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ORIGINAL ARTICLE

Identification and characterization of the rhizosphere phosphate-solubilizing bacterium *Pseudomonas frederiksbergensis* JW-SD2, and its plant growth-promoting effects on poplar seedlings

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Abstract The rhizospheric bacterium JW-SD2 was identified as *Pseudomonas frederiksbergensis* based on phenotypic features, the Biolog Identification System and 16S rRNA sequence analysis. The phosphate-solubilizing activity, acidification in culture media, growth rate and organic acid secretion of JW-SD2 were investigated during 192 h of cultivation. The phosphate solubilized by JW-SD2 reached 7.75 mM. The decrease in pH and increase of titratable acidity were closely correlated (Pearson's $r = -0.953$ and 0.969, respectively) with the phosphate-solubilizing activity. High concentrations of gluconic, 2-ketogluconic, pyruvic, maleic and malic acids were detected before 96 h of culture when the strain displayed a high level of phosphate-solubilizing activity, indicating that these organic acids were efficient components in phosphate solubilization. However, acetic acid did not affect phosphate solubilization as shown by a remarkable increase at 144 h of culture when the phosphate-solubilizing activity decreased. The phosphate-solubilizing ability of JW-SD2 was significantly ($P < 0.01$) affected by environmental factors. Over a broad ranges of temperature (20–35 °C), pH (4–9), salinity (0–3.0 %), and volume of medium (1/5 − 3/5 of flask volume), the phosphate solubilized by JW-SD2 remained above 4.00 mM, demonstrating good potential in adapting to a changing environment. The inoculation experiments indicated that JW-SD2 could significantly ($P < 0.05$) promote growth of poplar (*Populus euramericana* cv NL-895) in both sterilized and non-sterilized soils. The effects of plant growth promotion were greater in non-sterilized than in sterilized soil. During the 150 days of the trial, the effects of plant growth promotion by JW-SD2 first increased then decreased over time, suggesting that, in field applications, periodic supplementation of the strain into the rhizosphere should be considered.

Keywords *Pseudomonas frederiksbergensis* · Plant growth promotion · Phosphate solubilization · Organic acids · Poplar

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Introduction

Phosphorus (P) is an essential nutrient required for the growth and development of plants. However, most P in soil exists as insoluble compounds that cannot be absorbed and utilized by plants (Illmer and Schinner 1995). Phosphate deficiency in soil is a worldwide problem and 5.7 billion ha of land is deficient in soluble phosphate ($H_2PO_4^-$) and cannot achieve optimal crop production (Batjes 1997; Raghothama and Karthikeyan 2005). To circumvent the limitation of plant growth caused by phosphate deficiency, huge amounts of chemical phosphate fertilizers are used in agriculture. Nevertheless, the soluble phosphate applied to soil is quickly transformed into insoluble forms by combining with metal ions, such as, aluminum (Al^{3+}), ferric (Fe^{3+}) and calcium (Ca^{2+}), which are then not available to plants (Goldstein 1986). Repeated and inappropriate application of phosphate fertilizers leads not only to the degradation of soil fertility, but also the loss of biodiversity and bioactivity of soil microorganisms, eventually resulting in the reduction of plant yields (Khan et al. 2007).

Soil and rhizospheric microorganisms, known as phosphate-solubilizing microorganisms, can release soluble phosphate from insoluble phosphate compounds, and promote plant growth (Vassilev et al. 2012). As an important part of phosphate-solubilizing microorganisms, phosphate-

solubilizing bacteria (PSB) have attracted much attention (Rodríguez and Fraga 1999). Numerous PSB have been reported, including important genera of *Aerococcus*, *Alteromonas*, *Arthrobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Chryseobacterium*, *Chryseomonas*, *Delftia*, *Enterobacter*, *Erwinia*, *Gordonia*, *Klebsiella*, *Kluyvera*, *Paenibacillus*, *Pantoea*, *Phyllobacterium*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Xanthobacter* and *Xanthomonas* (Illmer and Schinner 1992; De Freitas et al. 1997; Vazquez et al. 2000; Chung et al. 2005; Chen et al. 2006; Ferandez et al. 2007; Liu et al. 2014). PSB strains have been considered as a novel alternative to phosphate fertilizers, and can promote plant growth while maintaining the long-term balance of the soil ecosystem (Richardson 2001). However, species of PSB strains isolated from different soils are quite different and show functional differences in their phosphate-solubilizing activity (Kucey 1983; Rodríguez and Fraga 1999). Therefore, identification of PSB strains and evaluation of their phosphate-solubilizing activity are important for further study. Moreover, the phosphate-solubilizing activities of PSB strains are affected by many environmental factors, such as temperature, pH, salinity and dissolved oxygen (Vassileva et al. 2010). Studies on how environmental factors affect the activity of phosphate solubilization would provide useful information on application of PSB strains in practice.

At present, most PSB have been isolated from the rhizosphere of field crops (Kim et al. 1997; Kuklinsky-Sobral et al. 2004; Srinivasan et al. 2012), but there are few reports of isolation from tree rhizospheres (Yao et al. 2013). PSB strains can facilitate the growth and development of many crops including maize (Hameeda et al. 2008), mustard (Ahemad and Khan 2012), sorghum (Srinivasan et al. 2012), soybean (Kuklinsky-Sobral et al. 2004) and wheat (Shaharoona et al. 2008). However, research on growth promotion of PSB strains on trees is still lacking.

Poplars (*Populus* spp.) are major trees used in afforestation in China. To date, the planting areas of poplar have exceeded 7 million ha in China (Fang 2008). Frequent harvests of poplar exhaust soil nutrients, leading to a subsequent reduction in soil fertility. Soil degradation has become a major problem in poplar plantations in China, and is greatly affecting the growth of poplar (Liu et al. 2007a), with phosphate deficiency an important factor for fertility reduction in the soils (Sun et al. 1995).

The PSB strain JW-SD2 was isolated and screened from the rhizosphere of *Populus euramericana* cv. Robust in our previous study (Liu et al. 2011). JW-SD2 showed potential to solubilize insoluble mineral phosphate. In this study, JW-SD2 was identified and its phosphate-solubilizing activity, organic acid secretion and acidification of media were investigated. In addition, the effects of environmental factors on phosphate solubilization and plant growth promotion of poplar were evaluated. The results will help in further understanding the mechanisms of phosphate solubilization and provide useful information on application of JW-SD2 as a biofertilizer in the forest industry.

Materials and methods

Characterization and identification of the bacterial strain

The strain JW-SD2 was isolated from the rhizosphere of *P. euramericana* cv. Robust in Yishui, Shandong, China (Liu et al. 2011). JW-SD2 was preserved in the China Center for Type Culture Collection (CCTCC) (accession no. CCTCC M 2015350). The culture was routinely grown and maintained on Luria–Bertani (LB) medium (10 g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl at pH 7.0), either as broth or solid medium (solidified with 1.5 % agar).

The biological characteristics of JW-SD2 were determined using the methods described in Shen and Chen (2007) including morphology of colony, cell, flagella, spore, capsule, Gram staining, KOH test and respiration type test. Silver nitrate and negative staining methods were used for flagella and capsule observation, respectively. A Zeiss AxioImager M2 epifluorescence microscope (Zeiss, Oberkochen, Germany) was used in observation of staining results under a 100× oil objective lens. Respiration type was defined based on the growth condition of JW-SD2 on an LB plate covered by a sterilized coverslip to cover the cross of inoculation streak immediately after inoculation. Carbon-source utilization patterns were determined using the Biolog Identification System (Biolog, Hayward, CA) as described by Li et al. (2013a).

Genomic DNA of JW-SD2 was extracted using the CTAB method (Wang and Qiao 2010). The 16S rRNA gene fragment was amplified by polymerase chain reaction (PCR) using bacterial universal primers 27 f (5′-GAGTTTGATCACTGGCTCAG-3′) and 1492 r (5′-TACGGCTACCTTGTTACGACTT-3′) (Byers et al. 1998). PCR was performed in a 25-μL reaction system containing: 1 μL template (50 ng), 2.5 μL 10× *Taq* DNA polymerase buffer, 2 μL $MgCl_2$ (25 mM), 2 μL dNTP mixture (2.5 mM each), 0.2 μL ExTaq DNA polymerase (5 U/μL) and 0.5 μL (10 μM) of each primer. Conditions of PCR were as follows: 94 °C for 3 min, followed by 30 cycles of 94 °C for 45 s, 54 °C for 45 s, and 72 °C for 1.5 min with a final extension at 72 °C for 4 min. The PCR products were purified using a DNA purification kit (Axygen Biosciences, Union City, CA) and sequenced at Majorbio Company, Limited (Shanghai, China). The sequence was BLAST searched against GenBank database using both NCBI Blastn program (http://www.ncbi.nlm.nih.gov) and Eztaxon Identify System (http://www.ezbiocloud.net/eztaxon/identify). The sequence was submitted to GenBank nucleotide sequence database with accession

Table 1 Basic characteristics of the soil for plant cultivation

Collecting site	Soil type[a]	Soil bulk density (kg/L)	Soil nutrient content (mg/kg)							pH
			Organic matter	Total N	P	K	Ca	Fe	Mg	
Purple Mountain, Nanjing, Jiangsu, China	Yellow brown soil / Cambisols	1.2	53.0	3.2	75.9	163.0	425.3	624.7	120.6	6.4

[a] The soil type is defined based on the China Soil Classification System and the World Reference Base for Soil Resources (Zhang et al. 2014)

no. KJ451557. Multiple sequence alignment was performed using ClustalW. A phylogenetic dendrogram was constructed by the neighbor-joining (NJ) algorithm, and tree topology was evaluated by performing bootstrap analysis with 1000 replicates using Molecular Evolutionary Genetics Analysis (MEGA 5.0) software (Tamura et al. 2011).

Evaluation of phosphate-solubilizing ability

In the phosphate solubilization experiments, the National Botanical Research Institute's Phosphate (NBRIP) growth medium [10 g/L glucose, 5 g/L $Ca_3(PO_4)_2$, 5 g/L $MgCl_2 \cdot 6H_2O$, 0.25 g/L $MgSO_4 \cdot 7H_2O$, 0.2 g/L KCl, and 0.1 g/L $(NH_4)_2SO_4$ at pH 7] (Nautiyal 1999) was used and tricalcium phosphate (TCP) was the sole phosphate source. The strain JW-SD2 was incubated in LB broth (180 rpm, 30 °C) for 18–20 h. Bacterial culture (1 mL of 10^8 CFU/mL) was aseptically harvested by centrifugation (2500 *g*, 20 °C for 3 min) and washed three times with 1 mL sterile saline solution. The bacterial cells were resuspended with 1 mL sterile saline. NBRIP liquid medium (50 mL) in a 100-mL Erlenmeyer flask was inoculated with 1 mL bacterial suspension. The flasks were incubated on a rotary shaker (180 rpm) at 30 °C. Bacterial growth, phosphate-solubilizing activity and pH in the media were assessed every 24 h with three replicates. Culture medium without bacterial inoculation served as a control. Bacterial growth was estimated by optical density measurements at 600 nm. The OD_{600nm} of the culture was measured using a Helios alpha spectrophotometer (Thermo Electron Corporation, Madison, WI). The culture supernatants were collected via centrifugation (10,000 *g*, 4 °C for 15 min) and filtered through 0.22-μm medical Millex-GP filters (Millipore, Bedford, MA). Soluble phosphate of the filtrates was measured using the ascorbate method (Ames 1966). The soluble phosphate released by JW-SD2 was calculated by subtracting the concentration of soluble phosphate of the relevant control from the concentration of soluble phosphate of the treatment. The pH value and titratable acidity were measured by basic pH meter (Sartorius, Germany) and acid–base titration, respectively.

Analysis of organic acids

The presence of acetic, citric, crylic, gluconic, lactic, maleic, malic, malonic, methanoic, oxalic, pyruvic, tartaric and 2-ketogluconic acids in the filtrates were analyzed by High Performance Liquid Chromatography (Agilent 1200 Liquid Chromatograph, Santa Clara, CA). The parameters used in analysis for gluconic acid were: chromatographic column: Agilent Zorbax SB-Phenyl, 4.6 × 250 mm, 5 μm; mobile phase: A: 0.1 % H_3PO_4; B: CH_3CN; flow rate: 1 mL/min; gradient: 0–4 min; B phase: 40 %; 5–7 min; B: 90 %; determination of wavelength: 203 nm; injection volume: 2 μL. The parameters used for other organic acids were: Chromatographic column: Thermo Hypersil Gold, 250 × 4.6 mm, 5 μm; Mobile phase: 50 mM KH_2PO_4, pH 2.5; Flow rate: 1 mL/min; Gradient: 0–8 min; B phase: 0 %; 8–13 min; B: 40 %; Determination of wavelength: 203 nm; Injection volume: 2 μL.

Effects of environmental factors on phosphate-solubilizing activity

The phosphate solubilization by JW-SD2 with respect to temperature, initial pH, salinity and volume of medium were tested with experiments of a single factor using a completely randomized design. The experiments were conducted in 100-mL Erlenmeyer flasks containing 50 mL sterilized NBRIP broth medium, respectively. Five temperatures (20 °C, 25 °C, 30 °C, 35 °C and 40 °C) were controlled using an Innova 40R benchtop incubator shaker (Eppendorf, Germany). Seven initial pH values (4, 5, 6, 7, 8, 9, and 10) were adjusted using 1 mM HCl or NaOH solution. Nine salinities (0 %, 1.0 %, 2.0 %, 3.0 %, 4.0 %, 5.0 %, 6.0 %, 7.0 % and 8.0 %, m/v) were conducted by adding pure NaCl. Five volumes of medium (1/5, 2/5, 1/2, 3/5 and 4/5 of the flask volume) were established in 100-mL Erlenmeyer flasks. The flasks were inoculated with the bacterial suspension (as discussed earlier) at inoculation proportion of 2 % and incubated at 30 °C with rotary shaking (180 rpm). Each treatment was inoculated with bacteria in triplicate. Culture media without bacterial inoculation served as control. After 72 h of incubating, the supernatants were collected by centrifugation and

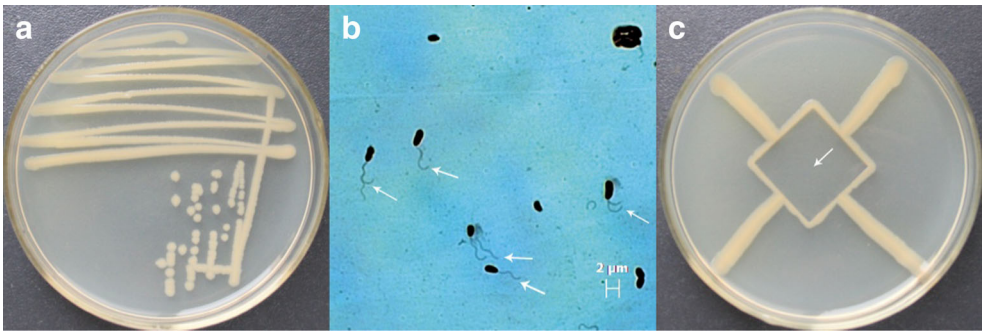

Fig. 1 Biological characteristics of strain JW-SD2. **a** Colony characteristics on Luria-Bertani (LB) plate. **b** Flagella staining results, *white arrows* flagella. **c** Respiration type test, *white arrow* coverslip

the soluble phosphate measured as described in the section 'Evaluation of phosphate-solubilizing ability'.

Inoculation on poplar seedlings

Soil for plant cultivation was collected from Purple Mountain in Nanjing, China (32°07′N, 118°83′E). The physical and chemical characteristics of the soil were analyzed in the Soil Testing Laboratory at Nanjing Forestry University (Table 1). The soil was heat-digested by H_2SO_4–$HClO_4$ (9:1, v/v) extract and the quantities of soil total nitrogen (N), P, potassium (K), Ca, Fe and magnesium (Mg) were determined by inductively coupled argon plasma spectrophotometry (ICP-AES Optima 2100DV, Perkin Elmer). Organic matter was measured using a Total Organic Carbon Analyzer (GE, http://www.geinstruments.com). Soil pH was determined potentiometrically in a 1:2.5 solution (m/v, soil:water). Ethanol disinfected 2.5-L plastic pots filled with 2.2 L sterilized soil (autoclaved at 121 °C for 60 min) or non-sterilized soil were prepared for cultivation. Cuttings of *P. euramericana* cv NL-895 (8–10 mm in diameter, 11–12 cm in length) taken from un-sprouted cauline branch (Yan et al. 2009) were inserted in soil to 5 cm depth for cultivation. The cuttings were incubated at 25 °C in a plant growth chamber under 16-h light and 8-h dark. The soil moisture was maintained within 40–60 % of maximum water holding capacity.

After 40 days incubation, the poplar cuttings were transferred to a greenhouse, and those of similar shoot height and ground diameter and with no sign of disease were selected for inoculation. The shoot height (H0) and ground diameter (D0) of the seedlings were measured. The height of sprouts was defined as the shoot height (H) and the diameter of the base of the sprouts was measured as the ground diameter (D). The seedling roots were inoculated with 10 mL of bacterial suspension (preparation methods identical to the section 'Evaluation of phosphate-solubilizing ability' above) using the root-drenching method (Ge et al. 2004), and the control seedlings were processed with 10 mL sterile saline (CK). The

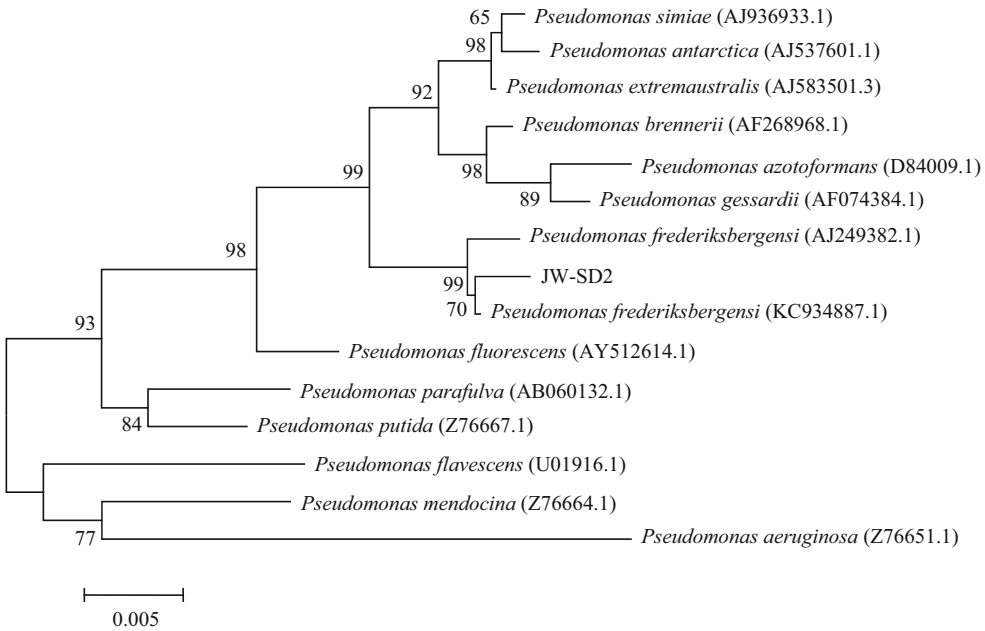

Fig. 2 Phylogenic tree showing the phylogenic relationship between JW-SD2 and other representative bacterial strains based on 16S rRNA sequence using the neighbor-joining (NJ) algorithm. Bootstrap values (50 %) were calculated based on 1000 replications shown at nodes of the tree (values < 50 not included). *Bar* is 0.005 substitutions per nucleotide position

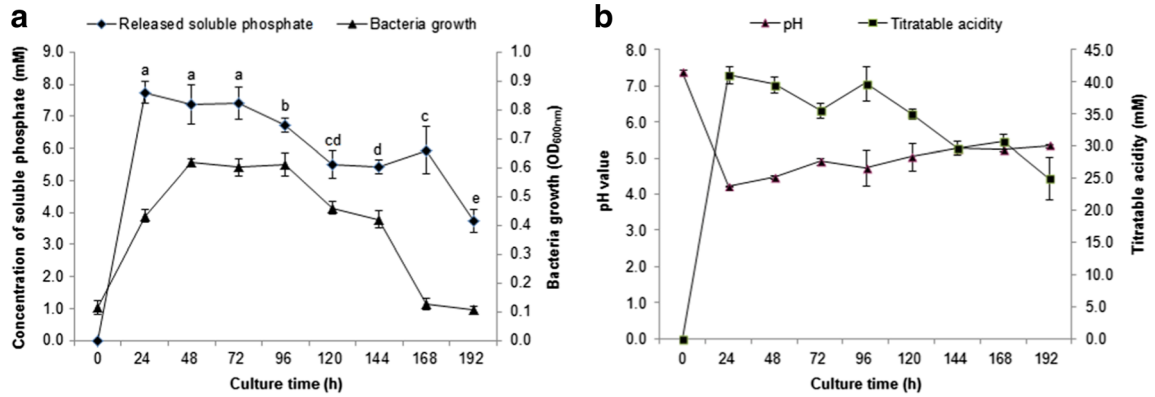

Fig. 3 Phosphate-solubilizing activity, growth of *Pseudomonas frederiksbergensis* JW-SD2 and acidification of medium during the phosphate solubilization. **a** The phosphate-solubilizing activity and growth of JW-SD2. **b** The pH value and titratable acidity in the culture medium. Lowercase letters indicate significant differences at $P < 0.05$. *Error bars* Standard deviation (SD)

seedling shoot heights (H50, H100 and H150), ground diameters (D50, D100 and D150) were measured at 50, 100 and 150 days after inoculation, respectively. The increase rates of shoot heights of each inoculation treatment and control were calculated following the formula: increase rate (%) = (Hn – H0) / H0 × 100 %, n = 50, 100 and 150. The increase rates of the ground diameters of each inoculation treatment and control were calculated following the formula: increase rate (%) = (Dn – D0) / D0 × 100 %, n = 50, 100 and 150. The increase rate of 50, 100 and 150 days after inoculation were defined as 'increase rate I', 'increase rate II' and 'increase rate III', respectively. The plant growth-promoting effect of JW-SD2 was indicated by subtracting the increase rate of the relevant control from the increase rate of the treatment. Five replicates for each inoculation treatment and control were grown at 25 ± 5 °C in a greenhouse with 16-h light and 8-h dark cycles, and watered appropriately.

Data analysis and processing

Microsoft Excel 2007 (Microsoft, Redmond, CA) and SPSS (version 19.0, IBM, New York, NY) were used to collate and analyze the data of phosphate solubilization and poplar growth experiments. Fisher's least significant difference (LSD) method was used to perform significant difference and multiple comparison analyses of phosphate-solubilizing

Table 2 Organic acid secretion by *Pseudomonas frederiksbergensis* JW-SD2 during phosphate solubilization. Values are given as mean ± SD of three replicates

Organic acid	Concentration of organic acids (μg/mL)		
	24 h	72 h	144 h
Gluconic acid	2155.25 ± 50.05	2102.54 ± 15.87	259.73 ± 48.89
2-Ketogluconic acid	171.14 ± 52.17	363.37 ± 93.55	1.67 ± 2.02
Pyruvic acid	329.29 ± 18.29	10.51 ± 7.44	5.15 ± 4.07
Maleic acid	224.81 ± 25.79	158.19 ± 637	1.36 ± 2.36
Malic acid	219.99 ± 82.73	1377.85 ± 235.21	39.65 ± 51.67
Lactic acid	103.75 ± 15.97	8.69 ± 10.32	ND [a]
Malonic acid	79.96 ± 16.47	9.03 ± 15.65	11.43 ± 19.80
Acetic acid	ND	16.70 ± 28.92	4638.55 ± 574.67
Methanoic acid	ND	ND	86.76 ± 15.11
Tartaric acid	ND	ND	18.53 ± 9.14
Oxalic acid	ND	ND	11.47 ± 10.13
Crylic acid	ND	ND	ND
Citric acid	ND	ND	ND
Total organic acids	3284.19	4046.88	5074.31

[a] Not detected

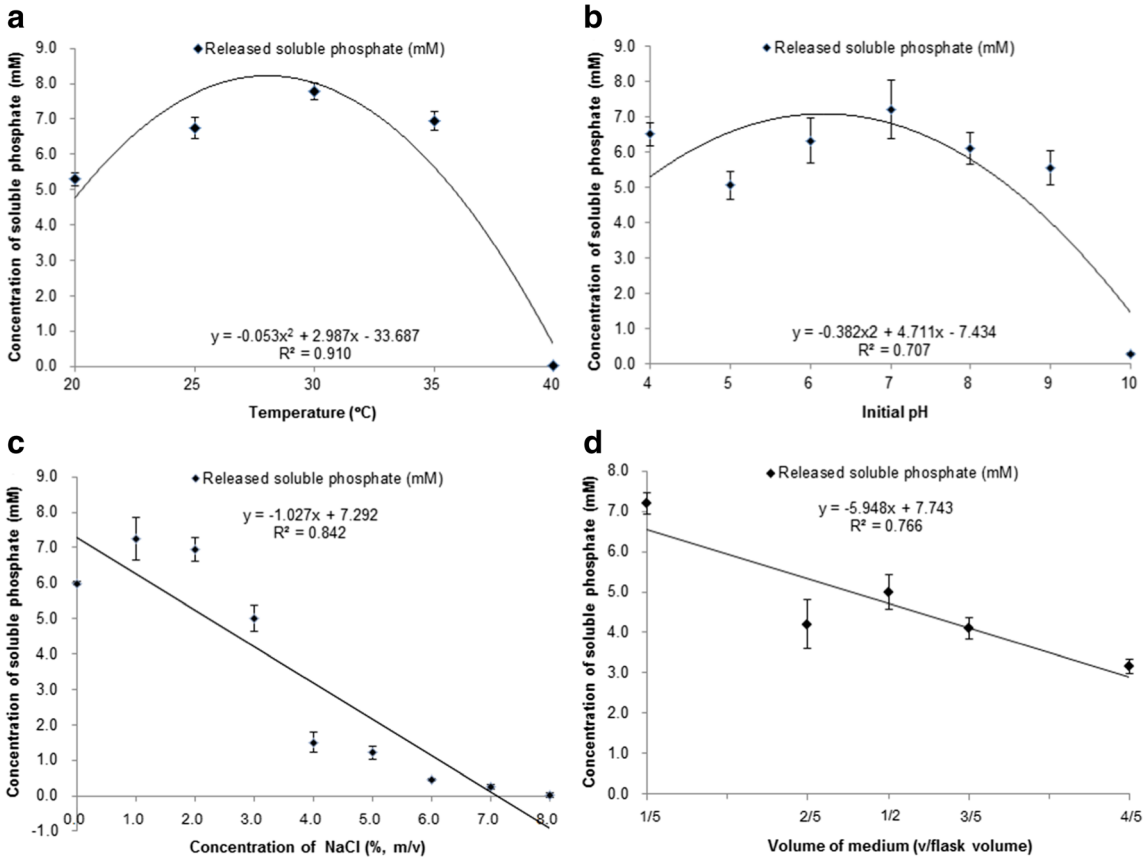

Fig. 4 Effects of different factors on phosphate solubilization by *P. frederiksbergensis* JW-SD2. **a** Temperature, **b** initial pH, **c** concentration of NaCl, and **d** volume of medium. *Error bars* SD

activity as well as the H and D of poplar seedlings. Statistically significant differences were determined as $P < 0.05$. Repeated measures and multivariate ANOVA were used to analyze the data of plant growth over time. Relationships between acidification in culture media and environmental factors with phosphate-solubilizing activity were analyzed with correlation and regression.

Results

Biological characterization

The colonies and biological characteristics of JW-SD2 were photographed and recorded 3 days after inoculation (Fig. 1a). The strain formed yellow, convex and opaque colonies with circular shape and neat edges. The surface of colonies was smooth, wet and glossy. The Gram reaction and the KOH test demonstrated that the strain was a Gram-negative bacterium.

The cells were rod or short-rod shaped with terminal flagella on one end. All cells had single or double long polar flagella showing a motile characteristic (Fig. 1b). The strain did not form spores or capsules. No growth of the strain was observed under the coverslip during the respiration type test (Fig. 1c), indicating that it was aerobic.

Identification by 16S rRNA sequence analysis

A 1460-bp 16S rRNA gene fragment was obtained from JW-SD2. A phylogenetic tree was constructed based on the alignment results compared with available data in GenBank (Fig. 2), and showed that the sequence had a high similarity of 99 % with *P. frederiksbergensis* (KC934887.1). The alignment analysis of the Eztaxon Identify System confirmed the sequence similarity with *P. frederiksbergensis* strain (AJ249382.1). The Biolog Identification System results showed that the strain belonged to the genus *Pseudomonas*. Therefore, PSB strain JW-SD2 was identified as *P. frederiksbergensis*.

Dynamics of phosphate solubilization

During the 192 h of cultivation, the phosphate-solubilizing activity of JW-SD2 showed dynamic changes (Fig. 3a). The phosphate solubilization increased quickly and the concentration of released soluble phosphate in the media reached a maximum of 7.75 mM at 24 h and remained stable until

Fig. 5 Effects of *P. frederiksbergensis* JW-SD2 on the growth of poplar seedlings during 150-day trial. Sterilized soil for **a** 50 days, **b** 100 days and **c** 150 days. Non-sterilized soil for **d** 50 days, **e** 100 days and **f** 150 days. Inoculation treatments (JW-SD2) are on the *left*, and control treatments (CK) on the *right*

120 h. The concentration of released soluble phosphate reduced gradually over time. In controls, the concentrations of soluble phosphate remained at a low level (0.18 ± 0.09 mM) over time. The bacterial growth results showed that JW-SD2 had the highest growth rate from inoculation to 24 h, and the stationary phase lasted from 48 h to 96 h. During the solubilization, the pH of the culture medium was negatively correlated (Pearson's $r = -0.953$) with the phosphate-solubilizing activity, while the titratable acidity was positively correlated (Pearson's $r = 0.969$) with the phosphate-solubilizing activity. The pH of the culture medium decreased during the first 24 h from the initial value of 7.38 to 4.23, and the titratable acidity increased to the maximum quantity of 41.03 mM (Fig. 3b). After this, pH showed an upward trend and titratable acidity displayed a slow downward trend.

Organic acid secretion during phosphate solubilization

Gluconic, 2-ketogluconic, pyruvic, maleic, malic, lactic and malonic acids were detected at 24 h (Table 2). The production of gluconic acid showed the highest level of 2155.25 μg/mL (65.6 % of total organic acids). When the culture time reached 72 h, 52.0 % of organic acid secretion corresponded to gluconic acid (2102.54 μg/mL) and 34.0 % to malic acid for a secondary high concentration of 1377.85 μg/mL. The remaining 14.0 % corresponded to 2-ketogluconic, pyruvic, maleic, lactic, malonic and acetic acids. When the culture time reached 144 h, the quantity of acetic acid quickly increased to 4638.55 μg/mL, accounting for 91.4 % of total organic acids, while only 5.1 % corresponded to gluconic acid.

The productions of gluconic, 2-ketogluconic, pyruvic, maleic and malic acids presented high concentrations at 24 h and 72 h of growth when JW-SD2 showed a high phosphate-solubilizing activity, and the secretion of these organic acids was restricted by 144 h. Nevertheless, the quantity of total organic acids increased from 3284.19 μg/mL to 5074.31 μg/mL over time.

Effects of environmental factors on phosphate-solubilizing activity

Temperature had significant effects on the phosphate-solubilizing activity of *P. frederiksbergensis* JW-SD2. Regression analysis showed a quadratic relationship between temperature and the concentration of the released soluble phosphate ($R^2 = 0.910$, $P < 0.01$) (Fig. 4a). The phosphate-solubilizing activity increased to a maximum at 28.2 °C and then decreased with further temperature rise. The concentration of released soluble phosphate remained above 5.93 mM between 20 and 35 °C. Along with the increase of pH, the concentration of released soluble phosphate reached a maximum at pH 6.1 and declined thereafter, and fit a quadratic model ($R^2 = 0.707$, $P < 0.01$) (Fig. 4b). The concentration of released soluble phosphate was more than 4.02 mM for pH 4–9. The relationship between the NaCl concentration and the concentration of released soluble phosphate fit a linear model ($R^2 = 0.842$, $P < 0.01$) (Fig. 4c). For the NaCl concentration of 1.0 – 3.0 % (w/v), the concentration of released soluble phosphate exceeded 4.21 mM. The volume of medium and the concentration of soluble phosphate were linearly related ($R^2 = 0.766$, $P < 0.01$) (Fig. 4d). As the volume of medium increased from one-fifth to one-third of the flask volume, the concentration of released soluble phosphate remained above 4.17 mM. In controls, the concentrations of soluble phosphate remained at low levels for different temperatures (0.10 ± 0.02 mM), pHs (0.33 ± 0.11 mM), NaCl concentrations (0.18 ± 0.03 mM), and volumes of medium (0.10 ± 0.07 mM).

Effects of JW-SD2 on growth of poplar

Repeated measures ANOVA revealed significant within-subjects effects ($P < 0.05$) of time and significant interactions ($P < 0.05$) between treatment and time in both shoot height and ground diameter. The subsequent simple-effect analysis suggested that, during the 150-day incubation, the seedlings inoculated with JW-SD2 showed significantly better growth ($P < 0.05$) of H and D than CK in both sterilized and non-sterilized soil (Fig. 5, Table 3). The seedlings also showed

Table 3 Effects of *P. frederiksbergensis* JW-SD2 on growth of poplar seedlings during the 150-day trial. Values are given as mean ± standard deviation of five replicates and lowercase letters within every single column of shoot heights or ground diameters indicate significant differences at $P < 0.05$

Soil	Treatments	Shoot height (cm)[d]						
		H0	H50	Increase rate I (%)[f]	H100	Increase rate II (%)[f]	H150	Increase rate III (%)[f]
Sterilized	*P. frederiksbergensis* JW-SD2	7.35 ± 0.90 a	32.57 ± 4.74 a	347.15 ± 70.76 a	108.88 ± 4.98 a	1393.45 ± 68.73 a	170.05 ± 6.26 a	2237.82 ± 192.80 a
	Control (sterile saline)	7.41 ± 0.61 a	24.46 ± 3.02 b	232.82 ± 54.44 b	85.62 ± 6.73 b	1060.82 ± 114.25 b	151.45 ± 6.22 b	1952.02 ± 122.90 b
Non-sterilized	*P. frederiksbergensis* JW-SD2	7.34 ± 0.74 a	31.46 ± 3.42 a	329.59 ± 40.11 a	91.44 ± 5.33 b	1152.24 ± 111.61 b	150.54 ± 8.90 b	1968.37 ± 254.85 b
	Control (sterile saline)	7.37 ± 0.48 a	22.06 ± 5.72 b	199.81 ± 71.97 b	66.19 ± 6.14 c	798.12 ± 67.30 c	126.48 ± 4.63 c	1622.74 ± 151.75 c

Soil	Ground diameter (mm)[e]						
	D0	D50	Increase rate I (%)[g]	D100	Increase rate II (%)[g]	D150	Increase rate III (%)[g]
Sterilized	3.04 ± 0.48 a	5.96 ± 0.91 a	101.08 ± 5.03 a	9.32 ± 0.89 a	217.60 ± 31.66 a	12.38 ± 0.82 a	322.52 ± 37.49 a
	2.83 ± 0.33 a	5.15 ± 0.22 b	83.16 ± 15.78 bc	7.78 ± 0.58 b	176.20 ± 11.47 bc	10.78 ± 0.62 b	282.73 ± 24.50 bc
Non-sterilized	2.73 ± 0.41 a	5.19 ± 0.46 b	91.07 ± 7.84 ab	8.13 ± 0.27 b	200.72 ± 27.11 ab	10.82 ± 0.63 b	299.44 ± 26.57 ab
	2.65 ± 0.31 a	4.52 ± 0.40 b	70.92 ± 9.49 c	6.76 ± 0.55 c	156.09 ± 21.38 c	9.47 ± 0.38 c	258.98 ± 25.13 c

[d] H0, H50, H100 and H150 are seedling shoot heights at 0, 50, 100 and 150 d after inoculation, respectively

[e] D0, D50, D100 and D150 are seedling ground diameters at 0, 50, 100 and 150 d after inoculation, respectively

[f] The increase rates of shoot heights of each inoculation treatment and control were calculated separately following the formula: increase rate (%) = (Hn – H0) / H0 × 100 %, n = 50, 100 and 150

[g] The increase rates of the ground diameters of each inoculation treatment and control were calculated separately following the formula: increase rate (%) = (Dn – D0) / D0 × 100 %, n = 50, 100 and 150

significantly better growth in sterilized than in non-sterilized soil ($P < 0.05$). Seedlings inoculated with JW-SD2 had significantly higher increase rates than CK over time ($P < 0.05$). The H and D of seedlings increased 2237.82 and 322.52 % at 150 day after inoculation in sterilized soil, respectively, and correspondingly 1968.37 and 299.44 % in non-sterilized soil. However, the growth-promoting effects of JW-SD2 were higher for seedlings in non-sterilized than in sterilized soil. In sterilized soil, increase rates of the inoculation treatments at 50 days, 100 days and 150 days were 114.33, 332.63 and 285.80 % higher, respectively, than CK for H and correspondingly 17.92 %, 41.40 % and 39.79 % higher for D. In non-sterilized soil, corresponding values were 129.78 %, 354.12 % and 345.67 % for H, and 20.15 %, 44.63 % and 40.46 % for D. During the 150-day trial, the growth-promoting effects initially increased after inoculation and then decreased in both soils.

Discussion

Phosphate solubilization is an important phenotype of plant growth-promoting rhizobacteria (PGPR) (Rodriguez et al. 2006). Much research has focused on the potential of PSB to enhance soil fertility. Species of the genus *Pseudomonas* have been shown to possess the ability to solubilize insoluble phosphate (Illmer et al. 1995; Vazquez et al. 2000; Pandey et al. 2006; Valverde et al. 2006; Ferandez et al. 2007; Rane et al. 2008). In the current study, the PSB strain JW-SD2 was identified as *P. frederiksbergensis* and its phosphate-solubilizing ability and mechanism were investigated.

PSB strains are always screened using NBRIP plates with halo/clear zone around the colonies. However, this method cannot accurately detect phosphate-solubilizing activity, and the NBRIP broth assay is more efficient in determining this activity (Nautiyal 1999). The strain JW-SD2 had a high level of activity in TCP solubilization (solubilized phosphate 7.75 mM) in NBRIP broth (Fig. 3) compared with other PSB strains (Gulati et al. 2008; Song et al. 2008; Collavino et al. 2010). In our study, *P. frederiksbergensis* JW-SD2 was isolated and screened from the north of China, where TCP is the main insoluble mineral phosphate (Shen and Jiang 1992), and hence the high activity of solubilizing TCP might have evolved to make use of P. China has the largest area of poplar plantations in the world (Fang 2008) and so JW-SD2 has high potential to increase the levels of available phosphate, and promote poplar growth in China. It also has been reported that the phosphate solubilization of mineral phosphate by PSB strain varies with the structural complexity of mineral phosphate (Bardiya and Gaur 1974). The activities of TCP and hydroxyapatite solubilization by PSB strains are different (Yadav et al. 2013), and are always higher than that of rock phosphate solubilization (Bardiya and Gaur 1974; Gulati et al. 2008). Hydroxyapatite and rock phosphate are also important components of calcium phospahtes; hence, solubilization of hydroxyapatite and rock phosphate by the JW-SD2 should be considered in further research.

During phosphate solubilization, 11 kinds of organic acids with more than 5000 μg/mL in total quantity were secreted by JW-SD2 (Table 2). Different PSB strains have different quantities and qualities in their production of organic acids during mineral phosphate solubilization (Vyas and Gulati 2009). Acetic, citric, gluconic, 2-ketogluconic, lactic, malonic, oxalic and pyruvic acids were detected in *Arthrobacter* sp. and *Bacillus firmus* ACB5 (Banik and Dey 1982), and *B. megaterium* CC-BC10 and *Serratia marcescens* CC-BC141 (Chen et al. 2006). The production of organic acids by PSB strains is their main mechanism of solubilizing mineral phosphate (Chen et al. 2006). In the present study, gluconic, 2-ketogluconic, pyruvic, maleic and malic acids were detected at high concentrations before 96 h of growth, and JW-SD2 displayed a high level of phosphate-solubilizing activity. With prolonged culture time, production of gluconic, pyruvic and maleic acids was reduced, showing the same trend as the phosphate-solubilizing activity. Therefore, production of these organic acids might be responsible for the phosphate solubilization. Among these five organic acids, gluconic acid was predominant, which is consistent with previous studies (Rodriguez et al. 2004; Patel et al. 2008; Vyas and Gulati 2009). Gluconic acid is generated from the direct oxidation pathway that occurs on the external surface of cytoplasmic membranes (Oubrie et al. 1999). Its role in phosphate solubilization was demonstrated in *Enterobacter intermedium* (Kim et al. 2003) and *P. fluorescens* (Vyas and Gulati 2009) by means of molecular biology. Thus, gluconic acid is likely to be the most effective organic acid in phosphate solubilization by *P. frederiksbergensis* JW-SD2. In contrast to some previous studies that showed no correlation between change of pH and phosphate solubilization (Illmer and Schinner 1995), we found that the drop of pH and increase of titratable acidity were closely correlated (Pearson's $r = -0.953$ and 0.969, respectively) with phosphate solubilization, consistent with Chen et al. (2006). Our results indicated that the production and secretion of organic acids leading to acidification of surroundings might be the mechanism of mineral phosphate solubilization by JW-SD2.

It is notable that the increase of total organic acids was not proportional to the phosphate-solubilizing activity (Table 2). Especially, methanoic, tartaric and oxalic acids were detected only in the later period of culture (144 h), and acetic acid accounted for 81.4 % of organic acid secretion. However, the activity of solubilizing phosphate in the medium declined to a low level by 144 h of culture. The phosphate-solubilizing ability has been shown to be linked with the nature of organic acids (Bolan et al. 1994). Therefore, our results suggest that the quantity of total organic acids was not correlated with the phosphate-solubilizing activity, and that some organic acids,

especially acetic acid, played no role in phosphate solubilization.

Both nutritional and environmental factors can affect the phosphate solubilization by PSB strains (Illmer and Schinner 1992). In the present study, the phosphate-solubilizing activity of *P. frederiksbergensis* JW-SD2 was significantly ($P < 0.01$) affected by temperature, initial pH, salinity and volume of medium (Fig. 4). At a constant shaking rate, dissolved oxygen (DO) in the medium is negatively related to volume of medium (Sen et al. 2008). In shaking flasks, the DO values in medium with lower volumes are significantly higher than for those with higher volume (Tsai et al. 1997). The temperature, pH, salinity and DO are the most important indexes of soil (Wu et al. 2001). Most land in China is located in temperate and subtropical areas, where soil temperature is 20–35 °C for nearly 6 months of the year (Zhang et al. 2009). The pH of soil ranges from weakly acidic in the south of China to weakly basic in the north (Yu et al. 2003). There are also large areas of saline-alkali soil in China (Liu et al. 2007b). These complicated soil environments require that plant growth-promoting microbes be broadly tolerant of a wide range of environmental variables for widespread application as biofertilizer. The strain JW-SD2 showed relatively high phosphate-solubilizing activity in different environmental conditions, suggesting it has high potential to cope with a changing soil environment. This provides useful information for future application of this strain in practice.

Evaluating the phosphate-solubilizing activity and how it was affected by environmental factors showed the plant growth-promoting potential of *P. frederiksbergensis* JW-SD2. The strain significantly ($P < 0.05$) promoted the growth of poplar seedlings (Fig. 5, Table 3). PSB strains can promote plant growth by increasing available phosphate in soil and phosphate uptake by plants (Gupta et al. 2012). The effective solubilization of mineral phosphate by JW-SD2 might contribute to the promotion of plant growth. The effects of plant growth promotion on poplar seedlings by JW-SD2 first increased and then decreased during the trial. Plant growth promotion by PGPR is based on the survival and rhizosphere colonization of the bacteria (Compant et al. 2010). Colonization of PGPR strains in the rhizosphere changes along with time after inoculation (Benizri et al. 2001). The population of PSB strain *Burkholderia multivorans* showed a down–up–down fluctuation after inoculation in the plant rhizosphere (Li et al. 2013b). Changes in the effects of JW-SD2 on plant growth promotion might be caused by changes in rhizosphere colonization. Therefore, supplements of the strain into the rhizosphere of plants at regular times should be adopted in future applications. PSB strains can also colonize tissues of plants (Montanez et al. 2012) and further study is necessary to investigate colonization of *P. frederiksbergensis* JW-SD2 in the rhizosphere.

The *P. frederiksbergensis* strain JW-SD2 showed better effects on the growth of poplar seedlings in non-sterilized soil. PSB strains can alter the composition of root secretions and phenotype, and this could have feedback that influences the colonization by rhizospheric microorganisms and plant development (Yu et al. 2011). PSB strains also have the ability to regulate interactions between microorganisms and plants to reduce the damage to plants from soil-borne plant pathogens (Kloepper et al. 1989). It would be of interest to test whether JW-SD2 also possesses any regulating ability to protect plants.

Conclusion

PSB strain *P. frederiksbergensis* JW-SD2 was identified through phenotypic features, the Biolog Identification System and 16S rRNA sequence analysis. The strain exhibited a high mineral phosphate solubilization activity. During the solubilization, large amounts of organic acids were produced and secreted, accompanied by a drop in pH and acidification of the surroundings. Gluconic, 2-ketogluconic, pyruvic, maleic and malic acids were the most effective components in phosphate solubilization. Although acetic acid had high levels of production, it did not play a role in phosphate solubilization. Environmental factors had significant effects on phosphate solubilization and JW-SD2 showed high potential in adapting to a changing environment. The high activity of phosphate solubilization would give a high plant growth-promoting ability to this strain, and the inoculation on poplar seedlings demonstrated its plant growth promotion potential. The effects of plant growth promotion by JW-SD2 first increased then decreased over time after inoculation, suggesting that, for better plant growth-promoting effects, the strain should be supplemented to the rhizosphere of plants at regular time intervals. These results will help a better understanding of the mechanisms of phosphate solubilization by PSB, and provide useful information for future application of this strain as a biofertilizer. Further research is needed to investigate the colonization and regulating ability of JW-SD2 in the rhizosphere. It is also necessary to measure the plant growth-promoting effects of JW-SD2 in field tests.

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References

Ahemad M, Khan MS (2012) Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica compestris*) rhizosphere. Chemosphere 86: 945–950. doi:10.1016/j.chemosphere.2011.11.013

Ames BN (1966) Assay of inorganic phosphate, total phosphate and phosphatase. Methods Enzymol 8:115–118

Banik S, Dey B (1982) Available phosphate content of an alluvial soil as influenced by inoculation of some isolated phosphate-solubilizing micro-organisms. Plant Soil 69:353–364. doi:10.1007/BF02372456

Bardiya S, Gaur AC (1974) Isolation and screening of microorganisms dissolving low-grade rock phosphate. Folia Microbiol 19:386–389. doi:10.1007/BF02872824

Batjes N (1997) A world dataset of derived soil properties by FAO–UNESCO soil unit for global modelling. Soil Use Manag 13:9–16

Benizri E, Baudoin E, Guckert A (2001) Root colonization by inoculated plant growth-promoting rhizobacteria. Biocontrol Sci Tech 11:557–574. doi:10.1080/09583150120076120

Bolan DNS, Naidu R, Mahimairaja S, Baskaran S (1994) Influence of low-molecular-weight organic acids on the solubilization of phosphates. Biol Fertil Soils 18:311–319. doi:10.1007/BF00570634

Byers HK, Stackebrandt E, Hayward C, Blackall LL (1998) Molecular investigation of a microbial mat associated with the great artesian basin. FEMS Microbiol Ecol 25:391–403. doi:10.1111/j.1574-6941.1998.tb00491.x

Chen Y, Rekha P, Arun A, Shen F, Lai W-A, Young C (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41. doi:10.1016/j.apsoil.2005.12.002

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37:1970–1974. doi:10.1016/j.soilbio.2005.02.025

Collavino MM, Sansberro PA, Mroginski LA, Aguilar OM (2010) Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote *Phaseolus vulgaris* growth. Biol Fertil Soils 46:727–738. doi:10.1007/s00374-010-0480-x

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678. doi:10.1016/j.soilbio.2009.11.024

De Freitas J, Banerjee M, Germida J (1997) Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biol Fertil Soils 24:358–364. doi:10.1007/s003740050258

Fang S-Z (2008) Silviculture of poplar plantation in China: a review. Chin J Appl Ecol 19:2308–2316

Ferandez LA, Zalba P, Gomez MA, Sagardoy MA (2007) Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43: 805–809. doi:10.1007/s00374-007-0172-3

Ge H-H, Guo J-H, Qi H-Y, Guo Y-H, Huang Y-X (2004) Biological control of Capsicum bacterial wilt by compound bacterial mixture AR99. Acta Pedol Sin 34:162–165. doi:10.3321/j.issn:0412-0914.2004.02.011

Goldstein AH (1986) Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agric 1:51–57. doi:10.1017/S0889189300000886

Gulati A, Rahi P, Vyas P (2008) Characterization of phosphate-solubilizing fluorescent pseudomonads from the rhizosphere of seabuckthorn growing in the cold deserts of Himalayas. Curr Microbiol 56:73–79. doi:10.1007/s00284-007-9042-3

Gupta M, Kiran S, Gulati A, Singh B, Tewari R (2012) Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of *Aloe barbadensis* Miller. Microbiol Res 167:358–363. doi:10.1016/j.micres.2012.02.004

Hameeda B, Harini G, Rupela O, Wani S, Reddy G (2008) Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Res 163:234–242. doi:10.1016/j.micres.2006.05.009

Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soils. Soil Biol Biochem 24: 389–395. doi:10.1016/0038-0717(92)90199-8

Illmer P, Schinner F (1995) Solubilization of inorganic calcium phosphates-solubilization mechanisms. Soil Biol Biochem 27:257–263. doi:10.1016/0038-0717(94)00190-C

Illmer P, Barbato A, Schinner F (1995) Solubilization of hardly-soluble $AlPO_4$ with P-solubilizing microorganisms. Soil Biol Biochem 27: 265–270. doi:10.1016/0038-0717(94)00205-F

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture-a review. Agron Sustain Dev 27:29–43. doi:10.1051/agro:2006011

Kim KY, Jordan D, Krishnan HB (1997) *Rahnella aquatilis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol Lett 153:273–277. doi:10.1111/j.1574-6968.1997.tb12585.x

Kim CH, Han SH, Kim KY, Cho BH, Kim YH, Koo BS, Kim YC (2003) Cloning and expression of pyrroloquinoline quinone (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. Curr Microbiol 47:457–461. doi:10.1007/s00284-003-4068-7

Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–44. doi:10.1016/0167-7799(89)90057-7

Kucey R (1983) Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678. doi:10.4141/cjss83-068

Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251. doi:10.1111/j.1462-2920.2004.00658.x

Li G-E, Wu X-Q, Ye J-R, Hou L, Zhou A-D, Zhao L (2013a) Isolation and identification of phytate-degrading rhizobacteria with activity of improving growth of poplar and Masson pine. World J Microbiol Biotechnol 29:2181–2193. doi:10.1007/s11274-013-1384-3

Li G-X, Wu X-Q, Ye J-R (2013b) Biosafety and colonization of *Burkholderia multivorans* WS-FJ9 and its growth-promoting effects on poplars. Appl Microbiol Biotechnol 97:10489–10498. doi:10.1007/s00253-013-5276-0

Liu F, Jiang Y, Wang H, Wang Y, Kong L (2007a) Soil productivity maintenance technique of poplar plantation under continuous croping. Sci Silvae Sin 43:58–64. doi:10.3321/j.issn:1001-7488.2007.z1.012

Liu Y, He W, He J, Shen Z (2007b) Progress of improvement and utilization of saline-alkali land. J Agric Sci 28:68–71. doi:10.3969/j.issn.1673-0747.2007.02.019

Liu H, Wu XQ, Ren JH, Ye JR (2011) Isolation and identification of phosphobacteria in poplar rhizosphere from different regions of China. Pedosphere 21:90–97. doi:10.1016/S1002-0160(10)60083-5

Liu F-P, Liu H-Q, Zhou H-L, Dong Z-G, Bai X-H, Bai P, Qiao J-J (2014) Isolation and characterization of phosphate-solubilizing bacteria from betel nut (*Areca catechu*) and their effects on plant growth and phosphorus mobilization in tropical soils. Biol Fertil Soils 50: 927–937. doi:10.1007/s00374-014-0913-z

Montanez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects in vitro. Appl Soil Ecol 58:21–28. doi:10.1016/j.apsoil.2012.02.009

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170:265–270. doi:10.1111/j.1574-6968.1999.tb13383.x

Oubrie A, Rozeboom HJ, Kalk KH, Olsthoorn AJ, Duine JA, Dijkstra BW (1999) Structure and mechanism of soluble quinoprotein glucose dehydrogenase. EMBO J 18:5187–5194. doi:10.1093/emboj/18.19.5187

Pandey A, Trivedi P, Kumar B, Palni LMS (2006) Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* (B0) isolated from a subalpine location in the Indian Central Himalaya. Curr Microbiol 53:102–107. doi:10.1007/s00284-006-4590-5

Patel DK, Archana G, Kumar GN (2008) Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. DHRSS in the presence of different sugars. Curr Microbiol 56:168–174. doi:10.1007/s00284-007-9053-0

Raghothama KG, Karthikeyan AS (2005) Phosphate acquisition. Plant Soil 274:37–49. doi:10.1007/s11104-004-2005-6

Rane MR, Sarode PD, Chaudhari BL, Chincholkar SB (2008) Exploring antagonistic metabolites of established biocontrol agent of marine origin. Appl Biochem Biotechnol 151:665–675. doi:10.1007/s12010-008-8288-y

Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct Plant Biol 28:897–906. doi:10.1071/PP01093

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339. doi:10.1016/S0734-9750(99)00014-2

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552–555. doi:10.1007/s00114-004-0566-0

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21. doi:10.1007/s11104-006-9056-9

Sen L, Tao Y, Wei Wei C (2008) Study on the xylitol production via fermentation by *Fusarium xysporum*. Modern Food Sci Technol 24:247–248. doi:10.3969/j.issn.1673-9078.2008.04.014

Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (*Triticum aestivum* L.). Appl Microbiol Biotechnol 79:147–155. doi:10.1007/s00253-008-1419-0

Shen P, Chen XD (2007) Microbiology experiment. Higher Education Press, Beijing, pp 69–84

Shen R, Jiang B (1992) Distribution and availablity of various forms of inorganic-P in calcareous soils. Acta Pedol Sin 29:80–86

Song O-R, Lee S-J, Lee Y-S, Lee S-C, Kim K-K, Choi Y-L (2008) Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. Braz J Microbiol 39:151–156. doi:10.1590/S1517-83822008000100030

Srinivasan R, Alagawadi AR, Yandigeri MS, Meena KK, Saxena AK (2012) Characterization of phosphate-solubilizing microorganisms from salt-affected soils of India and their effect on growth of sorghum plants [*Sorghum bicolor* (L.) Moench]. Ann Microbiol 62:93–105. doi:10.1007/s13213-011-0233-6

Sun CL, Zhu ZX, Wang Z, Tong CR (1995) Study on the soil degradation of the poplar plantation and the technique to preserve and increase soil fertility. Sci Silvae Sin 31:506–512

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. doi:10.1093/molbev/msr121

Tsai G-J, Tsai F-C, Kong Z-L (1997) Effects of temperature, medium composition, pH, salt and dissolved oxygen on haemolysin and cytotoxin production by *Aeromonas hydrophila* isolated from oyster. Int J Food Microbiol 38:111–116. doi:10.1016/S0168-1605(97)00094-9

Valverde A et al (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil 287:43–50. doi:10.1007/s11104-006-9057-8

Vassilev N, Eichler-Lobermann B, Vassileva M (2012) Stress-tolerant P-solubilizing microorganisms. Appl Microbiol Biotechnol 95:851–859. doi:10.1007/s00253-012-4224-8

Vassileva M, Serrano M, Bravo V, Jurado E, Nikolaeva I, Martos V, Vassilev N (2010) Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Appl Microbiol Biotechnol 85:1287–1299. doi:10.1007/s00253-009-2366-0

Vazquez P, Holguin G, Puente M, Lopez-Cortes A, Bashan Y (2000) Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol Fertil Soils 30:460–468. doi:10.1007/s003740050024

Vyas P, Gulati A (2009) Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing *fluorescent Pseudomonas*. BMC Microbiol 9:174. doi:10.1186/1471-2180-9-174

Wang YQ, Qiao XL (2010) Genomic DNA extraction and its identification of promoting bacteria from cotton roots. Genome Appl Biol 29:1005–1008. doi:10.3969/gab.029.001005

Wu Y, Liu Q, Qiao YK, Pan KW, Zhao CM, Chen QH (2001) Species diversity changes in subalpine coniferous forests of diferent restoration stages and their effects on soil properties. Chin J Plant Ecol 25:648–655

Yadav H, Gothwal RK, Nigam VK, Sinha-Roy S, Ghosh P (2013) Optimization of culture conditions for phosphate solubilization by a thermo-tolerant phosphate-solubilizing bacterium *Brevibacillus* sp. BISR-HY65 isolated from phosphate mines. Biocatalysis Agric Biotechnol 2:217–225. doi:10.1016/j.jssas.2012.07.002

Yan Y, Wang W, Zhu H, Shi X, Liu X, Zu Y (2009) Effects of salt-alkali stress on osmoregulation substance and active oxygen metabolism of Qingshan poplar (*Populus pseudo-cathayana* × *P. deltoides*). J Appl Ecol 20:2085–2091

Yao S et al (2013) Diversity of the phosphate solubilizing bacteria isolated from the root of tree peony (*Paeonia ostii*). In: Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture. Proceedings of 3rd Asian Conference on Plant Growth-Promoting Rhizobacteria (PGPR) and other Microbials, Manila, Philippines, 21–24 April 2013, Manila. Asian PGPR Society for Sustainable Agriculture, pp 469–474

Yu H, Huang J-K, Rozelle S, Brandt L (2003) Soil fertility changes of cultivated land in Eastern China. Geogr Res 22:380–388. doi:10.3321/j.issn:1000-0585.2003.03.015

Yu X, Liu X, Zhu TH, Liu GH, Mao C (2011) Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. Biol Fertil Soils 47:437–446. doi:10.1007/s00374-011-0548-2

Zhang H, Shi X, Yu D, Wang H, Zhao Y, Sun W, Huang B (2009) Seasonal and regional variations of soil temperature in China. ACTA Pedol Sin 46:227–234. doi:10.3321/j.issn:0564-3929.2009.02.006

Zhang W-L, Xu A-G, Zhang R-L, Ji H-L (2014) Review of soil classification and revision of China Soil Classification System. Sci Agric Sin 47:3214–3230. doi:10.3864/j.issn.0578-1752.2014.16.009