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Uptake of phosphate and promotion of vegetative growth in glucose-exuding rice plants (*Oryza sativa*) inoculated with plant growth-promoting bacteria

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ABSTRACT

We measured phosphorus uptake by rice plants inoculated with plant growth-promoting bacteria (PGPB) using *Pseudomonas* sp. strain PAC, *Serratia* sp. strain CMR165, and *Azospirillum brasilense* strain FT326. We measured plant growth parameters and phosphate solubilization and uptake. Results show that the ability to solubilize phosphates varied among PGPB strains. Strain FT326 was unable to solubilize phosphates. In the presence of glucose, PAC and CMR165 can solubilize inorganic tricalcium phosphate and organic calcium magnesium inositol hexaphosphate. Phosphate solubilization by strains PAC and CMR165 was different over time; FT326 was similar to the untreated control. Plants inoculated with PAC or CMR165 had higher concentrations of phosphates than those inoculated with FT326 and plants that were not inoculated. Glucose was the only sugar identified in rice root exudates. PAC and CMR165 promoted plant growth and uptake of phosphate and could be used as biofertilizers to optimize phosphate fertilization.

1. Introduction

After nitrogen, phosphorus (P) is the most limiting nutrient for plant growth. While abundantly present in many soils, it is immobilized by fixation in the form of insoluble Ca, Fe, or Al salts or Al and Fe oxides not available to plants (Rodríguez and Fraga, 1999; Richardson et al., 2001). Even in fertile soils, P concentration in the soil solution is not higher than 10 μM at pH 6.5 (Arnou, 1953). This phenomenon is dependent on pH and soil type (Dey, 1988).

According to Goldstein et al. (1993), P accumulated in agricultural soils can supply maximum yields of crops for 100 years. Approximately, 80% of inorganic P applied as fertilizer is quickly immobilized in the soil, becoming unavailable for plant absorption (Holford, 1997). Organic P in soil varies from 30% to 50% of total P in most soils. This fraction is formed mainly by inositol-phosphate (phytate) which is the most stable organic form of P (Dalal, 1977). Other types of organic P are phosphoesters, mainly of high molecular weight, that need to be converted to low-weight soluble forms to be readily available for plants (Goldstein, 1994).

Plant responses to P deficiency tend to increase its availability, absorption, and efficiency of use. Adaptation mechanisms to increase Pi availability include phosphatases, RNAases, and nuclease activities (for organic phosphates) and organic acid production (for inorganic phosphates) (Rodríguez et al., 2006). Root adaptations to low levels of P include mycorrhizal associations, alterations of root architecture, an increase in length and density of root hairs (Raghothama, 1999; Raghothama and Karthikeyan, 2005), and enhanced expression of high affinity phosphate transporters to increase P absorption (Kochian, 2000).

Conceptually, plant growth-promoting bacteria (PGPB) can affect plant growth and development either directly or indirectly (Bashan and Holguin, 1998). On the one hand, PGPB may decrease or prevent some effects of phytopathogenic organisms by producing antibiotics. On the other hand, these bacteria may directly provide plants with different compounds or facilitate incorporation by fixing nitrogen or solubilizing phosphorus. PGPB can solubilize phosphates effectively and enhance P uptake in poor, calcareous P soils or those fertilized with phosphoritic rock (Kundu and Gaur, 1984; Magda et al., 2003).

Microbial activity is one of the most important pathways of solubilizing P in soils (Halder et al., 1989; Gyaneshwar et al., 2002; Rodríguez et al., 2006). Among PGPB are phosphate-solubilizing bacteria (PSB). The phenotype exhibited by PSB is attributed to lower pH caused by low-molecular-weight organic acids or proton liberation to the medium (Gyaneshwar et al., 1998). Organic acids can dissolve Pi directly through anionic interchange or chelating ions associated with the Pi (Goldstein, 1994). Production of organic acids has been identified in PSB (Rodríguez and Fraga, 1999; Vyas

Abbreviations: BCIP, 5-bromo-4-chloro-3-indolyl phosphate; HPLC, high performance liquid chromatography; IP6, calcium magnesium inositol hexaphosphate; PGPB, plant growth-promoting bacteria; PSB, phosphate-solubilizing bacteria; TCP, tricalcium phosphate.

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and Gulati, 2009). These acids are produced in the periplasm of many Gram-negative bacteria through a direct oxidation pathway of glucose (Liu et al., 1992).

In turn, plants supply root-borne carbon compounds, mainly sugars that can be metabolized for growth of bacteria (Goldstein, 1994). Lynch and Whipps (1990) estimated that 4–29% of photosynthates can be transferred to the rhizosphere and available to microorganisms. Root exudates are important nutrients for soil microorganisms and are involved in chemotactic root colonization processes (Lynch and Whipps, 1990).

PGPB colonize the rhizosphere and adhere to the root surface (Kloepper and Schroth, 1978; Bashan and Holguin, 1998; Kloepper et al., 1999) or penetrate the roots to establish endophytic populations (Gray and Smith, 2005).

To convert organic P into forms readily available for plants, it must be mineralized into low-weight inorganic forms. Organic P is mineralized by microbial phosphatases (Bishop et al., 1994), whose enzymatic activities were detected in different types of soils (García et al., 1992).

This study measured phosphorus uptake by rice plants using isolated and characterized PGPB from Argentine soils and compost in our laboratory. Our objective was to assess the degree of phosphate solubilization and plant growth-promotion of isolated bacterial strains. We hypothesize that in the sole presence of insoluble phosphates, plant growth is promoted when the plants are inoculated with phosphate-solubilizing strains of bacteria.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The *Pseudomonas* sp. strain BNM 0521 (PAC) was isolated from compost of green leaves of *Platanus hispanica* Mill. After appropriate steps of isolating this bacteria, it was cultured to obtain pure cultures in Cetrimide agar. The *Serratia* sp. strain BNM0522 (CMR165) was isolated from rice roots. To isolate bacteria, plant roots were washed with distilled water and 1 cm fragments from the main roots were cut and incubated in NFb semisolid broth with malate as the carbon source. After incubation for 48 h at 33 °C, the number of CFU was determined by serial dilution plating on Congo Red nutrient agar (Rodríguez-Cáceres, 1982). Morphology and motility were determined for each isolate. Biochemical tests were also performed; the isolated bacteria were selected for its capacity to produce indol acetic acid (IAA) and nitrogenase activity according to Torres et al. (2000) and Ribaudo (personal communication).

Azospirillum brasilense strain FT326 (FT326) was provided by the Brazilian Enterprise for Research on Agriculture (EMBRAPA, Empresa Brasileira de Pesquisa Agropecuária) and used as a negative control strain. Strains were grown in NFb with malate as the carbon source, supplemented with NH_4Cl (1 g l^{-1}), at 35 °C under continuous agitation (100 rpm) for 48 h (Döbereiner et al., 1976).

2.2. Molecular characterization

Isolates were grown at 25 °C for 24 h in BHA; after lysing cells, the DNA was extracted using a microbial DNA isolation kit (Ultra Clean, Mo Bio Laboratories, Carlsbad, CA). The DNA was checked for purity using standard methods (Sambrook et al., 1989). DNA templates were amplified in a thermocycler (Genius, Techne, Staffordshire, UK), using universal primers amplifying a 1000 bp region of the 16S rDNA, 616F: 5′-AGA GTT TGA TYM TGG CTC AG-3′, 699R: 5′-RGG GTT GCG CTC GTT-3′ (Invitrogen, Carlsbad, CA). These primers are located at positions 8–25 and 1099–1113 (*Escherichia coli* numbering), respectively. The amplification mixture (100 μl) contained 2 μl (50 pmol μl^{-1}) each of the 616F and 699R primers, 0.5 μl (2 U μl^{-1}) of Taq DNA polymerase (F-5495, Finnzymes, Vantaa, Finland), 10 μl 5× reaction buffer (F-5495, Finnzymes, Vantaa, Finland), 10 μl dNTP mixture containing 1 mmol l^{-1} each of dATP, dGTP, dCTP, and dTTP (F-5605, Finnzymes, Vantaa, Finland), 70 μl sterile, filtered water (Milli-Q purification system, EMD Millipore, Billerica, MA) and 100 ng DNA template. Reactions were run for 10 min at 94 °C followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Controls devoid of DNA were included in the amplification process. The integrity of the PCR products was controlled through the development of a single band after electrophoresis in 2% (w/v) agarose gels in TBE buffer at 5 V cm^{-1} for 1 h. Amplicons were purified using an UltraClean PCR clean-up kit (Mo Bio Laboratories), and subsequently sequenced, using an automated sequencer (Abi Prism 3730, Applied Biosystems, Carlsbad, CA) using a cycle sequencing kit, premixed format (BigDye Terminator v3.1, Applied Biosystems). Sequencing primers were the same as those used in the amplification reaction, but diluted ten times (5 pmol l^{-1}).

The resulting 16S rDNA sequences were compared in a BLAST search with those in the National Library of Medicine database (Altschul et al., 1997). The 16S rDNA sequence for strain PAC was submitted to GenBank (accession number FJ851181) and compared with known sequences using BLASTN PROGRAM (Zhang et al., 2000); search showed a 99% identity for 16S ribosomal RNA gene of *Stenotrophomonas maltophilia* strain ZA-6 (accession number: FJ851181.1) and *Pseudomonas aeruginosa* strain PS1 (accession number FJ705886.1). The strain CMR165 was submitted to GenBank (accession number FJ851180); search showed a 99% identity with *Serratia marcescens* strain AGPim1A 16S ribosomal RNA gene (accession number JF683415.1).

2.3. Bacterial solubilization and phosphatase activity of TCP and IP6

The ability of isolates to solubilize phosphate was assessed qualitatively on agar plates containing an insoluble P (Goldstein and Liu, 1987). Bacterial suspensions (100 μl $OD_{600\ nm}$ = 0.2 in sterile 0.85%, w/v NaCl) were inoculated in a well in agar medium containing (g l^{-1}) NH_4Cl (5); NaCl (1), $MgSO_4 \cdot 7H_2O$. Malate, sucrose, lactose (5) or glucose (10) as alternative carbon sources and either $Ca_3(PO_4)_2$ or IP6 (5) mg ml^{-1} as insoluble P were added to the medium source in suspension. The pH was adjusted to 7.2. Plates were incubated at 37 °C for 48 h. Development of a clear zone around the colony was regarded as a positive indicator of P solubilization. To evaluate $[H^+]$, dependent P solubilization, methyl red (as a pH indicator) and 100 mM Tris–HCl buffer at pH 7.9 were added to the medium.

Stationary bacterial culture suspensions (2.5 ml, $OD_{600\ nm}$ = 0.2 in sterile 0.85%, w/v NaCl) were inoculated in Erlenmeyer flasks with 50 ml fresh broth (Liu et al., 1992). Flasks were incubated at 37 °C and agitated at 100 rpm. To compare bacterial and acid P solubilization, 0.5 m 1 M HCl was added to the medium to reach a similar pH to the inoculated cultures (Kim et al., 1997). At 0, 24, 48, 72, and 96 h after inoculation, 1 ml aliquots (duplicates) were centrifuged at 8000 × *g* for 10 min. The pellet was discarded and the $[H^+]$ (colorpHast, EMD Industries, Gibbstown, NJ) and soluble P were determined in the supernatant (Fiske and Subbarow, 1925). Phosphatase activity was determined according to Pond et al. (1989) in plates containing agar Luria Bertani and 200 μg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate (BCIP).

2.4. Bacterial inoculation and plant growth

The seeds of *Oryza sativa* (INTA Puitá CL variety) were surface-sterilized by agitation for 30 min in a solution containing 30% NaClO and 0.1% Triton X-100, followed by three 10-min washes with sterile water. Five seeds were transferred to a 170 ml culture flask containing 40 ml of 8 g l^{-1} agar dissolved in 0.5% (w/v) nutritive solution according to Hoagland and Arnon (1950), without soluble phosphorus and with 0.1% (w/v) NH_4Cl, 10 g l^{-1} glucose and 5 g l^{-1} $Ca_3(PO_4)_2$ as the sole P source. Plants were inoculated 72 h after sowing with 30 μl bacterial suspension ($OD_{600\ nm}$ = 0.2 re-suspended in sterile 0.85% (w/v) NaCl), in the range of 10^{10}–10^{13} CFU ml^{-1} between strains and experiments. The plants without bacteria (controls) received 30 μl 0.85% (w/v) NaCl solution. All plants were maintained in a growth chamber for 20 days at 20/25 ± 2 °C (day/night) with 16 h light exposure. Five replicates of all controls and inoculated treatments were run.

Growth parameters analyzed to assess the response to bacterial inoculation include plant height and main root length; each experiment being repeated twice. Soluble P was extracted from each sample according to Pieters et al. (2001) and its concentration was evaluated according to Fiske and Subbarow (1925).

ANOVA were used to test differences and, when appropriate, Tukey's test was used for comparison of means. All statistical analyses were performed with InfoStat software (Universidad Nacional de Cordoba, Argentina). Statistical significance was set at $P < 0.05$.

2.5. Root exudates and soluble sugar determination by HPLC

To obtain root exudates, an axenic hydroponic system was used, as described in Přikryl and Vančura (1980). The surface-sterilized seeds were placed in floating plastic mesh discs inside 170 ml flasks containing 40 ml of sterile milliQ water. Plants were grown as described above. Eight days after sowing, 500 μl of the bacterial suspension ($OD_{600\ nm}$ = 0.2 re-suspended in sterile 0.85% (w/v) NaCl) were injected into the hydroponic solution. All of the hydroponic solution (containing the radical exudates) was replaced at 0, 7 and 14 days after inoculation. These hydroponic solutions were filtered through a 0.2 μm cellulose-acetate filter (Sartorius, Goettingen, Germany) to remove bacterial cells and lyophilized for later analysis (Bacilio-Jiménez et al., 2003). Lyophilized exudates were washed three times with boiling ethanol 85% (v/v) in H_2O, concentrated to 3 ml in a rotavapor at 45 °C for 1 h. The concentrates were resuspended in 5 ml of milliQ water and filtered (Ø = 47 mm; 0.45 μm).

The soluble sugar content was analyzed according to Wills et al. (1982) using an HPLC (1100 Series, Agilent Technologies, Santa Clara, CA) with a carbohydrate analysis column (Agilent Zorbax, refraction index detector) at 30 °C, 1.4 ml min^{-1} acetonitrile:water flux (75:25).

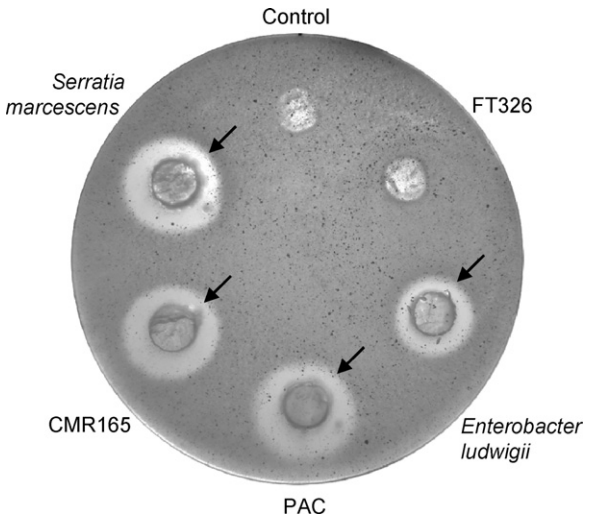

Fig. 1. Extracellular phosphate solubilization assay in agar plates. Bacterial suspensions were inoculated inside wells, and P-solubilization was measured after 48 h incubation at 37 °C. Control is trial without bacteria. Strain FT326, *Enterobacter ludwigii*, strain PAC, strain CMR165, and *Serratia marcescens* were inoculated inside wells performed on the agar surface. Arrows indicate solubilization halos.

3. Results

3.1. Solubilization of phosphate

Ability to solubilize phosphates varied among PGPB strains and was dependent on the P and carbon (C) source. Fig. 1 shows the strains growing in a plate containing insoluble phosphate suspended in the medium, as described in Section 2.3. Plate assay results are summarized in Table 1. Strain FT326 was unable to solubilize TCP or IP6 in the presence of malate, sucrose, lactose, or glucose as the C source. Strain PAC solubilized TCP and IP6 in the presence of glucose, but not with the other carbon sources. Strain CMR165 was able to solubilize both insoluble P sources in the presence of glucose, sucrose, and lactose, but not malate. The ability to use IP6 as the C source was measured. No strain was able to do so (data not shown). In the presence of malate in the culture medium, the strains could not solubilized P nor acidify the medium (Table 1).

To establish whether solubilization of P depended on changes in the medium's [H^+], a buffer solution and pH indicator were added to the culture medium. As shown in Table 1, the ability to solubilize P in strains PAC and CMR165 was diminished with the addition of a buffer solution.

Table 1
Extracellular solubilization of precipitated tricalcium phosphate (TCP) or calcium magnesium phytate (IP6) present in the medium at 5 g l^{-1}, with either malate, sucrose, lactose, or glucose as the sole carbon source.

P source	Bacterial strain	Malate		Sucrose		Lactose		Glucose		Glucose + buffer	
		P_{sol}	ΔpH	P_{sol}	ΔpH	P_{sol}	ΔpH	P_{sol}	ΔpH	P_{sol}	ΔpH
TCP	Control	–	NC	–	NC	–	NC	–	NC	–	NC
	FT326	–	NC	–	NC	–	NC	–	NC	–	NC
	PAC	–	NC	–	NC	–	NC	++	↓	–	NC
	CMR165	–	NC	+	↓	+	NC	++	↓	–	NC
IP6	Control	–	NC	–	NC	–	NC	–	NC	–	NC
	FT326	–	NC	–	NC	–	NC	–	NC	–	NC
	PAC	–	NC	–	NC	–	NC	++	↓	–	NC
	CMR165	–	NC	++	↓	++	NC	++	↓	–	↓

100 mM Tris–HCl buffer pH 7.2. Control without bacteria. Strains FT326, PAC, or CMR165 were inoculated inside wells on the agar surface. (–) No clear zone surrounding the colony, (+) <2 mm clear zone, (++) >2 mm clear zone, (↓) acidification of the medium, (NC) no change in pH.

Table 2
pH of the medium with tricalcium phosphate (TCP) and glucose or calcium magnesium inositol hexaphosphate (IP6) and glucose. Mean ($n = 2$).

P source	Bacterial strain	Time after inoculation (h)				
		0	24	48	72	96
TCP	Control	6.5	6.5	6.5	6.5	6.5
	FT326	6.5	6.5	6.5	6.5	6.5
	PAC	6.5	5.3	5.1	5.1	5.3
	CMR165	6.5	5.2	5.3	5.2	5.1
	HCl 0.01 M	7.0	5.5	5.5	5.5	5.0
IP6	Control	6.5	6.5	6.5	6.5	6.5
	FT326	6.5	6.5	6.5	6.5	6.5
	PAC	6.5	5.5	5.0	5.0	5.0
	CMR165	6.5	5.0	5.0	5.0	5.0
	HCl 0.01 M	7.0	5.5	5.5	5.5	5.0

The experiment was repeated twice with identical results.

Kinetics of phosphate solubilization coincides with the plate assay results. Table 2 shows the bacterial growth medium [H^+] at 0–96 h after inoculation. The pH of the medium's decreased below 5.5 after 24 h in cultures containing CMR165 or PAC; but cultures containing FT326 showed no change in the pH.

Fig. 2 shows the kinetics of P solubilization when TCP or IP6 were present as the insoluble P source in liquid culture. In the presence of TCP, CMR165 reached maximum solubilization of P at 24 h, close to 200 $\mu g\,ml^{-1}$ of soluble P content. Strain PAC acidified the medium similar to CMR165, but the kinetics of P solubilization were different. Solubilized P by strain PAC increased up to 55 h, and stayed stable in 150 $\mu g\,ml^{-1}$ afterwards, the highest value at that time. Strain FT326 did not solubilize TCP. When HCl was added to the growth broth, soluble P content was 100 $\mu g\,ml^{-1}$. When malate was used as the sole C source, the culture medium was alkalinized and no solubilization of P was observed in either treatment (data not shown).

When the insoluble P source in the culture medium was IP6, the control and FT326 treatments showed no change over time (50 $\mu g\,ml^{-1}$). Strains PAC and CMR165 reached soluble *P* values close to 75 and 200 $\mu g\,ml^{-1}$, respectively after incubation for 24 h and remained stable for the rest of the experiment. When HCl was added, the medium was acidified to the same extent as with PAC and CMR165 inoculated cultures; but the soluble P content was the same as in the control and FT326 culture assays, and significantly lower than in CMR165 and PAC inoculated cultures.

Phosphatase activity, evaluated as BCIP cleavage, was present in the culture of strains FT326 and CMR165, but not PAC.

3.2. Promotion of plant growth

Promotion of plant growth by bacteria on shoot and root length, and soluble P content was measured 20 days after inoculation (Fig. 3A and B). When rice plants grew with glucose and with TCP as the sole source of P, inoculation with PAC increased plant height by 48%, compared with uninoculated plants. Inoculation with CMR165 increased plant height by 28% and shoot length by 39%. Shoot and root length of plants inoculated with FT326 were the same as in uninoculated plants.

The concentration of soluble P was significantly higher in shoots of inoculated plants compared with plants that were not inoculated. Among strains, plants inoculated with PAC or CMR165 had higher soluble P than plants inoculated with FT326: 4.1, 3.4, and 2.4 mg P mg^{-1} fresh weight, respectively (Fig. 3C). Moreover, in plants inoculated with CMR165 or PAC, solubilization of P was visible as clear zones around the roots. No plant promotion was observed 20 days after inoculation, when glucose was absent in the culture medium.

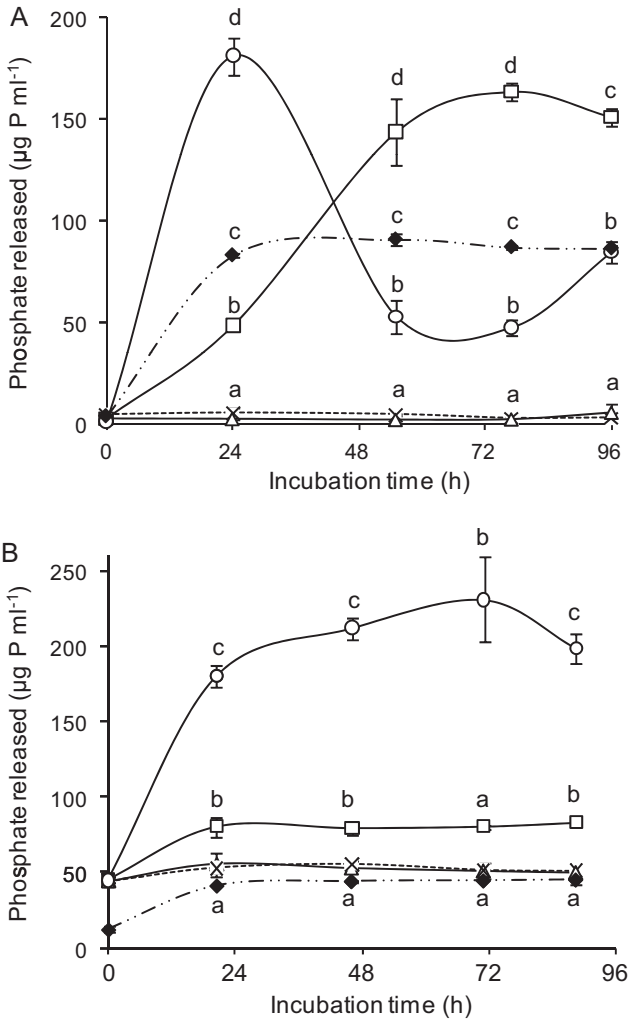

Fig. 2. Extracellular phosphate solubilization of inoculated liquid medium with glucose and 5 $g\,l^{-1}$ tricalcium phosphate (A) or calcium magnesium inositol hexaphosphate (B). X = control without bacteria; open triangle = strain FT326; open square = strain PAC; open circle = strain CMR165; black rhombus = control with 0.5 ml of 1 M HCl. The bar whiskers show standard deviation. Different letters indicate significant differences between treatments at a given incubation time (Tukey's test: $P < 0.05$).

3.3. Root exudates

Glucose was the only sugar identified in rice root exudates. No significant amounts of fructose, mannose, ramnose, sucrose, or xylose were detected. The glucose level was 9.22 mg $seedling^{-1}$ 8 days after sowing, and 6.54 mg $seedling^{-1}$ 15 days after sowing; no glucose was detected 22 days after sowing. Seven days after inoculation (15 days after sowing), glucose concentration in root exudates (mg ml^{-1}) was 3.14 ± 1.70 in control plants, 3.26 ± 1.72 in plants inoculated with strain FT326, 17.21 ± 2.84 with strain PAC, and 0.55 ± 0.78 with strain CMR165. Fourteen days after inoculation (22 days after sowing), glucose was only detected (at 0.42 ± 0.60 mg ml^{-1}) in root exudates of plants inoculated with strain CMR165.

4. Discussion

A. brasilense strain FT326 was not able to solubilize organic or inorganic phosphates. Mehnaz and Lazarovits (2005) obtained similar results using NBRIP medium (Nautiyal, 1999) for *A. brasilense* strain N8. However, Rodriguez et al. (2004) found that *A. brasilense*

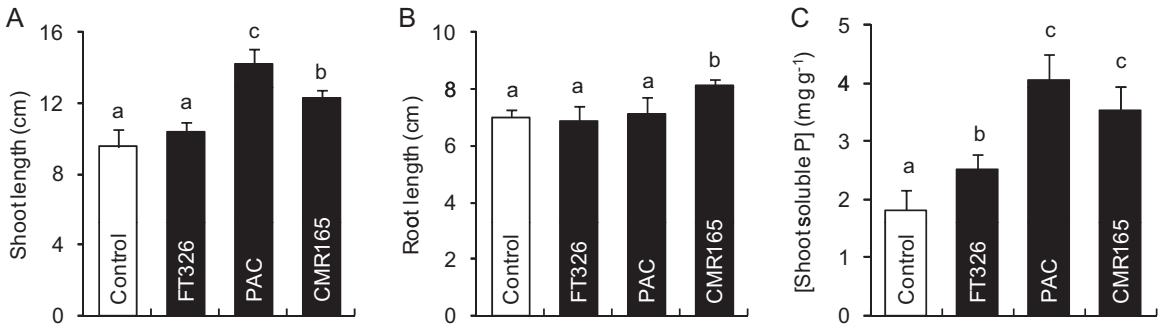

Fig. 3. Plant growth parameters in rice *Oryza sativa* inoculated with strains FT326, PAC, and CMR165 growing in culture medium with glucose and tricalcium phosphate as the sole source of phosphorus measured at 20 days after inoculation. Shoot length (A), main root length (B), and concentration of soluble phosphorus in shoots (C). White bars = control plants; black bars = inoculated plants. The bar whiskers indicate SD. Different letters indicate significant differences between inoculation treatments (Tukey's test: $P < 0.05$).

strains Cd and 8-I produced gluconic acid, which solubilized TCP when fructose and glucose were present in the culture medium.

Pseudomonas sp. strain PAC was able to solubilize TCP and IP6 only in the presence of glucose. Others have found P-solubilization ability in other *Pseudomonas* species (Molla et al., 1984; Babu-Khan et al., 1995; Peix et al., 2003; Vyas and Gulati, 2009). The enterobacteria *Serratia* sp. strain CMR165 solubilized TCP and IP6 in culture media containing glucose, sucrose, or lactose. Similar results were found for a *S. marcescens* strain (Krishnaraj and Goldstein, 2001; Chen et al., 2006).

Solubilization of P by bacteria varied with the source in the medium. Hameeda et al. (2006) measured solubilization of rock phosphate with different sources of C, finding the highest activity with glucose. Nautiyal (1999) found that the amount of glucose in the medium influenced the degree of solubilization of P by bacteria. None of the strains we tested were able to use IP6 as a source of C. Richardson and Hadobas (1997) found that <0.5% of cultivable soil bacteria were able to do this.

Strains CMR165 and PAC solubilized TCP, but their kinetics were different over time. Solubilized P in medium inoculated with PAC was greater than solubilized P from addition of HCl 48 h after incubation until the end of the experiment. The amount of solubilized P by strain CMR165 was comparable to addition of HCl, but varied over time. Kim et al. (1997) found that *Enterobacter agglomerans* acidifies the medium and solubilizes more hydroxyapatite than several acids at similar pH after 84 h of incubation.

PAC and CMR165 solubilized calcium and magnesium inositol hexaphosphate (phytic acid), but FT326 did not. Others have found phytase activity in strains of *Pseudomonas* and *Serratia* (Richardson and Hadobas, 1997; Mukesh et al., 2004), but Hameeda et al. (2006) found *S. marcescens* strain EB67 was not able to cleave sodium IP6.

Medium acidification by adding HCl solubilized TCP. No strain was able to solubilize phosphates in a pH 7.9-buffered solution, although CMR165 acidified the medium. Gyaneshwar et al. (1998) found similar results with *Bacillus coagulans* and *Citrobacter koseri*.

Phosphatase activity, measured as 5-bromo-4-chloro-3-indolyl phosphate cleavage was positive for FT326 and CMR165, but negative for PAC.

When glucose was added to the culture medium, solubilization of TCP occurred around roots inoculated with PAC and CMR165. Plants inoculated with FT326, PAC, and CMR165 had higher soluble P concentration in shoots, and those inoculated with PAC and CMR165 had longer and heavier shoots than control plants. There was no difference in main root length of control and inoculated plants. Only plants inoculated with CMR165 had heavier root systems compared with the controls and other inoculation treatments. Datta et al. (1982) inoculated rice plants with *Bacillus firmus* strain NCIM-2636, a P-solubilizing bacteria, and fertilized it with rock phosphate and superphosphate, and observed enhanced yield and P content in the grain.

When plants were grown without glucose in the culture medium, there were no significant differences in growth or P uptake between inoculated and control plants. This suggests that, even though glucose was detected in rice root exudates, its concentration may be insufficient to allow bacteria to solubilize P as Nautiyal (1999) points out.

Root exudation of sugar was highest one week after sowing, reaching undetectable amounts 4 weeks after sowing. This pattern is similar to the results of Přikryl and Vančura (1980) with wheat and Bacilio-Jiménez et al. (2003) with rice. The main sugar identified in rice root exudates was glucose. Similar results were found by Bacilio-Jiménez et al. (2003) in root exudates of 7–28 day old rice plants; they also found that rice root exudates exert greater chemotactic attraction over endophytic bacterias, compared with other rhizobacteria present in the rhizosphere.

Glucose exuded by rice roots could be used by bacteria that need this carbon source to solubilize phosphates, which in turn enhances P uptake. Rhizospheric microorganisms may promote exudation, as detected by Přikryl and Vančura (1980) in wheat plants inoculated with *Pseudomonas putida*.

5. Conclusions

In summary, plant growth and P uptake is promoted by *Pseudomonas* sp. strain PAC and *Serratia* sp. strain CMR165. This may be a promising approach for sustainable agriculture. These strains might be used as biofertilizers, minimizing chemical fertilization that is currently used to obtain high yields for profitable agriculture.

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