Brazilian Journal of Microbiology 45, 3, 937-943 (2014)
ISSN 1678-4405


Research Paper

# Dissolving mechanism of strain P17 on insoluble phosphorus of yellow-brown soil

Zhong Chuan-qing[1], Cao Guang-xiang[2,3], Huang Wei-yi[4], Luan Xing-she[1], Yang Yi-fei[1]

[1]College of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan, China.
[2]Shandong Medicinal Biotechnology Centre, Jinan, China.
[3]State Key Laboratory of Microbial Technology, Shandong University, Jinan, China.
[4]Life Science College, Nanjing Agricultural University, Nanjing, China.

Submitted: March 17, 2013; Approved: March 14, 2014.

## Abstract

Strain P17 was a bacterial strain identified as *Bacillus megaterium* isolated from ground accumulating phosphate rock powder. The fermentation broth of strain P17 and the yellow-brown soil from Nanjing Agricultural University garden were collected to conduct this study. The simulation of fixed insoluble phosphorous forms after applying calcium superphosphate into yellow-brown soil was performed in pots, while available P and total P of soil were extremely positive correlative with those of groundwater. Then the dissolving effect of strain P17 on insoluble P of yellow-brown soil was studied. Results showed that *Bacillus megaterium* strain P17 had notable solubilizing effect on insoluble phosphates formed when too much water-soluble phosphorous fertilizer used. During 100 days after inoculation, strain P17 was dominant. Until the 120th day, compared with water addition, available P of strain P17 inoculation treated soil increased by 3 times with calcium superphosphate addition. Besides available P, pH, activity of acid and alkaline phosphatase and population of P-solubilizing microbes were detected respectively. P-solubilizing mechanism of P-solubilizing bacteria strain P17 seems to be a synergetic effect of pH decrease, organic acids, phosphatase, etc.

**Key words:** calcium superphosphate, p-solubilizing microbes, acid phosphatase, alkaline phosphatase.

## Introduction

The supply of phosphorus (P) in adequate amounts is very critical for normal plant growth and development (Bhardwaj 2011; Bressan 2001; Marcos *et al.*, 2003). Although soils generally contain a large amount of total P, only a small proportion is immediately available for plant uptake (Keziah *et al.*, 2012). Soil P is mostly associated with particles, and the concentration in soil solution is low, while available P and total P of soil were extremely positive correlative with those of groundwater P must be released from the particles in order to be available to plants. Phosphate movement in soils is present in both dissolved and particulate forms (Zhang *et al.*, 2003). Once chemical fertilizer is added into the soil, chemical, biochemical, physical and biological reaction are taking place, which leading to transformation of configuration of fertilizer (Shen and Jiang, 1992). For example, the solubilization of monocalcium phosphate in soil solution is characterized by formation of $CaHPO_4 2H_2O$ and release of $H_3PO_4$ (Wang *et al.*, 2003). Fixed mechanism of P includes physical and chemical absorption, anion exchange, surface precipitation and separation of solid phase precipitation (Zhang and Li, 1998). Much phosphate is fixed, available P to meet the need of plants is probably low. Phenomenon of P deficiency in calciform soil of North China is ubiquitous. When available P of soil exceeds a certain value, P fertilizer infliction could lead to no effect, even to a reduction of output. The efficiency of P fertilizer mainly depends on available P, which have nothing to do with agrotype.

Phosphorus-solubilizing microbes (PSM) could activate phosphatic deposit in soil in order to meet the need of plants (Hilda 2000). Sharif (2011) reported that Arbuscular mycorrhiza (AM) increased the P uptake of capsicum annum L. in a low P fossil oxisol with a P concentration of

Send correspondence to Z. Chuan-qing. College of Municipal and Environmental Engineering, Shandong Jianzhu University, 250101 Jinan, China. E-mail: zhongchq@163.com.

0.15 μmol $L^{-1}$. The P- solubilizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) together could reduce P fertilizer application by 50% without any significant reduction of crop yield (Jilani *et al.*, 2007; Yazdani *et al.*, 2009). As a kind of functional microbes, a large amount of PSM exist in rhizosphere and pedosphere. Sundara (Sundara and Natarajan, 2002) reported that PSB application increased PSB population in rhizosphere and the plant available P status in the soil. PSM could excrete inorganic acids, organic acids and phosphatase. Inorganic acids can change the solubility of phosphatic deposit; organic acids can chelate metal ions of phosphatic deposit, so $PO_4^{3-}$ could be released; phosphatase mainly hydrolyze organic P compound, which offer P source for microbes living in environment without P. Phosphatase is a kind of enzyme that could catalyze the hydrolyzation of ester and anhydride in phosphoric acid. The distinct specialty of acid and alkaline phosphatase is their specificity (Hilda 2000). Solubilizing effects of *Bacillus megaterium* strain P17 on insoluble phosphatic deposit was simulated in this study.

## Materials and Methods

### Soil treatment and P17 inoculation

PSM strain No.17 (namely, P17) was isolated from ground accumulating phosphate rock powder and identified as *Bacillus megaterium* (Zhong and Huang, 2004). *Bacillus megaterium* strain P17 and yellow-brown soil from Nanjing Agricultural University garden were collected to conduct this study. Soil samples were milled and sifted out by 20-mesh sifter. Whole P and available P were determined. Put the soil into 48 pots by 1.5 kg $pot^{-1}$. Ascending caliber of pots was 16.1 cm, lower caliber was 10.9 cm and the height was 14.0 cm. 24 pots of soil were autoclaved intermittently in high pressure enduring polyethylene bags for three times, 121 °C maintaining 1 h each time. The soil was cooled after sterilization and put into pots after being detected sterile. Chose 12 pots in sterilized soil and unsterilized soil, respectively. Put 5.0 g calcium superphosphate into each pots and mixed equally. 3 pots were chosen to detect whole P and available P. Distilled water was irrigated by 90 mL in each pot every week during 7 months. Salvers were matted under each pot to prevent water leakage. Diluted P17 fermented liquid to 100 times by sterilized water 7 months later. At this time insufflating 1 mL diluted liquid into 3 pots of sterilized soil with calcium superphosphate added; sterilized soil without calcium superphosphate; air-dried soil with calcium superphosphate addition; air-dried soil without calcium superphosphate, respectively, which was called "inoculation" treatment. Then put 1 mL sterilized diluted liquid, 1 mL diluted blank liquid medium, 1 mL distilled water into another 12 pots with same tags above, respectively, which were "sterilized" treatment; "medium" treatment and "water" control. Sampled at 5$^{th}$ day, 10$^{th}$ day, 22$^{nd}$ day, 60$^{th}$ day, 100$^{th}$ day, 120$^{th}$ day, respectively and about 10 g each time.

### Characteristics of the yellow brown soil

Whole P, available P, organic matter content and pH were determined according to reference (Nanjing Agricultural University 1996). PSM were detected by diluting plate method. Acid and alkaline phosphatase activities was measured by the method provided by Peiqi and Mile (1991).

## Results

### Characteristics of the yellow brown soil

Some properties of soil are given in Table 1. Variational tendency of available P content in differently treated soils was shown as figures below. 8 months later, about 90% of available P were fixed.

### Solubilizing effect of *Bacillus megaterium* strain P17 on fixed-phosphorous of soil

#### *Dynamic change of Available P content*

After *Bacillus megaterium* strain P17 inoculation, taking one with another, available P content was elevatory, excluding control. Elevatory trend of available P content was the same. Nutrition might be supplied by medium. Dead thalli might be transformed into available P. Elevatory trend of available P with sterilized bacteria inoculation was larger than medium attachment in calcium superphosphate added soil after 100 days. To then part of dead thalli might have transformed into available P which could be absorbed by plants directly. PSB weren't brought into medium treated soil. Available P began to decrease, which showed that P was fixed. In strain P17 inoculation treated soil without calcium superphosphate, available P in sterilized soil was higher than that of air-dried soil, which showed that strain P17 had no inhibiting action on indigenous microbes. In soil with calcium superphosphate addition, available P in air-dried soil was more than that of sterilized soil during antecedent 100 days, and the former was lower than the latter in retral 100 days, which showed that in calcium superphosphate added soil, available aboriginal microorganisms were dominant. After 100 days, PSB strain P17 were dominant. Until the 120th day, compared with water addition, available P of strain P17 inoculation treated soil increased by 3 times with calcium superphosphate addition, while available P of soil without calcium superphosphate but with strain P17 inoculation the available P doubled. Accordingly, more fixed phosphates could stimulate activation of insoluble phosphates. All results showed that PSB strain P17 played an important role in activation of insoluble phosphates. Data are shown in Figures 1-4.

Solubilization of phosphate rock powder from different sources and insoluble phosphates in erlenmeyer flask in triplicate by strain P17 were studied (Zhong and Huang, 2004). The screening medium was NBRIP medium. As a

**Table 1** - Characteristics of soil before treatments.

| Soil treatment | Phosphatase activity (μg hydroxybenzene $g^{-1}$ $h^{-1}$) | | Population of PSM (cfu $mL^{-1}$) | Available p (g $kg^{-1}$) | Whole p (g $kg^{-1}$) | Organic matter (g $kg^{-1}$) |
|---|---|---|---|---|---|---|
| | Acid | Alkaline | | | | |
| Calcium superphosphate+sterilized soil | 0 ± 0.002 | 0 ± 0.004 | 0 | 0.608 ± 0.32 | 1.22 ± 0.03 | 21.8 ± 0.66 |
| Sterilized soil | 0 ± 0.003 | 0 ± 0.006 | 0 | 0.384 ± 0.213 | 0.99 ± 0.009 | 20.3 ± 0.54 |
| Calcium superphosphate+air-dried soil | 105 ± 1.02 | 115 2.05 | $(1.68 \pm 0.5)10^5$ | 0.993 ± 0.22 | 1.359 ± 0.072 | 20.2 ± 0.65 |
| Air-dred soil | 92 ± 0.09 | 102 ± 1.99 | $(1.93 \pm 0.7)10^5$ | 0.401 ± 0.157 | 0.97 ± 0.106 | 19.8 ± 0.45 |

Note: Before inoculation, sterilized soil with calcium superphosphate added was called "Calcium superphosphate +sterilized soil"; air-dried soil with calcium added was called "Calcium superphosphate + air-dried soil"; sterilized soil that without calcium superphosphate was called "sterilized soil"; air-dried soil without calcium superphosphate was called "air-dried soil".

kind of microbiological fertilizer, applying effect is important. Results suggested that under pot conditions, after calcium superphosphate was transformed into insoluble phosphates, *Bacillus megaterium* strain P17 had preferably effects on those phosphates.

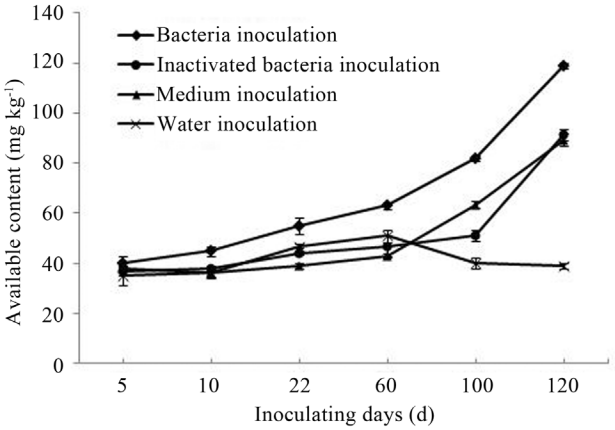


**Figure 1** - Available P of different treatments in sterilized soil with calcium superphosphate addition.

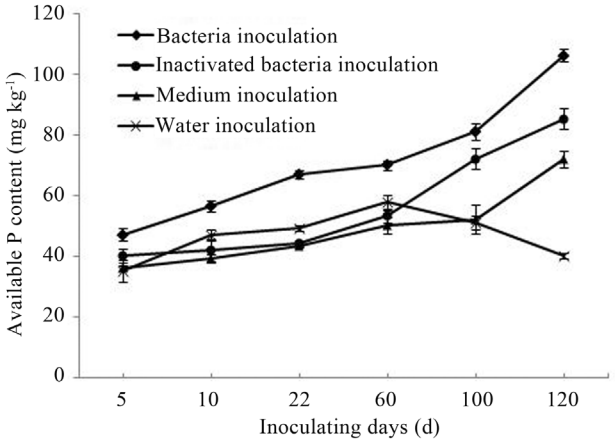


**Figure 2** - Available P of different treatments in air-dried soil with calcium superphosphate addition.

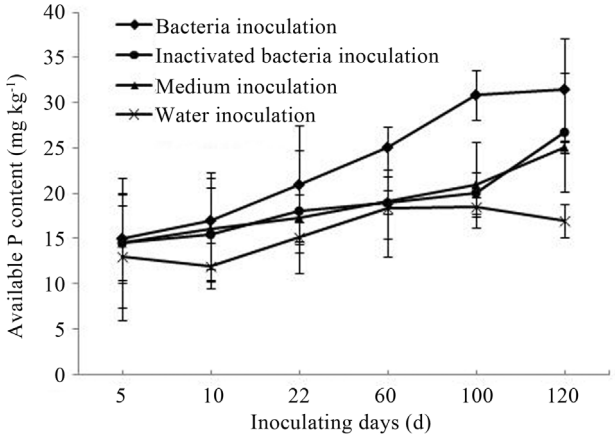


**Figure 3** - Available P of different treatments in sterilized soil without calcium superphosphate.

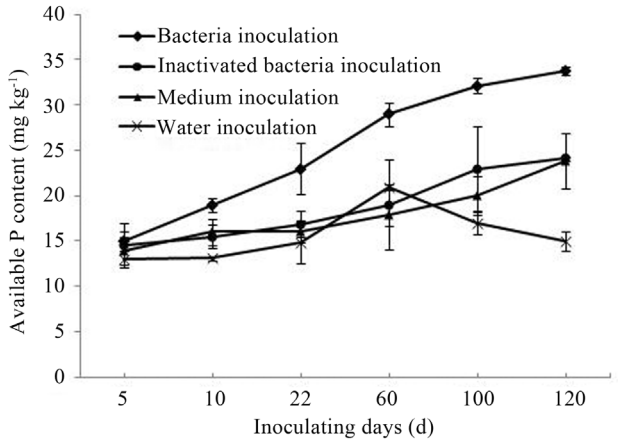


**Figure 4** - Available P of different treatments in air-dried soil without calcium superphosphate.

### *Population of PSM*

Population of PSM was the least in control, as the data were shown in Figures 5-8. After inactivated strain P17 and medium were added into the soil, nutrition of soil might be increased and growth of PSM stimulated. When the soil was inoculated by strain P17, not only nutritional components but also high active PSB were brought into the soil. Compared with "sterilized" and "medium" treated soil, population of PSM increased largely. During the period of 22 days after the soil was inoculated by strain P17, population of PSM was aggrandized; after 60 days, population of PSM reduced lightly. Results suggested that PSM needed to be inoculated continuously. Otherwise energy sources such as organic fertilizer require inflicting to offer necessary nutrition. When population of PSM was calculated by diluting plates method, PSM were mostly bacteria.

### *pH of soil*

Calcium superphosphate is a kind of water-soluble P fertilizer. Its main components include Ca $(H_2PO_4)_2H_2O$ and insoluble $CaSO_4$. After calcium superphosphate was inflicted into the soil, pH of soil decreased insignificantly. With time prolonged, the tendency of pH decrease was increscent. As the data were shown in Table 2. Differences between "inoculation" treatment and others were inconspicuousness. There was no positive correlation between decrease of pH and increase of available P. This suggested that during the period of transforming insoluble P into available P, decrease of pH was not the single factor. pH of "water" treated soil changed indistinctly. Veresoglou *et al.*

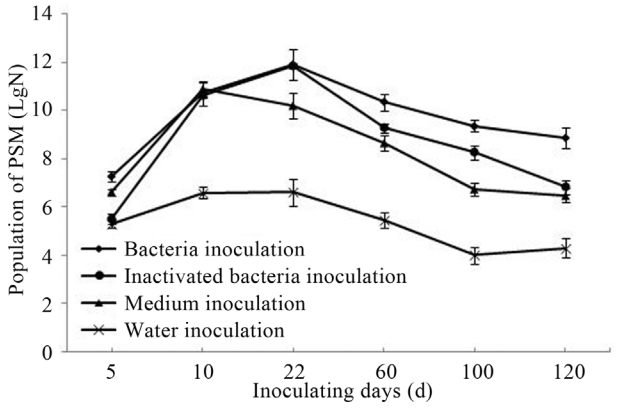


**Figure 5** - Population of PSM in sterilized soil with calcium superphosphate addition.

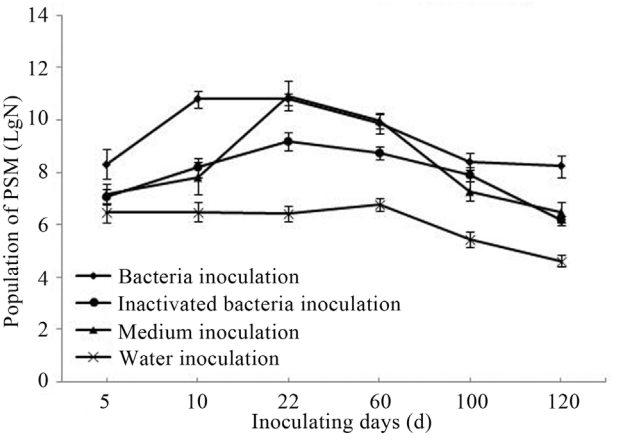


**Figure 6** - Population of PSM in sterilized soil without calcium superphosphate.

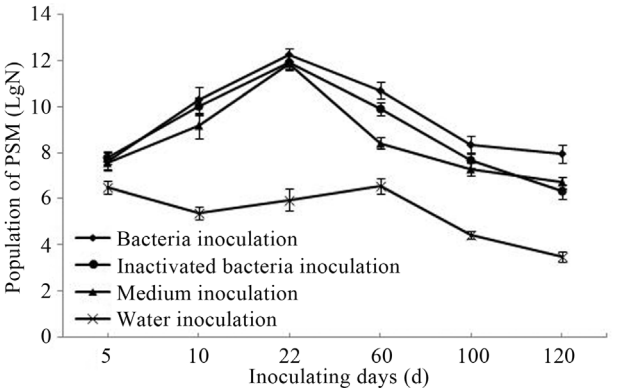


**Figure 7** - Population of PSM in air-dried soil with calcium superphosphate addition.

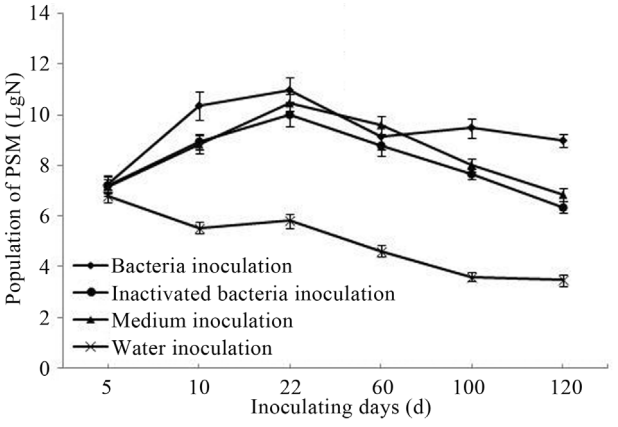


**Figure 8** - Population of PSM in air-dried soil without calcium superphosphate.

**Table 2** - pH of soil during different treatments.

| Soil types | Treatment | Day of assay after inoculation | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 22 | 60 | 100 | 120 |
| Calcium superphosphate+sterilized soil | Inoculation | 8.47 ± 0.15 | 7.09 ± 0.07 | 6.12 ± 0.12 | 6.30 ± 0.10 | 5.10 ± 0.01 | 4.50 ± 0.21 |
| | Sterilized | 8.09 ± 0.22 | 7.02 ± 0.01 | 7.08 ± 0.19 | 7.88 ± 0.10 | 5.43 ± 0.03 | 4.29 ± 0.12 |
| | Medium | 9.12 ± 0.09 | 7.82 ± 0.10 | 6.49 ± 0.10 | 6.70 ± 0.17 | 5.29 ± 0.09 | 4.19 ± 0.08 |
| | Water | 8.31 ± 0.18 | 8.25 ± 0.08 | 8.12 ± 0.19 | 8.08 ± 0.12 | 7.47 ± 0.30 | 7.90 ± 0.17 |
| Sterilized soil | Inoculation | 8.72 ± 0.27 | 8.59 ± 0.16 | 7.20 ± 0.07 | 6.82 ± 0.17 | 6.00 ± 0.08 | 4.50 ± 0.14 |
| | Sterilized | 8.23 ± 0.12 | 8.10 ± 0.18 | 7.79 ± 0.11 | 6.07 ± 0.07 | 4.87 ± 0.01 | 4.58 ± 0.06 |
| | Medium | 8.93 ± 0.18 | 8.28 ± 0.15 | 7.99 ± 0.05 | 7.10 ± 0.10 | 5.58 ± 0.23 | 4.10 ± 0.06 |
| | Water | 8.30 ± 0.11 | 8.16 ± 0.20 | 8.57 ± 0.08 | 8.49 ± 0.22 | 8.20 ± 0.13 | 8.09 ± 0.12 |
| Calcium superphosphate+air-dried soil | Inoculation | 8.68 ± 0.28 | 7.19 ± 0.10 | 7.04 ± 0.01 | 7.90 ± 0.17 | 7.76 ± 0.27 | 4.39 ± 0.13 |
| | Sterilized | 8.37 ± 0.23 | 7.01 ± 0.16 | 6.52 ± 0.09 | 7.52 ± 0.08 | 5.20 ± 0.09 | 4.10 ± 0.08 |
| | Medium | 8.99 ± 0.18 | 7.63 ± 0.12 | 6.90 ± 0.08 | 7.00 ± 0.01 | 5.89 ± 0.10 | 4.62 ± 0.09 |
| | Water | 8.68 ± 0.11 | 8.19 ± 0.20 | 7.95 ± 0.18 | 8.05 ± 0.15 | 8.37 ± 0.02 | 8.24 ± 0.27 |
| Air-dried soil | Inoculation | 9.18 ± 0.23 | 8.30 ± 0.10 | 7.50 ± 0.23 | 6.21 ± 0.09 | 5.40 ± 0.09 | 3.87 ± 0.12 |
| | Sterilized | 8.80 ± 0.15 | 8.60 ± 0.09 | 7.89 ± 0.15 | 6.20 ± 0.14 | 5.34 ± 0.09 | 4.19 ± 0.06 |
| | Medium | 8.40 ± 0.14 | 8.12 ± 0.19 | 7.60 ± 0.15 | 6.52 ± 0.08 | 5.22 ± 0.15 | 4.06 ± 0.01 |
| | Water | 8.30 ± 0.10 | 8.67 ± 0.19 | 8.07 ± 0.15 | 7.93 ± 0.07 | 7.60 ± 0.12 | 8.23 ± 0.20 |

(2011) found that the increased soil fertility appeared detrimental to the typical pH productivity relationship that would be normally expected on the basis of more speciation events under typical growth conditions.

### *Activity of acid and alkaline phosphatase in differently treated soils*

Chhonkar and Tarafdar (1984) reported that enzyme activities, including soil phosphatase activity, could be compared not only with soil physical and chemical properties, but also with other biological factors (*e.g.*, microbial biomass, the level of adenosine triphosphate, etc.). Results from Table 3 showed that phosphatase activities of bacteria inoculation, sterilized bacteria and medium treated soil were higher than those of water control; phosphatase activities of bacteria inoculation were higher than inactivated bacteria and medium treated soils; there was no distinct difference between phosphatase activities of inactivated bacteria addition and medium treated soil. Phosphatase activities of sterilized soil was low during prophase of sampling. With time prolonging, population of PSM was increasing, phosphatase activities enhanced. Phosphatase is possibly derivational enzyme; its activities had something to do with not only population and abilities of PSM, but also available P content of soil. Phosphatase activities were decreased slightly probably because of increase of available P.

**Table 3** - Activity of acid phosphatase and alkaline phosphatase in different treatments.

| Soil types | Treatment | Phosphatase | Day of assay after inoculation (μg hydroxybenzene $g^{-1}$ $h^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5 | 10 | 22 | 60 | 100 | 120 |
| Calcium superphosphate+sterilized soil | Inoculation | Acid | 102 ± 1.93 | 136 ± 1.42 | 162 ± 1.20 | 268 ± 1.86 | 556 ± 2.21 | 330 ± 1.29 |
| | | Alkaline | 119 ± 1.49 | 178 ± 1.24 | 283 ± 2.46 | 293 ± 3.24 | 1091 ± 8.52 | 736 ± 4.97 |
| | Sterilized | Acid | 58 ± 1.26 | 118 ± 2.19 | 146 ± 2.85 | 260 ± 6.09 | 519 ± 4.07 | 356 ± 4.39 |
| | | Alkaline | 92 ± 1.40 | 160 ± 6.38 | 224 ± 3.08 | 212 ± 4.56 | 1086 ± 7.12 | 630 ± 3.02 |
| | Medium | Acid | 52 ± 1.03 | 120 ± 2.38 | 138 ± 1.60 | 230 ± 1.98 | 501 ± 4.64 | 312 ± 2.27 |
| | | Alkaline | 69 ± 0.75 | 139 ± 1.09 | 220 ± 5.28 | 230 ± 2.09 | 1080 ± 8.90 | 689 ± 4.01 |
| | Water | Acid | 53 ± 0.99 | 79 ± 1.02 | 168 ± 2.80 | 168 ± 1.20 | 178 ± 2.11 | 168 ± 2.39 |
| | | Alkaline | 52 ± 1.03 | 42 ± 1.36 | 188 ± 2.88 | 170 ± 1.39 | 167 ± 1.54 | 160 ± 4.00 |
| Sterilized soil | Inoculation | Acid | 80 ± 2.21 | 119 ± 1.22 | 128 ± 2.57 | 190 ± 2.48 | 507 ± 3.08 | 439 ± 2.19 |

**Table 3 (cont.)**

| Soil types | Treatment | Phosphatase | Day of assay after inoculation (μg hydroxybenzene $g^{-1}$ $h^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 5 | 10 | 22 | 60 | 100 | 120 |
| | | Alkaline | 70 ± 1.21 | 82 ± 1.74 | 92 ± 1.09 | 394 ± 3.19 | 1076 ± 5.56 | 809 ± 1.98 |
| | Sterilized | Acid | 48 ± 1.68 | 98 ± 1.20 | 110 ± 1.67 | 96 ± 1.70 | 350 ± 2.99 | 313 ± 2.32 |
| | | Alkaline | 69 ± 2.12 | 112 ± 1.36 | 120 ± 2.98 | 289 ± 2.43 | 998 ± 3.21 | 957 ± 3.78 |
| | Medium | Acid | 31 ± 0.39 | 99 ± 1.78 | 118 ± 1.08 | 139 ± 1.80 | 608 ± 3.09 | 159 ± 3.79 |
| | | Alkaline | 75 ± 1.90 | 123 ± 5.56 | 176 ± 1.67 | 298 ± 3.45 | 1178 ± 3.96 | 367 ± 6.89 |
| | Water | Acid | 38 ± 1.92 | 54 ± 2.09 | 89 ± 1.99 | 123 ± 3.77 | 167 ± 3.68 | 115 2.68 |
| | | Alkaline | 49 ± 0.91 | 59 ± 2.68 | 98 ± 2.68 | 178 ± 3.88 | 256 ± 8.99 | 200 ± 6.88 |
| Calcium superphosphate+air-dried soil | Inoculation | Acid | 123 ± 2.56 | 160 ± 8.68 | 198 ± 6.89 | 298 ± 6.00 | 561 ± 3.78 | 460 ± 7.68 |
| | | Alkaline | 149 ± 5.88 | 216 ± 6.78 | 325 ± 8.89 | 430 ± 8.21 | 1148 ± 8.38 | 950 ± 6.30 |
| | Sterilized | Acid | 112 ± 1.35 | 135 ± 8.68 | 116 ± 6.89 | 230 ± 3.97 | 400 ± 6.70 | 356 ± 2.56 |
| | | Alkaline | 138 ± 0.68 | 168 ± 6.80 | 180 ± 6.00 | 382 ± 5.60 | 768 ± 4.68 | 680 ± 4.65 |
| | Medium | Acid | 112 ± 1.48 | 119 ± 6.80 | 130 ± 5.09 | 212 ± 4.00 | 370 ± 4.65 | 298 ± 4.98 |
| | | Alkaline | 128 ± 1.50 | 180 ± 6.78 | 270 ± 3.88 | 379 ± 3.78 | 728 ± 4.56 | 690 ± 6.20 |
| | Water | Acid | 80 ± 1.24 | 90 ± 6.00 | 180 ± 2.70 | 180 ± 2.68 | 219 ± 2.45 | 279 ± 9.12 |
| | | Alkaline | 72 ± 1.48 | 122 ± 3.43 | 198 ± 6.87 | 199 ± 6.00 | 216 ± 4.80 | 149 ± 3.10 |
| Air-dried soil | Inoculation | Acid | 112 ± 1.26 | 119 ± 4.68 | 149 ± 5.26 | 328 ± 4.89 | 380 ± 4.56 | 279 ± 3.05 |
| | | Alkaline | 129 ± 1.25 | 193 ± 5.79 | 223 ± 5.67 | 435 ± 7.98 | 989 ± 5.00 | 896 ± 6.88 |
| | Sterilized | Acid | 87 ± 0.86 | 116 ± 8.90 | 130 ± 6.80 | 345 ± 5.00 | 390 ± 3.99 | 340 ± 6.80 |
| | | Alkaline | 110 ± 1.97 | 178 ± 5.89 | 189 ± 8.78 | 509 ± 6.46 | 819 ± 6.60 | 800 ± 6.89 |
| | Medium | Acid | 88 ± 1.68 | 110 ± 2.12 | 96 ± 3.13 | 299 ± 3.90 | 309 ± 9.99 | 189 ± 8.99 |
| | | Alkaline | 120 ± 1.81 | 169 ± 7.07 | 120 ± 6.13 | 419 ± 5.69 | 840 ± 3.91 | 799 ± 6.03 |
| | Water | Acid | 90 ± 1.32 | 87 ± 1.89 | 66 ± 4.56 | 179 ± 7.10 | 218 ± 4.79 | 190 ± 6.80 |
| | | Alkaline | 88 ± 1.00 | 78 ± 0.80 | 70 ± 0.87 | 187 ± 0.96 | 258 ± 1.98 | 188 ± 2.12 |

## Discussion

The physiology of phosphate solubilization has not been studied thoroughly. In addition some studies indicate that certain mineral elements play important roles in this process. A critical K concentration is necessary for optimum solubilization rates (Beever and Burns, 1980; Illmer and Schinner, 1992), while Mg and Na seem to be important in some fungi (Beever and Burns, 1980), but not in *Pseudomonas sp.* Illmer and Schinner, 1992). The role of N and P uptake remains controversial (Cabala and Wild, 1982). So components of medium also play important roles in metabolism products of microbes, which may do good to P-solubilization. Some microbes can also produce hormones such as IAA which could stimulate growth of plants. Nevertheless, buffering capacity of the medium reduce the effectiveness of PSB in releasing P from tricalcium phosphates (Stephen and Jisha, 2009). Khan and Joergensen (2009) reported that although there were many PSB strains in the soils tested, only a few (5%) were effective in terms of their phosphate-solubilizing ability. So it is necessary to inoculate PSM in order to promote the growth of plant.

## Acknowledgments

The research was sponsored by the doctor foundation of Shandong Jianzhu University (XNBS1232).

## References

Beever RE, Burns DJ W (1980) P uptake, storage and utilization by fungi. Adv Bot Res 8:127-219.

Bhardwaj RL (2011) Effect of water regimes, nitrogen and P on crop growth, yield and economics of aloe (*Aloe barbadensis*). Indian J Agr Sci 81:44-49.

Bressan W (2001) The interactive effect of P and nitrogen on “in vitro” spore germination of *Glomus etunicatum* Becker & Gerdemann, root growth and mycorrhizal colonization. Braz J Microbiol 32:276-280.

Cabala RP, Wild A (1982) Direct use of low grade phosphate rock from Brazil as fertilizer II. Effects of mycorrhizal inoculation and nitrogen source. Plant Soil 65:363-373.

Chhonkar PK, Tarafdar JC (1984) Accumulation of phosphatases in soils. Indian Soc Soil Sci 32:266-272.

Hilda R, Reynaldo F (1999) Phosphate solubilizing bacteria and their roles in plant growth promotion. Bio technol Adv 17:319-339.

Hilda R (2000) Isolation of a gene from *Burkholderia cepacia IS-16* encoding a protein that facilitates phosphatase activity. Curr Microbiol 40:362-366.

Illmer P, Schinner F (1992). Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 24:389-395.

Jilani G, Akram A, Ali RM, Hafeez FY, Shamsi IH, Chaudhry AN, Chaudhry A G (2007) Enhancing crop growth, nutrients availability, economics and benefical rhizosphere microflora through organic and biofertilizers. Ann Microbiol 57:177-183.

Keziah W, Ndung'u-Magiroi, Laetitia H (2012) Occurrence and genetic diversity of phosphate-solubilizing bacteria in soils of differing chemical characteristics in Kenya. Ann Microbiol 62:897- 904.

Khan KS, Joergensen RG (2009) Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. Bioresour Technol 100:303-309.

Marcos AB, Aline Elesbão N, Wanderley S, Kazutaka FGM (2003) Effects of P on polyphosphate accumulation by *Cunninghamella elegans*. Braz J Microbiol 34:363-372.

Nanjing Agricultural University (1996) Soil Agricultural Chemistry Analysis. 2nd Ed. (in Chinese). Agricultural Publishing Company, Beijing.

Peiqi AL, Mile RH (1991). Soil analysis methods (in Chinese). Chinese Agricultural Science and Technology Publishing Company, Beijing.

Sharif M, Claassen N (2011) Action Mechanisms of Arbuscular Mycorrhizal Fungi in P Uptake by *Capsicum annuum L.* Pedosphere 21:502-511.

Shen RF, Jiang BF (1992) Modal distribution and its validity of limy soil. Acta Pedologica Sinica (in Chinese) 29:80-85.

Stephen JM, Jisha S(2009) Buffering reduces phosphate solubilizing ability of selected strains of bacteria. World J Agric Sci 5:135-137.

Sundara B, Natarajan VH (2002) Influence of P solubilizing bacteria on the changes in soil available P and sugarcane and sugar Fields. Field Crop Res 77:43-49.

Veresoglou SD, Voulgari OKR, Sen Mamolos AP, Veresoglou DS (2011) Effects of nitrogen and P fertilization on soil pH-plant productivity relationships in upland grasslands of Northern Greece. Pedosphere 21:750-752.

Wang HY, Zhou JM, Chen XQ, Li ST, Du CW (2003) Interaction of NPK fertilizers during their transformation in soils I. Dynamic changes of soil pH. Pedosphere. 13:257-262.

Yazdani MA, Bahmanyar H, Pirdashti EMA(2009) Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of Corn (Zea mays L.). Proc World Acad Science Eng. Technol 37:90-92.

Zhang BG, Li GT (1998) Effects of soil organisms on enhancement of plant availability of soil P. Acta Pedologica Sinica (in Chinese) 35:104-111.

Zhang M K, Jiang H, Liu XM. (2003) P concentration and forms in surface and subsurface drainage water from wetland rice fields in the Shaoxing Plain. Pedosphere 13:239-248.

Zhong, CQ, Huang WY (2004) Effects and mechanism of P-solubilizing bacillus strain P17 on P solubilization of different phosphate rocks. Acta Pedologica Sinica (in Chinese) 41:931-937.

## Abbreviations

PSM: P-solubilizing microbes.
AM: Arbuscular mycorrhiza.
PSB: P-solubilizing bacteria.
NBRIP: National botanical research institute's phosphate growth medium.
PGPR: Plant growth promoting rhizobacteria.