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PHOSPHATE SOLUBILIZING ENDOPHYTIC BACTERIA AND THEIR ROLE IN MAIZE PLANT GROWTH PROMOTION

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ABSTRACT: *Phosphate solubilizing bacteria were isolated from maize roots and their beneficial effects on plant growth were studied. Among 9 isolates, 6 showed high phosphate solubilization efficiency. These isolates were investigated for maximum phosphate solubilization in regard to different pH and incubation periods. Results showed high solubilization efficiency at pH 7 and incubation periods between 6- 12 days. Based on solubilization levels, three isolates 4PC, 5PC and 6PC were selected and studied for their possible growth promoting potential for maize (Zea mays L.) in pot experiment. Plants sown in soil containing rock phosphate and inoculated with phosphate solubilizing isolates, recorded a significant increase in root length and plant height, fresh and dry weight of root and shoot as well as number of leaves / plant, photosynthetic pigments and N, P and K concentrations as compared to uninoculated plants. The isolate 6PC and 4PC exhibited better performance and therefore they identified based on 16S rDNA as Planococcus sp. and Bacillus cereus, respectively. This study showed that these isolates can be applied as phosphate solubilizers in the soil containing insoluble form of phosphate.*

Key words: *Phosphate solubilization, endophytes, maize, biofertilizer, Plant growth promoting bacteria.*

INTRODUCTION

Phosphate is major essential macronutrient required for plant growth to optimize yield. Its, the second major plant nutrient is an integral part of plants generally deficient in soils (Batjes, 1997) due to its speedy fixation. Phosphate anions ($H_2PO_4^-$, HPO_4^{2-}) are extremely reactive and form metal complexes with Ca in calcareous soils (Lindsay *et al*., 1989) and Fe3+ and Al3+ (Norrish and Rosser, 1983) in acidic soils. These metal ion complexes precipitated the 80% of added P fertilizer (Stevenson, 1986; Goldstein, 1986). In the agricultural traditions, phosphorus is added to the soil as synthetic super phosphate. Given the negative environmental impacts of chemical fertilizers and increasing costs. Use of alternative indigenous resources and minerals such as rock phosphate are growing importance to alleviate the dependence of imported or closely commercial fertilizers (Badr *et al*., 2006). Phosphate solubilizing bacteria (PSB) could convert these insoluble phosphates into available forms for plant via the process of acidification, chelating, exchange reactions, and production of gluconic acid (Chung *et al*., 2005; Gulati *et al*. 2010). Utilization of PSB is advantageous for sustainable agricultural practices (Gyaneshwar *et al*., 2002). The use of plant growth promoting rhizobacteria (PGPR) including phosphate solubilizing bacteria as biofertilizer was suggested as a sustainable solution to improve plant nutrient and production (Vessey 2003). Many bacteria *Pseudomonas fluorescens, Bacillus megaterium* and *Azospirillum* spp. isolated from the rhizosphere of maize and rice solubilized Ca_3PO_4 *in vitro*, *Pseudomonas fluorescens* and *Bacillus megaterium* strains were the most powerful phosphate solubilizers on Pikovskaya (PVK) plates and liquid medium (El-Komy, 2005). Bacterial strains *Azotobacter vinelandii* and *Bacillus cereus* when tested *in vitro* were found to solubilize Phosphate and thus help in the growth of plant (Husen, 2003). Phosphate solubilizing microorganisms solubilize insoluble Phosphate by producing various organic acids, Plants take up this available P (Sujatha *et al.,* 2004).

Abou El-Yazeid and Abou-Aly (2011) found a significant positive effect on tomato (in regard to vegetative characteristics and mineral contents (N, P and K),) after addition of rock phosphate with phosphate solubilizing microorganisms (*Bacillus megaterium* var. *phosphaticum, Paenibacillus polymyxa*) when compared with uninoculated treatments. Increasing the bioavailability of P in soils with inoculation of PGPR or/and rock material, may lead to increasing P uptake and plant growth (Sahin *et al*, 2004; Girgis, 2006 and Eweda *et al*., 2007). PGPR strains use one or more direct or indirect mechanisms to enhance the growth and health of plants. PGPR have been reported to directly enhance plant growth by a variety of mechanisms: fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores, and synthesis of plant growth hormones i.e. Indole-3- acetic acid (IAA), gibberellic acid, cytokinins, and ethylene (Nelson, 2004). Indirect mechanisms involves the biological control of plant pathogens and deleterious microbes, through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and siderophores or through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield (Khan, 2006).

The present study was conducted to evaluate the solubilization potential of endophytic isolates to solubilize insoluble phosphate *in vitro* and impact of their application on plant growth parameters in pot experiment.

MATERIALS AND METHODS

1. Isolation of Phosphate solubilizing bacteria:

Phosphate solubilizing bacteria were isolated from healthy maize roots grown in El menoufyia Governorate agricultural field (Egypt). The roots were washed with tap water then surface sterilized for 2 min with 70% ethanol and 2 min with 0.53% NaOCl (Mejia *et al*., 2008), then macerated with a sterile mortar and pestle. Serially diluted (up to 10^{-6}) aliquots of the macerates were spread onto plates containing Pikovskaya agar medium (Pikovskaya, 1948) consists of (10 g l^{-1}), $(NH4)_2SO_4$ (0.5 g l^{-1}), NaCl (0.3 g l^{-1}), KCl (0.3 g l^{-1}), $MgSO_4.7H_2O$ (0.3 g l^{-1}), $FeSO_4.7H_2O$ (0.03 g l^{-1}), $MnSO_4.4H_2O$ (0.03 g l^{-1}), $Ca_3(PO_4)_2$ (5 g l^{-1}) and amended with 1.8% agar. The colonies with clear halo were selected from the plates and considered as PSB (Nautiyal, 1999), and kept on 4°c. Results of zone diameter in relation to growth diameter are expressed as solubilization efficacy (SE) according to the equation of Nguyen *et al*., (1992).

$$\text{Solubilization efficiency (SE)} = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \text{ X } 100$$

2. Quantitative determination of P solubilization:

Quantitative assessment of solubilization was carried out using National botanical research institute's phosphate growth medium (NBRIP broth) (Nautiyal 1999) which include; glucose (10 g l^{-1}), $MgCl_2.6H_2O$ (5 g l^{-1}), $MgSO_4.7H_2O$ (0.25 g l^{-1}), KCl (0.2 g l^{-1}), $(NH_4)_2SO_4$ (0.1 g l^{-1}), $Ca_3(PO_4)_2$ (5 g l^{-1}). Tricalcium phosphate (TCP) was autoclaved separately, then, mixed with other sterile ingredients after autoclaving. The final pH was adjusted to 7.0. Erlenmeyer flasks (150 ml) containing 50 ml of the medium were inoculated in triplicate with an inoculum of (2×10^8 CFU ml^{-1}). Autoclaved uninoculated medium served as control. The flasks were incubated for 6 days at 30°C in a shaking incubator at 150 rpm. The cultures were harvested by centrifugation at 4,000 rpm for 30 min. soluble phosphate in culture supernatant was determined by the molybdenum blue method (Murphy and Riley 1962). Simultaneously, the pH of the medium was recorded (Anandham *et al.* 2007).

3. Effect of pH and incubation period on phosphate solubilization:

3.1. pH:

To study the effect of pH on the efficiency of tricalcium phosphate solubilization, the isolates were grown on NBRIP medium agar plates at 30°C and at different pH 4, 5, 6, 7, 8, 9 and 10 for 4 days. Clear zone diameter and the growth diameter of colony were recorded and efficiency of solubilization was calculated as described earlier.

3.2. Incubation period:

Bacterial isolates were grown on NBRIP agar medium and incubated on 30°C at different incubation periods (up to 16 days). The halo and growth colony diameter were measured after 2, 4, 6, 8, 10, 12, 14 and 16 days of incubation. The results are expressed as solubilization efficiency (SE) as described earlier.

4. Enzymes activity:

Cellulase activity: The bacteria were grown on CMC agar medium (Ariffin *et al.*, 2006), and incubated at 30°C for 5 days to allow for the secretion of cellulase. At the end of the incubation, the agar medium was flooded with an aqueous solution of Congo red (1% w/v) for 15 minutes. The Congo red solution was then poured off, and the plates were flooded with 1M NaCl for 15 minutes. The formation of a clear zone of hydrolysis indicates cellulose degradation.

Pectinase activity test: The isolates were spot-inoculated on the pectin agar plates and incubated for 4 days at 30°C. The plates were then flooded with iodine-potassium iodide solution (1.0g iodine, 5.0g potassium iodide and 330ml H_2O) to detect clearance zones (Fernandes-Salomao *et al.*, 1996). The presence of clear halo around the colonies was indicative of the degradation of pectin.

5. Application of PSB as possible PGPR in pot experiment:

Pot experiments were carried out at glasshouse at Shibin El-Kom farm, Egypt to determine the effectiveness of PGPR inoculation on the growth of maize (*Zea mays* L.), Giza-10 maize single cross hybrid. Plastic Pots at 25 cm diameter were filled with sterilized mixed soil of sand and clay in a mass ratio of 1:1 (10 Kg / pot). Three isolates namely 4PC, 5PC and 6PC were used as phosphate solubilizing inoculants. These isolates were cultivated in nutrient broth medium for 2 days. The cultures were centrifuged and the bacteria were then suspended in saline solution to prepare inoculum of 10^8. The seeds were surface sterilized using 0.1 % mercuric chloride and 70 % ethanol and washed with distilled water (Subba rao, 1993), then inoculated with Vermiculate based inoculants. Five seeds of maize were sown in each pot, after complete germination (7 days after sowing); pots were thinned to three seedling. Mineral fertilizers of N and K were added at the rates of 100% as recommended by the Egyptian Ministry of Agriculture, and rock phosphate (as a source of phosphate) were added as one part before sowing during the preparation of soil (100g / pot).

The treatments were prepared as the following:

1- Control1 (without fertilizer application and without inoculation)
2- Control2 (N+ K+ Rock phosphate and without inoculation).
3- N+ K+ Rock phosphate with 4PC inoculant.
4- N+ K+ Rock phosphate with 5PC inoculant.
5- N+ K+ Rock phosphate with 6PC inoculant.

6. Determination of plant growth characteristics:

Three plants (for each replicate) of 3 replicates for each treatment were collected after 50 days of sowing to determine their growth characteristics; root and shoot length, fresh and dry weight of shoot and roots, number of leaves.

7. Determination of photosynthetic pigments:

Chlorophyll a, b and carotenoids were determined by spectrophotometer at the

wavelengths of 440, 644 and 662 nm as described by Wettestein (1957).

8. Estimation of total nitrogen, phosphorus and potassium (NPK):

A known weight of dry matter of shoots was wet digested with concentrated sulphuric and perchloric acids according to Jackson (1973). Total N, P and K contents were determined in the digested solutions of shoot. N content was determined by microKjeldahl method according to Page (1982). Phosphorus content was determined using stannous chloride and ammonium molybdate. The blue color formed was measured spectrophotometrically at 700 nm (Chapman and Pratt, 1978). Potassium concentration was determined by flame photometer (A.O.A.C. 2005).

9. Identification of the effective PSB isolates by 16S rDNA sequence:

Based on results of pot experiment, bacterial isolates that showed high phosphate solubilization (4PC and 6PC) were identified using 16S rDNA sequencing analysis. Bacterial DNA was extracted using gene jet genomic DNA purification kit (Fermentas). Amplification of 16s rDNA was performed in a 50 µl final volume containing 2 µl of total DNA, 1 µl of Eub27F (5′–3′: AGAGTTTGATCCTGGCTCAG), 1 µl of primer Eub1492R (5′–3′: ACGGCTACCTTGTTACGACTT), 4 µl of each dNTP, 5 µl of $MgCl_2$, and 1 µl of Taq DNA polymerase (Weisburg *et al*., 1991). The reaction conditions were carried out according to SIGMA scientific serves company instructions. The PCR products were run in 1.0% (w/v) agarose gel and stained with ethidium bromide. Sequence data were aligned and compared with available standard sequences of bacterial lineage in the National Center for Biotechnology Information Gen Bank (http:// www.ncbi.nlm.nih.gov/) using BLAST (Chen *et al*., 2006).

10. Statistical analysis

The obtained data were subjected to statistical analysis using program Costat. Analysis of variance (ANOVA) and L.S.D test was carried out for the obtained data according to Steel and Torrie (1980).

RESULTS AND DISCUSSION:

1. Isolation of Phosphate solubilizing bacteria:

Six bacterial isolates showed halo formation on PVK medium indicating that they are PSB (Table 1). In the plate assay, isolate 6PC showed the highest P solubilization efficiency which was 800 % compared to other isolates, followed by isolate 5PC (366.666%), while the lowest P solubilization efficiency recorded by isolate 1PC which was 160 %. In this connection, El-Komy, (2005) found that *Pseudomonas fluorescens* and *Bacillus megaterium* strains were able to solubilize phosphate effectively, and recorded higher solubilization efficiency up to 350 and 185%, respectively, than different *Azospirillum* strains.

Table (1): Phosphate solubilization assay of bacterial isolates *in vitro*.

Bacterial isolates	Growth diameters (mm)	Solubilization diameter (mm)	Solubilization efficiency %
1PC	10	16	160.00
2PC	9	15	166.67
3PC	7	19	271.43
4PC	6	20	333.33
5PC	6	22	366.67
6PC	3	24	800.00

Many considered that contrary to indirect measurement of phosphate solubilization by plate assay, the direct measurement of phosphate solubilization in NBRIP broth assay always resulted in reliable results (Johri *et al*., 1999 and Nautiyal 1999). Thus, the bacterial isolates were further screened for its ability to solubilize phosphates in the liquid medium (Table 2). However, the results obtained from both measurements revealed that isolate 6PC exhibited a high solubilization efficiency, and also showed the highest amount of soluble P(284.83 µg/ µg/mL) after 6 days in liquid medium for this strain. The solubilization of TCP in the liquid medium by different isolates was accompanied by a drop in pH (to 5.14 and 5.67) from an initial pH (7.0) after 6 days. The highest levels of P solubilization were accompanied by a maximum drop in pH. Inorganic phosphate is solubilizing by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cation such as (Ca^{++}) and decrease the pH in basic soils (Kpomblekou & Tabatabai, 1994 and Stevenson, 2005). The present results are in line with those obtained by Yu *et al*., (2011) who found that the solubilization of TCP in the liquid medium by different strains was accompanied by a significant drop in pH (to 5.1 and 6.0) from an initial pH of 7.0 after 72 h.

2. Effect of pH and Incubation period on phosphate solubilization:

2.1. pH:

Optimum pH for better phosphate solubilization was almost 7 for all the isolates, while phosphate solubilization was less at pH 6, 8 and 9, except 6PC which showed high p-solubilization at pH 6 (Fig. 1). All the isolates were able to grow in pH ranging from 6 to 9 except 5PC showed an inability to grow at pH 9. At pH 4, 5 and 10 no growth and solubilization was observed while at pH 8 and 9 all the isolates, except 6PC were unable to solubilize tri-calcium phosphate. The results indicated that the isolate (6PC) could be strong candidates for the improvement of phosphate solubilization in a wide range of soil pH.

Similar results were obtained by Shahab and Ahmed (2008) who found that pH 7 was the most favorable pH for solubilization while at pH 4 no growth and solubilization were seen. They found that all isolates solubilized Zinc phosphate in the range of pH 5 – 7 and at pH 8 and 9, all the isolates, except *Pseudomonas* sp. (CMG859) were unable to solubilize zinc phosphate, although all are growing at pH 8 and 9.

Table (2): Quantitative determination of soluble phosphate among phosphate solubilizing isolates and its effect on pH medium.

PSB Strains	pH medium			Soluble P (µg /mL)
	2 days	4 days	6 days	
1PC	6.23	5.53	5.33	54.16
2PC	6.63	5.91	5.67	37.03
3PC	6.13	5.61	548	91.34
4PC	5.99	5.49	5.38	203.61
5PC	5.71	5.30	5.23	216.79
6PC	5.50	5.22	5.14	284.83

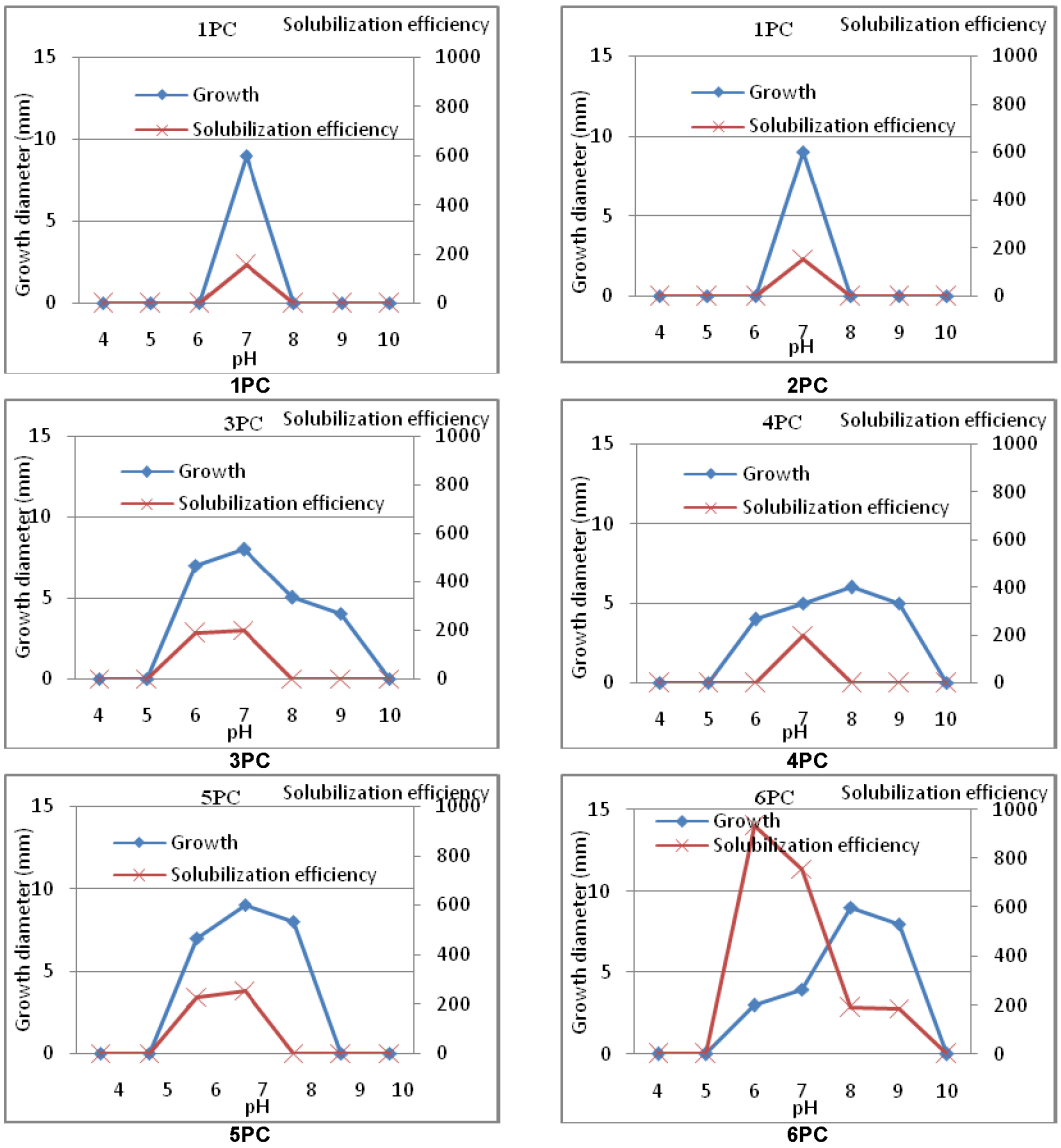

Fig. (1): Effect of pH on phosphate solubilization and growth of bacterial isolates.

2.2. Incubation period:

Results in Fig. (2) indicated that the optimum incubation period over the release of phosphate for phosphate dissolving isolates fluctuated between 6- 12 days. The isolates 1PC, 3PC and 4PC showed the highest increase in solubilization efficiency (SE) at 8 days of incubation, while 2PC was the highest after 6 days, then SE was decreased for all isolates afterwards. In this respect, El-Komy, (2005) who found that Solubilization efficiency (SE) for *Azospirillum lipoferum, Azospirillum brasilense, Pseudomonas fluorescens* strain 201 and *Bacillus megaterium* strain 98 was increased after 2 and 4 days of incubation, and then started to decrease after 6 days of incubation. While the isolates 5PC and 6PC recorded maximum solubilization efficiency at 12 days of incubation and then decreased afterwards. This may be due to the solubilization stopped, although the colony was still growing (El-Komy, 2005). Sahu *et al.*, (2007) found that the optimum incubation period over the release of phosphate for phosphate solubilizing Actinomycetes was 13 days.

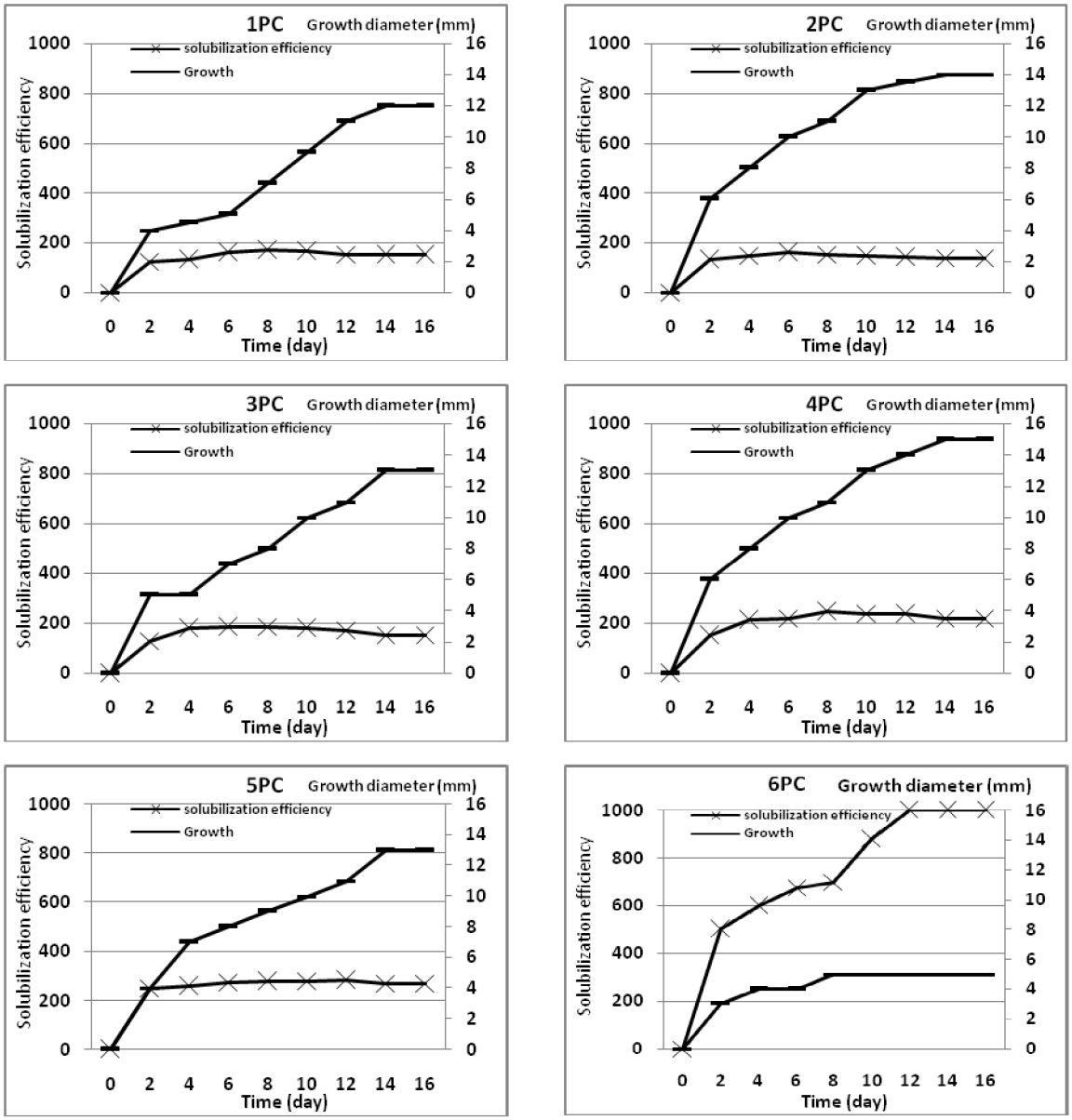

Fig. (2): Effect of incubation period on phosphate solubilization by bacterial isolates.

3. Enzymes activity:

Results clearly exerted that 6PC exhibited a cellulytic activity, which is reflected by forming a clear zone. When these isolates grown on pectin medium, the isolates 3PC, 4PC and 6PC gave a clear zone of hydrolysis on pectin agar plate (Table 3). The results indicated that these isolates were able to grow and utilizing the C-source with the production of cellulase or/ and pectinase enzymes. Hydrolytic enzymes, pectinase and cellulase may play a role in the mechanisms by which endophytic bacteria penetrate into and persist in the host plant (Hallmann *et al*., 1997; Reinhold-Hurek and Hurek, 1998).

4. Identification of PSB by 16S rDNA sequence:

Molecular analysis based on 16S rDNA sequencing identified the isolates 4PC and

6PC as *Bacillus cereus* and *Planococcus sp.* respectively. *Bacillus cereus* was isolated as endophytes from 14 maize cultivars (Gao *et al.,* 2004). Altalhi (2009) isolated a total of 111 different isolates of endophytic bacteria from grapevine (*Vitis vinifera* L.) belong to fourteen bacterial genera and *Planococcus* spp. is an among these genera.

5. Response of maize plant to inoculation with P- solubilizing bacteria:

5.1. Root length:

Data presented in Table (4) indicated that P-solubilizing PGPR significantly increased root length of maize as compared to both uninoculated controls. Generally, uninoculated controls gave a low root length comparing to other treatments. All isolates showed significantly high root length. However the isolate 6PC showed maximum increase (33.9 cm) by 109 % in root length of maize, as compared to both controls. The results are in agreement with obtained by Yasmin *et al.,* (2012) who found that seven rhizobacteria isolated from rhizosphere soil of rice and designated as 3PKR, 4PKR, 6PKR, 7PKR, 8PKR, 12PKR and 13PKR variably showed P-solubilization abilities and significant increase in root length of maize as compared to uninoculated control. The variable degree of stimulatory effect among individual or consortium PSB treatments on plant growth may be due to diverse interactions of inoculated PSB with plant roots or with native micro flora, which often results in the promotion of key processes benefiting plant growth and health (Braeken *et al*., 2008 and Barea *et al.,* 2005).

Table (3): Cellulase and pectinase activity of bacterial isolates.

Bacterial isolates	Cellulas activity	pectinase activity
1PC	-	-
2PC	-	-
3PC	-	+
4PC	-	+
5PC	-	-
6PC	+	+

Table (4): Effect of inoculation with P- solubilizing isolates on maize roots growth.

Treatments	Root length (cm/ plant)	Root fresh weight (g/ plant)	Root dry weight (g/ plant)
Control 1	10.7b	0.838d	0.131c
Control2 (N+ RP+ K)	16.2b	1.081cd	0.245bc
N+ RP+ K +4PC	33.5a	2.727b	0.471b
N+ RP+ K + 5PC	31.4a	2.022bc	0.497b
N+ RP+ K +6PC	33.9a	4.147a	1.124a

Control1= no inoculation and without fertilizers; RP= Rock phosphate. Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

5.2. Root fresh and dry weights:

Data presented in Table (4) showed the effect of inoculation of P- solubilizing isolates on root fresh and dry weight of maize. The results showed that root fresh and dry weight of maize plants increased by inoculation with P- solubilizing isolates but these increases was significant only by inoculation with 6PC in both fresh and dry weight (283 and 358% respectively), while 4PC showed significant increase in root fresh weight up to 152% over the control2 (no inoculation and with N+ K + rock phosphate). P- Solubilizing bacteria significantly increased root fresh and dry weight of maize by 50 and 45.9% as compared to control, respectively (Yasmin *et al*., 2012).Cakmakci *et al.,* (2007) suggest that seed inoculation of barley with N_2-fixing and P-solubilizing bacteria increased barley root weight by 17.9%-32.1% as compared to the control.

5.3. Plant height:

Different bacterial isolates had variable effects on plant height of maize. The results showed increase in plant height by all used isolates in comparison with both un-inoculated controls (Table 5). Maximum increase up to 23.3 % (105.1 cm) in plant height of maize was recorded by inoculation with isolate 4PC. The lowest increase in plant height of maize over uninoculated controls was recorded with the isolate 5PC. Yosefi *et al.,* (2011) reported that the highest plant height was obtained by application of biological fertilizer + 50 kg/ha P_2O_5 with micronutrient foliar application, and the lowest plant height was obtained in control treatment (non micronutrients foliar and fertilizer application). These results are in harmony with Yasmin *et al.,* (2012) who reported that, among 7 rice rhizosphere isolates strain 13PKR showed maximum increase by 43 % in shoot length of maize as compared to control.

5.4. Shoot fresh and dry weight:

Data in Table (5) showed shoot fresh and dry weight of maize plants as affected by inoculation with different P- solubilizing isolates. The results showed that shoot fresh and dry weight of maize plant increased by inoculation with P- solubilizing isolates. Inoculation with 6PC recorded maximum increase in shoot fresh and dry weight of maize that reached 25.72 (181.7%) and 3.801 (126.3%) g/ plant, respectively.

Cakmakci *et al*., (2007) found that bacterial inoculations significantly affected the growth of barley as the shoot weight increased to a greater degree with P-solubilizing *Bacillus* M-13 than with P fertilization. These findings are supported by the past studies of Zahir *et al*., (2004) who found that inoculating maize seeds with *Azotobacter* and *Pseudomonas* increased the shoot dry weight.

Table (5): Effect of inoculation with P- solubilizing isolates on growth of maize.

Treatments	Plant height (cm/ plant)	shoot fresh weight (g/ plant)	shoot dry weight (g/ plant)	Number of leaves / plant
Control 1	65.1d	4.97e	0.741d	5.67d
Control2 (N+ RP+ K)	85.2c	9.13d	1.679c	7.00c
N+ RP+ K +4PC	105.1a	19.84b	2.486b	8.00ab
N+ RP+ K + 5PC	95.2b	12.94c	2.285bc	7.33bc
N+ RP+ K +6PC	101.2ab	25.72a	3.801a	8.67a

Control1= no inoculation and without fertilizers; RP= Rock phosphate. Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

5.5. Number of leaves:

The data on the leaf number are presented in Table (5). The results showed that all isolates significantly increased the number of leaves in maize plants and the highest significant values recorded with rock phosphate inoculated with 6PC which was 8.67 leaves / plant. While the lowest value of leaf number was recorded with rock phosphate inoculated with 5PC in maize plants. Abou El-Yazeid and Abou-Aly (2011) revealed that number of leaves /plant had significant high values under application of phosphate solubilizing microorganisms combined with rock phosphate treatments compared to control. Zahir *et al.,* (2004) also reported the enhancement of maize leaves number inoculated with *Azotobacter* and *P. fluorescens*.

5.6. Photosynthetic pigments:

Data in Table (6) indicated that different photosynthetic pigments i.e., chlorophyll a, b and carotenoids in maize leaves were positively responded to inoculation with P-solubilizing isolates. Generally, these results are considered as a good explanation to the obtained data regarding the favorable role of biofertilizer on growth parameters that enhanced photosynthetic efficiency. Results showed that 6PC caused maximum significant increase in chlorophyll a and b concentrations that was 66.5 and 67% higher than control2 (no inoculation and with N+ K + rock phosphate), respectively. Maximum significant increase in carotenoids concentration was recorded by 4PC (up to 43.3% increase over control2). Similar results were reported by Han *et al.,* (2006) who found that the integrated treatment of P-solubilizers and application of rock-P significantly increased the photosynthetic pigments of leaves over the control. Abou El-Yazeid and Abou-Aly (2011) showed that, the enhancing effect of rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium* as phosphate dissolvers on chlorophyll content may be due to that rock phosphate inoculated with these bacteria increased total sugar and the latter are essential for chlorophyll formation.

5.7. N P K concentrations in maize shoots:

Data presented in Table (7) showed that inoculation with PSB isolates increased N, P and K concentrations in shoot of maize. All isolates significantly increased P and K in shoot of maize plants as compared to both uninoculated controls. While, inoculation with 6PC significantly increased N in shoot of maize. The highest significant increase in N, P and K of maize (up to 29.6, 260.7 and 21% over the control2, respectively) was obtained by 6PC. In this connection, Abou El-Yazeid and Abou-Aly (2011) showed that maximum enhancement for chemical constituents (N, P and K) of tomato plants was observed in plants treated with rock phosphate inoculated with *Paenibacillus polymyxa* and *Bacillus megaterium.* The effect of phosphate solubilizing bacteria on growth may be due to the activity of P solubilization caused by the used strain and increased further mineral availability uptake. Also, Premsekhar and Rajashree (2009) and El-Tantawy and Mohamed (2009) showed that the increase in growth characters might be due to the fact that inoculated plants with phosphate solubilizing bacteria were able to absorb nutrients from solution at faster rates than uninoculated plants resulting in accumulation more N, P and K in the leaves.

Table (6): Effect of inoculation with P- solubilizing isolates on Photosynthetic pigments (chlorophyll a, b and carotenoids).

Treatments	Chlorophyll a (mg/ g D. W.)	chlorophyll b (mg/ g D. W)	Carotenoids (mg /g D. W.)
Control 1	1.59d	1.60c	0.67b
Control2 (N+ RP+ K)	2.48c	1.85c	0.83b
N+ RP+ K + 4PC	3.65a	2.52b	1.19a
N+ RP+ K + 5PC	3.10b	2.57b	1.14a
N+ RP+ K + 6PC	4.13a	3.09a	1.11a

Control1= no inoculation and without fertilizers; RP= Rock phosphate. Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

Table (7): Effect of inoculation with P- solubilizing isolates on N, P and K concentrations in maize shoots.

Treatments	N%	P%	K%
Control 1	1.03c	0.016c	2.71c
Control2 (N+ RP+ K)	1.62b	0.028c	4.13b
N+ RP+ K + 4PC	1.96ab	0.077b	4.69a
N+ RP+ K + 5PC	1.96ab	0.100ab	4.97a
N+ RP+ K + 6PC	2.10a	0.101a	5.00a

Control1= no inoculation and without fertilizers; RP= Rock phosphate. Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

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بكتيريا الإندوفيت المذيبة للفوسفات و دورها في تنشيط نمو نباتات الذرة

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الملخص العربى

تم عزل البكتريا المذيبة للفوسفات من جذور الذرة و تم دراسة التأثير المفيد على نمو نبات الذرة. و وجد أن من بين 9 عزلات ، اظهرت 6 عزلات كفاءة عالية لإذابة الفوسفات.و أوضحت الدراسة أن اقصى إذابة للفوسفات بواسطة هذه العزلات فيما يتعلق بتأثير الحموضة و فترات التحضين المختلفة كانت عند pH = 7 و مدة التحضين من 6 – 12 يوم. على أساس مستويات الإذابة ,تم اختيار 3 عزلات (4PC, 5PC و 6PC) لدراسة قدرتها على تنشيط نمو الذرة فى تجربة الأصص. سجلت نباتات الذرة المزروعة فى تربة تحتوى على صخر فوسفات و الملقحة بالعزلات المذيبة للفوسفات زيادة معنوية فى طول الجذور و طول النبات, الوزن الغض والجاف للجذور و المجموع الخضرى، عدد الأوراق لكل نبات ، الصبغات النباتية و محتوى النيتروجين والفوسفور و البوتاسيوم مقارنة بالنباتات الغير ملقحة. أظهرت العزلتان 6PC و 4PC أفضل تأثير، و لذلك عرفتا على اساس تواليات 16S rDNA و كانتا *Planococcus* sp. و *Bacillus cereus* على التوالى. و وجد من هذه الدراسة أن هذه العزلات يمكن أن تستعمل كمذيبات للفوسفات فى الأراضى المحتوية على فوسفات غير ذائب.