

Research Article

The potential of phosphate solubilizing bacteria isolated from sugarcane wastes for solubilizing phosphate

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Abstract: Most of P in agricultural soils is in unavailable forms for plant growth. Phosphate solubilizing bacteria can increase soil P availability. This study was aimed to isolate phosphate solubilizing bacteria from sugarcane waste compost and to test ability of the isolated bacterial to dissolve phosphate. The bacteria were isolated from three types of sugarcane waste, i.e. filter cake compost, bagasse compost, and a mixture of filter cake + bagasse + trash biomass compost. The potential colony was further purified by the Pikovskaya method on selective media. Eight isolates of phosphate solubilizing bacteria were obtained from all wasted studied. Amongst them, T-K5 and T-K6 isolates were superior in dissolving P from $\text{Ca}_3(\text{PO}_4)_2$ in the media studied. The two isolates were able to solubilize P with solubilizing index of 1.75 and 1.67 for T-K5 and T-K6, respectively. Quantitatively, T-K6 isolate showed the highest P solubilization (0.74 mg / L), followed by T-K5 isolate (0.56 mg / L), while the lowest P solubilization (0.41 mg / L) was observed for T-K4 isolate. The increase of soluble P was not always followed by the decrease in pH.

Keywords : *organic waste, phosphorus, phosphate solubilizing bacteria*

Introduction

Phosphorus (P) is the second important nutrient after N that affects plant growth and metabolism processes (Widawati and Suliasih, 2006). Mobility of phosphate ions in the soil is very low due to their high retention in soil. Stevenson (1986) and Holford (1997) reported that the recovery rate of P fertilizer by plants is only about 10-30 %. The remaining 70-90 % is accumulated in soil or in the form of immobile that is bound by Al or Fe in acid soils, or Ca and Mg in alkaline soils (Prochnow et al., 2006; Yang et al., 2010). As plants cannot absorb P in bound form, the P must be converted into available form.

Phosphate solubilising bacteria (PSB) can play an important role in dissolving both of fertilizer P and bound P in the soil that is environmentally friendly and sustainable (Khan et al., 2007). Phosphate solubilisation effect is mainly through the reaction between organic acids excreted from organic matters with phosphate

binders such as Al, Fe, and Ca, or Mg to form stable organic chelates to free the bound phosphate ion (Arcand and Schneider, 2006; Gupta et al., 2012). Exploration of phosphate solubilizing bacteria has been conducted by many researchers from soils (Chen et al., 2006; Widawati et al., 2008; Gupta et al., 2012), mangrove (Vazquez et al., 2000; Holguin et al., 2001), and rhizosphere (Chung et al., 2005; Poonguzhali et al., 2008; Oliveira et al., 2009). From such explorations, various types of phosphate solubilizing bacteria have been successfully identified. Another potential material as a source of phosphate solubilising bacteria isolates is compost. Isolation of phosphate solubilizing bacteria from compost of agricultural wastes (rice straw, maize, groundnut, gliricidia leaf, and macrofauna dung) has been conducted by Hameeda et al. (2008) and Nuraini et al. (2011). The use of compost as a source of the phosphate solubilising bacteria isolates has a dual function, i.e. as a source of soil organic matter and

as a source of energy and a carrier for the corresponding phosphate solubilising bacteria. This study was aimed to isolate phosphate solubilizing bacteria from compost of sugarcane wastes and to test examine their ability to dissolve phosphate.

Materials and Methods

Isolation of phosphate solubilizing bacteria

Isolation of phosphate solubilizing bacteria was performed on three types of sugarcane wastes, i.e. filter cake compost, bagasse compost and a mixture of filter cake compost + bagasse compost + sugarcane plant residue. Ten grams of each of the air-dried composts was placed in a 250 mL beaker glass containing 90 mL of sterile distilled water. The mixture was then shaken for 1 hour on a shaker at 120 rpm. The extract was then diluted to 10^{-7} (1 mL of compost extract was mixed with 9 mL of sterile distilled water) and shaken. From each series of diluted solution, 0.1 mL solution was then poured on a petridish containing Pikovskaya media having composition of 2.5 g $\text{Ca}_3(\text{PO}_4)_2$, 0.5 g $(\text{NH}_4)_2\text{SO}_4$, 0.2 g NaCl, 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 g KCl, 10 g glucose, 0.5 g of yeast extract, 20 g agar, small amounts of MnSO_4 and FeSO_4 , and 1000 mL distilled water (Rao, 1982). The culture media was then stored upside down for 5-7 days in an incubator with a temperature of about 30° C, and observed daily. Colonies which appeared with a surrounding clear zone were potential phosphate solubilizing bacteria that dissolved $\text{Ca}_3(\text{PO}_4)_2$. The potential colonies were further purified by the scratch method on selective Pikovskaya media. The purification was aimed to obtain a desired pure culture without any contaminants from other microorganisms. The purified isolates were then collected in a slant agar and stored at 4° C to be used for subsequent examination.

Examination of dissolving ability of phosphate solubilizing bacteria to dissolve P in solid Pikovskaya media

Isolates of solubilising bacteria colonies were collected using a needle and placed on sterile solid Pikovskaya media. $\text{Ca}_3(\text{PO}_4)_2$ was used as a source of phosphate. Observations were made until the formation of a clear zone around the colonies of bacteria that indicated the occurrence of phosphate dissolution. The qualitative test was done by calculating the clear zone formed using Solubilization Index by dividing the clear zone diameter with the colony diameter (Premono et al., 1996). Bacteria that formed the fastest clear

areas with the greatest diameter indicate the most superior phosphate solubilizing bacteria.

Examination of dissolving ability of phosphate solubilizing bacteria to dissolve P in liquid Pikovskaya media

Isolates of solubilizing bacteria colonies were mixed with 100 mL of liquid Pikovskaya media and placed in a 250 mL beaker glass. A source of P used in the media was 5 g $\text{Ca}_3(\text{PO}_4)_2/\text{L}$. The mixture was then shaken at 120 rpm for 5 days. Every day, 15 mL of sample was collected for pH measurement. Another 6 mL of sample was also collected for measurement of P solubilization after centrifugation at 1000 rpm for 10 minutes. Soluble P in the supernatant was measured by molybdenum-blue method using a spectrophotometer at a wavelength of 882 nm (Watanabe and Olsen, 1965).

Results and Discussion

Isolated phosphate solubilizing bacteria

Eight potential phosphate solubilizing bacteria were obtained from sugarcane waste composts. The isolates were found capable of forming a clear zone on solid media Pikovskaya with different characters (Table 1). From the eight isolates, 6 isolates were obtained from filter cake compost, 1 isolate from bagasse compost, and 1 isolate from mixture of bagasse+filter cake+trash biomass compost.

Ability of phosphate solubilizing bacteria to dissolve P in solid Pikovskaya media

The ability of each isolate in dissolving P varied in terms of both speed and magnitude of solubility index. The T-K5 formed the clear zone at hour 24 (day 1), T-K3, G-K1 and M-K1 isolates at hour 48, and T-K1, T-K2, T-K4, T-K6 isolates at hour 72. At the end of observation, isolates T-K5 and T-K6 exhibited higher P solubilisation index of 1.75 and 1.67, respectively, than other isolates. The lowest P solubilization index was observed for T-K1 isolate (Figure 1).

Ability of phosphate solubilizing bacteria to dissolve P in liquid Pikovskaya media

P dissolved of phosphate solubilizing bacteria in a liquid medium increased with increasing time (Figure 2). Compared with the initial observation (hour 4), at the end of observation (hour 96), the bacteria increased 84.20%-91.61% of P dissolution.

Table 1. Morphology of phosphate solubilizing bacteria isolated from composts of sugarcane wastes

| Compost | Isolate | Colony Morphology | | | | | | |
|---|---------|-------------------|---------------|-----------|------------|------------|--------|------------------------|
| | | Shape | Configuration | Elevation | Luster | Topography | Colour | Optical Characteristic |
| Filter cake | T-K1 | Irregular | Heave | Convex | Glossy | Sleek | White | Opaque |
| | T-K2 | Irregular | Heave | Convex | Glossy | Sleek | White | Translucent |
| | T-K3 | Round | Comprehensive | Convex | Glossy | Sleek | Yellow | Opaque |
| | T-K4 | Irregular | Heave | Flat | Glossy | Sleek | White | Translucent |
| | T-K5 | Round | Heave | Convex | Glossy | Sleek | White | Translucent |
| | T-K6 | Round | Heave | Convex | Not Glossy | Sleek | Yellow | Opaque |
| Bagasse | G-K1 | Round | Comprehensive | Flat | Glossy | Sleek | White | Translucent |
| Mixture of filter cake+bagasse+ trash biomass | M-K1 | Round | Comprehensive | Flat | Glossy | Sleek | White | Opalescent |

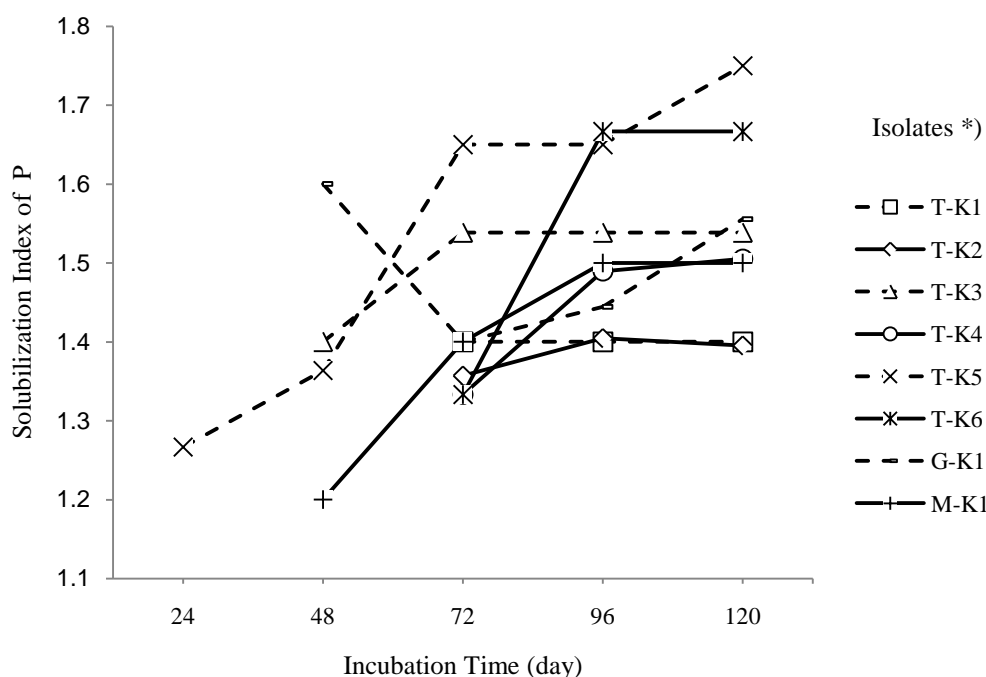


Figure 1. P solubilization index of eight phosphate solubilizing bacteria isolates in solid Pikovskaya medium. *) see Table 1

At the end of incubation the highest soluble P was observed T-K6 isolate (0.74 mg / L) followed by T-K5 (0.56 mg / L) and the lowest by T-K4 isolates (0.41 mg / L) (Figure 2). Results of quantitative calculation of P solubilization were similar to that of qualitative calculation (solid Pikovskaya media), especially for T-K6 and T-K5 isolates that showed high P solubilization compared to other isolates.

Phosphate solubilization mechanism is closely related to the release of organic acids by the phosphate solubilising bacteria that form a chelate with cations (Al, Fe, or Ca) that bind P through hydroxyl and carboxyl chelation, thus turning it into available forms (Kpombekou and Tabatabai, 1994; Goldstein, 1995; Kim et al., 1997). According to Gyaneshwar et al. (2002), organic acids that play roles in the release of P include acetate, lactate, malate, oxalate, succinate, citrate, gluconate, and ketogluconate acids. Tri-carboxylic acids (*cis*-aconitic and citric acids) and the di-carboxylic acids (oxalic, malonic, fumaric, and tartaric acids) were more effective than mono-carboxylic acids (glycolic, pyruvic, and salicylic acids) in releasing P (Kpombekou and Tabatabai, 2003). Phosphate solubilization may also occur due to the release of protons such as H^+ where the mechanism of phosphate solubilization is similar to tricalcium phosphate [$Ca_3(PO_4)_2$] that can be

described as follows : $Ca_3(PO_4)_2 + 2H^+ = 2CaHPO_4 + Ca^{2+}$ (Bashan et al. 2013).

Media pH changes

Incubation of eight isolates in liquid Pikovskaya media influenced the change in pH of the media (Figure 3). The T-K1, T-K3, T-K4 T-K6, G-K1, and M-K1 isolates showed pH decrease over time. These results are similar to those reported by Rashid et al. (2004). In contrast, the T-K2 and T-K5 isolates showed pH decrease only at the beginning of incubation, after which an increase in the pH of the media, even at the end of the observation was higher than at the beginning of incubation.

There is a significant relationship between pH and solubilized P dissolved in the culture media by the isolates tested. In the T-K1, T-K3, T-K4, T-K6, G-K1 and M-K1 isolates, the increase of available P was linearly followed by the decrease in pH (Figure 4). The pH decrease is thought to be related to the release of organic acids by the phosphate solubilizing bacteria which resulted in changes to the pH decrease in the culture medium (Khan et al., 2009; Tao et al., 2008; Rashid et al., 2004; Chen et al., 2006). According to Chen et al. (2006), bacteria that synthesize organic acids strongly reduce pH that

in turn increase P solubilization. In general, high levels of P solubilization was followed by media pH decrease. In the case of T-K2 and T-K5 isolates, however, the high P solubilization was not followed by by pH decrease. These results were

similar to those obtained by Yu et al. (2011) that high P solubilization by W4 bacterial strain was achieved at high pH (pH 6.0). However, P solubilisation is not always comparable to the decrease in pH (Behera et al., 2014).

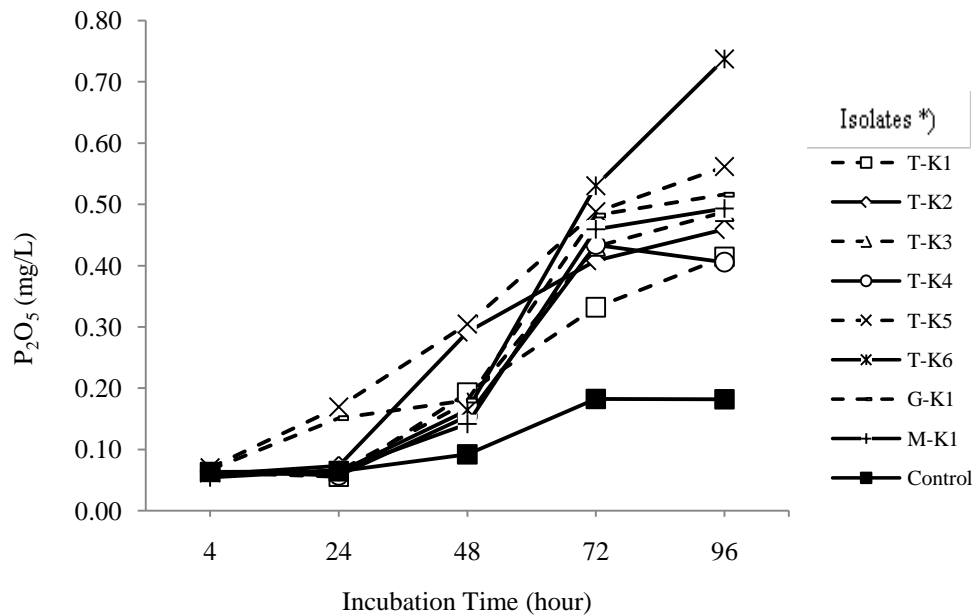


Figure 2. Solubilization of P by eight phosphate solubilizing bacteria isolates incubated in liquid Pikovskaya media. *) see Table 1

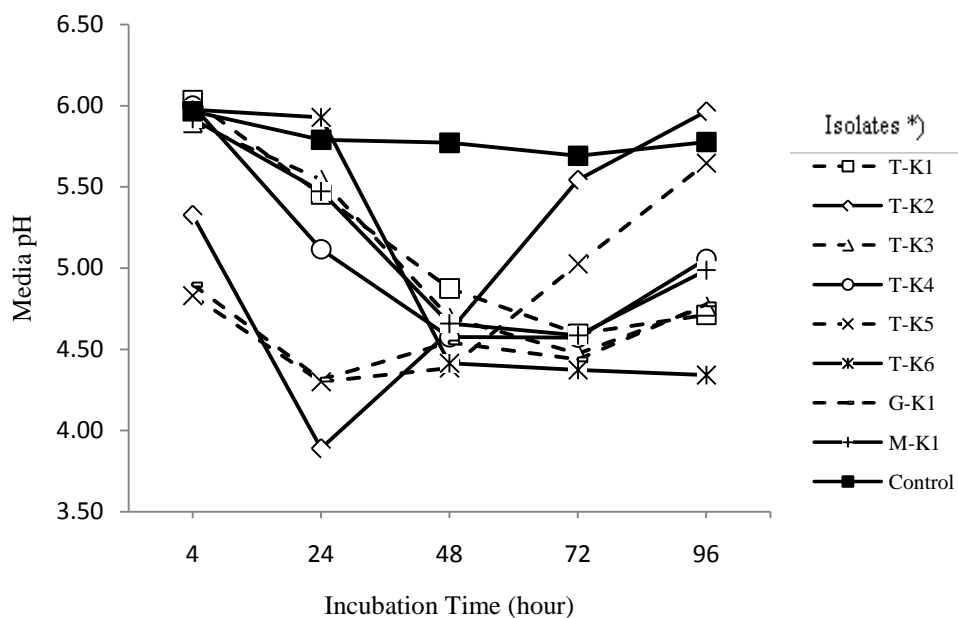


Figure 3. Changes in pH of Pikovskaya liquid media inoculated with eight phosphate solubilizing bacteria. *) see Table 1

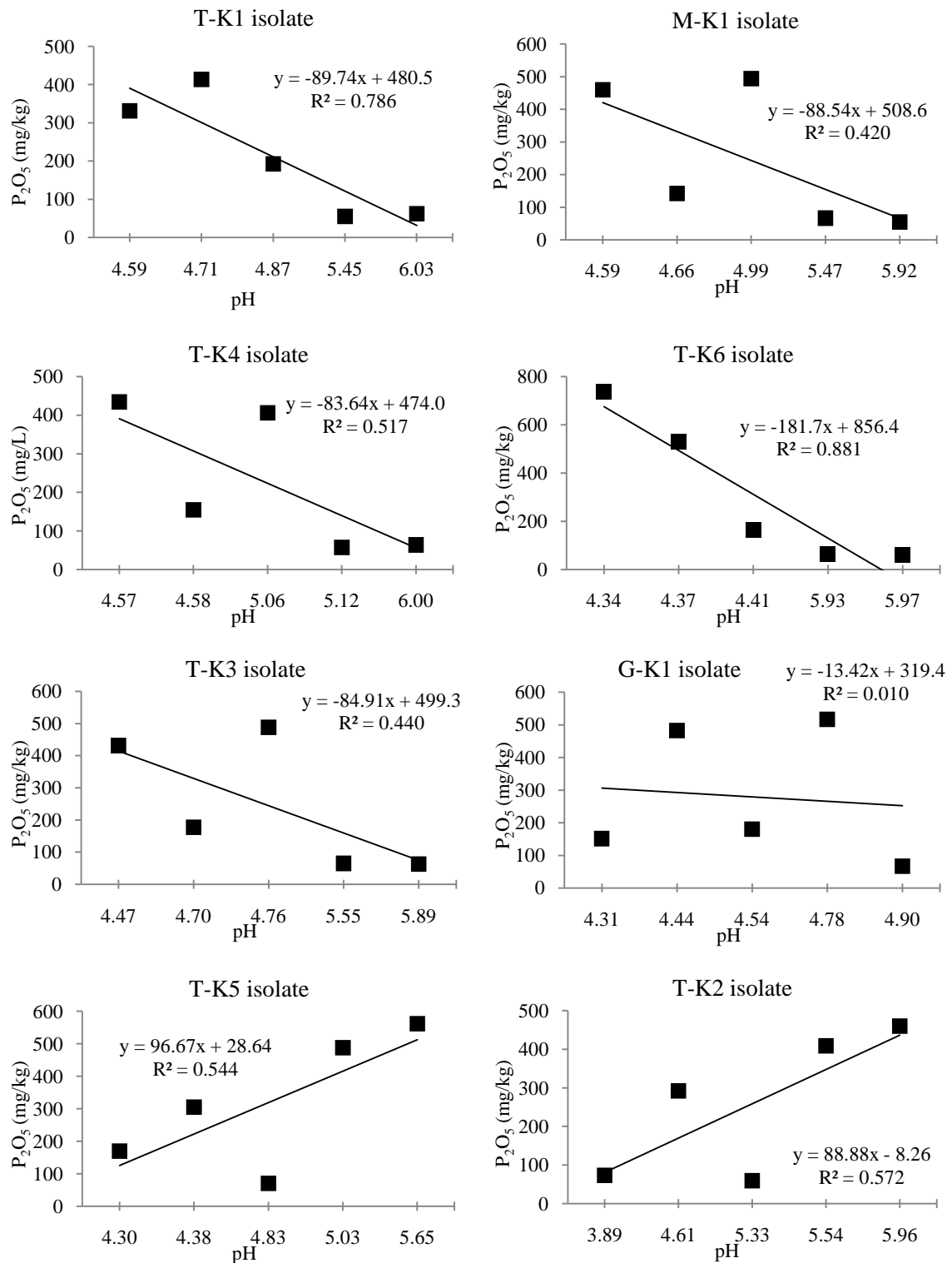


Figure 4. Correlations between pH and available P of eight phosphate solubilization bacteria

Conclusion

Isolation of phosphate solubilizing bacteria from composts of sugarcane waste resulted in eight potential phosphate solubilizing bacteria isolates. Two isolates (T-K5 and T-K6), were quantitatively and qualitatively very potential in solubilizing P from $\text{Ca}_3(\text{PO}_4)_2$ in Pikovskaya media. In general, the increase of P solubilisation was linearly correlated to the decrease of pH media.

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References

- Arcand, M.M. and Schneider, K.D. 2006. Plant-and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. *Anais da Academia Brasileira de Ciências* 78(4): 791–807.
- Bashan, Y., Kamnev, A.A. and de-Bashan, L.E. 2013. Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. *Biology and Fertility of Soils* 49: 465–479.
- Behera, B.C., Singdevsachan, S.K., Mishra, R.R., Dutta, S.K. and Thatoi, H.N. 2014. Diversity, mechanism and biotechnology of phosphate solubilizing microorganism in mangrove: A review. *Biocatalysis and Agricultural Biotechnology* 3(2): 97–110.
- Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Applied Soil Ecology* 34: 33–41.
- Chung, H., Park, M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H. and Sa, T. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biology and Biochemistry* 37: 1970–1974.
- Goldstein, A.H. 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilisation by Gram-negative bacteria. *Biological Agriculture and Horticulture* 12: 185–193.
- Gupta, M., Kiran, S., Gulati, A., Singh, B. and Tewari, R. 2012. Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of *Aloe barbadensis* Miller. *Microbiological Research* 167: 358–363.
- Gyaneshwar, P., Kumar, N.J., Pareka, L.J. and Podle, P.S. 2002. Role of soil microorganisms in improving P nutrition of plants. *Plant and Soil* 245(1):83–93.
- Hameeda, B., Harini, G., Rupela, O.P., Wani, S.P. and Reddy, G. 2008. Growth promotion of maize by phosphate solubilizing bacteria isolated from composts and macrofauna. *Microbiological Research* 163: 234–242.
- Holford, I.C.R. 1997. Soil phosphorus its measurement and its uptake by plants. *Australian Journal of Soil Research* 35: 227–239.
- Holguin, G., Vazquez, P. and Bashan, Y. 2001. The role of sediment microorganisms in the productivity, conservation and rehabilitation of mangrove ecosystems: an overview. *Biology and Fertility of Soils* 33: 265–278.
- Khan, A.A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S. and Rasheed, M. 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production. *Journal of Agriculture and Biological Science* 1(1): 48–58.
- Khan, M.S., Zaidi, A. and Wani, P.A. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review. *Agronomy and Sustainable Development* 27: 29–43.
- Kim, K.Y., Jordan, D. and McDonald, G.A. 1997. Solubilization of hydroxyl apatite by *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. *Biology and Fertility of Soils* 24: 347–352.
- Kpombekou-A, K. and Tabatabai, M.A. 2003. Effect of low-molecular weight organic acids on phosphorus release and phytoavailability of phosphorus in phosphate rocks added to soils. *Agriculture, Ecosystems and Environment* 100: 275–284.
- Kpombekou-A, K. and Tabatabai, M.A. 1994. Effect of organic acids on release of phosphorus from phosphate rocks. *Soil Science* 158: 442–453.
- Nuraini, Y., Abadi, A.L., Soemarno. and Ardyati, T. 2011. Potensial of legume and maize to stimulate population of nitrogen-fixing bacteria, phosphate-solubilizing bacteria and indole acetic acid production. *Journal of Agriculture and Food Technology* 1(12): 218–226.
- Oliveira, C.A., Alves, V.M.C., Marriel, I.E., Gomes, E.A., Scotti, M.R., Carneiro, N.P., Guimaraes, C.T., Schaffert, R.E. and Sa, N.M.H. 2009. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado Biome. *Soil Biology and Biochemistry* 41: 1782–1787.
- Poonguzhali, S., Madhaiyan, M. and Sa, T. 2008. Isolation and identification of phosphate solubilizing bacteria from chinese cabbage and their effect on growth and phosphorus utilization of plants. *Journal of Microbiology and Biotechnology* 18(4): 773–777.
- Premono, M.E., Moawad, A.M. and Vleck, P.L.G. 1996. Effect of phosphate solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indonesian Journal of Crop Science* 11:13–23
- Prochnow, L. I., Fernando, J., Quispe, S., Artur, E., Francisco, B. and Braga, G. 2006. Effectiveness of phosphate fertilizers of different water solubilities in relation to soil phosphorus adsorption, 1(August), 333–340.

- Rao, N.S.S. 1982. Phosphate solubilization by soil microorganisms. In N.S. Rao (ed.) *Advanced in Agricultural Microbiology*. New Delhi: Oxford and IBH Publishing Co.
- Rashid, M., Khalil, S., Ayub, N., Alam, S. and Latif, F. 2004. Organic acids production and phosphate solubilization by phosphate solubilization microorganisms (PSM) under in vitro conditions. *Pakistan Journal of Biological Sciences* 7(2): 187-196.
- Stevenson, F.J. 1986. *Cycles of Soil Carbon, Nitrogen, Phosphorus, Sulphur and Micronutrients*. Wiley, New York.
- Tao, G.C., Tian S.J., Cai, M.Y. and Xie, G.H. 2008. Phosphate-solubilizing and mineralizing abilities of bacteria isolated from soils. *Pedosphere* 18(4): 515-523.
- Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. and Bashan, Y. 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils* 30 (5-6): 460-468.
- Watanabe, F.S. and Olsen, S.R. 1965. Test of an ascorbic acid method for determining phosphorous in water and NaHCO₃ extracts from soil. *Soil Science Society of America*
- Widawati, S. and Suliasih. 2006. Augmentation of potential phosphate solubilizing bacteria (PSB) stimulate growth of green mustard (*Brasica caventis* Oed.) in marginal soil. *Biodiversitas* 7(1): 10-14.
- Widawati, S., Nurkanto, A. and Sudiana, I.M. 2008. Phosphate solubilizing activities of Actinomycetes isolated from Waigeo, Raja Ampat islands, West Papua. *Biodiversitas* 9(2): 87-90.
- Yang, M., Ding, G., Shi, L., Xu, F. and Meng, J. 2010. Detection of QTL for phosphorus efficiency at vegetative stage in Brassica napus. *Plant and Soil* 339 (1-2): 97-111. doi:10.1007/s11104-010-0516-x.
- Yu, X., Liu, X., Zhu, T.H., Liu, G.H. and Mao, C. 2011. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biology and Fertility of Soils* 47: 437-446.