Characterisation of Phosphate Solubilising Bacteria in Sandy Loam Soil Under Chickpea Cropping System

Machiavelli Singh · N. Tejo Prakash

Received: 17 April 2010 / Accepted: 10 July 2010 / Published online: 17 August 2011
© Association of Microbiologists of India 2011

Abstract With the aim to explore the possible role of phosphate-solubilizing bacteria (PSB) in phosphorus (P) cycling in agricultural soils, we isolated PSB inhabiting naturally in the sandy loam soils under chickpea cropping of Patiala (Punjab State). A total of 31 bacterial isolates showing solubilizing activities were isolated on Pikovskaya agar plates. The potent phosphate solubilizers were selected for further characterization. These isolates were shown to belong to the genera *Pseudomonas* and *Serratia* by partial sequencing analysis of their respective 16S rDNA genes. ERIC-PCR based fingerprinting was done for tracking the survival of introduced populations of the PSB during mass inoculation of these strains under chickpea plots. The results showed positive correlation ($r^2 = 0.853$) among soil phosphatase activity and phosphate solubilizers population, which was also positively correlated ($r^2 = 0.730$) to available phosphorus. Identification and characterization of soil PSB for the effective plant growth-promotion broadens the spectrum of phosphate solubilizers available for field application.

Keywords Agricultural soils · Chickpea · Phosphatase · Phosphorus · *Pseudomonas* · *Serratia*

Introduction

In natural environments, i.e., the rhizosphere of different plant species, phosphate-solubilizing bacteria (PSB) are considered to play an important ecophysiological role: indeed, PSB mobilize insoluble inorganic phosphates from their mineral matrix to the bulk soil where they can be absorbed by plant roots. In turn, the plants supply rootborne C compounds, mainly sugars, which can be metabolized for bacterial growth [1, 2]. Furthermore, the organisms possessing a phosphate-solubilizing ability can also convert the insoluble phosphatic compounds into soluble forms [3, 4] in soil and make them available to the crops. The crop microbial ecosystem can thus be energized in sustainable agriculture with considerable ecological stability and environmental quality. The PSB increase the availability of soluble phosphate and can enhance plant growth by increasing the efficiency of biological nitrogen fixation or enhancing the availability of other trace elements such as iron, zinc, etc., and by production of plant growth-promoting regulators [5, 6]. The discovery of this mutual relationship between plants and PSB encouraged the development of new technologies, such as the use of PSM for biofertilization to improve crop yield [7–9]. This work reports the efficacy of Phosphate Solubilising Bacteria (PSB) in improving the organic fertility and nutritional status of sandy loam soil under chickpea cropping during plot studies.

Materials and Methods

Isolation and Screening of PSB

Soil samples were collected from chickpea (*Cicer arietinum* L.) agricultural fields of Patiala District (Punjab,

M. Singh (✉) · N. Tejo Prakash
Department of Biotechnology and Environmental Sciences,
Thapar University, Patiala 147004, India
e-mail: machiavellisingsh@gmail.com

Present Address:

M. Singh
Department of Applied Sciences and Humanities, Jind Institute
of Engineering and Technology (JIET), Panipat Road, Jind
126102, India

India). All samples were collected randomly at from 0 to 30 cm of depth from nine different sites and three composites samples were prepared, air-dried and pulverized for soil physico-chemical parameters by standard methods [10]. The soil biochemical parameter was monitored by estimating soil phosphatase [11] taken at the initial time (0 week) and after the harvest of crop (16th week). Soils were processed the same day for microbial and enzymatic activities and stored at 4°C for soil analysis. For isolation of PSB, serial soil dilutions were spread plated on modified Pikovskaya agar [12] and the colonies were counted (cfu g⁻¹ dry wt of soil). Phosphate-solubilization by each PSB isolate was screened in Pikovskaya liquid media. The flasks were incubated at 37 ± 2°C for 72 h and the contents were centrifuged at 10,000 rpm for 10 min. The supernatant was analyzed for soluble P-content using the method as described by Jackson [10]. The pH of the supernatant was also recorded. The culture broth having approximately 10⁸ cfu/ml was spotted on Pikovskaya plates for determination of the P solubilization efficiency. The plates were incubated at 37 ± 2°C for 48 h and the diameter of the colony as well as the halo zone was measured. P-solubilizing index (PSI) was calculated as:

$$\text{PSI (\%)} = (Z - C)/C \times 100$$

where Z = Halo zone diameter, C = colony diameter.

Molecular Studies

The isolation of chromosomal DNA, PCR amplification, and digestion of 16S rDNA gene fragments were performed as described. Bacterial isolates were identified by amplification of 16S rDNA gene sequence with the following primers: Forward 5'-AGA GTTTGATCCTGGCT-CAG-3' and reverse 5'-ACGGGCGGTGTGTTC-3' [13]. DNA amplification was performed with Genamp PCR system (Applied Biosystem, USA). Reaction mixture for the PCR contained 1 × PCR buffer (Invitrogen, USA), each deoxynucleotide triphosphate at a concentration of 200 μM, 1.5 mM magnesium chloride, 0.1 μM of each primer and 2.5 U of Taq DNA polymerase (Invitrogen, USA) in a final volume of 100 μl. PCR reaction sequence was as follows: Preheating at 92°C for 2 min, 36 cycles of 92°C for 1 min, 48°C 30 s and 72°C for 2 min and final extension 72°C for 6 min. Amplified DNA was verified by electrophoresis of aliquots of PCR product (5 μl) in 1.0% agarose gel in 1 × TAE buffer. The product was cloned into the pDrive-cloning vector and transformed into *E. Coli* DH5α [14]. The PCR products were digested with the restriction enzymes *EcoRI*, *HinfI*, *RsaI* and *MboI* (New England Biolabs) and analysed on 1.5% agarose gel and stained with ethidium bromide. The restriction profiles were scored and documented using a Biorad Gel

Documentation system. Partial 16S rDNA sequences similarities of soil isolates were calculated and compared with those available in GenBank database using BLAST program [15] and SEQMATCH of RDP-II [16]. The sequences of closely related bacteria were retrieved from RDP-II and aligned using multiple alignments CLUSTALW program [17]. The evolutionary distance was calculated by Kimura 2 parameter and phylogenetic dendograms were constructed by neighbor-joining using MEGA3.0 software [18]. For analysis, 500 bootstrap replicates were performed to assess the statistical support for the tree. Enterobacteriaceae Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was carried out to obtain DNA fingerprints of the PSB. PCR primer sequences [19], ERIC-IR (5'-3') -ATGTAAGCTCCTGGGGAATCAC- and ERIC-2 (5'-3') -AAGTAAGTGAAGTGGGTGAGCG- were obtained from Life Technologies, USA. Single isolated colonies were picked at random from the LA plates, suspended in 50 μl water, and lysed by heating for 10 min at 95°C. Cell lysate was centrifuged (9000 × g, 3 min) at 4°C, and 2 μl of the supernatant was used in the reaction mixture. The reaction mixture (25 μl) contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl₂, 0.01% gelatin (w/v), 0.25 mM each of dNTPs, 0.375 μM each of primers ERIC-1R and ERIC-2, and 2.0 units of Taq polymerase (Life Technologies, USA). The amplification was done on Genamp PCR system (Applied Biosystem, USA). The PCR conditions were as follows: initial denaturation at 94°C for 4 min followed by 41 cycles at 94°C for 1 min, at 50°C for 1 min and at 72°C for 2 min. The final extension was done at 72°C for 5 min. The reaction was terminated by using a loading dye (1 μl) containing 15% Ficoll, 0.25% bromophenol blue, and 0.25% xylene cyanol. Each PCR product was resolved by electrophoresis on a 1.5% agarose gel.

Evaluation of PSB Activities Under Plot Studies

Plot studies were conducted under sandy loam agricultural soil. Randomized block design was used for layout of the plots which included one control and three plots inoculated with *MSK1*, *MSK13* and *MSK24*, respectively. Chemical composition of this soil was organic carbon, 0.78 ± 0.03%; total nitrogen, 0.0823 ± 0.005%; and available phosphorus 44.00 ± 5.6 mg/kg. Randomized block design was adopted to study the efficacy of inoculated PSB strains in the plots (4 m²) sown with chickpea (*Cicer arietinum* L.) crop. Cultures were grown in 10 l shake flasks containing LB medium and incubated at 37°C at 120 rpm. After 36 h, the cells were harvested and washed with 10 mM phosphate buffer (pH 6.8). Pellets were re-suspended in buffer and inoculated in plots at cell concentration of approximately 2 × 10⁸ cfu/g soil. The

prescribed package of practices [20] was followed and monitored during the cultivation of the chickpea. At regular intervals of time during 0, 4, 8, 12 and 18 week soil samples were processed to evaluate the population dynamics and other soil parameters. PSB were isolated by plating serial soil dilutions plated on Luria agar (LA) containing ampicillin (120 µg/ml). Colonies grown were scored and their DNA was isolated for fingerprinting protocol based on ERIC-PCR.

Simultaneously, along with the monitoring of PSB colonization over the duration of the study, the available P was also measured following ascorbic acid method [21].

Data Analysis

Statistical analysis was performed by ANOVA and were tested by the Newman Keuls test with a critical range of 5% using COSTAT software. All values are the means of three replicates.

Results and Discussion

Biochemical Characterisation and Screening of Phosphate Solubilising Bacterial Isolates

In the present study, 167 isolates of phosphate solubilising bacteria were isolated by plating soil dilutions on Pikovskaya agar, of which 31 isolates showed the development of sharp phosphate solubilization zones, ranging from 5 to 29 mm on modified Pikovskaya agar with phosphate solubilisation index. The maximum solubilization index was

observed with the isolate *MSK13* and the minimum solubilization with the isolate *MSK2*. Nine isolates showed the phosphate solubilisation index greater than 400 were selected for quantification of phosphate solubilization in liquid media. The results of phosphate solubilization were significantly higher among nine isolates namely *MSK1*, *MSK2*, *MSK9*, *MSK13*, *MSK14*, *MSK19*, *MSK23* and *MSK24* (Fig. 1). The TCP solubilization by these isolates ranged from 125 to 413 µg/ml compared with 450 from the alkaline soils of tropical India [22]. Johri et al. [23] have reported 510 µg/ml TCP solubilization by the best bacterial strain from the alkaline soils. These isolates appear to be among the most efficient phosphate-solubilizing bacterial strains in comparison with the earlier reports. Similarly, TCP solubilization ranged from 96 to 139 µg/mL by the 13 best isolates clustered under the genera *Enterobacter*, *Pantoea*, and *Klebsiella* from Korean soils [24]. A significant decline in the pH of medium was recorded during the solubilization of TCP, which suggested secretion of organic acids by the bacterial isolates [25, 26]. The release of soluble phosphates is not necessarily correlated with soil pH [27]. In the present study the quantity of phosphate solubilization and decline in pH during solubilization of TCP was observed which is reported in earlier findings on the solubilization of rock phosphates by some efficient microbial strains. Bardiya and Gaur [28] investigated that phosphate solubilization varies greatly with the nature of phosphate substrates available and the organisms. These three best phosphate solubilisers were chosen for further morphological and biochemical characterisation. *MSK1*, *MSK13* and *MSK24* were Gram negative, non-spore forming, and positive to extracellular CMCase and

Fig. 1 Secondary screening of PSB isolates in liquid Pikovskaya broth for Phosphate solubilising activity and reduction in media pH

xylanase activity. *MSK1* and *MSK24* showed catalase positive, oxidase and nitrate reduction negative reaction, but *MSK13* was catalase negative, oxidase positive and nitrate reduction positive. Antibiotic screening showed that *MSK1*, *MSK13* and *MSK24* were sensitive to norfloxacin, gentamicin, chloramphenicol, cefuroxime, ciprofloxacin, resistant to ampicillin. These bacterial isolates were subjected to 16S rDNA amplification using universal primers, and 1.5 kb amplicon was observed. These isolates were identified by partial sequencing of 16S rDNA. Homology search of 16S rDNA revealed that *MSK1*, *MSK13* and *MSK24* were 99% similar to *Serratia sp.*, *Pseudomonas sp.* and *Serratia marcescens* respectively.

ERIC Fingerprinting and Survival Efficacy of Bacterial Strains

Genetic variability among the phosphate solubilising bacterial strains assessed using ERIC-PCR fingerprints showed high level of polymorphism in the banding profile varied from ten to fourteen and their size varied 0.1–11 kb for these strains (Fig. 2). Although 16S rDNA sequence analysis showed that *MSK1* and *MSK24* to have close homology with *Serratia sp.*, ERIC-PCR enabled us to differentiate between these isolates. ERIC-PCR showed results population of *Serratia sp. MSK1*, *Pseudomonas sp. MSK13* and *Serratia sp. MSK24* showing a survival of 40.2, 34.4 and 56.8%, respectively, after 16 weeks (Table 1). The strains of *Serratia sp.* and *Pseudomonas sp.* were found to be stable at the end of 16 weeks in the treated plots which is presumed to be due to their adaptability to local soil environment from where these were isolated. Among all the three treatments tested, significantly high values of total microbial count and soil phosphatase activities were observed in plots inoculated with

Serratia sp. MSK24 compared to control (Table 2). It was observed that the conventional plate counting method resulted in lower rates of survival of these isolates (41%) as compared to the survival rate obtained from PCR fingerprinting (58%). In absence of proper control, mere decrease in antibiotic counts cannot be related to the survival of specific inoculated bacterial isolate in a complex environment like agricultural soil. Since in natural environment several microorganisms have no specific-drug resistance [29, 30] and therefore are selected on ERIC-PCR fingerprinting. The results of investigation also support the hypothesis that indigenous isolates will results in greater adaptation to the system as well promote the microbial population and activities in the soil sustainability.

Fig. 2 ERIC-PCR fingerprint of unknown bacterial isolates on 1.5% agarose gel. Bacterial colonies were isolated from soil in the plots. Pure colonies were picked, heat lysed, and whole genomic DNA was subjected to ERIC-PCR to get the fingerprint. Lanes 1–10 fingerprints of different isolates obtained from selected plates containing 120 µg/ml of Ampicillin, Lane 11 standard bacterial strain of *Pseudomonas sp. MSK13*, Lane 12 standard bacterial strain of *Serratia sp. MSK1*, Lane 13 standard bacterial strain of *Serratia sp. MSK24*, Lane 14 500 bp ladder and Lane 15 negative control

Table 1 Survival of introduced phosphate solubilisers population during growing chickpea crop in plots

Colony forming units ($\times 10^7$ cfu/g of dry wt. soil)						
Time (weeks)	0	4	8	12	16	
Control						
Ampicillin count	2.2	1.8	1.5	1.7	1.3	
PCR count	0	0	0	0	0	
<i>Serratia sp. MSK1</i>						
Ampicillin count	15.0	13.0	10.0	8.5	8.0	
PCR count	11.7	10.4	7.2	5.8	4.7	
<i>Pseudomonas sp. MSK13</i>						
Ampicillin count	13.0	12.0	8.8	7.0	6.0	
PCR count	10.2	9.9	5.6	5.1	3.5	
<i>Serratia sp. MSK24</i>						
Ampicillin count	15.0	14.0	11.5	10.5	9.0	
PCR count	13.4	12.0	9.3	8.7	7.6	

Soil suspension diluted in physiological saline was plated on LA-ampicillin plate and genomic DNA was amplified by ERIC-PCR

Table 2 Microbiological and Bio analytical results in soils of chickpea plot. Total microbial count, phosphate solubilisers count, soil phosphatase activity, available phosphorus and organic carbon were determined at 0 week (Chickpea sowing stage) and 16 weeks (Chickpea harvest stage) in plot soils

Treatment	Total count (CFU $\times 10^6$ /g dry wt. soil)		Phosphate solubilisers (CFU $\times 10^6$ /g dry wt. soil)		Soil phosphatase activity (μ M PNP/g dry wt. soil/hr)		Available phosphorus (mg/kg)		Organic carbon (%)	
	0 week	16 week	0 week	16 week	0 week	16 week	0 week	16 week	0 week	16 week
Control	84.91 $\pm$ 8.16a	115.10 $\pm$ 3.74b	13.00 $\pm$ 0.96a	26.76 $\pm$ 1.43b	0.167 $\pm$ 0.004a	0.554 $\pm$ 0.016c	44.00 $\pm$ 5.68a	70.33 $\pm$ 6.11a	0.340 $\pm$ 0.030a	0.554 $\pm$ 0.016c
<i>Serratia</i> sp. MSK 1	86.06 $\pm$ 5.18a	192.73 $\pm$ 11.91a	17.09 $\pm$ 1.28a	40.37 $\pm$ 5.41ab	0.171 $\pm$ 0.004a	0.662 $\pm$ 0.019b	47.00 $\pm$ 6.24a	98.66 $\pm$ 7.05a	0.388 $\pm$ 0.030a	0.662 $\pm$ 0.019b
<i>Pseudomonas</i> sp. MSK 13	87.10 $\pm$ 4.43a	193.10 $\pm$ 8.36a	14.09 $\pm$ 2.10a	44.99 $\pm$ 4.38ab	0.161 $\pm$ 0.013a	0.711 $\pm$ 0.041ab	48.66 $\pm$ 4.97a	96.66 $\pm$ 14.31a	0.360 $\pm$ 0.036a	0.711 $\pm$ 0.041ab
<i>Serratia</i> sp. MSK 24	87.80 $\pm$ 7.20a	232.66 $\pm$ 19.19a	14.37 $\pm$ 0.71a	53.14 $\pm$ 6.28b	0.157 $\pm$ 0.009a	0.836 $\pm$ 0.045a	48.00 $\pm$ 8.50a	99.66 $\pm$ 11.89a	0.368 $\pm$ 0.028a	0.836 $\pm$ 0.045a

Tested by the Student–Newman–Keuls test with a critical range of 5%. Data characterized by the same letter are not significantly different. Above table represents mean $\pm$ standard error of mean (SEM)

Soil Nutrient and Enzyme Activities

The present study also indicated increase in availability of phosphorus and organic carbon assumed to be facilitated through enhanced enzyme activity. The increased phosphate activity in the soil drawn from the plots amended with *Pseudomonas* sp. MSK 13 could be related to the metabolic versatility of this microbial group that facilitates increasing activity of phosphatases when P is a limiting nutrient [31]. The plots treated with *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 showed significantly enhanced soil phosphatase activity ranging from 0.858 μ M PNP g⁻¹ dry wt soil h⁻¹ in plots inoculated with *Serratia* sp. (MSK13) to 1.497 μ M PNP g⁻¹ dry wt soil h⁻¹ in plots augmented with *Serratia* sp. (MSK24) (Table 2). The increased population of phosphate solubilizers showed the increased availability of available phosphorus significantly high as compared to the control plot, to 98.66, 96.66 and 99.66 mg/kg in the *Serratia* sp. MSK1, *Pseudomonas* sp. MSK13 and *Serratia* sp. MSK24 inoculated plots respectively. A positive correlation ($r^2 = 0.853$) was also observed among soil phosphatase activity and phosphate solubilizers population (Fig. 3), which was also positively correlated ($r^2 = 0.730$) to available phosphorus (Fig. 4). Similar investigations have earlier shown that legumes secrete more phosphatase enzymes than cereal [32]. This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. The reports on the efficacy of *Bacillus* sp. showed enhance nodulation, plant dry matter and grain yield on co-inoculation with *Rhizobia* in other legumes [33]. Bacterial strains isolated from the maize rhizosphere including *Bacillus*, *Pseudomonas* and *Serratia* were reported to improve the yield by 9–14% [34]. In addition to these traits, phosphate solubilising bacterial strains were found to competent, able to survive and colonize in the sandy

Fig. 3 Correlation between the phosphatase activity and phosphate solubilisers population in soil during the chickpea plot experiments

Fig. 4 Correlation between the phosphatase activity and available phosphorus in soil during the chickpea plot experiments

loam soil. Fortunately, the interaction between PSB and chickpea plants was observed to be much stable even after 16 weeks of crop. The good results obtained produced under field conditions. It is expected that inoculation with other rhizobacteria containing plant growth promoting characteristics consequently promote root and shoot growth as well as nodulation. Further evaluation of the isolates exhibiting multiple plant growth promoting traits on soil–plant system is needed to uncover their efficacy as effective bioinoculants.

Acknowledgment The authors acknowledge the supervision of Prof. Sunil Khanna, NIIT University, Neemrana-Rajasthan for this research work; and grant support provided by the UNDP-TIFAC, Govt. of India under the IT-SAP project.

References

- Goldstein AH (1995) Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram-negative bacteria. *Biol Agri Hort* 12:185–193
- Deubel A, Gransee A, Merbach W (2000) Transformation of organic rhizodeposits by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphate. *J Plant Nutr Soil Sci* 163:387–392
- Kang SC, Ha CG, Lee TG, Maheshwari DK (2002) Solubilization of insoluble inorganic phosphate by a soil inhabiting fungus *Fomitopsis* sp. PS102. *Curr Sci* 82:439–442
- Pradhan N, Sukla LB (2005) Solubilization of inorganic phosphate by fungi isolated from agriculture soil. *Afr J Biotechnol* 5:850–854
- Kucey RMN, Janzen HH, Legett ME (1989) Microbially mediated increase in plant available phosphorus. *Adv Agron* 42:198–228
- Ponmurugan P, Gopi C (2006) In vitro production of growth regulators and phosphate activity by phosphate solubilising bacteria. *Afr J Biotech* 5:348–350
- Richardson AE (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust J Plant Physiol* 28:897–906
- Niranjan RS, Shetty HS, Reddy MS (2006) Plant growth promoting rhizobacteria: potential green alternative for plant productivity. In: Siddiqui ZA (ed) *PGPR: biocontrol and biofertilization*. Springer, Netherlands, pp 197–216
- Saghir Khan M, Zaidi A, Wani PA (2007) Role of phosphate solubilising microorganisms in sustainable agriculture—a review. *Agron Sustain Dev* 27:29–43
- Jackson MH (1967) *Soil chemical analysis*. Prentice-Hall of India, New Delhi, India
- Tabatabai MA, Bremner JM (1969) Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1:301–307
- Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Microbiology* 17:362–370
- Weisberg WA, Barns SM, Pelletier DA, Lane DJ (1991) 16S rDNA amplification for phylogenetic study. *J Bacteriol* 173:697–703
- Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* 166:557–580
- Altschul SF, Hadden TL, Schaffer AA, Zhang J, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Cole JR, Chai B, Marsh TL, Farris RJ, Wang Q, Kulam SA, Chandra S, McGarrell DM (2003) The ribosomal database project (RDP-II): previewing a new autoaligner that allow a regular updates and the new prokaryotic taxonomy. *Nucleic Acid Res* 31:442–443
- Thompson JD, Gibson TJ, Plewniale F, Jeanmaugin F, Higgins DG (1997) The CLUSTAL X windows interface flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25:4876–4882
- Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings Bioinform* 5:150–163
- Versalovic J, Koeuth T, Lupski JR (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 24:6823–6831
- Anonymous (2005) *Package of practices for crops of Punjab-Rabi*. 2005–06. PAU, Ludhiana
- Bray RH, Kurtz LT (1945) Determination of total, organic and available forms of phosphorus in soils. *Soil Sci* 59:39–45
- Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R, Verma D (2000) Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiol Lett* 182:291–296
- Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH and temperature-tolerant phosphate-solubilizing bacteria in alkaline soils. *Curr Microbiol* 39:89–93
- Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilisation bacteria from the rhizosphere of crop plants of Korea. *Soil Biol Biochem* 37:1970–1974
- Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *App Soil Ecol* 34:33–41
- Tripura C, Sashidhar B, Podile AR (2007) Ethyl methanesulfonate mutagenesis-enhanced mineral phosphate solubilization by groundnut-associated *Serratia marcescens* GPS-5. *Curr Microbiol* 54:79–84
- Asea PEA, Kucey RMN, Stewart JWB (1988) Inorganic phosphate solubilization by two *Penicillium* species in solution culture and soil. *Soil Biol Biochem* 20:459–464
- Bardiya S, Gaur AC (1974) Isolation and screening of microorganisms dissolving low-grade rock phosphate. *Folia Microbiol* 19:386–389

29. Nakaune R, Adachi K, Nawata O, Tomiyama M, Akutsu K, Hibi T (1998) A novel ATP-binding cassette transporter involved in multidrug resistance in phytopathogenic fungus *Penicillium digitatum*. Appl Environ Microbiol 64:3983–3988
30. Mathew AG, Saxon AM, Upchurchand WG, Chattin SE (1999) Multiple antibiotic resistance pattern of *Escherichia coli* isolates from swine farms. Appl Environ Microbiol 65:2770–2772
31. Tadano T, Ozowa K, Satai M, Osak M, Matsui H (1993) Secretion of acid phosphatase by the roots of crop plants under phosphorus deficient conditions and some properties of the enzyme secreted by lupine roots. Plant Soil 156:95–98
32. Yadav RS, Tarafdar JC (2001) Influence of organic and inorganic phosphorous supply on the maximum secretion of acid phosphatase by plants. Biol Fert Soils 34:140–143
33. Sivaramaiah N, Malik DK, Sindhu SS (2007) Improvement in symbiotic efficiency of chickpea (*Cicer arietinum*) by coinoculation of *Bacillus* strains with *Mesorhizobium* sp. Cicer Indian J Microbiol 47:51–56
34. Lalande R, Bissonnette N, Countlee D, Antoun H (1989) Identification of rhizobacteria from maize and determination of their plant growth promoting potential. Plant Soil 115:7–11