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# Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant

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## KEYWORDS

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*Acromobacter* sp.

**Abstract** This study was conducted with a view to isolate bacteria associated with the roots of sweet potato plants (*Ipomoea batatas* (L.) Lam.) and to assess their functional potentialities in relation to plant growth promoting activities. Seven bacterial isolates namely P18, P19, P31, P32, P35, P39, and P42 were obtained from surface sterilized healthy roots of sweet potato. The isolates were tested for morphological and biochemical characteristics. The results of in vitro assays showed that all isolates can produce IAA, while four isolates i.e. P18, P31, P35, and P42) solubilize rock phosphate. These isolates having abilities for IAA production and phosphate solubilization were tested as bioinoculant to potato tubers. The results of inoculated plants showed significant differences in vegetative growth parameters as well as photosynthetic pigments and N, P, and K concentrations compared with control. Consequently, the more efficient isolates namely P31 and P35 were identified by 16S ribosomal DNA sequencing analysis as *Bacillus cereus* and *Achromobacter xylosoxidans*, respectively. These can be recommended as biofertilizers for reducing the dependence on chemical fertilizers and providing a step forward toward sustainable agriculture.

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## Introduction

In both managed and natural ecosystem, beneficial plant associated bacteria play a key role in supporting and/or increasing plant health and growth (Compant and Sessitsch, 2010).

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Following rhizosphere and rhizoplane colonization, some soil borne microorganisms, can enter roots and establish subpopulations ranging from  $10^5$  to  $10^7$  CFU  $g^{-1}$  FW (Hallmann, 2001). These endophytes are more likely to show plant growth promoting effects than bacteria exclusively colonizing the rhizosphere (Conn et al., 1997; Chanway et al., 2000).

Sweet potato is grown world wide as source of carbohydrates, and its commercial production requires high input of chemical fertilizers which is high cost. Plant growth promoting rhizobacteria (PGPR) is being used as biofertilizer and bioenhancer for different crop plants as an alternative source of chemical fertilizer. PGPR has been known to improve plant root growth and nutrition (Egamberdiyeva and Hoflich, 2004).

Different mechanisms were found to be involved in plant growth promotion, either by rhizosphere or endophytic bacteria. Plant growth regulators produced by some bacteria are signal molecules acting as chemical messengers and play a fundamental role as growth and development regulators in the plant (Martinez-Viveros et al., 2010). Five of the six pathways for auxin biosynthesis in bacteria rely on tryptophan as the main IAA precursor (Patten and Glick, 1996; Spaepen et al., 2007). On the other hand, phosphate solubilizing bacteria could convert insoluble phosphates into available forms for plant via the process of acidification, chelation, exchange reactions, and production of gluconic acid (Chung et al., 2005; Gulati et al., 2010).

Various bacterial strains were inhabited or associated with different plant roots (Khan et al., 2009) isolated eleven different bacteria belonging to the genera, *Enterobacter*, *Rahnella*, *Rhodanobacter*, *Pseudomonas*, *Stenotrophomonas*, *Xanthomonas*, and *Phyllobacterium*, from sweet potato as an endophytes. Among them four strains were shown to produce indole acetic acid hormone.

Farzana et al. (2009) isolated PGPR strains from sweet potato rhizosphere. Fifteen rhizobacterial (PGPR) strains were screened for phosphate solubilizing activity. Six isolates which represent about 40% were able to solubilize in-soluble phosphate as evident by production of clear zone on calcium phosphate medium. *Erwinia cyripedi* had the highest ability for phosphate solubilization, while *Pseudomonas fuscovaginae* had the lowest ability.

Inoculation with PGPR has a great effect on plant growth. A field study was conducted to determine the effects of different local strains of PGPR and nitrogen fertilizer on growth and yield of sweet potato. Four PGPR strains i.e. *Klebsiella* sp. UPM SP9, *Erwinia* sp. UPM SP10, *Azospirillum brasilense* SP7, and *Bacillus sphaericus* UPMB 10 and a non-inoculated control and three levels of nitrogen fertilizer (0, 33, and 100 kg N ha<sup>-1</sup>) were used. Plants inoculated with the PGPR together with 1/3 of the normal rate (33 kg N ha<sup>-1</sup>) gave the highest storage root dry weight compared to non-inoculated control plants. Inoculation also increased the concentrations of N, P, and K in shoots and storage root (Farzana et al., 2007).

The present study was carried out to isolate endophytic bacterial isolates from the healthy roots of sweet potato and to investigate their impact on potato plant growth parameters.

## Materials and methods

### Isolation of cultural endophytic bacteria

Freshly collected roots were carefully washed with tap water for removing adhering soil. The roots were surface sterilized using 70% ethanol for 30 s and 2% sodium hypochlorite for 5 min and then washed twice with sterilized distilled water (Elbeltagy et al., 2000). The sterilized roots were aseptically cut into 1–2 cm sections, macerated with 0.8% saline solution and quartz sand, and then decimally diluted in 0.8% saline solution. Sterility check test was done to insure that the isolated strains were from root inside (endophytes). The last dilutions were used to spread on different specific cultural media, namely yeast extract mannitol agar medium (YEMA) (Vincet,

1970), The National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999) and Pikovskaya's (PVK) agar medium (Pikovskaya, 1948) were used for isolation of IAA and phosphate solubilizing bacteria respectively.

The isolates were subcultured on their specific media for purification and maintained as a stock culture at 4–5 °C for further studies.

### Characterization of bacterial isolates

Morphological characteristics, such as shapes, Gram reaction (Hucker and Conn, 1923), spore formation, motility, and catalase activity (Whittenbury, 1964) of all isolates, were performed by standard procedures. Motility of bacteria was observed by hanging drop method as described by Bertrand et al. (2001). The method to determine spore forming bacteria was followed as described by Harrigan and MacCance (1976).

### Screening of bacterial isolates for their indole acetic acid production

The ability of endophytic bacterial isolates to produce IAA was determined qualitatively and on Yeast Extract Mannitol broth medium (YEM) (Vincet, 1970) amended with tryptophan (0.1 g/l). This broth medium was inoculated in triplicate with standard inoculum 350 × 10<sup>5</sup> CFU/ml. The cultures were incubated in dark at 30 °C for 7 days, and then, the cultures were centrifuged at 3000 rpm for 30 min. Two ml of the supernatant was mixed with 2 drops of orthophosphoric acid, 4 ml of Salkowski's reagent (50 ml, 35% perchloric acid; 1 ml 0.5 M FeCl<sub>3</sub>) was added, and the mixture was allowed to stand for 15 min as described by Gordon and Weber (1951). The intensity of the produced rose color was measured at 530 nm using spectrophotometer. For quantitative determination, color was also developed in standard solution of pure indole-3-acetic acid for drawing a standard curve (Sarwart et al., 1992).

### Evaluation of phosphate solubilizing ability of bacterial isolates

#### On agar plates

Bacterial isolates were screened for their phosphate solubilizing ability on NBRIP and PVK media, respectively. These media were supplemented with insoluble phosphate i.e. tri-calcium phosphate Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> at final concentration of 0.5%. The solubilization zone and colony diameters were measured after 120 h of incubation at 30 °C. The results were expressed according to Nguyen et al. (1992) as follows:

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

### Quantitative assessment of phosphate solubilization

#### In liquid culture

Erlenmeyer flasks (100 ml) contained 20 ml of NBRIP broth medium (Nautiyal, 1999) were inoculated in triplicate with an inoculum of (350 × 10<sup>5</sup> CFU/ml) and then incubated at 28 °C for 7 days. The cultures were harvested by centrifuging at 5000 rpm for 20 min. and the phosphorous content in culture supernatant was determined by the para molybdenum blue method (Olsen and Sommers, 1982). The resulted color

was estimated by spectrophotometer at 600 nm (Naik et al., 2008). For quantitative determination, standard solution of potassium monohydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) was used for drawing a standard curve.

#### *Effects of rhizobacterial inoculum on potato plants*

Pot experiments were carried out at glass house of Shibin El-com farm, Egypt. For determining the effectiveness of the isolated bacterial isolates as bioinoculants, plastic pots (12 cm width) were filled with mix of sterilized soil/sand in 1:1 ratio, and 100 g of sterilized vermiculite and 4 g of rock phosphate were added to sterilized mixture soil (3 kg). Potato tubers were cut into pieces and left to dry for 2 days, coated with the selected isolates grown in liquid culture medium for 15 min using 10% Arabic gum, and then left to dry for 15 min. Potato tubers were sown in pots under soil surface and irrigated weakly. After 30 days of planting, potato plants were collected and measured for different growth parameters; shoot and root length, fresh and dry weight for shoot and root. Chlorophyll a, chlorophyll b, and carotenoids were determined using spectrophotometer at the wavelengths of 440, 644, and 662 nm (Faeel, 1962).

Nitrogen, phosphorus, and potassium contents were determined according to the methods described by AOAC (2005), APHA (1992), and AOAC (2005), respectively.

#### *Bacterial identification using 16S rRNA sequences*

The most efficient endophytic bacterial isolates were completely identified by using 16S rRNA sequences technique as the following: The isolate was grown in nutrient broth on a rotary shaker (120 rpm) at 28 °C for 24 h. Bacterial genomic DNA Mini-Prep Kit (Axygen cat. No. V110440-05) was used to isolate DNA according to SIGMA company instructions. The universal 16S primers used were as follows:

F15'AGAGTTT(G/C)ATCCTGGCTCAG 3'R1 5' ACG-G(A/C)TACCTTGTTACGACTT3'.

Primers were checked for specificity using the PROBE CHECK function of the Ribosomal Database and the BLAST search facility at the National Center for Biotechnology Information. Amplification was confirmed by analyzing 5 µl of PCR reaction mixture on 1% agarose gel (Promega). The resulting PCR product sizes were ranged from 1450 to 1500 bp.

The PCR product was purified using QIAquick PCR Purification Kit (Qiagen) and sequenced using automatic ABI 310 DNA Sequencer, Big Dye Terminator Cycle Sequencing Ready Reaction Kit, and Perkin Elmer. The sequencing was performed in two directions using the previously described

primers (Lane et al., 1985; Lane, 1991). Sequencing data were analyzed by two different computer alignment programs, DNASTar (DNASTAR, Inc., USA) and Sequence Navigator (Perkin, Corp., USA).

The BLAST database (Altschul et al., 1997) of National Center for Biotechnology Information was used to compare resolved sequence of the unknown isolates with known 16S rDNA sequences. Determination of phylogenetic relationships was analyzed by the program Phylogenetic Analysis megAlign of DNASTar version 7.

#### *Statistical analysis*

Analysis of variance (ANOVA) and LSD test were carried out for the obtained results according to Steel and Torrie (1980).

### **Results and discussion**

#### *Characterization of bacterial isolates*

The data presented in Table 1 showed some characteristics of the obtained bacterial isolates. According to morphological characteristics, four bacterial isolates were short rods and Gram -ve, while two isolates were long rods. Three isolates, namely P18, P31, and P39, were spore forming bacteria. All isolated bacteria produced catalase enzyme which used by all aerobic microorganisms. All the isolates were motile except isolates p19 and p39. The motility of these plant associated bacteria may confer an advantage for intercellular ingress and spreading as endophytes within the host plant (Elbeltagy et al., 2000).

#### *Quantitative analysis of IAA reduction and phosphate solubilization efficiency*

The data presented in Table 2 revealed that all the bacterial isolates produce IAA. The produced mounts of IAA ranged from 10.73 to 0.6 µg/ml. The highest production was observed by P19, while the lowest production was detected by P39. These results are in harmony with Chaiharn and Lumyong (2011) who found that 18.05% from 216 bacterial strains isolated from rice rhizospheric soils in Northern Thailand produced IAA and identified the best IAA producer by biochemical testing and 16s rDNA sequence analysis as *Klebsiella* SN 1.1. This strain produced the highest amount of IAA (291.97 ± 0.19 ppm) in culture media supplemented with L-tryptophan. Furthermore, data in Table 2 clearly indicated that the isolates i.e. P18, P31, P35, and P42 solubilize rock phosphate on PVK agar medium, P31 showed the highest

**Table 1** Morphological characteristics of the endophytic bacterial isolates from potato roots.

Bacterial isolate	Shape	Gram reaction	Catalase production	Motility	Sporulation
P18	Long rod	+	+	+	+
P19	Short rod	-	+	-	-
P31	Long rod	+	+	+	+
P32	Short rod	-	+	+	-
P35	Short rod	-	+	+	-
P39	Short rod	-	+	-	+
P42	Small cocci	-	+	-	-

**Table 2** Quantitative determination of IAA and phosphate solubilization efficiency of plant growth promoting bacteria isolated from potato plant.

Bacterial isolate	IAA production (µg/ml)	Phosphate solubilization efficiency (%) <sup>a</sup>	Phosphate concentration (µg/ml) <sup>b</sup>
P18	7.27	250.9	293.4
P19	10.73	ND	105.7
P31	4.91	350	354.3
P32	0.6	ND	110.6
P35	8.38	108.3	240.6
P39	3.06	ND	101.1
P42	5.36	150.3	266.9

ND: not detectable.

<sup>a</sup> PVK agar media.

<sup>b</sup> NBRIP broth media.

phosphate solubilization efficiency which was 350%, and this isolate also showed the highest amount of soluble P (354.3 µg/ml) after 6 days of incubation in liquid medium.

These results are in agreement with Park et al. (2010) who isolated nineteen phosphate solubilizing bacteria (PSB) from various soils samples and six strains solubilized more than 250 mg/l of P from tri-calcium phosphate amended with National Botanical Research Institute's Phosphate (NBRIP) medium. Also, El-Komy (2005) found that *Pseudomonas fluorescens* and *Bacillus megaterium* strains were able to solubilize phosphate effectively and recorded higher solubilization efficiency on PVK plates up to 350 and 185, respectively.

#### Evaluation the effect of inoculation with rhizobacterial isolates on potato growth parameters

To further confirm plant growth promoting characteristics, plant inoculation assay was performed. Earlier reports have shown that PGPR can improve the growth of sweet potato (Radziah and Zulkifli, 2003).

Data in Table 3 indicated that all the tested isolates showed significant increase in all vegetative parameters (shoot length, root length, shoot fresh and dry weight and root fresh and dry weight) as compared to control. Isolate P35 showed the highest value in all growth parameters by approximately 1.9, 1.79, 2.6, 2.02, 2.47, and 2.3 times in shoot length, root length shoot fresh and dry weight and root fresh and dry weight respectively higher than control.

**Table 3** Effect of inoculation with PGPR on some vegetative growth parameters of potato.

Isolate	Shoot length (cm/plant)	Root length (cm/plant)	Shoot fresh wt (g/plant)	Shoot dry wt (g/plant)	Root fresh wt (g/plant)	Root dry wt (g/plant)
Control	8.2 <sup>n</sup>	10.167 <sup>m</sup>	2.9 <sup>n</sup>	0.041 <sup>k</sup>	1.7 <sup>l</sup>	0.019 <sup>j</sup>
P18	12.8 <sup>f</sup>	16.8 <sup>d</sup>	6.2 <sup>e</sup>	0.053 <sup>f</sup>	3.1 <sup>fg</sup>	0.032 <sup>d</sup>
P19	11.3 <sup>i</sup>	15.3 <sup>h</sup>	5.88 <sup>f</sup>	0.049 <sup>h</sup>	2.9 <sup>gh</sup>	0.033 <sup>d</sup>
P31	15.4 <sup>b</sup>	16.3 <sup>f</sup>	6.99 <sup>c</sup>	0.057 <sup>c</sup>	4.1 <sup>d</sup>	0.041 <sup>b</sup>
P32	12.2 <sup>h</sup>	14.4 <sup>j</sup>	6.8 <sup>d</sup>	0.056 <sup>c</sup>	3.2 <sup>f</sup>	0.028 <sup>f</sup>
P35	15.6 <sup>a</sup>	18.3 <sup>c</sup>	7.6 <sup>a</sup>	0.083 <sup>a</sup>	4.2 <sup>cd</sup>	0.044 <sup>a</sup>
P39	12.8 <sup>f</sup>	15.7 <sup>g</sup>	5.7 <sup>g</sup>	0.047 <sup>i</sup>	3.9 <sup>e</sup>	0.031 <sup>de</sup>
P42	12.4 <sup>g</sup>	13.7 <sup>l</sup>	5.2 <sup>h</sup>	0.047 <sup>i</sup>	2.7 <sup>hi</sup>	0.029 <sup>ef</sup>

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 4** Effect of inoculation with PGPR isolates on photosynthetic pigments (chlorophyll a, b and carotenoids) after 30 days of cultivation.

Isolate	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)
Control	8.21 <sup>n</sup>	5.35 <sup>o</sup>	1.01 <sup>g</sup>
P18	10.57 <sup>f</sup>	9.91 <sup>b</sup>	0.044 <sup>p</sup>
P19	9.01 <sup>l</sup>	8.07 <sup>g</sup>	0.05 <sup>p</sup>
P31	11.57 <sup>d</sup>	5.59 <sup>m</sup>	0.12 <sup>n</sup>
P32	9.39 <sup>k</sup>	6.243 <sup>k</sup>	1.42 <sup>c</sup>
P35	12.23 <sup>a</sup>	8.78 <sup>d</sup>	2.22 <sup>d</sup>
P39	9.78 <sup>j</sup>	8.63 <sup>f</sup>	3.81 <sup>c</sup>
P42	11.48 <sup>c</sup>	6.73 <sup>i</sup>	0.38 <sup>k</sup>

FW = fresh weight. Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

These results are in harmony with Zaghoul (2002) who reported that the growth characteristics i.e. plant height, leaves number/ plant, and branches number/plant were significantly increased potato tuber inoculated by *B. megaterium* var. *phosphaticum* 002E.

#### Photosynthetic pigment production by PGPR

The obtained result in Table 4 clearly indicated that P35 and P18 showed significant increase in chlorophyll a and chlorophyll b by approximately 32.8% and 46.01%, respectively, over control, whereas P39 showed significant increase in carotenoids by approximately 73.49% over control.

These results are in accordance with the findings of Abou El-Yazeid and Abou-Aly (2011) they reported that rock phosphate inoculated with *Paenibacillus polymyxa* and *B. megaterium* increased total sugar and chlorophyll formation. Also, Eleiwa et al. (2012) found that inoculation of wheat grains with biofertilizers, *Bacillus polymyxa*, or *Azospirillum brasilense* that produced auxin significantly increased the chlorophyll a, chlorophyll b, and carotenoids as compared with uninoculated treatment.

#### Effect of rhizobacterial inoculation on NPK content of potato plant

The data presented in Table 5 showed that all the isolates showed significant increase in (N, P, and K contents than

**Table 5** Effect of inoculation with PGPR isolates on N, P and K percentage in potato shoot after 30 days of cultivation.

Isolate	N%	P%	K%
Control	2.22 <sup>l</sup>	0.4 <sup>h</sup>	6.82 <sup>o</sup>
P18	2.1 <sup>m</sup>	0.59 <sup>c</sup>	9.64 <sup>d</sup>
P19	3.17 <sup>e</sup>	0.49 <sup>f</sup>	7.1 <sup>m</sup>
P31	3.51 <sup>b</sup>	0.703 <sup>b</sup>	8.51 <sup>h</sup>
P32	3.08 <sup>f</sup>	0.47 <sup>f</sup>	9.64 <sup>d</sup>
P35	4.48 <sup>a</sup>	0.58 <sup>c</sup>	13.19 <sup>b</sup>
P39	3.12 <sup>f</sup>	0.48 <sup>f</sup>	9.64 <sup>d</sup>
P42	2.96 <sup>g</sup>	0.47 <sup>f</sup>	7.67 <sup>k</sup>

Values within the same vertical area with the same letter are not significantly different at 5% probability level by Duncan's Multiple Range test.

control). P35 isolate showed significant increase in nitrogen and potassium content by approximately 50.5% and 48.3%, respectively, comparing with control, while P31 had significant increase in phosphorus content by approximately 43.1% over control.

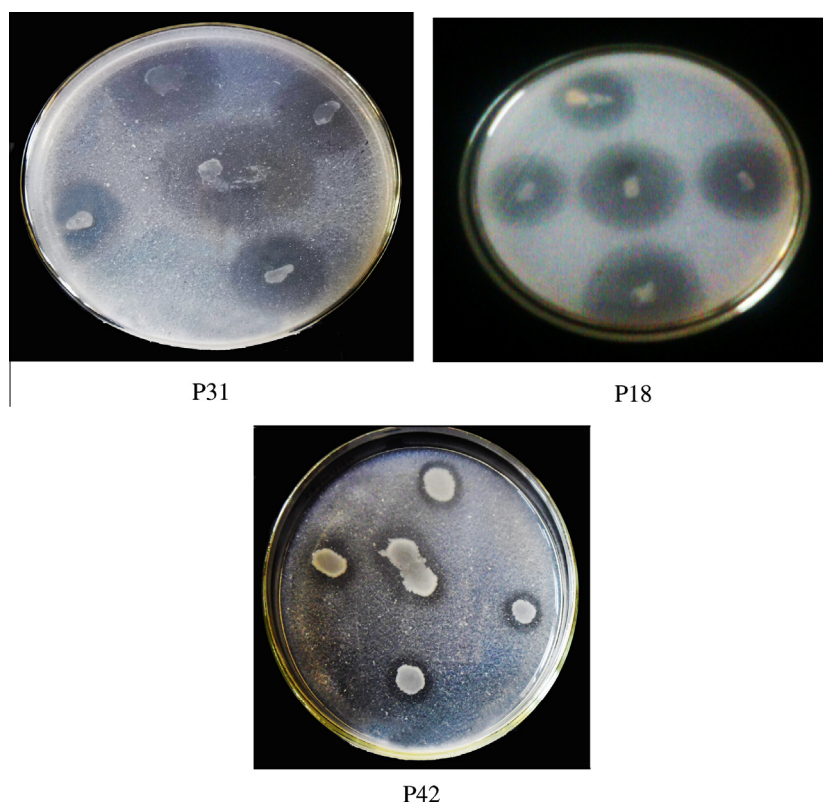
These results are in agreement with those obtained by Mahendran et al. (1996) they reported that inoculation with *Azospirillum* sp and *B. megaterium* in combination, significantly increased the N, P, and dry matter contents and NPK uptake by different plant parts of potato. Also, Farzana and Radizah (2005) determined the influence of rhizobacterial isolates on the response of sweet potato plant growth. A total of five rhizobacterial isolates capable of producing IAA were used. Four of the isolates were collected from sweet potato rhi-

zosphere, and one isolate was imported. Three of the isolates significantly increased the plant growth and the N, P, K, Ca, and Mg uptake of sweet potato cultivar after 60 days of planting.

#### Identification of selected bacterial isolates by 16S rDNA sequencing

Among all the isolates tested, isolate p31 and p35 appeared to be better in terms of P solubilization, IAA production. These isolates showed good results as biofertilizers. So, we identified and characterized these isolates in greater detail. On the basis of 16S rDNA sequence, p35 isolate was identified as *Achromobacter xylosoxidans*, while p31 was identified as *Bacillus cereus*. These results are in harmony with Jha and Kumar (2009) they isolated *A. xylosoxidans* as plant growth promoting endophytic bacteria from wheat. Also, Zhao et al. (2011) isolated the plant growth promoter *B. cereus* from *Sophora alopecuroides* root nodules.

This study showed that some isolated bacteria were able to produce IAA and solubilize phosphate as well. Solubilization of inorganic phosphate could be detected by increasing the phosphorus content in the shoot system and stimulating potato growth. Although four bacterial isolates have a potential for such double function characteristics, the isolates P31 and P35 showed a high plant growth promoting capacities when used as bioinoculants. Therefore, these isolates can be recommended as bioinoculants for reducing the dependence on chemical fertilizers and providing a step forward toward sustainable agriculture (see Figs. 1–3).



**Fig. 1** In vitro assay for phosphate solubilization by some bacteria isolated from potato.

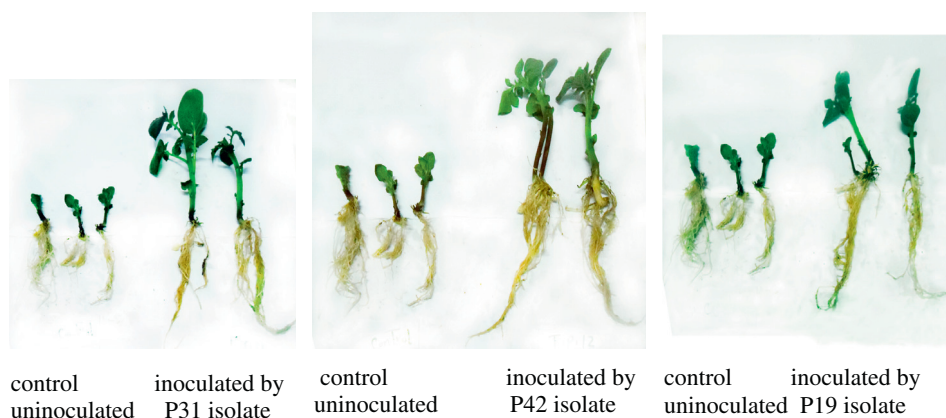


Fig. 2 Effect of inoculation with different bacterial isolates on potato growth.

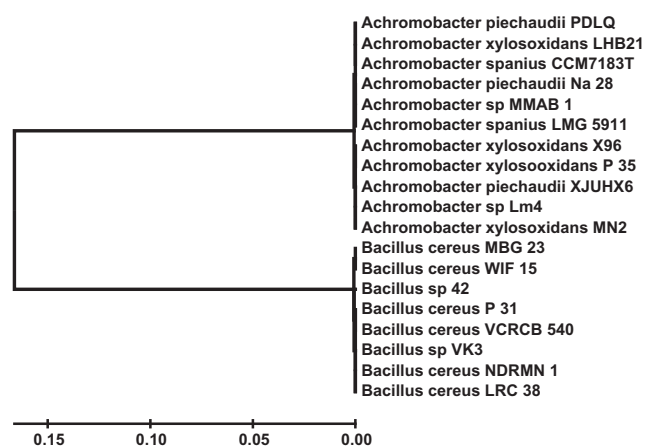


Fig. 3 Evolutionary relationships between identified isolates: p31 and p35 and their relatives in the Gene Bank as inferred by the UPGMA method (Sneath and Sokal, 1973). The optimal tree with the sum of branch length = 2.41306942 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004).

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