ISOLATION AND CHARACTERIZATION OF SOME RHIZOBACTERIAL STRAINS WITH PHOSPHORUS SOLUBILIZING CAPABILITIES

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Abstract: It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to solubilize P. The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR (plant growth promoting rhizobacteria) that increase nutrient availability to host plants. Because intensive agriculture entails the risk of excessive fertilization, microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible. The objective of the present work was to assess P solubilizing potential of several bacterial strains isolated from soybean rhizosphere and rizoplane in order to be used in the future studies as biofertilizers. In the regions surrounding soybean roots is developing a population of bacteria less diversified from a micro-morphologic point of view. Approximately 31 % of the tested strain presented P solubilizing capabilities, the majority being isolated from soybean rhizosphere.

INTRODUCTION

Phosphorus (P) is second only to nitrogen in mineral nutrients most commonly limiting the growth of terrestrial plants. Ironically, soils may have large reserves of total P, but the amounts available to plants is usually a tiny proportion of this total (Stevenson and Cole, 1999). The low availability of P to plants is because the vast majority of soil P is found in insoluble forms, and plants can only absorb P in two soluble forms, the monobasic (H_2PO^{4-}) and the diabasic (HPO_4^{2-}) ions (Glass, 1989).

It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere, are able to solubilize P. Secretion of organic acids and phosphatases are common method of facilitating the conversion of insoluble forms of P to plant-available forms (Kim et al., 1998). The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR (plant growth promoting rhizobacteria) that increase nutrient availability to host plants (Richardson, 2001). Examples of studied associations include *Bacillus circulans* and *Cladosporium herbarum* and wheat (Singh and Kapoor, 1999), *Bacillus* sp. and five crop species (Pal, 1998), *Enterobacter agglomerans* and tomato (Kim, et al., 1998), *Pseudomonas chlororaphis* and *P. putida* and soybean (Cattelan et al., 1999).

Because intensive agriculture entails the risk of excessive fertilization, microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible (Çakmakçi et al., 2006). Therefore, the use of rhizobacteria as biofertilizers or control agents for agriculture improvement has been a focus of numerous researchers for a number of years (Kloepper, 1994, Lemanceau, 1992, Suslov, 1982). This group of bacteria is represented by genera such as *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium.*

Phosphate-solubilizing bacteria are common in rhizosphere (De Freitas et al., 1997, Illmer et al., 1995). However, the ability to solubilize P by no means indicates that a rhizospheric bacterium will constitute a PGPR. For example (Cattelan, et al., 1999) found only two of five rhizospheric isolates positive for P solubilization actually had a positive effect on soybean seedling growth. Likewise, not all P solubilizing PGPR increase plant growth by increasing P availability to the hosts. For example, (De Freitas, et al., 1997) found a number of P solubilizing *Bacillus* sp. isolates and a *Xanthomonas maltophilia* isolate from canola (*Brassica napus* L.) rhizosphere which had positive effects on plant growth, but no effects on P content of the host plants.

The objective of the present work was to assess P solubilizing potential of several bacterial strains isolated from soybean rhizosphere and rizoplane in order to be used in the future studies as biofertilizers.

MATERIALS AND METHODS

Isolation of rhizobacteria

Bacteria were isolated from the rhizosphere and rizoplane of field-grown soybean crop from Ezareni, jud. Iasi. Plants and roots were collected by removing 20-cm^2 blocks of soil, which were kept in plastic bags at 4 °C for 12 h until processing. Roots were separated from bulk soil. Soil remained on the surface roots after moderate shaking was used for isolation of rhizospheric microorganism. After the separation of rhizospheric soil, roots were soaked in sterile phosphate-buffered saline (PBS, pH 7.2, 10 mM K_2PO_4-KH_2PO_4, 0.14 M NaCl) for 10 min, chopped into 5 g pieces and suspended in 45 ml PBS. Rhizospheric soil and root samples were blended in a sterile Waring blender at high speed for 1 min and serial dilutions (1/10) were made in PBS. Aliquots (0.1 ml) were plated on Bunt Rovira nutrient medium and incubated at 28°C for 7 days. 29 rhizobacteria isolates were selected to represent distinct types based on differences in colony morphology including: colony form, elevation, opacity and pigment production. Isolates were re-streaked on Bunt Rovira nutrient medium, checked for purity, and stored on slants at 4^0 C.

Microscopic examination was conducted on Gram stained smears (Dunca et al., 2004) using an Olympus CH 30 optical microscope.

P solubilizing assay

The ability of isolates to solubilize P was assessed qualitatively using potato-dextrose yeast extract agar (PDYA, pH 7.0) containing freshly precipitated calcium phosphate: 50 ml sterile 10% (wt·vol^{-1}) K_2HPO_4 and 100 ml sterile 10% (wt·vol^{-1}) $CaCl_2$ were added per sterile PDYA liter to produce a precipitate of $CaHPO_4$ (Katznelson and Bose, 1959). Each bacterial culture was streaked in the center of a PDYA-CaP plate and incubated at 28^0 C. Phosphate solubilization was assessed up to 14 days by measuring the zone of clearing (area of solubilization) surrounding the developed bacterial colony.

RESULTS AND DISCUSSIONS

Morphological characterization

The main micro-morphological characters of the isolated bacterial strains are presented in Table 1. Our studies have revealed that in the areas surrounding soybean roots is developing a morphologically less diversified bacterial population (Fig. 1) represented predominantly by sporulated and non-sporulated Gram positive bacilli (Photo 1, Photo 2), also by cocci (Photo 3) and cocobacili (Photo 4). The morphological structure of the soybean rhizosphere microbiota described in our studies largely corresponds with the data reported in the literature (Liu and Sinclair, 1993, Lynch, 1990, Sylvia et al., 1999).

Table 1 - Micro-morphological characterization of strains isolated from soybean rhizosphere and rizoplane

Strain	Micro-morphological characters
R1	Sporulated Gram positive bacilli, subterminal non-deformant spores
R2	Sporulated Gram positive bacilli, subterminal non-deformant spores
R3	Sporulated Gram positive bacilli, central non-deformant spores
R4	Non-sporulated Gram negative bacilli, short chains
R5	Non-sporulated Gram positive bacilli, long chains
R6	Sporulated Gram positive bacilli, central non-deformant spores
R7	Non-sporulated Gram negative bacilli, short chains
R8	Micelian hyphae
R9	Sporulated Gram positive cocobacilli, subterminal non-deformant spores, short chains
R2.1	Sporulated Gram positive bacilli, central non-deformant spores, short chains
R2.2	Isolated non-sporulated Gram negative bacilli
R2.3	Non-sporulated Gram positive bacilli

R2.4	Sporulated Gram positive bacilli, central non-deformant spores
R2.5	Sporulated Gram positive bacilli, central non-deformant spores, short chains
R2.6	Isolated non-sporulated Gram positive cocci
Rzp1	Non-sporulated Gram positive cocci, tetrades
Rzp2	Isolated sporulated Gram positive bacilli, subterminal non-deformant spores
Rzp3	Sporulated Gram positive cocobacilli, subterminal non-deformant spores, short chains
Rzp4	Isolated non-sporulated Gram positive bacilli
Rzp5	Isolated non-sporulated Gram negative cocci
Rzp6	Isolated non-sporulated Gram positive cocci
Rzp7	Isolated non-sporulated Gram negative bacilli
Rzp8	Isolated non-sporulated Gram positive bacilli, short chains
Rzp2.1	Isolated non-sporulated Gram positive bacilli, short chains
Rzp2.2	Isolated non-sporulated Gram positive bacilli
Rzp2.3	Isolated non-sporulated Gram negative bacilli
Rzp2.4	Isolated non-sporulated Gram positive bacilli
Rzp2.5	Sporulated Gram positive cocobacilli, subterminal non-deformant spores, short chains
Rzp2.6	Isolated non-sporulated Gram negative bacilli

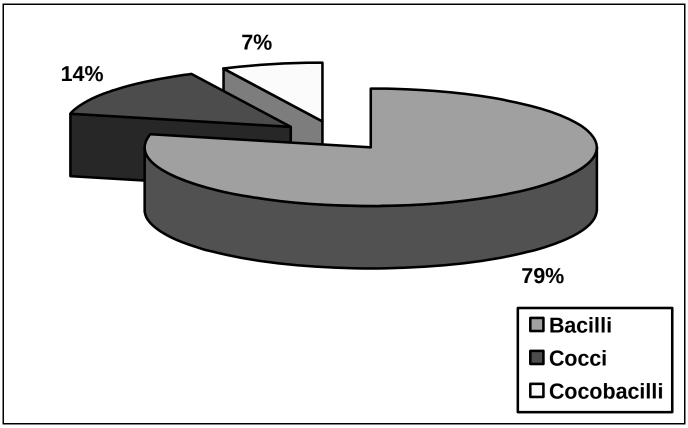

Fig. 1 - The main morphological types isolated from soybean roots

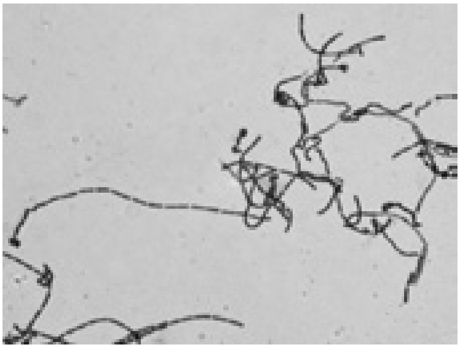

Photo 1 – Strain R2.1 (1000 x)

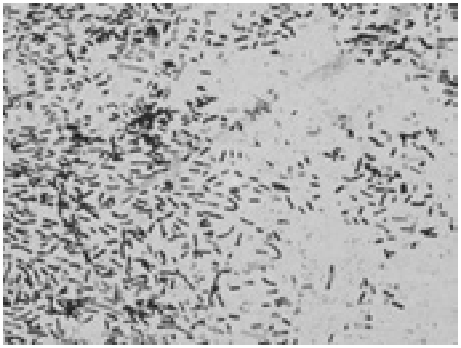

Photo 2 – Strain R5 (1000 x)

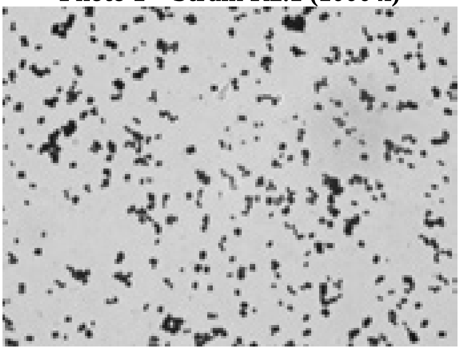

Photo 3 – Strain RZP1 (1000 x)

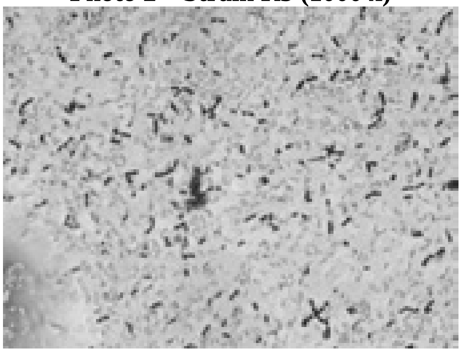

Photo 4 – Strain RZP3 (1000 x)

Screening for phosphate-solubilizing bacteria

Qualitative screening of the P solubilizing microorganisms was possible using a plate methods, which show clearing zones around the microbial colonies in media containing insoluble mineral phosphates as single P source. The method can be regarded as generally reliable for isolation and preliminary characterization of phosphate-solubilizing microorganisms (Gupta et al., 1994, Illmer and Schinner, 1992). Our results showed that 31.03% of the isolates presented P solubilizing capabilities (Fig. 2): R2, R7 (Photo 5), R8, R2.2 (Photo 6), R2.6 (Photo 7), RZP4 (Photo 8), RZP5, RZP2.3, RZP2.6.

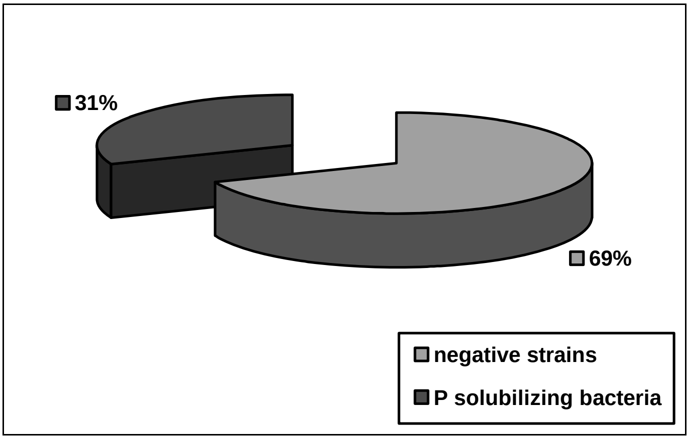

Fig. 2 – Classification of bacterial strains isolated from soybean rhizosphere and rizoplane according to their P solubilizing potential

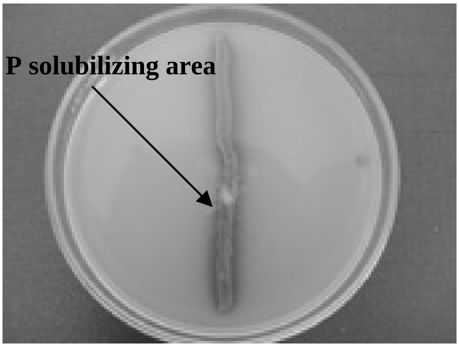

Photo 5 – R7 strain isolated from soybean rhizosphere

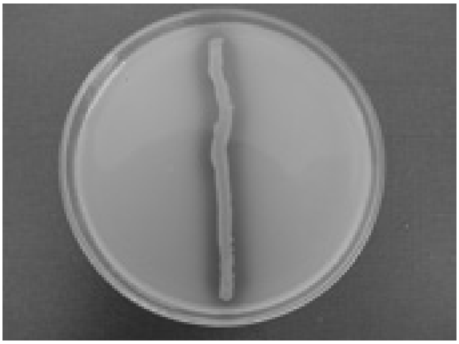

Photo 6 – R2.2 strain isolated from soybean rhizosphere

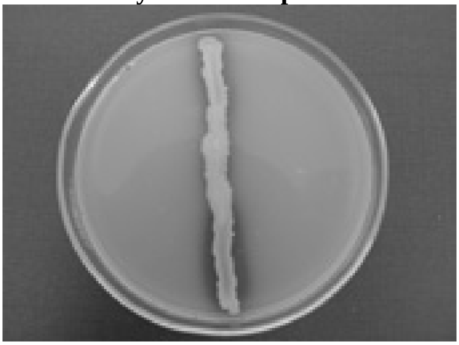

Photo 7 – R2.6 strain isolated from soybean rhizosphere

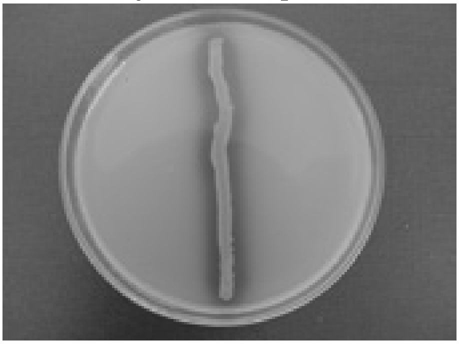

Photo 8 – RZP4 strain isolated from soybean rizoplane

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate (Goldstein, 1986, Richardson, 1994, Singh and Kapoor, 1999). There are many populations of P solubilizing bacteria in soil, but considerably higher concentration of this microorganisms are commonly found in the rhizosphere (Katznelson et al., 1962, Laheurte and Berthelin, 1988, Raghu and MacRae, 1966, Richardson, 1994). As our results revealed, the majority of the strains presenting P solubilizing capabilities were isolated from soybean rhizosphere (Fig. 3).

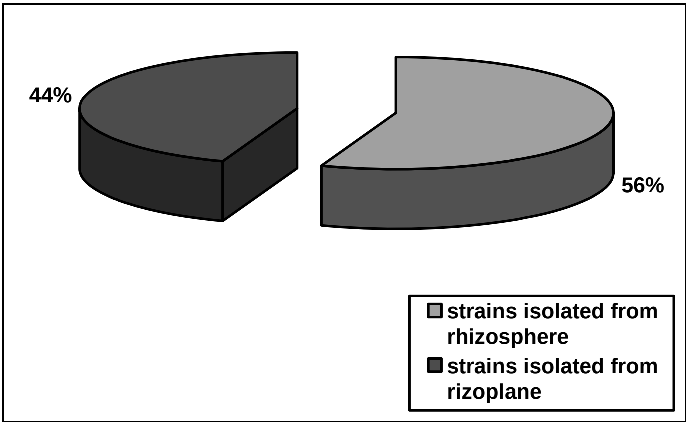

Fig. 3 – Isolation sources of bacteria with P solubilizing potential

The presence of P solubilizing bacteria in soybean rhizosphere may be correlated with the special nutritional needs of this plant species and with the complex interrelations that are set in the roots zone. Thus, it is considered that phosphorus promotes development of soybean nitrogen fixing nodules by stimulating the development of nitrogen-fixing bacteria via a mechanism that includes increased P availability (De Freitas, et al., 1997). Considering that P availability is a limiting step in plant nutrition (Goldstein, 1986), this evidence suggests a fundamental contribution of phosphate-solubilizing bacteria to plant nutrition and to the improvement of plant growth performance (Rodríguez and Fraga, 1999), therefore our strains present a potential capacity to be used in further experiments as PGPR.

CONCLUSIONS

In the regions surrounding soybean roots is developing a population of bacteria less diversified from a micro-morphologic point of view. Approximately 31 % of the tested strain presented P solubilizing capabilities, the majority being isolated from soybean rhizosphere. Because these strains can produce other metabolites beneficial to the plant, such as phytohormones, antibiotics, or siderophores, among others, further investigations are necessary in order to be more precisely about the specific role of phosphate solubilization bacteria in plant growth and their use as PGPR.

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