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ORIGINAL ARTICLE

Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya

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Abstract This study focused on the isolation, identification (sequencing of 16S rDNA gene) and determination of the phosphorus (P)-solubilizing efficiency of native populations of phosphate-solubilizing bacteria (PSB) in 13 Kenyan soils with differing chemical characteristics. Air-dried soil samples were serially diluted and plated on NBRIP media and enumerated. Their phosphate-solubilizing efficiency was assessed on Frioni's agar. Pearson correlation coefficients were determined between PSB populations and soil properties. The PSB populations varied among the sites tested and had a positive and significant correlation ($p \leq 0.05$) with organic carbon ($r=0.76$), exchangeable calcium ($r=0.93$) and exchangeable magnesium ($r=0.92$). A total of 150 isolates were identified to the genus and species level. Among the isolates, *Bacillus megaterium*, *Bacillus* sp. and *Arthrobacter* sp. were the most abundant and well-distributed strains. However, only 5% of the total isolates were efficient in terms of phosphate-solubilizing efficiency. The results indicate that although there were many PSB strains in the soils tested, only a few (5%) were effective in terms of their phosphate-solubilizing ability. It is therefore unlikely that native PSB contribute significantly to solubilizing phosphate in the soils tested, which would ultimately benefit plant growth. Therefore, inoculation with effective strains with a high P solubilization potential is necessary.

Keywords Frioni's agar · Phosphorus solubilization efficiency · Microbial diversity · Kenya

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Introduction

Phosphorus (P) is an essential element in plant development. Although soils generally contain a large amount of total P, only a small proportion is immediately available for plant uptake. This explains why P deficiency is a major constraint to crop production in sub-Saharan Africa. In most Kenyan soils, P deficiency is mainly due to fixation by aluminum (Al) and iron (Fe) oxides and hydroxides, a process which deprives plants of up to 80% of the added P (Goldstein 1986). To meet the crop demand, farmers apply up to three- to fourfold the required amount of P to crops, causing a substantial increase in production costs. Due to the high cost of mineral fertilizers, most smallholder farmers opt not to use fertilizers, which ultimately results in poor yields. The use of phosphate rocks (PR) in acid soils has been advocated by many researchers since it is a cheaper means of P fertilization (Okalebo et al. 1994;

Ndung'u et al. 2006). In fact, an economic evaluation of Minjingu PR and its residual effects under farming conditions over four maize cropping seasons clearly indicated that the direct application of Minjingu PR could be more profitable in comparison with conventional fertilizers (Bationo et al. 1997; Ndung'u et al. 2006). However, most PR have a low solubility and may be ineffective in soils with a pH>5.5. Free-living bacteria and fungi are able to mobilize orthophosphate (predominantly as HPO_4^{2-} and $H_2PO_4^-$) from either organic or inorganic P sources (Richardson 2007) or PR. Phosphate-solubilizing microorganisms (PSM) are characterized by their capacity to solubilize precipitated forms of P, such as tri-calcium phosphate (TCP), the main form of P in PR. Several studies conducted under greenhouse and field conditions have demonstrated improvements in P availability and crop yields when PSM are used together with PR due to improved solubilization (see review by Richardson et al. 2009). This approach could be a more viable and cost-effective means of providing P to plants when insoluble PR is added (Stamford et al. 2007). Furthermore, various sources of PR exist within the East African region that can be used to improve crop yields.

Mechanisms for the dissolution of TCP in PR include the production of inorganic and organic acids and/or a decrease in the soil pH, which releases available phosphate that can be taken up by plants (Richardson et al. 2009). Among the bacterial genera, *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Flavobacterium* and *Erwinia* spp. have been reported to enhance plant growth promotion as well as inorganic and organic P solubilization from soil (Kämpfer 2007). However, their diversity, population densities and bioactivity vary from soil to soil depending on the nutritional status of the soil [carbon (C), nitrogen (N) and P], their efficiency in P solubilization and soil conditions (Nahas 2007; Panhwar et al. 2009). To date, no study has been performed to ascertain the occurrence and P solubilization efficiency of phosphate-solubilizing bacteria (PSB) capable of solubilizing PR in Kenyan soils of varying soil chemical properties. The aim of the study reported here was, therefore, to assess the diversity and P-solubilizing efficiencies of indigenous PSB in Kenyan soils and evaluate the relationship between these PSB and soil chemical properties.

Materials and Methods

Soil samples were collected from 13 different sites distributed across parts of Kenya in high-potential regions of the Central, Nyanza, Rift Valley, Western, and Coast provinces of Kenya (Fig. 1). The sites were experimental fields under soybean cultivation at the time of sampling. At each site, one composite soil sample was collected from the 0- to 15-cm soil layer within control (no input) plots. Samples were stored in plastic bags. After air drying, a portion of each sample was ground to pass through a 2-mm sieve. Chemical analyses were performed as described by Okalebo et al. (2002) and Anderson and Ingram (1993). The initial soil characteristics are as shown in Table 1. The remainder of the sample was put in sterile zip locks and immediately used for the isolation of PSB.

Isolation of PSB

Phosphate-solubilizing bacteria were isolated by serial dilutions of soil suspension. Ten grams of soil was suspended in 90 ml sterile physiological water (9 g l^{-1} NaCl) and mixed thoroughly for 45 min using a mechanical shaker. Serial dilutions were then prepared up to 10^{-7}. A 100-µl aliquot of each serial dilution was plated evenly on National Botanical Research in Phosphate medium (NBRIP) agar plates [glucose, 10 g l^{-1}; $Ca_3(PO_4)_2$, 5 g l^{-1}; $MgCl_2\ 6H_2O$, 0.5 g l^{-1}; $MgSO_4\ 7H_2O$, 0.25 g l^{-1}; KCl, 0.2 g l^{-1}; $(NH_4)_2SO_4$, 0.1 g l^{-1} and agar, 15 g l^{-1}; adjusted to pH 7.0 using 1 M NaOH before autoclaving; Mehta and Nautiyal 2001]. Both phosphate-solubilizing fungi (PSF) and PSB were able to grow in this medium. The samples were incubated for 3 days at 28°C±2. Both the PSB and PSF populations were enumerated. The number of PSM was considered to be the sum of all the colonies (PSB + PSM). The populations were expressed as colony-forming units (CFU):

$$\text{CFU } g^{-1}\text{soil} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of the aliquot}}$$

The PSB colonies were identified according to their morphology and colony characteristics. Effective PSB were detected by the presence of clear zones of solubilization surrounding or underneath the colony. All of the morphologically different colonies were purified severally in NBRIP agar plates and finally in nutrient agar (beef extract, 1 g l^{-1}; yeast extract, 2 g l^{-1}; peptones, 5 g l^{-1}; NaCl 5 g l^{-1}; agar 15 g l^{-1}; adjusted to pH 7.4 before autoclaving) prior to preservation in sterile glycerol (20%) at −80°C.

Determination of P solubilization efficiency

The P solubilization efficiency was determined using Frioni's agar (yeast extract, 2 g l^{-1}; glucose, 20 g l^{-1}; TCP 2 g l^{-1}; agar 15 g l^{-1}; adjusted to pH 7.0 before autoclaving; Frioni 1999; Taurian et al. 2010]. Although most soils used in this study had a pH<6.0 (with a predominance of Al–P and Fe–P), a TCP media was used for the isolation and testing of the P solubilization

Fig. 1 Map of the soil sampling sites in Kenya

efficiency so as to establish strains capable of solubilizing insoluble PR.

A 10-μl sample of liquid culture containing approximately $1–2\times10^7$ CFU ml^{-1} of a 2-day culture was used to inoculate the center of the plates (in triplicate). After the fifth day of growth, the diameter of the colonies (n) and that of the halo zone (z) were measured and the solubility index (SI) determined as described by Fankem et al. (2006):

$$\text{Solubilization index}(\text{SI}) = \frac{\text{n} + \text{z}}{\text{n}}$$

Three reference strains, namely, MAN ID, PER 3A and PER 3C, acquired from Argentina (Fernandez et al. 2007),

Table 1 Selected topsoil (depth 0–15 cm) properties of the study sites

Sampling site	pH (water)	Organic C (%)	Total N (%)	Extractable P (mg/kg)	Exchangeable Ca (cmol /kg)	Exchangeable Mg (cmol/kg)	Exchangeable K (cmol/kg)
Nyanza							
Kisii	5.33	1.40	0.13	4.36	5.09	1.95	0.45
Migori	5.60	1.50	0.14	3.07	1.90	0.33	0.40
Bondo	5.87	3.67	0.25	3.00	25.99	12.53	0.82
Rachuonyo	5.57	1.40	0.11	3.91	4.74	2.26	0.48
Western Kenya							
Bungoma	5.56	1.50	0.18	2.39	6.23	1.80	0.15
Butere Mumias	5.92	1.40	0.14	1.67	3.71	1.30	0.23
Teso	6.36	1.50	0.14	2.05	5.94	1.90	0.23
Siaya	5.04	3.50	0.30	3.67	20.00	9.80	0.95
Rift Valley							
Nakuru	6.03	2.69	0.28	4.66	10.03	3.27	1.28
Uasin Gishu	5.63	1.40	0.10	7.08	3.68	1.55	0.55
Central Kenya							
Meru South	5.68	2.60	0.23	8.50	6.98	2.54	0.88
Mitunguu	5.58	1.90	0.25	7.31	5.45	2.02	0.42
Coast							
Kilifi	6.93	0.98	0.08	7.00	4.04	1.71	0.55

were used for comparing the efficiency of the isolated indigenous PSB strains.

Molecular characterization of isolated strains

DNA extraction from liquid cultures

Cells were harvested from a 2-day-old actively growing culture in nutrient broth, and total genomic DNA was extracted as described by Wilson (1987). The isolated DNA was checked for quality by electrophoresis in a 2% agarose gel (pre-stained with ethidium bromide—0.116 μg ml^{-1}), then visualized on a UV trans-illuminator and photographed (Bio-Rad Gel Doc Software; Bio-Rad, Hercules, Ca). The DNA was stored at −20°C for further analysis.

Polymerase chain reaction

The PCR analysis was performed using a Bio-Rad iCycler thermocycler. The PCR reactions were conducted using PCR beads (containing 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U Taq DNA polymerase and dNTP; 200 μM in 10 mM Tris–HCl, pH 9.0, at room temperature) in a final volume of 25 μl. The primers used were fD1 (5′-AGAGTTTGATCCTGGCT CAG-3′ and rD1 (5′-AAGGAGGTGATCCAGCC-3′) (Weisburg et al. 1991). The PCR cycling conditions consisted of pre-heating at 95°C for 3 min, 35 cycles of denaturing at 95°C for 1 min, annealing at 55°C for 1 min and extension 72°C for 2 min, and a final extension at 72°C for 3 min (Fankem et al. 2006). The PCR products (approx. 1500 bp) were confirmed by electrophoresis on a 2% agarose gel (prestained with ethidium bromide—0.116 μg ml^{-1}), then visualized on a UV trans-illuminator and photographed (Bio-Rad Gel Doc Software).

Strain identification

Strains were identified by sequencing the 16S rDNA region and performing a BLAST analysis of the sequences. Sequencing reactions were performed using the BigDye terminator Cycle Sequencer (ABI, Foster City, CA). The sequences were analyzed using the gapped BLASTn search algorithm (http://www.ncbi.nlm.nih.gov).

Statistical analysis

An analysis of variance (ANOVA) was conducted to ascertain differences in SI between various strains using the general linear model (GLM procedure; SAS 2006). Pearson correlation coefficients between PSM, PSB and soil properties were computed using the Correlation Procedure of the SAS software.

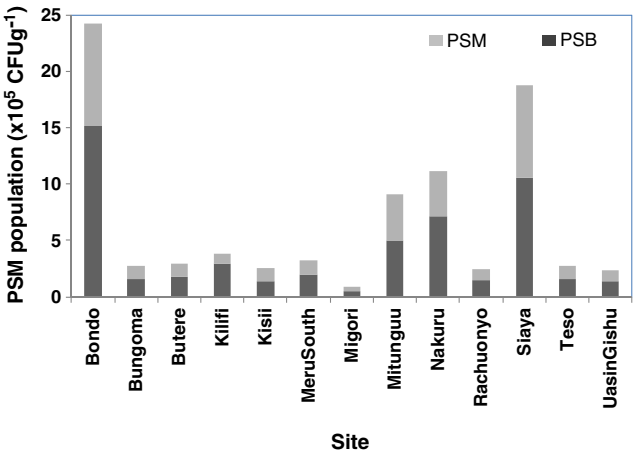

Fig. 2 Population of phosphate-solubilizing microorganisms (*PSM*) and phosphate-solubilizing bacteria (*PSB*) isolated from different soils of Kenya. *CFU* Colony-forming units

Results and discussion

The PSM population ranged from 5.4×10^4 to 1.5×10^6 CFU g^{-1} soil while the PSB population varied between 3.8×10^4 and 9.1×10^5 CFU g^{-1} in the soils from Migori and Bondo, respectively (Fig. 2). The results further showed a variation in the proportion of PSB/PSM. Soils from Mitunguu had the highest proportion of PSB (84%) while the proportion of PSB was only 25% in the Kilifi soils; in the other soil samples the PSB proportion varied between 52 and 79%. It is well known that the interplay of very many factors, such as physicochemical properties of the soil, vegetation, crop rotation and environmental conditions, greatly influence the quantity and composition of soil microbial flora (Jha et al. 1992; Setiadi 1989). In this study, soil factors may have influenced the populations of PSB. The Pearson correlation coefficient of selected soil properties with PSB population showed a positive and highly significant ($p<0.001$) correlation between PSB and PSM populations ($r=0.98$), exchangeable Ca ($r=0.93$), exchangeable Mg ($r=0.92$) and organic C ($r=0.76$), while pH and extractable P did not correlate with the PSB population (Table 2). The high population of PSB in soils from Bondo and Siaya were most likely due to the high organic C and exchangeable Ca levels. PSB are heterotrophic bacteria which depend on external C sources to solubilize P. They also require energy and essential nutrients, including Ca, to grow and reproduce. The C in these soils is mainly associated with decomposing organic matter, and the higher the soil content of decomposing organic matter, the more active and prolific the soil microbes (John et al. 2001). Vikram et al. (2007) also reported high populations of heterotrophic PSB in Vertisols with high organic C, demonstrating the need to improve soil organic matter in soils through improved soil fertility management. No significant correlations between PSB/PSM populations and available P were observed, most likely due to the low range of available P levels in those soils, since the soils were collected from cultivated plots which had not received P inputs during the past two seasons.

A total of 150 strains were isolated from soil samples collected from the tested soils. Sequencing of the 16S rDNA gene of the strains using the NCBI database revealed that all of the isolated strains were PSB belonging to the *Bacillus*, *Arthrobacter*, *Pseudomonas*, *Paenibacillus*, *Burkholderia*, *Micrococcus*, *Microbacterium*, and *Curtobacterium* genera. *Bacillus megaterium*, *Bacillus* sp., *Arthrobacter* sp. and *Paenibacillus* sp. were the most abundant and well-distributed PSB strains, occurring in 92, 75, 51 and 50% of the soils, respectively (Table 3). These genera are able to adapt to a wide range of different soils and climate and have

Table 2 Pearson correlation coefficients of soil properties in relation to the populations of phosphate-solubilizing bacteria

	PSM	PSB	pH	ExCa	ExMg	ExK	ExtP	OrgC	TotN
PSM	1.00								
PSB	0.98***	1.00							
pH	0.08	−0.05	1.00						
ExCa	0.95***	0.93***	0.03	1.00					
ExMg	0.93***	0.92***	0.01	0.99**	1.00				
ExK	0.48	0.42	−0.07	0.39	0.36	1.00			
ExtP	−0.03	−0.02	−0.05	−0.15	−0.14	0.36	1.00		
OrgC	0.79**	0.78**	−0.29	0.76**	0.75**	0.68*	0.18	1.00	
TotN	0.71**	0.739**	−0.32	0.59*	0.56*	0.69*	0.38	0.92***	1.00

*, **, *** indicate significance at $p<0.05$, $p<0.01$ and $p<0.001$, respectively

PSM, Phosphate-solubilizing microorganisms; PSB, phosphate solubilizing bacteria; pH, soil pH; ExCa, exchangeable Ca; ExMg, exchangeable Mg; ExtP, extractable P; OrgC, organic C; TotN, total N

Table 3 Occurrence (%) of PSB strains in the study soils from various sites in Kenya

PSB strain	Sampling sites													Occurrence[a] (%)
	Bungoma	Bondo	Rachuonyo	Butere-Mumias	Siaya	Meru South	Teso	Kilifi	Kisii	Uasin Gishu	Migori	Mitunguu	Nakuru	
Bacillus sp.	−	−	+	−	+	−	+	+	+	+	+	+	+	75
Bacillus megaterium	+	+	+	+	+	+	+	−	+	+	+	+	+	92
Bacillus flexus	−	−	−	−	−	−	−	−	+	−	−	−	−	8
Bacillus subtilis	−	−	−	−	+	−	−	−	−	−	−	−	−	8
Pseudomonas sp.	+	−	−	−	−	−	−	+	−	−	−	−	−	17
Pseudomonas oryzihabitans	−	−	−	−	+	−	−	−	−	−	−	−	−	8
Paenibacillus sp.	−	−	+	−	+	+	−	−	−	+	+	+	−	50
Paenibacillus glycanilyticus	−	−	−	−	+	−	−	−	−	+	+	+	+	42
Paenibacillus polymyxa	−	−	−	−	+	−	−	−	−	−	−	−	−	8
Arthrobacter sp.	−	−	+	−	+	−	+	+	−	+	−	+	−	50
Arthrobacter globiformis	−	−	+	+	−	−	−	−	−	−	−	+	−	25
Arthrobacter ramosus	−	−	−	+	−	−	−	−	−	−	−	−	−	8
Burkholderia sp.	−	+	−	−	+	−	−	−	−	−	−	−	−	8
Williamsia sp.	−	−	−	−	−	−	−	+	−	−	−	−	−	8
Micrococcus luteus	−	−	−	−	−	−	−	−	−	−	+	−	−	8
Microbacterium sp.	−	+	−	−	+	−	−	−	−	−	−	−	−	8

+, Strain is present in the soil; −, strain absent in the soil

[a] Number of soils with the strain × 100/total number of soils

been found to be predominant in many ecological niches (Suliash 2005; Taha et al. 1969; Vikram et al. 2007). On the other hand, *Bacillus flexus*, *Bacillus subtilis*, *Pseudomonas oryzihabitans*, *Paenibacillus polymyxa*, *Arthrobacter ramosus*, *Bulkhoderia* sp., *Williamsia* sp., *Micrococcus luteus* and *Microbacterium* sp. were the least abundant, occurring in only one soil each. Most of the strains isolated in this study are predominantly spore formers, although we cannot exclude the possibility that isolation from air-dried soils could have favored the spore-forming Gram-positive bacteria and fungi. However, these results are in agreement with the work of Taha et al. (1969) and Suliash 2005. Spore formers are well known to be tolerant of adverse conditions, such as the high temperature and dryness as found in the bulk dry soils used in this study. PSB that can withstand such unfavorable conditions are also able to compete with other indigenous microbes, and their effective colonization of the rhizosphere may be important for use as biofertilizers.

The identity of PSB strains as determined by 16S rDNA sequences, after their comparison to reference strains using NCBI analysis, and their solubility indices are given in Table 4. After several purification processes in NBRIP media, most of the isolates lost their solubilization potential. Therefore, only the effective strains that maintained their P solubilization potential were posted to the gene bank and assigned accession numbers. The PCR amplicons had between 96 and 98% homology to the *Bacillus*, *Burkholderia*, *Paenibacillus* and *Williamsia* sp., respectively. The value of the SI indicates the activity of the strains: the greater the value, the higher the activity of the tested strains. The results show that strain TSBF 699 was the most efficient P solubilizer from the soils, with a SI of 4.9, while among the reference strains, strain MAN ID had the highest solubility index of 5.6.

Microbes have different tolerances to water stress, and the phosphate solubilizing activity can be indirectly affected by changes in the nutrient supply due to moisture-related changes. Although the continuous wetting and drying of soils in the field may not significantly influence microbial community diversity and composition (Carney and Matson 2006), such variations in soil moisture content are bound to change the phosphate solubilizing ability (Whitelaw 2000). The aim of this study was therefore to ascertain the presence of PSB capable of maintaining their P solubilization efficiency even under dry conditions. Although the number of PSB identified in this study as being able to maintain their P solubilization efficiency is quite low, some strains (TSBF 699, 739, 723 and 845; about 5% of isolates) were found to persist and form large halos in TCP medium after repeated subculturing, indicating their potential to release soluble inorganic phosphates from PR for plant uptake. Gyaneshwar et al. (2002) and Kucey (1983) have shown that PSB lose their in vitro P solubilizing capacity upon repeated subculturing. This may explain why, despite the fact that many native PSB are found in soils, the ability of these PSB to liberate sufficient P from the soils or from sparingly soluble PR is limited. This also explains why these rhizobacteria are unable to support crop P uptake. Hence, inoculation with a high concentration of effective PSB strains could be more beneficial for phosphate solubilizing activity in the root environment. To ascertain the performance of the efficient strains identified in this study and their interaction with other plant growth promoting rhizobacteria (PGPR) in the soil environment, greenhouse trials are currently being conducted on soybean and maize growth and P uptake.

Table 4 Phosphate solubilization potential and identification of efficient PSB isolated from Kenyan soils by 16S rDNA based on the output results from the NCBI database analysis

Strains	Source	Organism identified	Percentage identity (%)	Gene accession number	Solubility index[a]
TSBF699	Siaya (Kenya)[b]	*Burkholderia* sp.	98	HM637291	4.9 b
TSBF723	Siaya (Kenya)[b]	*Bacillus* sp.	97	HM637290	2.3 f
TSBF739	Mitunguu (Kenya)[b]	*Bacillus* sp.	98	HM637292	2.1 f
TSBF828	Uasin Gishu (Kenya)[b]	*Bacillus megaterium*	98	HM776635	2.2 f
TSBF845	Uasin Gishu (Kenya)[b]	*Paenibacillus* sp.	97	HM776636	2.6 e
PER 3A	Argentina[c]	*Burkholderia* sp.	98		4.7 b
MAN ID	Argentina[c]	*Burkholderia* sp.	98		5.6 a
PER 3C	Argentina[c]	*Burkholderia* sp.	96		4.4 c

[a] Values followed by same lower-case letter are not significantly different ($p<0.05$) using Tukey's test

[b] Strains isolated in this study

[c] Reference strains from Fernandez et al. (2007)

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References

Anderson JM, Ingram JS (1993) Tropical soil biology and fertility: a handbook of methods. CAB International, Wallingford

Bationo A, Ayuke ED, Ballo D, Kone M (1997) Agronomic evaluation of Tilemsi phosphate rocks in different agro-ecological zones of Mali. Nutr Cycl Agroecosyst 48:179–189

Carney KM, Matson PA (2006) The influence of tropical plant diversity and composition on soil microbial communities. Microbial Ecol 52:226–238

Fankem H, Nwaga D, Deubel A, Dieng L, Merbach W, Etoa FX (2006) Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. Afr J Biotechnol 5:2450–2460

Fernandez LA, Zabla P, Gómez MA, Sargardoy 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

Frioni L (1999) Procesos microbianos. Tomus I y II. Fundación de UNRC. Rio Cuarto Córdoba, 282 y 286p

Goldstein AH (1986) Bacterial mineral phosphate solubilization: Historical perspective and future prospects. Am J Altern Agric 1:57–65

Gyaneshwar P, Naresh KG, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93

Jha DK, Sharma GD, Mishra RR (1992) Ecology of soil micro-flora and mycorrhizal symbionts. Biol Fert Soils 12:272–278

John L, Herms D, Stinner B, Hostink H (2001) Mulch effect on soil microbial activity, nutrient cycling, and plant growth in ornamental landscape. Ornamental Plant Annual Report and Research Reviews 2001. The Ohio State University, Columbus

Kämpfer P (2007) Taxonomy of phosphate solubilizing bacteria. In: Velazquez E, Rodriguez-Barrueco C (eds) First Int Meet microbial phosphate solubilization. Springer SBM, Dordrecht, pp 101–106

Kucey RMN (1983) Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678

Mehta S, Nautiyal SC (2001) An efficient method for qualitative screening of phosphate-solubilizing bacteria. Current Microbiol 43:51–56

Nahas E (2007) Phosphate solubilizing microorganisms: effects of carbon, nitrogen and phosphorus. In: Velazquez E, Rodriguez-Barrueco C (eds) First Int Meet microbial phosphate solubilization. Springer SBM, Dordrecht, pp 111–115

Ndung'u KW, Okalebo JR, Othieno CO, Kifuko MN, Kipkoech AK, Kimenye LN (2006) Residual effectiveness of Minjingu phosphate rock and improved fallows on crop yield and financial returns in western Kenya. Exp Agric 42:323–336

Okalebo JR, Schiner F, Lekasi, JK (1994) Maize response to fertilizer and rock phosphate in a high phosphate fixing soil of Malava, Kakamega. In: Kapkiyai JJ, Okalebo JR, Maritim HK. Proc PrePREP Workshop held in Moi University. Moi University, Eldoret, pp 77–82

Okalebo JR, Gathua KW,Woomer PL (2002) Laboratory methods of soil and plant analysis. A working manual. Marvel EPZ, Nairobi

Panhwar QI, Radziah O, Sariah M, Ismail MR (2009) Solubilization of different phosphate forms by phosphate solubilizing bacteria isolated from aerobic rice. Int J Agric Biol 11:667–673

Richardson AE (2007) Making microorganisms mobilize soil phosphorus. In: Velazquez E, Rodriguez-Barrueco C (eds) First International Meeting on microbial phosphate solubilization. First Int Meet microbial phosphate solubilization. Springer SBM, Dordrecht, pp 85–90

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339

SAS Institute (2006) SAS Users' guide statistics. SAS Institute, Cary

Setiadi Y (1989) Pemanfaatan Mikroorganisme dalam Kehutanan. Institut Pertanian Bogor, Bogor

Stamford NP, Santos PR, Santos CE, Freitas AD, Dias SHL, Lira MA (2007) Agronomic effectiveness of biofertilizers with phosphate rock, sulphur and *Acidithiobacillus* for yam bean grown on a Brazilian tableland acidic soil. Bioresour Technol 98:1311–1318

Suliasih WS (2005) Isolation and identification of phosphate solubilizing and nitrogen fixing bacteria from soil in Wamena biological garden, Jayawijaya, Papua. Biodiversitas 6(3):175–177

Taha SM, Mahmoud SAZ, Halim A, Damaty E, El Hafez AMA (1969) Activity of phosphate dissolving bacteria in Egyptian soils. Plant Soil 31:149–160

Taurian T, Anzuay SM, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Vikram A, Alagawadi AR, Hamzehzarghani H, Krishnaraj PU (2007) Factors related to the occurrence of phosphate solubilizing bacteria and their isolation in Vertisols. Int J Agric Res 2 (7):571–580

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Whitelaw MA (2000) Growth promotion of plants inoculated with phosphate-solubilizing fungi. Adv Agron 69:99–151

Wilson KJ (1987) Preparation of genomic DNA from bacteria. In: Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) Current Protocols in Molecular biology. Wiley Interscience, New York, pp 241–245