Influence of *Streptomyces* sp. Kp109810 on Solubilization of Inorganic Phosphate and Growth of Maize (*Zea mays* L.)

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**Abstract:** Phosphorus plays a major role in plant growth, but only a small portion of phosphorus in soil is taken up by plants and the remaining part becomes unavailable for plants. Phosphate solubilizing microorganisms play a vital role in dissolving the complex form of phosphates to the accessible forms. The present paper reports the solubilization of tricalcium phosphate (TCP), single super phosphate (SSP), rock phosphate (RP), iron phosphate (FeP) and aluminum phosphate (AlP) by *Streptomyces* sp. KP109810 (S) with the production of organic acids as well as acid phosphatase. The amount of phosphate released (562 mg l\(^{-1}\)) was found to be the highest in the case of single super phosphate, as compared to the other phosphate sources. The highest phosphatase activity was observed in the presence of iron phosphate (0.033 Uml\(^{-1}\)). The proposed thin layer chromatography (TLC) method was used to analyze the organic acids in culture broths of the following P sources. The numbers of these organic acids were six organic acids from SSP, seven from RP, four organic acids from FeP and two for each of AlP and TCP. The effects of *Streptomyces* sp. KP109810 on P solubilization from different P sources, plant biomass production, and P content of maize (*Zea mays* L.) were examined in a greenhouse study. A complementary greenhouse experiment was conducted in pots by growing maize as a test crop. Our findings showed a great efficient promotion of maize growth and P content compared to uninoculated plants. Furthermore, single supper phosphate showed better results than rock phosphate, the latter performed comparably upon *Streptomyces* inoculation. These findings demonstrate that *Streptomyces* sp. KP109810 can improve crop growth and P nutrition.

**Keywords:** Streptomyces, TLC, Organic acids, Maize, PSB

**INTRODUCTION**

Phosphorus is one of the major essential plant macronutrient which plays a significant role in the development of roots, flowers and seed formation, helps in crop maturity and provides resistance to plant diseases (Khan and Jilani, 2009).

The function of certain key enzymes that are responsible for regulating metabolic pathways also depends on the availability of phosphorus (Tallapragada and Seshachala, 2012). Despite the high total soil P content, plant P availability is often reported to be limited (Collavino *et al.*, 2010). As a result, plants can utilize 10 to 15% of the P added as fertilizers during a year of application (Antoun, 2012). The remaining fractions are also rapidly converted into unavailable forms in the rhizosphere around the roots, without the expected impact on agricultural yield (Fernández *et al.*, 2012). These problems not only increase production costs, but also pollute the environment. Therefore, proper utilization of different P sources and improvements in their P-utilization efficiency are required to ensure sustainable food production and environmental protection across the globe.

The use of bioinoculants may be a better alternative and a complement to mineral fertilizers, since biological fertilizers could help to increase the availability of soil phosphorus, increase plant yield, minimization of harmful effects of phosphate fertilizers, reduce environmental pollution and promote sustainable agricultural development (Chen *et al.*, 2006). In the rhizosphere, a group of soil microorganisms known as phosphate solubilizing microorganisms (PSMs) play a key role to solubilize inorganic phosphates (Illmer and Schinner, 1995; Whitelaw, 1999). PSMs are divided into two groups (i) phosphate solubilizing bacteria (PSB) and (ii) phosphate solubilizing fungi (PSF) (Tallapragada and Seshachala, 2012). There are several previous reports about efficiency of PSBs alone or in combination with organic manures on different P sources for improving P release capacity or P solubilization (Harinasut *et al.*, 2003; Fernández *et al.*, 2007; Jain *et al.*, 2010; Harikrishnan *et al.*, 2014). Phosphate solubilizing bacteria (PSB), which are rhizobacteria have ability to convert complex form of phosphates to the accessible form through acidification, chelation, phosphatase enzyme and production of organic acids (Rodríguez and Fraga, 1999). Some organic acids produced by streptomycete strains were fumaric, tartic and succinic acids (Encheva-Malinova *et al.*, 2014). These organic acids chelate cations (Al, Fe, Ca) bound to mineral phosphate forms and convert them into soluble forms available to plants through their hydroxyl and carboxyl groups (Panhwar *et al.*, 2012). Many works recorded that the seed inoculation with PSB belonging to the genera *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Bacillus*, *Flavobacterium*, *Enterobacter*, *Serratia*, *Pseudomonas* and *Acinetobacter* improved growth, yield and P uptake in several crops (Hu *et al.*, 2010; Castagno *et al.*, 2011; Yu *et al.*, 2012).

The present study was undertaken to (i) establish relative potential rates of P solubilization of various phosphate sources by replacing tricalcium phosphate (present in Pikovskaya’s medium) with rock phosphate (RP), single super phosphate (SSP), iron phosphate (FeP) and aluminum phosphate (AlP) by *Streptomyces* sp. KP109810. (ii) Examine the contribution of organic acids produced by *Streptomyces* sp. KP109810 in solubilization of phosphates. (iii) explore the role of...
Phosphate solubilization in a liquid medium:

To determine phosphate solubilization, *Streptomyces* sp. KP109810 was screened on Pikovskaya’s (PKV) broth medium with five different phosphate sources tricalcium phosphate (TCP), single super phosphate (SSP), rock phosphate (RP), iron phosphate (FeP) and aluminum phosphate (AlP) at initial pH adjusted to 7.0 and incubated at 30°C for 15 days in a rotary shaker at 125 rpm. Un-inoculated flasks were served as controls. In order to remove bacterial cells and other insoluble materials, cultures were centrifuged at 10000 rpm for 20 min (Liu et al., 2011). Parameters such as phosphate solubilization, production of organic acid and phosphatase activity have been determined.

Phosphate estimation:

The Amount of phosphate released was determined by to Fiske-Subbarow method (Fiske and Subbarow, 1925). The supernatant (0.5 ml) was mixed with 1 ml of 2.5 M sulphuric acid and 2.5% ammonium molybdate. To the above mixture 1.0 ml of the reducing agent (0.2 g of 1.0 amino-2-naphthol-4 sulfonylic acid and 1.2 g of sodium sulfate in 100 ml distilled water) was added. The available phosphorus was calculated at 650 nm by a spectrophotometer and calibrated with a standard KH$_2$PO$_4$ curve. Phosphate concentration was expressed in term of mg/ml phosphate released in culture.

Determination of organic acids by thin-layer chromatography (TLC):

Qualitative detection of organic acids produced in the media was performed by TLC with a procedure described by Lee et al. (2001). A volume of 50 μl from supernatant of Streptomyces sp. KP109810 culture and several organic acids were spotted in the bottom of silica gel plate's 0.25 mm silica gel plate (20 x 20 cm, aluminum oxide 60 F254). Separation was performed with acetone-water-chloroform-ethanol-ammonium hydroxide (60:2:6:10:22) as the solvent system. The spotted TLC plate was then placed in the bottom of the chromatographic chamber to ensure a sufficient supply of solvent vapor and the chamber was closed. The development of the chromatogram was allowed to proceed until the solvent had traveled 6-7 cm beyond the starting line for 20 min at the room temperature. Finally, the plate was dried at 120°C for 15 min. The organic acids were detected by spraying an indicator solution of 0.25 g of methyl red and 0.25 g of bromophenol blue in 100 ml of 70% methanol and the color was developed by brief heating (1–3 min) in a hot dry oven 165°C.

Enzyme activity:

Phosphatase activity was determined spectrophotometrically by using p-nitrophenyl phosphate as substrate as described by Eivazi and Tabatabai (1977). Five hundred microlitres of the bacterial cell free culture supernatant was mixed with 1.0 ml of pNPP solution along with 1.0 ml of sodium acetate buffer pH 5.3. The reaction mixture was incubated at 40°C for 30 minutes. The reaction was inhibited by the addition of 2.0 ml of 0.05 M NaOH and the absorbance was measured in triplicate at 410 nm. The enzyme activity is defined as the amount of enzyme required to release p-nitrophenol per ml, per minute under standard conditions.

Greenhouse Experiment:

The greenhouse experiment was conducted in the summer season 2020 at the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. This experiment was conducted to evaluate the effect of *Streptomyces* sp. KP109810 as bio-inoculants on maize (*Zea mays L.*) fertilized with different P sources. Six treatments were used in this investigation: control (C), *Streptomyces* sp. KP109810 (S+C), single super phosphate (SSP), rock phosphate (RP), S+SSP and S+RP. Inoculum of *Streptomyces* sp. KP109810 (S) was prepared in Luria–Bertani liquid medium at 28°C for 48 h to get 10$^8$ CFU ml$^{-1}$. Maize seeds (*Zea Mays L.*) were surface sterilized with 1.0% sodium hypochlorite solution for 5 min, rinsed thrice in sterile distilled water and the seeds were soaked in 20 ml of inoculum for 1.0 h under a gentle shaking. During the same period of time the control seeds were submerged in deionized sterilized water. The soil used in this experiment was sieved through a 2 mm sieve then was autoclaved and filled in plastic pots (25 cm diameter and 35 cm depth) at the rate of 4 Kg pot$^{-1}$. For the experimental group, 200 ml of *Streptomyces* sp. KP109810 suspensions (10$^8$ CFU/mL) were mixed thoroughly with the soil for 2 min. Non-inoculated soil served as a control. Inoculated and non-inoculated seeds were planted in plastic pots (25 cm diameter and 35 cm depth) each pot was filled with loamy sand soil. Each pot was planted with 5 seeds and then thinned to three plants after 14 days. A completely randomized design with three replications was used. Phosphorus from different sources including RP and SSP was applied at the rate equivalent to 150 mg P kg$^{-1}$ soil.

The data on various growth parameters including, plant dry weight (g), plant height (cm), stem length (cm), root length (cm), plant fresh weight (g) and P-content (mg/plant) were determined after 45 days from sowing. Plant P-content in the vegetative tissue of a plant (shoot + leaves) was determined by the vanadomolydbdate phosphoric blue color method (Olsen and Sommers,1982).

Statistical Analysis:

Preliminary data for all experiments were statistically analyzed using the appropriate analysis of variance according to (Steel, 1997) using the one way analysis of variance. A Computer program software CoStat version 6.311 was used to analysis the data of all experiments. Least significant difference (LSD) at 5% level was used separately to evaluate the response of each character in each experiment.
RESULTS AND DISCUSSION

Phosphate solubilization:
Phosphorus solubilization efficiency of Streptomyces sp. KP109810 was performed in Pikovskaya’s broth supplemented with five phosphate sources TCP, SSP, RP, FeP and AlP after incubation period of 15 days. Figure (1), represents the ability of Streptomyces sp. KP109810 to dissolve insoluble phosphates for all the substrates. The results showed that the inoculation with Streptomyces sp. KP109810 has a positive effect on the five substrates. The amount of phosphate liberated reached its highest with SSP (562 mg l⁻¹) followed by TCP (423 mg l⁻¹). Rock phosphate was found to be better compared to FeP and AlP. These findings are in a good agreement with those obtained by Hamdali et al. (2008), who found that the levels of phosphate solubilized by Streptomyces griseus and Streptomyces cavourensis were 58.9 and 83.3 mg/100 ml respectively. In this respect, (Zhen Dong et al., 2013) isolated four P-solubilizing bacteria from the plant Anaphalis lacteal rhizosphere and their P-dissolving rates ranged from 65.24 to 315.36 mg/l. Similar studies have been reported earlier on solubilization of TCP, RP and AlP by different microorganisms (Kang et al., 2002; Nath et al., 2012). The solubilization of triple super phosphate by two Bacillus spp. PSB 9 and PSB 16 was reported by Panwar et al. (2016). Son et al. (2006) isolated a P-dissolving Pantoea from the soybean rhizosphere, and soluble P in its culturing medium reached to 900 mg/l.

![Fig (1): phosphate solubilization by Streptomyces sp. KP109810 in Pikovskaya's (PVK) broth media during 15 days. Tricalcium phosphate (TCP), single super phosphate (SSP), rock phosphate (RP), iron phosphate (FeP) and aluminum phosphate (AlP)](image)

Organic acid secretion during phosphate solubilisation:
Detection of organic acids in the culture broth of Streptomyces sp. KP109810 was observed through TLC during the solubilization of SSP, TCP, RP, FeP and AlP. Table (1) shows that the number and type of organic acids differed depending on the different sources of P. Numbers of organic acids produced during the solubilization of SSP, TCP, RP, FeP and AlP were 6, 2, 7, 4 and 2 acids, respectively. The production of organic acids during phosphate solubilization is a common phenomenon, and the type of acid produced depends on the type of phosphate source (Vyas and Gulati, 2009; Mardad et al., 2013). Our findings are consistent with Vazquez et al. (2000), who also reported the production of succinic acid, lactic acid, etc. Many papers reported that major organic acids were produced by actinobacteria such as citric acid, gluconic acid, lactic acid, malic acid, and oxalic acid (Chen et al., 2006; Jog et al., 2014; Yin et al., 2017).

Phosphatase activity
Phosphatase enzymes are believed to be very important in the uptake of phosphorus by maize plants. The highest phosphatase activity was found in the media amended with RP (0.091 Uml⁻¹), followed by AlP (0.071 Uml⁻¹), SSP (0.062 Uml⁻¹), TCP (0.042 Uml⁻¹) and the lowest activity was found by FeP (0.033 Uml⁻¹) (Fig. 2). Singh et al. (2000) stated that P. indica produced acid phosphatases to dissolve complex forms of phosphate present in rhizosphere to be more accessibility by plants. Roca et al. (2013) found that the presence of insoluble phosphate for which PSB can generate enzymes and organic acids to solubilize phosphorus was the reason behind greater alkaline phosphatase enzyme activity.
Increased plant height, plant fresh weight, shoot dry weight and root dry weight of maize plants were recorded with the plants inoculated with *Streptomyces* sp. KP109810. The plant height and plant weights (fresh and dry weights) were significantly ($p \leq 0.05$) increased by the applied P fertilizers in the presence or absence of *Streptomyces* sp. KP109810 (Fig. 3). The total biomass (shoot and root weights) of the plants grown in soil amended with S+RP was significantly ($p \leq 0.05$) greater compared to RP alone. Similarly, SSP with S displayed higher values compared to their sole application without S. It was observed that for most of the growth characteristics of the plants co-inoculated with S and amended with SSP displayed significantly ($p \leq 0.05$) higher values than those supplemented with inorganic P fertilizer (RP+S). An increase in P content was observed in plants inoculated with S. The P-uptake of maize was significantly ($p \leq 0.05$) higher in plants treated with RP compared to those grown in the control (Fig. 4). Application of S with different P sources showed significantly ($p \leq 0.05$) increase in plant P content compared to the treatments without S. It was recorded that plants treated with SSP displayed higher P-content compared to those treated with RP (Fig. 4). The P-content in plants treated with RP without S was 6.8 mg plant$^{-1}$ that had been increased to 11.5 mg plant$^{-1}$ when treated with S. Increased growth and P content of several crop plants due to PSB inoculation have been reported in many studies conducted under both growth chamber and greenhouse conditions (Tao et al., 2017; Yadav et al., 2017; Danso Marfo et al., 2019). Yin et al. (2017) also reported detection of seven to eight organic acids following the application of PSBs that resulted in the solubilization of P and also had a positive effect on plant growth. Afzal and Bano, (2008) who reported that wheat (*Triticum aestivum*) inoculated with *Pseudomonas* sp. strain 54RB significantly increased plant height, root and shoot weight, spike length, grain and P uptake compared with the un-inoculated control. Similar results were also found in cowpea (*Vigna unguiculata*) which enhance the nodulation, root and shoot biomass, straw and grain yield and P and N uptake of plants inoculated with *Glucoseacetobacter* sp. and *Burkholderia* sp. (Linu et al., 2009).
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**Fig (3):** The effect of *Streptomyces* sp. KP109810 (A) plant height (cm), (B) plant fresh weight (g), (C) shoot dry weight (g) and (D) root dry weight (g) of maize plants with different phosphate sources after 50 days from inoculation. Control (C), *Streptomyces* sp. KP109810 (S+C), single super phosphate (SSP), rock phosphate (RP).
Yield and P utilization efficiency of applied P of P solubilizing bacteria are recommended as a high cost chemical P fertilizers, and environmental benefits for avoiding environmental issues caused due to mineral P fertilizer application. Hence, application of P solubilizing bacteria are recommended as a sustainable way for increasing P solubilization from insoluble RP and other P sources, and improving crop yield and P utilization efficiency of applied P fertilizers. These results are recommended to be evaluated under various soils and environmental conditions before using this actinobacterial strain as a biofertilizer.

CONCLUSION

From the results presented here, it is reported the solubilization of five different inorganic phosphate sources namely TCP, RP, SSP, FeP and AlP by the actinobacterial strain Streptomyces sp. KP109810 differed significantly depending on the nature of phosphate source. Many organic acids present in the medium were detected by TLC and the organic acids production differed according to the different sources of P. Phosphatase activity was reported in Streptomyces sp. KP109810 with a maximum activity of 0.091 U/mL by RP sample. Our results suggest that Streptomyces sp. KP109810 were effective in solubilization of RP, thus, improving its capacity to release P for cultivated plants. The application of this actinobacterial strain had beneficial effects on growth, yield and P nutrition on maize plants. These results have an agronomic importance for crop cultivation, economic importance for saving money involved in high cost chemical P fertilizers, and environmental benefits for avoiding environmental issues caused due to mineral P fertilizer application. Hence, application of P solubilizing bacteria are recommended as a sustainable way for increasing P solubilization from insoluble RP and other P sources, and improving crop yield and P utilization efficiency of applied P fertilizers. These results are recommended to be evaluated under various soils and environmental conditions before using this actinobacterial strain as a biofertilizer.

REFERENCES

Afzal, A. and A. Bano (2008). Rhizobium and Phosphate Solubilizing Bacteria Improve the Yield and Phosphorus Uptake in Wheat (Triticum aestivum), Int. J. Agri. Biol., 10: 1560-8530.

Antoun, H. (2012). Beneficial Microorganisms for the Sustainable Use of Phosphates in Agriculture, Procedia Engineering, 46: 62-67.

Castagno, L., M. Estrella, A. Sannazzaro, A. Grassano, and O. Ruiz (2011). Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by Pantoea eucalypti isolated from Lotus tenuis rhizosphere in the Salado River Basin (Argentina), Journal of Applied Microbiology, 110 (5): 1151-1165. doi: 10.1111/j.1365-2672.2011.04968.x.

Chen, Y. P., P. D. Rekha, A. B. Arun, F. T. Shen, W.-A. Lai, and C. C. Young (2006). Phosphate-solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities, Applied Soil Ecology, 34(1): 33-41. doi: 10.1016/j.apsoil.2005.12.002.

Collavino, M. M., P. A. Sansberro, L. A. Mrogsinski, and O. M. Aguilar (2010). Comparison of in vitro solubilization activity of diverse phosphate-solubilizing bacteria native to acid soil and their ability to promote Phaseolus vulgaris growth, Biology and Fertility of soils, 46(7): 727-738.

Danso Marfo, T., R. Datta, V. Vranová, and A. Ekielski (2019). Ecotone Dynamics and Stability from Soil Perspective: Forest-Agriculture Land Transition.

Eivazi, F. and M. A. Tabatabai (1977). Phosphatases in soils, Soil Biology and Biochemistry, 9(3): 167-172, doi: 10.1016/0038-0717(77)90070-0.

Encheva-Malinova, M., M. Stoyanova, H. Avramova, Y. Pavlova, B. Gocheva, I. Ivanova, and P. Moncheva (2014). Antibacterial potential of streptomyecete strains from Antarctic soils, Biotechnology & Biotechnological Equipment, 28(4): 721-727. doi: 10.1080/13102818.2014.947066.

Fig (4): The effect of Streptomyces sp. KP109810 inoculation mineral P addition on P-content (mg/plant) of maize plants with different phosphate sources after 50 days from inoculation. This investigation; control (C), Streptomyces sp. KP109810 (S+C), single super phosphate (SSP), rock phosphate (RP) *, Different letters mean significant difference between treatments
Influence of *Streptomyces* sp. Kp109810 on Solubilization of Inorganic Phosphate and Growth of Maize (*Zea mays L.*)

Fernández, L., B. Agarás, P. Zalba, L. G. Wall and C. Valverde (2012). *Pseudomonas* spp. isolates with high phosphate-mobilizing potential and root colonization properties from agricultural bulk soils under no-till management, Biology and fertility of soils, 48(7): 763-773.

Fernández, L. A., P. Zalba, M. A. Gómez and M. A. Sagardoy (2007). Phosphate-solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions, Biol Fertil Soils, 43(6): 805-809, doi: 10.1007/s00374-007-0172-3.

Fiske, C. H. and Y. Subbarow (1925). The Colorimetric Determination of Phosphorus, J. Biol. Chem., 66(2): 375-400.

Hamdali, H., B. Bouzizgarne, M. Hafidi, A. Lebrihi, M. J. Virolle and Y. Ouhdouch (2008). Screening for rock phosphate solubilizing Actinomycetes from Moroccan phosphate mines, Applied Soil Ecology, 38(1): 12-19. doi: 10.1016/j.soilbio.2007.08.007.

Haririkrishnan, H., V. Shanmugaiah, N. Balasubramanian, M. P. Sharma and S. O. Kotchoni (2014). Antagonistic potential of native strain *Streptomyces aureantiogriseus* VSMGT1014 against sheath blight of rice disease, World J Microbiol Biotechnol., 30(12): 3149-3161, doi: 10.1007/s11274-014-1742-9.

Harinasut, P., D. Poonsopa, K. Roengmongkol and R. Charoensataporn (2003). Salinity effects on antioxidant enzymes in mulberry cultivar, Science Asia, 29(2): 109-113.

Hu, X.-J., Z.-J. Li, Y.-C. Cao, J. Zhang, Y.-X. Gong and Y.-F. Yang (2010). Isolation and identification of a phosphate-solubilizing bacterium *Pantoea stewartii* subsp. stewartii g6, and effects of temperature, salinity, and pH on its growth under indoor culture conditions, Aquaculture International, 6(18): 1079-1091, doi: 10.1007/s10499-010-9325-8.

Illmer, P. and F. Schinner (1995). Solubilization of inorganic calcium phosphates-Solubilization mechanisms, Soil Biology and Biochemistry, 27(3): 257-263. doi: 10.1016/0038-0717(94)00190-C.

Jain, R., J. Saxena and V. Sharma (2010). The evaluation of free and encapsulated *Aspergillus awamori* for phosphate solubilization in fermentation and soil-plant system, Applied Soil Ecology, 46(1): 90-94. doi: 10.1016/j.soilbio.2010.06.008.

Jog, R., M. Pandya, G. Nareshkumar and S. Rajkumar (2014). Mechanism of phosphate solubilization and antifungal activity of *Streptomyces* spp. isolated from wheat roots and rhizosphere and their application in improving plant growth, Microbiology (Reading, England), 160(Pt 4): 778-788. doi: 10.1099/mic.0.074146-0.

Kang, S. C., C. G. Ha, T. G. Lee and D. K. Maheshwari (2002). Solubilization of insoluble inorganic phosphates by a soil-inhabiting fungus *Fomitopsis* sp. PS 102, Current Science, 82(4): 439-442.

Khan, A. and G. Jilani (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. Agric. Biol. Sci., 1(1): 48-58.

Lee, K.-Y., J. -S. So and T. -R. Heo (2001). Thin layer chromatographic determination of organic acids for rapid identification of bifidobacteria at genus level, Journal of Microbiological Methods, 45(1): 1-6. doi: 10.1016/S0167-7012(01)00214-7.

Lin, M. S., J. Stephen and M. S. Jisha (2009). Phosphate solubilizing *Glucosacetobacter* sp., *Burkholderia* sp. and their potential interaction with cowpea (*Vigna unguiculata* (L.) Walp.), International Journal of Agricultural Research, 4(2): 79-87.

Liu, H., X.-Q. Wu, J.-H. Ren and J.-R. Ye (2011). Isolation and Identification of Phosphobacteria in Poplar Rhizosphere from Different Regions of China, Pedosphere, 1(21): 90-97. doi: 10.1006/s1016/010(10)00383-5.

Mardad, I., A. Serrano and A. Soukri (2013). Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit., African Journal of Microbiology Research, 7(8): 626-635.

Nath, R., G. D. Sharma and M. Barooah (2012). Efficiency of Tricalcium Phosphate Solubilization by Two Different Endophytic *Penicillium* sp. Isolated from Tea (*Camellia sinensis* L.), Eur. J. Exp. Biol., 2(4): 1354-1358.

Olsen, S. R. and L. E. Sommers (1982). Phosphorus in AL Page, (Ed). Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monographs, 9(2).

Panhwar, Q. A., R. Othman, Z. A. Rahman, S. Meon and M. R. Ismail (2012). Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice., African Journal of Biotechnology, 11(11): 2711-2719.

Panwar, M., R. Tewari and H. Nayyar (2016). Native halo-tolerant plant growth promoting rhizobacteria *Enterococcus* and *Pantoea* sp. improve seed yield of Mungbean (*Vigna radiata* L.) under soil salinity by reducing sodium uptake and stress injury, Physiol. Mol. Biol. Plants, 22(4): 445-459, doi: 10.1007/s12298-016-0376-9.

Roca, A., P. Pizarro-Tobías, Z. Udaondo, M. Fernández, M. Matilla, A. Molina-Henares, L. Molina, A. Segura, E. Duque and J.-L. Ramos (2013). Analysis of the plant growth-promoting properties encoded by the genome of the rhizobacterium *Pseudomonas putida* BIRD-1, Environmental Microbiology, 15(3): 780-794, doi: 10.1111/1462-2920.12037.

Rodriguez, H. and R. Fraga (1999). Phosphate solubilizing bacteria and their role in plant
Tallapragada, P. and U. Seshachalla (2012). Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India., Turkish Journal of Biology. 36(1).

Tao, J., X. Liu, Y. Liang, J. Niu, Y. Xiao, Y. Gu, L. Ma, D. Meng, Y. Zhang, W. Huang, et al. (2017). Maize growth responses to soil microbes and soil properties after fertilization under controlled environment by phosphate in vitro and plant growth promotion in maize. Microbiology and Biotechnology, 101(3): 1289-1299.

Vyas, P. and A. Gulati (2009). Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing microbes. Applied Microbiology and Biotechnology, 82(3): 959-966.

Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India., Turkish Journal of Biology. 36(1).

S. Cha, and M. R. Kim (2013). Screening, identification and characterization of fluorescent Pseudomonas isolated from the rhizosphere of Zea mays (maize). Acta Prataculturae Sinica, 22(6): 150-158.

Ma, D. Meng, Y. Zhang, W. Huang, et al. (2017). Maize growth responses to soil microbes and soil properties after fertilization under controlled environment by phosphate in vitro and plant growth promotion in maize. Microbiology and Biotechnology, 101(3): 1289-1299.

Vyas, P. and A. Gulati (2009). Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing microbes. Applied Microbiology and Biotechnology, 82(3): 959-966.

Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India., Turkish Journal of Biology. 36(1).

S. Cha, and M. R. Kim (2013). Screening, identification and characterization of fluorescent Pseudomonas isolated from the rhizosphere of Zea mays (maize). Acta Prataculturae Sinica, 22(6): 150-158.

Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India., Turkish Journal of Biology. 36(1).

S. Cha, and M. R. Kim (2013). Screening, identification and characterization of fluorescent Pseudomonas isolated from the rhizosphere of Zea mays (maize). Acta Prataculturae Sinica, 22(6): 150-158.

Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India., Turkish Journal of Biology. 36(1).

S. Cha, and M. R. Kim (2013). Screening, identification and characterization of fluorescent Pseudomonas isolated from the rhizosphere of Zea mays (maize). Acta Prataculturae Sinica, 22(6): 150-158.