Saudi Journal of Biological Sciences (2012) **19**, 55–63

King Saud University
Saudi Journal of Biological Sciences
www.ksu.edu.sa
www.sciencedirect.com

الجمعية السعودية لعلوم الحياة
SAUDI BIOLOGICAL SOCIETY

ORIGINAL ARTICLE

Impact of rock materials and biofertilizations on P and K availability for maize (*Zea Maize*) under calcareous soil conditions

I.I. Abou-el-Seoud [a,b], **A. Abdel-Megeed** [c,d,*]

[a] *Department of Soil and Agriculture Chemistry, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt*
[b] *The National Plan for Sciences and Technology, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[c] *Department of Botany and Microbiology, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[d] *Department of Plant Protection, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt*

Received 18 May 2011; revised 12 September 2011; accepted 17 September 2011
Available online 24 September 2011

KEYWORDS
Maize plant;
Phosphate and potassium solubilizing bacteria;
Rock P and K

Abstract The present work evaluated the synergistic effects of soil fertilization with rock P and K materials and co-inoculation with P and K-dissolving bacteria [PDB (*Bacillus megaterium* var. *phosphaticum*) and KDB (*Bacillus mucilaginosus* and *B. subtilis*)] on the improvement of P and K uptake, P and K availability and growth of maize plant grown under limited P and K soil conditions (calcareous soil). The experiment was establishment with eight treatments: without rock P and K materials or bacteria inoculation (control), rock P (RP), rock K (RK), RP + PDB, RK + KDB and R(P + K) + (P + K)DB. Under the same conditions of this study, co-inoculation of PDB and KDB in conjunction with direct application of rock P and K materials (R(P + K)) into the soil increased P and K availability and uptake, and the plant growth (shoot and root growth) of maize plants grown on P and K limited soils.

1. Introduction

Deficiency in plant-available phosphorus and Potassium is considered to be a major limiting factor to food production in many Agricultural soils (Xie, 1998). Most of the Egyptian desert soils are poor or marginal in nutrient status and require adequate fertilization to sustain high productivity (Badr et al., 2006). Phosphate and potassium are major essential macronutrients for plant growth and development and soluble P and K fertilizers are commonly applied to replace removed minerals and to optimize yield. When phosphate is added into soils as a fertilizer in relatively soluble and plant-available forms, it is easily converted into insoluble complexes with calcium carbonate, aluminum and iron oxides, and crystalline and amorphous aluminum

* Corresponding author at: Department of Botany and Microbiology, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia. Tel.: +966 531053638.
E-mail address: mfarrag83@yahoo.com (A. Abdel-Megeed).

Peer review under responsibility of King Saud University.
doi:10.1016/j.sjbs.2011.09.001

ELSEVIER
Production and hosting by Elsevier

silicate (Sample et al., 1980). Consequently, to achieve optimum crop yields, soluble phosphate fertilizers have to be applied at high rates which cause unmanageable excess of phosphate application and environmental and economic problems (Brady, 1990; Akande et al., 2008). Potassium is essential in all cell metabolic processes. Consequently, K deficiencies become a problem because K decreases easily in soils due to crop uptake, runoff, leaching and soil erosion (Sheng and Huang, 2002). Direct application of rock phosphate (rock P) and potassium (rock K) materials may be agronomically more useful and environmentally more feasible than soluble P and K (Rajan et al., 1996; Ranawat et al., 2009). Rock P and K materials are cheaper sources of P and K; however, most of them are not readily available to a plant because the minerals are released slowly and their use as fertilizer often causes insignificant yield increases of current crop (Zapata and Roy, 2004). The use of plant growth promoting rhizobacteria (PGPR), including phosphate and potassium solubilizing bacteria (PSB and KSB) as biofertilization, was suggested as a sustainable solution to improve of plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors (Vessey, 2003; Sheng, 2005; Setiawati and Handayanto, 2010; Ekin, 2010). On the other hand, use of biofertilization on Egyptian soils has decreased the pH, which had led to increased availability of trace elements that enhance plant growth (Mahfouz and Sharaf-Eldin, 2007). PSB have been used to improve rock P value because they convert insoluble rock P into soluble forms available for plant growth (Nahas et al., 1990; Bojinova et al., 1997; Shivay, 2010). This conversion is through acidification, chelation and exchange reactions (Gerke, 1992) and produces, in the periplasm, strong organic acids (Alexander, 1997), which have become indicators for routine isolation and selection procedures of PSB (Illmer et al., 1995). The PDB may release several organic acids (including oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, glyconic, fumaric, adipic, and 2-ketogluconic acid (Moghimi et al., 1978; Leyval and Berthelin, 1989); inorganic acids, as well as CO_2 (Zayed, 1998), and acid phosphatase which play a major role in the mineralization of organic phosphorus in soil (Rodriguez and Fraga, 1999; Marschner et al., 2010). *Bacillus megaterium* var. *phosphaticum* is known for its ability to solubilize rock P material (Schilling et al., 1998; Rodriguez and Fraga, 1999). In addition *B. megaterium* can also improve root development and increase the rate of water and mineral uptake (Alexander, 1997).

Fundamentally, KSB is a heterotrophic bacteria which is obtaining all their energy and cellular carbon from pre-existing organic material. Thus, they are important in the formation of humus in soil, the cycling of other minerals tied up in organic matter, and the prevention of build up of dead organic materials (Zakaria, 2009). Besides, KSB is an aerobic bacteria which play an important role in maintaining soil structure by their contribution in the formation and stabilization of water-stable soil aggregates (Zakaria, 2009). In addition, these Gram positive bacteria can produce substance that stimulates plant growth or inhibits root pathogens (Zakaria, 2009). Moreover, KSB are able to solubilize rock K mineral powder, such as micas, illite and orthoclases (feldspar), also through production and excretion of organic acids (Friedrich et al., 1991; Ullman et al., 1996) or chelate silicon ions to bring the K into solution (Groudev, 1987; Friedrich et al., 1991; Ullman et al., 1996; Bennett et al., 1998). It was shown that KSB, such as *Bacillus mucilaginosus*, increased K availability in soils and increased mineral content in plant (Sheng et al., 2002; Zakaria, 2009). Also, Vandevivere et al. (1994) proposed that *B. mucilaginosus* increases the dissolution rate of silicate and alumino-2 silicate minerals and releases the K^+ and SiO_2 from the crystal lattice primarily by generating organic acids. However, this hypothesis is controversial and *B. mucilaginosus* is also thought to accelerate the dissolution of a variety of silicates by the production of extracellular polysaccharides (EPS) (Welch and Ullman, 1999). The dispute about the mechanism by which *B. mucilaginosus* decomposes silicate minerals and releases K^+ and SiO_2 may have severely limited the use of the organism in Agriculture as a form of biological K fertilizer. Recently, Liu et al. (2006) proved that the polysaccharides strongly adsorbed the organic acids and attached to the surface of the mineral, resulting in an area of high concentration of organic acids near the mineral. They indicated also that the EPS adsorbed SiO_2 and this affected the equilibrium between the mineral and fluid phases and led to the reaction toward SiO_2 and K^+ solubilization.

An integrated application of rock P and K materials with co-inoculation of bacteria that solubilize them might provide faster and continuous supply of P and K for optimal plant growth (Girgis, 2006; Eweda et al., 2007). However, little is known about the combined effects of rock materials and co-inoculation of PSB and KSB on mineral availability in calcareous soils (with high pH and high $CaCO_3$ content), mineral content and growth of maize (*Zea maize*).

2. Materials and methods

The present work evaluated the synergistic effects of soil fertilization with rock P and K materials and co-inoculation with P and K-solubilizing bacteria (PSB and KSB) on the improvement of P and K uptake, P and K availability and growth of maize plant grown under limited P and K soil conditions.

2.1. Soil preparation

The soil used in this work was collected from Burg El-Arab City, Alexandria, Egypt, from the surface layer to plow depth (down to 20 cm). The soil was air-dried, sieved through a 2 mm sieve to homogenize and separate roots from soil. The chemical properties of the soil were as follows: pH (1:1 *w/v* water) 8.12, EC (1:1) 2.30 dS/m, organic matter 0.41%, $CaCO_3$ 26.18%, available nitrogen 17.20 mg/kg soil, available P 6.5 mg/kg soil (Olsen), total P 0.45 g/kg soil, available K 49.65 mg/kg soil (1 M NH_4-OAc) and total K 3.10 g/kg soil. The soil properties were determined according to the methods described by Black (1965). Basal applications of N and Mg fertilizers were corporate with each kg soil at a rate of 150 mg N as NH_4NO_3, 40 mg Mg as $MgSO_4$ per kg soil. The N fertilizer was applied in splits as required in the time course for plant growth at the rate of 50 mg/20 ml water for each pot.v

2.2. Bacteria materials

We used two strains of plant growth promoting rhizobacteria (PGPR) in these experiments. For PSB, *B. megaterium* var. *phosphaticum* (a commercial product) was obtained from Hanover, Germany, added at the rate of 50 ml/plant (200 g powder/100 l water as recommended). For KDB, *B. mucilaginosus* and *B. subtilis* (a commercial product) were obtained from Hanover, Germany, added at the rate of 100 ml/plant (200 g powder/100 l water as recommended).

2.3. Rock P and K materials

A low grade rock phosphate (RP) and rock potassium (RK) samples from a sedimentary rock materials deposit supplied as raw mining are after grinding to a fine powder to pass through a 400- mesh standard sieve by Al Ahram mining and natural fertilizer company in Egypt. RP as apatite powder contains (total P 11.29% and total K 0.16%). RK as feldspar and illite powder contains (total K 5.29% and total P 0.03%).

2.4. Pot experiment

Each plastic pot (Ø = 15 cm and 12 cm deep) was uniformly filled with 1000 g of the prepared soil and compacted to bulk density of about 1.37 g cm^{-3}. One week before planting, all pots were watered to the volumetric moisture content 0.25 cm^3 cm^{-3}, which corresponded to the field capacity. The experiment was established with 8 treatments: without rock P and K materials or bacteria inoculation (control), rock P (3 g/kg soil), rock K (3 g/kg soil), rock P + PSB, rock K + KSB and R (P + K) + (P + K)SB. Rock materials were mixed thoroughly with the soil in a plastic pot. Uniform seedlings of corn (*Zea maize*) each having two or three pairs of fully grown leaves, were selected and transplanted into the pots at the rate of two seedlings per pot. All plants were harvested 23 days after transplanting and the shoots were separated from roots.

2.5. Nutrient content

The shoots were then dried at 70 °C/48 h (Steyn, 1959) to constant weight in a forced-draft oven for 48 h and then weighted (recorded) and milled for analysis. Samples of plant material were wet digested with H_2SO_4–H_2O_2 (Lowther, 1980). Phosphorus content was determined by the vanadomolybdophosphoric method (Jackson, 1967). Potassium content was determined using the Flame spectro-photometer (Jackson, 1973).

Phosphorus was extracted from soil samples at harvest by the sodium bicarbonate (0.5 N) method according to Olsen et al. (1954) and determined by the ascorbic acid–molybdenum blue method at wave length of 406 nm as described by Murphy and Riley (1962). Available K was extracted from soil samples at harvest by the 1 M NH_4-OAc (pH 7) method and determined using the Flame spectro-photometer (Jackson, 1973).

2.6. Quantifying roots length

Plant roots were removed from each pot and separated from soil by washing them under a jet of tap water on a 0.5 mm sieve. Excess moisture was blotted from the cleaned roots by wrapping up the roots in layers of paper towel for 3 min (Schenk and Barber, 1979). For each pot three samples of 0.3 g fresh weight were used for determination of root length by the line intersect method of Tannant (1975).

$$RL = \frac{11}{14} \times N \times G$$

where RL = root length, N = sum of horizontal and vertical crossing, G = length of the grid unit (2 cm or 1 cm). Surface area of a 1 cm root cylinder (SAC) was calculated as follows:

$$\mathrm{SAC} = 2\pi \times r_0$$

where SAC = surface area of the root cylinder, r_0 = root radius. Mean half distance between neighboring roots (r_1) was calculated according to the following formula (Schenk and Barber, 1979):

$$r_1 = \sqrt{\frac{V}{\pi RL}}$$

where V = volume of the soil in the pot (cm^{-3}), RL = root length per pot (cm).

2.7. Statistical methods

The experiment was structured following a randomized complete block design (RCBD) with five replications. Data were analyzed by using analysis of variance in SAS (SAS institute Inc., Cary, USA, 1996). The Tukey test was used to compare treatment means. A significance level of $\alpha = 0.05$ was used in all analysis.

3. Results and discussions

3.1. Available P and K in the soil

The term available nutrients is often used to describe the amount of soil nutrients that can be extracted from solution or taken up by plant roots and utilized by the plant to growth and develop during its life cycle (Setiawati and Handayanto, 2010).

All the treatments under the present study increased the available P and K significantly compared with the untreated plants (control) except, the rock potassium (RK) treatment with available P and rock phosphate (RP) treatment with available K where there was a slight non-significant increase (Fig. 1). The available P of the following treatments (RP, PDB, KDB, RP + PDB, RK + KDB, R(P + K) + (P + K)DB) was increased by about 9%, 65%, 24%, 75%, 51% and 117%, respectively, as compared with the control (without the bacterial inoculum and without the rock material fertilizer). On the other hand, the available K of (RK, PDB, KDB, RP + PDB, RK + KDB, R(P + K) + (P + K)DB) was increased by about 32%, 6%, 58%, 8%, 80% and 82%, respectively, as compared with the control. From the previous results, applied together, mix-inoculation and rock P and K minerals, resulted in the highest P and K available in the soil compared with the other treatments (Fig. 1).

Increasing the bioavailability of P and K in the soils with combined inoculation and rock materials has been reported by many researchers (Schilling et al., 1998; Lin et al., 2002; Han and Lee, 2005; Han et al., 2006; Marschner, 2009), which may lead to increased P and K uptake and plant growth (Han et al., 2006; Chen et al., 2006; Eweda et al., 2007; Jorquera et al., 2008; Sabannavar and Lakshman, 2009). Several mechanisms have been proposed to explain the phosphate solubilization by these microorganisms; they are associated with the release of organic and inorganic acids (Richardson, 2001; Marschner et al., 2010). In addition, the release of phosphatase enzymes that mineralize organic phosphate compounds has also been suggested as another mechanism involved (Marschner, 1995; Takano et al., 2006). Since, microbial produced organic ligands include metabolic byproducts, extracellular

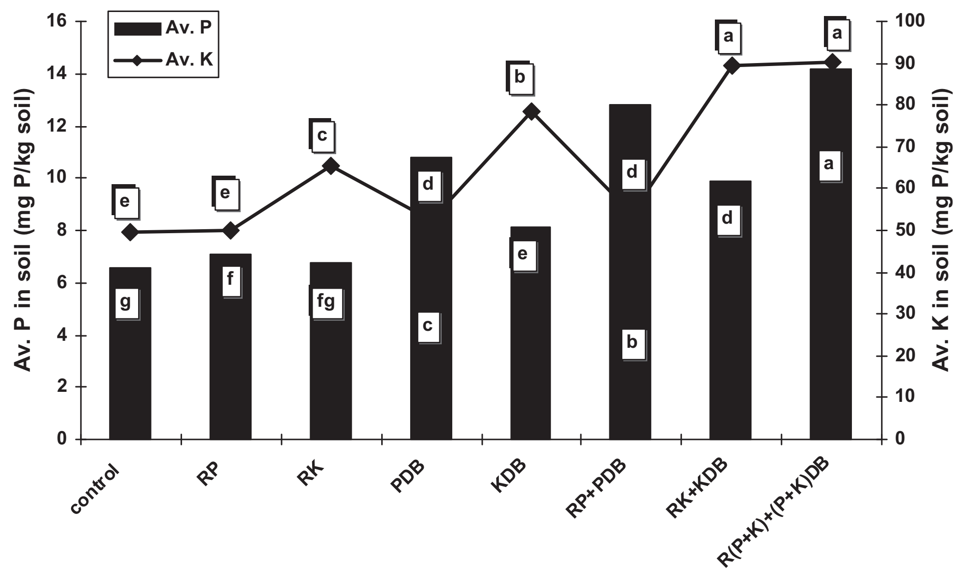

Figure 1 Available phosphorus and potassium in soil (mg/kg soil) as affected by rock minerals and inoculation with P and K dissolving bacteria (Av. = available; RP = rock phosphate; RK = rock potassium; PDB = phosphate dissolving bacteria and KDB = potassium dissolving bacteria; different letters indicate significant differences, $P \leqslant 0.05$).

enzymes, chelates, and both simple and complex organic acids. These substances can influence feldspar dissolution rates either by decreasing pH (Richardson, 2001; Chen et al., 2006), forming framework destabilizing surface complexes, or by complexing metals in the solution (Stillings et al., 1996). Vandevivere et al. (1994) proposed that *B. mucilaginosus* increases the dissolution rate of silicate and alumino-silicate minerals and releases the K^+ from the crystal lattice primarily by generating organic acids. However, this hypothesis is controversial and *B. mucilaginosus* is also thought to accelerate the dissolution of a variety of silicates by the production of extracellular polysaccharides (EPS) (Welch and Ullman, 1999).

The dispute about the mechanism by which *B. mucilaginosus* decomposes silicate minerals and releases K^+ may have severely limited the use of the organism in agriculture as a form of biological K fertilizer.

In general, phosphate and potassium solubilizing bacteria (PSB) and/or (KSB) play an important role in reducing nutrients deficiency in soil (Aipova et al., 2010).

3.2. Plant growth

3.2.1. Shoot dry weight

The shoot dry weight of all treatments of maize plants were increased significantly compared with the control (Fig. 2). The treatment which combined both bacteria and mineral rocks R(P + K)+(P + K)DB was obtained with the highest significant increase compared with the control and other treatments. Similarly, Han et al. (2006) showed, an integrated application of rock P and K materials with co-inoculation of both bacteria (P + K)DB that solubilize them and might provide faster and continuous supply of P and K for optimal plant growth. This treatment was increased by 52% over the control, and followed by (RP + PDB) and (RK + KDB) which increased by about 38% and 28%, respectively, compared with the control. In the same line, Han et al. (2006) found that combined PSB inoculation with application of rock P consistently increased shoot and root dry weight as compared to control. Furthermore, the dry weight of maize plants inoculated with bacteria alone (PDB) and KDB increased by 26% and 23%, respectively, compared to non-inoculated treatment. Growth enhancement by bacteria may relate to its ability to produce extensive root length (Sheng and Huang, 2001) and can also improve root development and increase the rate of water and mineral uptake (Alexander, 1997; Saghir et al., 2007). On the other hand, Ibrahim et al. (2010) discussed the increase in the growth of the biofertilized trees may be due to the ability of *B. megaterium* to produce some growth promoting substances such as IAA, gibberellins and abscisic acid, it is also well known that *B. megaterium* produces organic, inorganic acids and CO_2 which lead to increase in soil acidity and consequently convert the insoluble forms of phosphorus into soluble ones (Alexander, 1997; Wani et al., 2007). In addition, increasing plant dry matter due to inoculation with PDB was attributed to the reduction of media pH and hence the solubility of phosphates (Kucey, 1988; Adesemoye and Kloepper, 2009).

Also, Badr et al. (2006) found that the dry matter of sorghum plants inoculated with silicate dissolving bacteria (SBS strain) and supplied with minerals (feldspar and rock phosphate) increased by 48%, 65% and 58% for clay, sandy and calcareous soil, respectively, compared to the plants supplied with minerals alone. These results are in agreement with those of Gad (2001) who reported that biofertilization on plants increased growth and yield.

3.2.2. Root growth

Plants having long and extensive root system can explore large volume of the soil and take up more nutrients than those with short roots. Generally, a healthy root growth is the basis for maintaining a high efficiency of nutrient acquisition, shoot growth and yield. The present data in Fig. 3 indicated that, there was highly significant increase in root length of maize plants treated with rock minerals alone (RP or RK), bacterial strain (PDB or KDB) and combined both bacteria and mineral

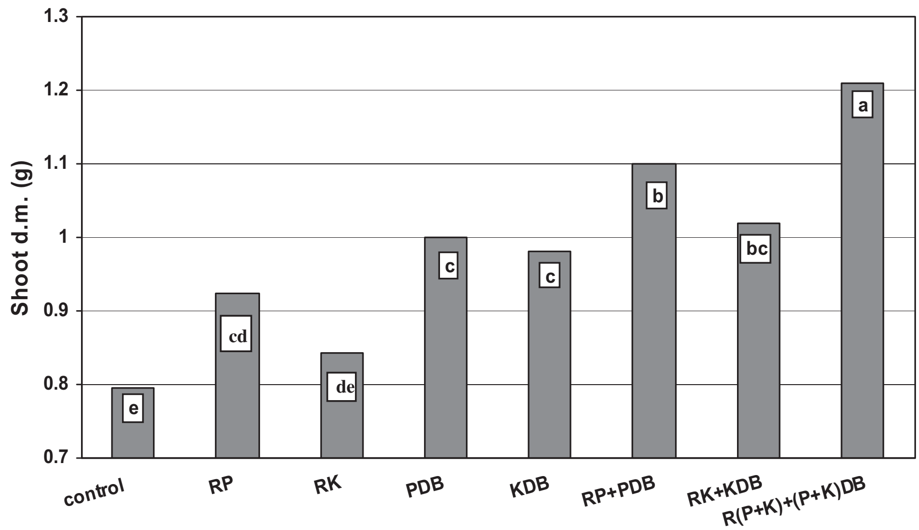

Figure 2 Shoot dry weight of maize plant as affected by rock minerals and inoculation with P and K-dissolving bacteria (d.m. = dry matter; RP = rock phosphate; RK = rock potassium; PDB = phosphate dissolving bacteria and KDB = potassium dissolving bacteria; different letters indicate significant differences, $P \leqslant 0.05$).

rocks compared with the untreated plants (control). The root length of maize plants treated with RP, RK, PDB, KDB, (RP + PDB), (RK + KDB), and R(P + K) + (R + K)DB increased by about 7%, 4%, 16%, 13%, 23%, 22.6%, and 30%, respectively, compared with control. Similarly, Artursson et al. (2006) and Marschner et al. (2010) reported that the treatments inoculated with bacteria significantly increased root growth, compared with their controls which were not inoculated. Also, Abou El Seoud et al. (2009) reported that the PDB have a significant effect on root yield. The root yield of sugar beet varieties Lados and TWS 1436 inoculated with PDB were higher root yield by about 19.8% and 20.2% than Lados and TWS 1436 without inoculation, respectively.

On the other hand, there was no significant difference between root length of maize plants treated with (RP + PDB) and (RK + KDB), between (PDB and KDB), and between (RP and RK). In other words, there was slight non-significant increase in root length between maize plants treated with (RP + PDB) and (RK + KDB), between (PDB and KDB), and between (RP and RK) (Fig. 3). The results in Table 1 clearly show that root surface area (SA, cm^2) of maize plants treatments increased compared with the untreated plants (control). The root surface area of maize plants treated with R(P + K) + (P + K)DB increased by about 40% compared with the control. Similarly, Amer et al. (2010) the increase in root surface area of common bean plants inoculated with

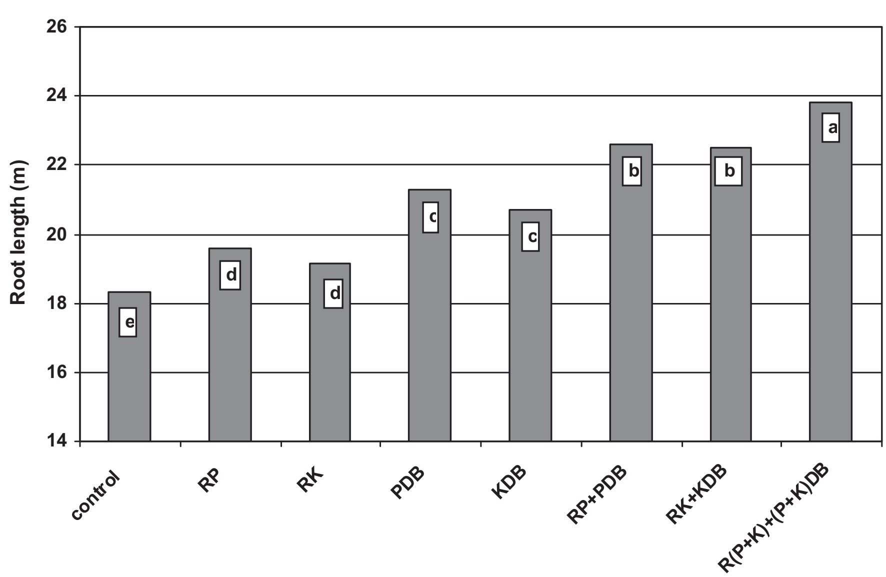

Figure 3 Root length (*m*) of maize plant as affected by rock minerals and inoculation with P and K-dissolving bacteria (RP = rock phosphate; RK = rock potassium; PDB = phosphate dissolving bacteria and KDB = potassium dissolving bacteria; different letters indicate significant differences, $P \leqslant 0.05$).

Table 1 Root surface area (SA), root radius (r0) and mean half distance between roots (r1) of maize plants as affected by rock minerals and inoculation with P and K dissolving bacteria (RP = rock phosphate; RK = rock potassium; PDB = phosphate dissolving bacteria and KDB = potassium dissolving bacteria).

Treatments	SA (cm^2)	r_1 (cm)
1 – control	297	0.35
2 – RP	334	0.34
3 – RK	312	0.34
4 – PDB	362	0.33
5 – KDB	354	0.33
6 – RP + PDB	391	0.32
7 – RK + KDB	375	0.32
8 – R(P + K) + (P + K)DB	417	0.31

B. subtilis was about 1.6-fold compared with the common bean plants without inoculation. In general, a large root surface area is of key importance for nutrient acquisition by roots (Marschner, 1995). An increase in root surface area can be either an inherent property or deficiency induced, such as N or P deficiency.

In contrast, the mean half distance between roots (r_1, cm) of the treatments of maize plants decreased compared with the control. Abou El Seoud (2005) reported that when r_1 is larger than the depletion zone around roots, part of this nutrient would be unavailable. In other words, when the root length density increased, the r_1 decreased. This result leads to increase in the nutrient depletion zone of the soil. And then, the plant with less r_1 will get more yields compared with the other plant with high r_1 value. The amount of nutrients which will take up from soil depends on the size of the root system and its distribution in the soil profile. The amount of nutrients each root segment absorbs will depend on the soil volume it can exploit, which is measured by the average distance between the root segment and any neighboring root segment (r_1) and the morphological and physiological properties of the root (Abou El Seoud, 2005).

3.3. Plant nutrients uptake

Results showed that, rock potassium materials (RK) did not significantly increase P uptake in maize plants compared with the untreated plants (control) (Fig. 4). Significantly increase of P uptake in the shoot generally occurred in the other treatments, i.e. RP, PDB, KDB, (RP + PDB), (RK + KDB), and R(P + K) + (P + K)DB compared with the control plants, which increased by about 84%, 187%, 124%, 2.5-fold, 140%, and 3-fold, respectively. Many investigators have explained the role of *B. megaterium*, which increases the availability of phosphorus in the soil. Consequently there is an increase in phosphorus absorption as well as phosphorus accumulation in plant tissues (Chen et al., 2006; Mahfouz and Sharaf-Eldin, 2007; Marschner et al., 2010). Similarly, Han et al. (2006) reported that, Soil inoculation with PSB or KSB significantly increased N, P and K uptake in pepper and cucumber plants, especially when the respective rock P or rock K were added. Application of insoluble rock phosphate alone in the soil caused limited increase in the P uptake, this increase in P content could be due to the role of indigenous microorganisms in the soil. This result is agreement with Abarchi et al. (2009) who studied the legumes, *Mucuna pruriens* (L.) and *Lablab purpureus* (L.) treated with or without rock phosphate (RP), and found that application of RP led to higher P contents in both legumes.

On the other hand, application of RP combined with PDB results in increase of P uptake by about 89% compared with RP alone (Fig. 4). This might be because these bacteria have been used to convert insoluble rock P material into soluble forms available for plant growth (Nahas et al. 1990; Bojinova et al., 1997). This conversion is through acidification by producing strong organic acids (Schilling et al., 1998). In the same line, Amer et al. (2010) reported that the P uptake of common bean treated with of *B. subtilis* and *P. fluorescens* was increased by about 1.2-fold and 97%, respectively, in comparison to the control.

From Fig. 4 we can notice that all treatments increased K uptake significantly compared with untreated plants (control).

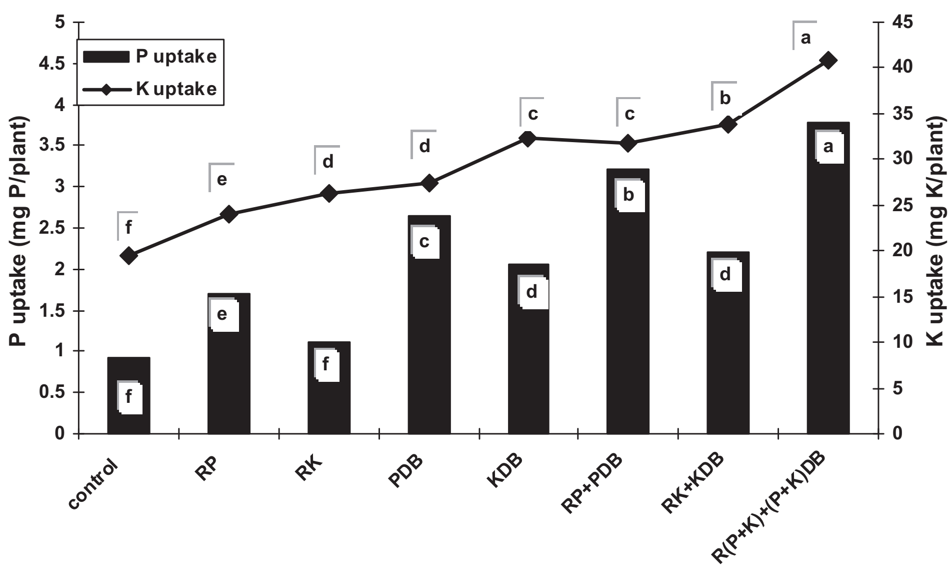

Figure 4 Phosphorus (P) uptake and potassium (K) uptake (mg/plant) of maize plant as affected by rock minerals and inoculation with P and K-dissolving bacteria (RP = rock phosphate; RK = rock potassium; PDB = phosphate dissolving bacteria and KDB = potassium dissolving bacteria; different letters indicate significant differences, $P \leqslant 0.05$).

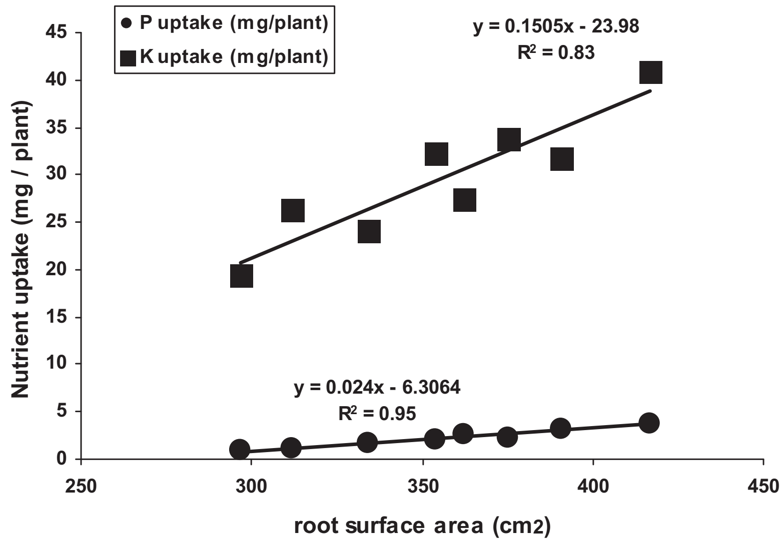

Figure 5 Relationship between nutrients uptake (P and K uptake) (mg/plant) and root surface area (SA, cm^2) of maize plant.

The treatments RP, RK, PDB, KDB, (RP + PDB), (RK + KDB), and [R(P + K)+(P + K)DB] increased by 24%, 35%, 41%, 68%, 63%, 74%, and 111%, respectively, compared with the control. The treatment (RK + KDB) was significantly increased by 29% compared with RK treatment. This increasing was due to fact that KDB release organic acids which solubilize the insoluble rock K materials (Friedrich et al., 1991; Ullman et al., 1996).

Similarly, Styriakova et al. (2003) reported that the activity of potassium dissolving bacteria played a pronounced role in the release of K from Feldspar. Also, Badr et al. (2006) found that potassium uptake improved markedly with inoculation of bacteria in the tested soils compared to corresponding controls. In contrast to RK with P uptake, rock phosphate materials (RP) significantly increase K uptake by maize roots compared with the untreated plants. That could be due to the rock phosphate materials containing a little amount of K (total K 0.16%). This amount of K helps the plant to develop the K uptake of maize plants as compared with the untreated plants.

The relationship between root surface area (SA) of maize plants and nutrient uptake (P and K uptake) was analyzed to determine the effect of root growth on the nutrient uptake. A highly significant correlation ($R^2 = 0.95$, $p < 0.05$) between root surface area and P uptake indicated that root growth contribute to the acquisition of P from the soil (Fig. 5). The increase in P uptake is largely due to increased absorption of P from soil solution by plant's root. Similarly, Gao et al. (2005) reported a significantly positive correlation between nutrient uptake and root surface area, indicating the importance of root growth for nutrient acquisition in rice. In contrast, the relatively low correlation of K uptake (compared to P uptake) explained percentage ($R^2 = 0.83$, $p < 0.05$) suggests additional mechanisms other than root growth which might play a major role in determining the capacity of maize plants to take up K from the soil.

In conclusion, under the same conditions of this study, co-inoculation of PSB and KSB in conjunction with direct the application of rock P and K materials into the soil increased P and K availability and uptake, and the plant growth of maize plants grown on P and K limited soils.

Acknowledgment

The authors extended their appreciation to the Deanship of Scientific Research at King Saud University for funding their work through the research group project No. RGP-VPP-010.

References

Abarchi, I., Zhang Zhan-Yu, B., Xiang-Ping, V.G., Wei-Mu, W., Ong'or, B.T., Timbely, D., 2009. Effects of plant age and rock phosphate on quality and nutrient release of legume residue. Pedosphere 19 (1), 78–85.

Abou El Seoud, I.I.A., 2005. Influence of mycorrhizae and phosphate mobilizing bacteria on P nutrition of some vegetable crops. Ph.D., Faculty of Agriculture (Saba Basha), Alexandria University, Egypt, p. 174.

Abou El Seoud, I.I.A., Elham, A.B., El Shimaa, A.E., 2009. Response of two sugar beet varieties to chicken manure and phosphorine application. Alex. Sci. Exch. J. 30 (4), 433–444.

Adesemoye, A.O., Kloepper, J.W., 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85, 1–12.

Aipova, R., Aitkeldiyeva, A., Kurmanbayev, A., Sadanov, K., Topalova, B., 2010. Assessment of biotechnological potential of phosphate solubilizing bacteria isolated from soils of Southern Kazakhstan. Nat. Sci. 2 (8), 841–845.

Akande, M.O., Adediran, J.A., Oluwatoyinbo, F.I., Makinde, E.A., Adetunji, M.T., 2008. Suitability of poultry manure amended Sokoto rock phosphate on growth, nutrient uptake and yield of chilli pepper (*Capsicum fruitscens* L). Niger. J. Soil Sci. 18, 167–174.

Alexander, M., 1997. Introduction to Soil Microbiology, second ed. John Wiley & Sons Inc., New York.

Amer, M.A., Abou El Seoud, I.I.A., Rasmy, M.R., Manar, M., 2010. Biological control of *Sclerotinia sclerotiorum*, the casual agent of white basal rot disease of beans (*Phaseolus vulgaris* L.). Alex. Sci. Exch. J. 30 (4), 334–339.

Artursson, V., Finlay, R.D., Jansson, J.K., 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ. Microbiol. 8, 1–10.

Badr, MA., Shafei, AM., Sharaf El-Deen, SH., 2006. The dissolution of K and P-bearing minerals by silicate dissolving bacteria and their effect on sorghum growth. Res. J. Agric. Biol. Sci. 2 (1), 5–11.

Bennett, P.C., Choi, W.J., Rogera, J.R., 1998. Microbial destruction of feldspars. Miner. Manage. 8 (6), 149–150.

Black, C.A., 1965. Methods of soils analysis parts 1 and 2, agronomy monograph No. 9, Madison, Wisconsion.

Bojinova, D., Velkova, R., Grancharov, I., Zhelev, S., 1997. The bioconversion of Tunisian phosphorite using *Aspergillus niger*. Nutr. Cycle Agroecosyst. 47, 227–232.

Brady, N.C., 1990. The Nature and Properties of Soils. Macmillan, New York, USA, pp. 351–380.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C., 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. 34, 33–41.

Ekin, Z., 2010. Performance of phosphate solubilizing bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertilizer. Afr. J. Biotech. 9 (25), 3794–3800.

Eweda, W.E., Selim, S.M., Mostafa, M.I., Abd El-Fattah, D.A., 2007. Use of *Bacillus circulans* as bio-accelerator enriching composted Agricultural wastes identification and utilization of the microorganism for compost production. In: Proceedings of the 12th Conference of the Microbiology. Organized by The Egyptian Soc. of App. Micro. (ESAM), Giza, Egypt, 18–20, pp. 43–65.

Friedrich, S., Platonova, N.P., Karavaiko, G.I., Stichel, E., Glombitza, F., 1991. Chemical and microbiological solubilization of silicates. Acta Biotech. 11, 187–196.

Gad, W.M., 2001. Physiological studies on *Foeniculum vulgare* Mill and *Anethum graveolens* L. M.Sc. Thesis, Faculty of Agriculture, Kafr El-Sheikh, Tanta University, Egypt. p. 214.

Gao, X., Zou, C., Zhang, F., vander Zee, E., Hoffland, E., 2005. Tolerance to zinc deficiency in rice correlates with zinc uptake and translocation. Plant Soil 278, 253–261.

Gerke, L., 1992. Phosphate, aluminum, and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Z. Pfl.-Ernähr. Bodenkde 155, 17–22.

Girgis, M.G.Z., 2006. Response of wheat to inoculation with phosphate and potassium mobilizers and organic amendment. Ann. Agric. Sci. 51 (1), 85–100.

Groudev, S.N., 1987. Use of heterotrophic microorganisms in mineral biotechnology. Acta Biotech. 7, 299–306.

Han, H.S., Lee, K.D., 2005. Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. Res. J. Agric. Biol. Sci. 1 (2), 176–180.

Han, H.S., Supanjani, E., Lee, K.D., 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ. 52 (3), 130–136.

Ibrahim, H.I.M., Zaglol, M.M.A., Hammad, A.M.M., 2010. Response of Balady guava trees cultivated in sandy calcareous soil to biofertilization with phosphate dissolving bacteria and/or VAM fungi. J. Am. Sci. 6 (9), 399–404.

Illmer, P., Barbato, A., Schinner, F., 1995. Solubilization of hardly-soluble AlPO with P-4 solubilizing microorganism. Soil Biol. Biochem. 27, 265–270.

Jackson, M.L., 1967. Soil Chemical Analysis. Prentice-Hall, Inc., Englewood Cliffs, USA.

Jackson, M.L., 1973. Soil Chemical Analysis. Prentice-Hall India Private Limited, New Delhi.

Jorquera, M.A., Hernández, M.T., Rengel, Z., Marschner, P., Mora, M.L., 2008. Isolation of culturable phosphobacteria with both phytate mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biol. Fertil. Soils 44, 1025–1034.

Kucey, R.M.N., 1988. Effect of *Penicillium biloji* on the solubility and uptake of P and micronutrients from soil by wheat. Can. J. Soil 68, 261–267.

Leyval, C., Berthelin, J., 1989. Interactions between *Laccacia laccata*, *Agrobacterium radiobacter* and beech roots: influence on P, K, Mg, and Fe mobilization from minerals and plant growth. Plant Soil 117, 103–110.

Lin, Q.M., Rao, Z.H., Sun, Y.X., Yao, J., Xing, L.J., 2002. Identification and practical application of silicate-dissolving bacteria. Agric. Sci. China 1, 81–85.

Liu, W., Xu, X., Wu, X., Yang, Q., Luo, Y., Christie, P., 2006. Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. Environ. Geochem. Health 28, 133–140.

Lowther, J.R., 1980. Use of a single H_2SO_4–H_2O_2 digest for the analysis of pinus radiate needles. Commun. Soil Sci. Plant Anal. 11, 175–188.

Mahfouz, S.A., Sharaf-Eldin, M.A., 2007. Effect of mineral vs. biofertilizer on growth, yield, and essential oil content of fennel (*Foeniculum vulgare* Mill.). Int. Agrophy 21, 361–366.

Marschner, A., Crowley, D.B., Rengel, Z., 2010. Interactions between rhizosphere microorganisms and plants governing iron and phosphorus availability. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, second ed. Academic Press, London, UK, p. 889.

Marschner, P., 2009. The role of rhizosphere microorganisms in relation to P uptake by plants. In: White, P.J., Hammond, J.P. (Eds.), The Ecophysiology of Plant–Phosphorus Interactions, Plant Ecophysio. Series. Springer, Heidelberg, pp. 165–176.

Moghimi, A., Tate, M.E., Oades, J.M., 1978. Characterization of rhizosphere products especially 2-ketogluconic acid. Soil Biol. Biochem. 10, 283–287.

Murphy, J., Riley, J.P., 1962. A modified single solution for the determination of phosphate in natural waters. Anal. Chem. Acta 27, 31–36.

Nahas, E., Banzatto, D.A., Assis, L.C., 1990. Fluorapatite solubilization by *Aspergillus niger* in vinasse medium. Soil Biol. Biochem. 22, 1097–1101.

Olsen, S.C.C., Watanable, F., Dean, L.A., 1954. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. U.S.D.A. Circular No. 939.

Rajan, S.S., Watkinson, J.H., Sinclair, A.G., 1996. Phosphate rock of for direct application to soils. Adv. Agron. 57, 77–159.

Ranawat, P., Kumar, K.M., Sharma, N.K., 2009. A process for making slow-release phosphate fertilizer from low-grade rock phosphate and siliceous tailings by fusion with serpentinite. Curr. Sci. 96 (6), 843–848.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorous by plants. Aust. J. Plant Physiol. 28, 897–906.

Rodriguez, H., Fraga, R., 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 17, 319–339.

Sabannavar, S.J., Lakshman, H.C., 2009. Effect of rock phosphate solubilization using mycorrhizal fungi and phosphobacteria on two high yielding varieties of *Sesamum indicum* L. World J. Agric. Sci. 5 (4), 470–479.

Saghir, K.M., Zaidi, A., Wani, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture: a review. Agron. Sustain. Dev. 27, 29–43.

Sample, E.C., Soper, R.J., Racz, G.J., 1980. Reactions of phosphate fertilizers in soils. In: Khasawneh, F.E., Sample, E.C., Kamprath, E.J. (Eds.), The Role of Phosphorus in Agricultures. American Society of Agronomy, Madison, Wisconsin, USA, pp. 263–310.

SAS, 1996. SAS Stat User's Guide. SAS Institute Inc., Cary, NC, USA.

Schenk, M.K., Barber, S.A., 1979. Phosphate uptake by corn as affected by soil characteristics and root morphology. Soil Sci. Soc. Am. J. 43, 880–883.

Schilling, G., Gransee, A., Deubel, A., Lezovic, G., Ruppel, S., 1998. Phosphorus availability, root exudates, and microbial activity in the rhizosphere. Z. Pflanzenernahr. Bodenkd. 161, 465–478.

Setiawati, A., Handayanto, E., 2010. Role of phosphate solubilizing bacteria on availability phosphorus in Oxisols and tracing of phosphate in corn by using ^{32}P. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia.

Sheng, X.F., 2005. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. Soil Biol. Biochem. 37, 1918–1922.

Sheng, X.F., Huang, W.Y., 2001. Physiological characteristics of strain NBT of silicate bacterium. Acta Pedol. Sin. 38, 569–574.

Sheng, X.F., Huang, W.Y., 2002. Mechanism of potassium release from feldspar affected by the strain NBT of silicate bacterium. Acta Pedol. Sin. 39, 863–871.

Sheng, X.F., He, L.Y., Huang, W.Y., 2002. The conditions of releasing potassium by a silicate-dissolving bacterial strain NBT. Agric. Sci. China 1, 662–666.

Shivay, Y.S., 2010. Effect of diammonium phosphate and mussoorie rock phosphate on productivity and economics of potato (*Solanum tuberosum*). Indian J. Agric. Sci. 80 (4), 329–332.

Steyn, W.J.A., 1959. Leaf analysis, errors involved in the preparative phase. J. Agric. Food. Chem. 7, 344–348.

Stillings, L.L., Drever, J.L., Brantley, S.L., Sun, Y., Oxburgh, R., 1996. Rates of feldspar dissolution at pH 3–7 with 0–8 mM oxalic acid. Chem. Geol. 132, 79–90.

Styriakova, I., Styriak, I., Galko, I., Hradil, D., Bezdicka, P., 2003. The release of iron-bearing minerals and dissolution of feldspar by heterotrophic bacteria of Bacillus species. Acta Pedol. Sin. 47 (1), 20–26.

Takano, Y., Mori, H., Kaneko, T., Ishikawa, Y., Marumo, K., Kobayashi, K., 2006. Phosphatase and microbial activity with biochemical indicators in semi-permafrost active layer sediments over the past 10, 000 years. Appl. Geochem. 21, 48–57.

Tennant, D., 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63, 995–1001.

Ullman, W.J., Kirchman, D.L., Welch, S.A., 1996. Laboratory evidence for microbially mediated silicate mineral dissolution in nature. Chem. Geol. 132, 11–17.

Vandevivere, P., Welch, S.A., Ullman, W.J., Kirchman, D.L., 1994. Enhanced dissolution of silicate minerals by bacteria at near-neutral pH. Microb. Ecol. 27, 241–251.

Vessey, K.J., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571–586.

Wani, P.A., Khan, M.S., Zaidi, A., 2007. Synergistic effects of the inoculation with nitrogen-fixing and phosphate-solubilizing rhizobacteria on the performance of field-grown chickpea. J. Plant Nutr. Soil Sci. 170, 283–287.

Welch, S.A., Ullman, W.J., 1999. The effect of microbial glucose metabolism on bytownite feldspar dissolution rates between 5 and 35 °C. Geochem. Cosmochim. Acta 63, 3247–3259.

Xie, J.C., 1998. Present situation and prospects for the world's fertilizer use. Plant Nutr. Fert. Sci. 4, 321–330.

Zakaria, A.A.B., 2009. Growth optimization of potassium solubilizing bacteria isolated from biofertilizer. Bachelor of Chem. Eng. (Biotech.), Fac. of Chem., Natural Resources Eng. Univ., Malaysia Pahang, p.40.

Zapata, F., Roy, R.N., 2004. Use of Phosphate Rock for Sustainable Agriculture. FAO and IAEA, Rome, Italy.

Zayed, G., 1998. Can the encapsulation system protect the useful bacteria against their bacteriophages? Plant Soil 197, 1–7.