*P. luteola*) in soil or in a number ten times higher than the number of native PSM in soil (*P. luteola* × 10). Shoot numbers; average and total shoot lengths; contents of P, K, Mg and Ca in plant material; contents of available P, K and total Mg in non-rhizosphere soil, changes in the number of cfu (colony forming units) of microorganisms (PSM, cophiotrophs, oligotrophs and fungi) in non-rhizosphere soil and in the rhizosphere of the apple trees were studied. When the higher number of *P. luteola* BN0834 was introduced into soil without a mineral fertilizer added near the surface of the roots, positive correlations were found between the number of PSM in the apple tree rhizosphere and the content of available P in non-rhizosphere soil and also between the number of PSM in the apple tree rhizosphere and the amount of P, K and Ca in plant leaves. The highest total shoot length was also obtained from *P. luteola* × 10 application.

1. Introduction

The mechanisms by which rhizobacteria can promote plant growth include the ability to produce plant hormones, such as auxins, gibberellins, cytokinins (Ponmurugan and Gopi, 2006; Taurian et al., 2010); the ability to reduce ethylene synthesis (Glick et al., 1998); N_2 fixation (Lucy et al., 2004); antagonism against pathogenic microorganisms by production of siderophores (Ahmad et al., 2006; Taurian et al., 2010), antibiotics and enzymes; and the ability to increase the supply of plant mineral nutrients from soil sources (Poonguzhali et al., 2008; Yazdani et al., 2009; Zaidi et al., 2009; Singh et al., 2011).

Phosphorus compounds are one of the major nutrients that limit plant growth and crop productivity (Wissuwa, 2003; Khan et al., 2007). Phosphorus occurs in soil mostly in insoluble inorganic and organic forms (approximately 95–99% of total P in soil) (Pradhan and Sukla, 2005), and soluble compounds, the only ones which can be taken up by plants, represent below 1% of total P in soil (Khan and Bhatnagar, 1977; Kucey, 1983; Chang and Yang, 2009). Concentrations of phosphorus ions in most soil solutions range from 0.1 to 1 $\mu g\,g^{-1}$ of soil. In agricultural soils, chemical fertilizers are the main source of plant-available P, but a large portion (even up to 80%) of this element (Wu et al., 2009) rapidly reacts with soil constituents, becoming unavailable to plants (Sundara et al., 2002). For that reason, at the beginning of every vegetative season a new portion of chemical fertilizers has to be applied. On the other hand an amount of total phosphorus in soil increasing over time (Kucey et al., 1989). Apart from phosphorus, phosphate fertilizers also contain heavy metals (*e.g.*, Cd in a range from 1 to 641 μg^{-1} kg) which are natural components of phosphate rocks and apatite rocks, the raw materials used in the production of these fertilizers (Mc Langhlin et al., 1996). Additionally phosphate rock is a non-renewable natural source and its world's reserves of it should last around 100 years, that is why phosphorus is called a "disappearing

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http://dx.doi.org/10.1016/j.scienta.2013.09.012

nutrient" (Gilbert, 2009). One of the solutions to this problem is an increased use of phosphate-biofertilizers that could reduce the dose of mineral phosphate fertilizers and improve their efficiency (Karlidag et al., 2007).

There are several groups of microorganisms that have the potential to mobilize P from its inorganic and organic pools in soil (Chen et al., 2006). There are many reports concerning increased concentrations of bioavailable P in soil resulting from application of microbial inoculants (Thomas and Shantaram, 1986; Toro et al., 1997; Pal, 1998; Sundara et al., 2002; Aslantas et al., 2007; Gupta et al., 2012). The most efficient of those microorganisms, called phosphate solubilizing microorganisms (PSM), export organic acids and protons into the environment and dissolve insoluble phosphorus compounds in soil. This activity of PSM can also decrease immobilization of P applied with mineral fertilizers, thus increasing their efficiency (Zaidi et al., 2003). Solubilization of K-bearing minerals by microorganisms is also often associated with their ability to export organic acids to the environment (Tu et al., 2007). Release of P and K from soil minerals can be enhanced by biosurfactants. These metabolites can change cell surface properties of microorganisms and regulate cell attachment to surfaces of minerals (Liu et al., 2006; Yi et al., 2008; Hua et al., 2010). Direct contact of microorganisms with mineral surfaces can enhance the action of acidic metabolites. Biosurfactants can also enhance the growth of bound microorganisms on mineral surfaces by better dispersion of the minerals in water (Ron and Rosenberg, 2001; Girgis et al., 2008).

Some plants may have low levels of P in their tissues despite high concentrations of bioavailable P in soil (Kucey et al., 1989). Many environmental conditions such as soil structure, pH, moisture, temperature and fertility as well as root morphology of plant species and their cultivars have a strong influence on P uptake by plants (Schachtman et al., 1998; van Arkel et al., 2007; Desnos, 2008). The high concentration of available P is very important during root development which is crucial for P uptake by plants (Schachtman et al., 1998). Phosphorus is accumulated mainly during the vegetative growth and later this macroelement is translocated into generative organs. The high concentration of phosphorus in tissues of apple trees is responsible for good color and firmness of the fruits (Marcelle, 1995).

There are only a few reports on the application of PSM in orchard systems (Aslantas et al., 2007; Karlidag et al., 2007; Nadeem et al., 2012). Besides the above mentioned abiotic factors, the success of bacterial inoculation also depends on biotic factors such as soil microbial communities (Strigul and Kravchenko, 2006). The dose of applied PSM must conform to the number of native soil microorganisms with the same abilities (Kucey et al., 1989; Strigul and Kravchenko, 2006). We identified the strain solubilizing inorganic and organic compounds of phosphorus as *Pseudomonas luteola* BN0834 and characterized its plant growth promoting activities. The aim of the present study was also to evaluate the effect of application of *P. luteola* BN0834 in two doses, on growth and mineral nutrition of young apple trees and also on microbial population in the rhizosphere and in the non-rhizosphere soil.

2. Materials and methods

2.1. Bacterial strain and culture conditions

From the soil sampled in Central Poland 23 bacterial strains were isolated on the basis of their ability to utilize nonsoluble phosphorus compounds (calcium phosphate, zinc phosphate, hydroxyapatite and calcium phytate). The most effective strain, a Gram-negative, non-spore forming rod producing a yellow pigment during growth on Pikovskaya medium (Pikovskaya, 1948), was identified as *Pseudomonas* sp. The strain BN0834 showed fluorescence on King's B medium (Atlas, 1995) and was positive for catalase, oxidase and urease production and for casein and gelatin hydrolysis. The standard biochemical characteristic was re-evaluated by 16SrDNA sequence analysis. A DNA sequence similarity search performed using the BLAST program with Blastn 2.2.18+ (Zhang et al., 2000) showed that this bacterium was identical to *P. luteola*. This strain BN0834 has been deposited at the Netherlands Culture Collections of Bacteria as *P. luteola* strain NCCB 100257.

For long term storage at −20 °C, the *P. luteola* BN0834 strain was maintained in MBS liquid medium (Babich and Stotzky, 1977) enriched with 10% of glycerol. For the experiment, *P. luteola* BN0834 was grown in MBS liquid medium on an orbital shaker at 150 rpm for 24 h at 28 ± 2 °C. Cells were harvested by centrifugation. Washed and pelleted cells were resuspended in sterile distilled water. The density of the bacterial suspension was measured as the absorbance of the suspension at 600 nm, with reference to a standard curve calibrated by plate enumeration. When the soil was inoculated with the quantity of bacteria ten times higher than the number of native PSM in soil, the density of the cell suspension for bacterial inoculum was adjusted to about 2.0×10^8 cfu mL^{-1}. When the soil was inoculated with the quantity of bacteria equal to the number of native PSM in soil, the cell suspension was diluted with water to about 2.0×10^7 cfu mL^{-1}.

2.1.1. Plant growth-promoting properties of P. luteola BN0834

The ability of *P. luteola* BN0834 to produce indole acetic acid (IAA) was tested on Czapek–Dox (1% glucose) medium without tryptophan or with an addition of 500 μg tryptophan ml^{-1}. The concentration of IAA produced by *P. luteola* BN0834 was measured using the Salkowski reagent according to the method of Glickmann and Dessaux (1995). Siderophore production was tested on the Chrome azurol S agar medium (Shwyn and Neilands, 1987). To quantify hydroxamate and catechol siderophores produced by the investigated strain, methods described by Arnow (1937) and Csaky (1948) were used, respectively. Asbey's Nitrogen Free Agar (Atlas, 1995) was used to test the ability of the strain to grow without added N.

Plate assays using Pikovskaya medium supplemented with 0.1% (w/v) of different P sources (Pikovskaya, 1948) were carried out to test the ability of the *P. luteola* BN0834 to solubilize hydroxyapatite, zinc phosphate and calcium phytate (Saravanan et al., 2007). To quantify solubilization of tricalcium phosphate, Pikovskaya's broth (Pikovskaya, 1948) was used.

The ability of the strain to produce surfactants was tested by the oil spreading technique (Youssef et al., 2004) on LB medium (Bric et al., 1991).

2.2. Pot experiment

One-year-old apple trees, cultivar Ligol (rootstock M 26), were grown for 14 weeks (from 1st June to 1st September) in a pot house located in Skierniewice (Central Poland). The pots were kept in the pot house under natural conditions of sunlight. June mean temperature was 17.0 °C (± 4.3); July 22.3 °C (± 2.8) and August 17.1 °C (± 2.1). June mean air humidity was 80% (± 7.6), July 64% (± 7.5) and August 91% (± 5.8). A nonsterilized soil classified as a sandy loam soil with properties described in Table 1 was used to fill the pots. Each pot contained 2 kg of soil.

2.2.1. Fertilization

In all treatments, a mineral fertilizer (1 g of fertilizer kg^{-1} of soil) containing 15% total N, including 7.9% N in the amidic form and 7.1% N in the nitrate form, and 12% K_2O was added as a solution to the surface of the soil pots after planting the trees. Additionally, mineral