EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ON GROWTH CHARACTERISTICS OF MAIZE, BEANS AND GROUNDNUT SEEDLINGS IN POTTED SOIL

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Abstract: Growth effects of phosphate solubilizing bacterial strains on maize, beans and groundnut seedlings in potted soil samples were investigated. The bacterial strains were isolated from uncultivated agricultural farmland on nutrient agar medium. The isolates were tested for their phosphate solubilizing potential using National Botanical Research Institute Phosphate (NBRIP) agar containing tricalcium phosphate as the sole phosphate source. The isolates showed varying phosphate solubilization index (PSI) ranging from 1.8 to 3.1. Among the isolates tested for phosphate solubilization, *Pseudomonas* sp. PSBA2, *Pseudomonas* sp. PSBN1 and *Bacillus* sp. PBSC1 showed higher phosphate solubilization index (PSI) values of 2.8, 3.1 and 1.9 respectively. The best growth performances (31.2 cm, 38.4 cm^2, and 2.93 cm for plant height, leave area and shoot length respectively) were observed in maize inoculated with *Pseudomonas* sp. PSBN1 while the least performances with values of 6.4 cm, 2.8 cm^2, and 2.9 cm for plant height, leave area and shoot length respectively was observed in beans inoculated with *Bacillus* sp. PBSC1. Results of this study are suggestive of the potential for the use of phosphate solubilizing bacteria as a biofertilizer.

Keywords: Potted soil, Phosphate-solubilizing bacteria, Plant seedlings

INTRODUCTION:

Phosphorus is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al.*, 2002). Phosphorus has been reported as one of the key elements in crop production which is associated with several vital functions and is responsible for many characteristics of plant growth such as nodule formation, cell division and organization, fat formation and transfer of heredity. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Qureshi *et al.*, 2012; Saxena and Sharma, 2003). Phosphorus is second only to nitrogen in mineral nutrients most commonly limiting the growth of crops. Phosphorus is an essential element for plant development and growth making up about 0.2 % of plant dry weight. Plants acquire phosphorus from soil solution as phosphate anions (Yadav *et al.*, 2011).

However, phosphate anions are extremely reactive and may be

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immobilized through precipitation with cations such as calcium, magnesium, iron and aluminium ions, depending on the particular properties of a soil. In these forms, phosphorus is highly insoluble and unavailable to plants. As a result, the amount of phosphorus available to plants is usually in small proportion (Ezawa *et al.*, 2002).

Although microbial inoculants have been in use for improving soil fertility during the last century, however, only few studies have been reported on phosphorus solubilization compared to nitrogen fixation. Large amount of phosphorus applied as fertilizer enters into the immobile pools through precipitation reactions with highly reactive elements such as Al^{3+} and Fe^{3+} in acidic soils and Ca^{2+} in calcareous soils (Gyaneshwar *et al.*, 2002; Hao *et al.*, 2002). Soil microorganisms play a key role in soil phosphorus solubilization thereby making phosphate ions available to plants. Inorganic forms of phophorus are solubilized by a group of heterotrophic microorganisms excreting organic acids that dissolve phosphatic minerals or chelate cationic partners of the phosphate ion directly, releasing phosphorus into solution (He *et al.*, 2002). Release of phosphorus in insoluble and adsorbed forms by phosphate solubilizing bacteria (PSB) is an important aspect regarding phosphorus availability in soils. There are strong evidences that soil bacteria are capable of transforming soil phosphorus to the forms available to plant. Microbial biomass assimilates soluble phosphorus and prevents it from adsorption onto soil particles (Khan and Joergesen, 2009). Microorganisms enhance the phosphorus availability to plants by mineralizing organic phosphorus in soil and by solubilizing precipitated phosphates (Chen *et al.*, 2006). These bacteria in the presence of labile carbon serve as a sink for phosphorus by rapidly immobilizing it even in low phosphorus soils (Bünemann *et al.*, 2004). Subsequently, PSB become a source of phosphorus to plants upon its release from their cells. The PSB and plant growth promoting rhizobacteria (PGPR) together could reduce phosphorus fertilizer application by 50 % without any significant reduction of crop yield (Jilani *et al.*, 2007). It indicates that phosphate solubilizing bacteria inoculants hold great prospects for sustaining crop production with optimized phosphate fertilization. Beneficial effects of PSB have often been evaluated on the basis of faster seed germination, better seedling emergence and increased plant growth.

The present study is aimed at assessing the effect of phosphate solubilizing bacterial strains isolated from uncultivated agricultural farmland on the growth of maize, beans and groundnut in potted soil under a controlled environment.

Material and methods

Sample collection:

Soil samples were collected from the rhizosphere of weeds growing in an uncultivated agricultural farmland in Asaba Delta state of Nigeria and were pooled together. The samples were collected in sterile polythene containers and transported within 6 h under aseptic conditions to the laboratory for isolation of phosphate solubilizing bacteria.

Isolation and purification of organisms

The pooled soil samples was weighed and transferred into 9 ml normal saline in 20 ml screw capped glass test tubes. The tubes were vigorously vortexed for 1 min and allowed to settle for 15 min. One milliliter (1.0 ml) of the resulting supernatant was used to carry out serial dilutions upto 10^{-6}. One tenth (0.1 ml) of 10^4 dilution was plated out by spread technique using sterile bent glass rod on nutrient agar (composed of g/l: Peptone, 5.0; Beef extract, 3.0; NaCl, 5.0 and Agar, 15.0) and incubated at 30°C for 48 h. Thereafter, colonies that developed were enumerated and expressed as colony forming unit per mililitre (CFU/ml). Morphologically distinct colonies were selected, purified by repeated culturing and maintained on nutrient agar slants at 4°C.

Screening of isolates for phosphate solubilization

The isolates were screened for their ability to solubilize phosphate using bromophenol blue (BPB) (2.5 µg/ml) amended National Botanical Research Institutes Phosphate (NBRIP) medium (Nautiyal, 1999). The NBRIP has the following composition (g/l): glucose, 10.0; Ca_2 $(PO_4)_2$, 5.0; $MgCl_2.6H_2O$, 5.0; $MgSO_4.7H_2O$, 0.25; $(NH_4)_2SO_4$, 0.1; KCl, 0.2 and agar, 15.0. Purified isolates inoculated onto NBRI-BPB plates were incubated at room temperature (28 ± 2)°C for 5 days. Isolates with halo zones around them were selected and the phosphate solubilization capacity was calculated in terms of phosphate solubilization index (PSI). The phosphate solubilization index (PSI) of the selected isolates was estimated using the expression (Edi–Premono *et al.*, 1996):

$$Phoshate\ solubilization\ index, PSI = \frac{Colony\ diameter + halozone\ diameter}{Colony\ diameter}$$

The isolates showing PSI > 2 were considered as phosphate solubilizing organisms and were identified based on morphological and biochemical characteristics following the schemes of Holt *et al.*, (1994).

Inoculum preparation

A single colony of each strain on agar slants was transferred with sterile wireloop into a 50-ml flask, containing nutrient broth and grown aerobically in flasks for 48 h on a rotating shaker (120 rpm) at 28 ± 2°C. Bacteria cells were harvested by centrifugation at 6000 rpm for 10 min and the cell pellets obtained were suspended in sterile distilled water to optical density of 0.6 at 540nm by diluted with sterile distilled water to a final cell concentration of 10^8 cell/ml.

Pot experiment

Sterile soil preparation

The soil sample from the uncultivated agricultural farmland with its physicochemical characteristics as shown in Table 1 was used in this assay. The assay was carried out as described by Ashrafuzzaman *et al.*, (2009). Sterile farm soil sample was prepared by autoclaving the sample for 20 min at 120°C. This procedure produced sterile soil for the pot experiment. This was followed by transfer of the soil sample into sterile plastic pots (of 6 kg soil capacity) set up in duplicates. The containers were labeled accordingly with the corresponding seedlings and controls.

Table 1: Properties of soil sample used in the study

Parameter				
Soil pH	Total nitrogen (%)	Total organic carbon (%)	Available phosphorus (ppm)	Exchangeable potassium (ppm)
7.4	2.34	11.8	11.9	152.6

Seed bacterization

Seed bacterization was carried out as described by Sharifi *et al.*, (2011) with little modifications. Healthy seedlings of maize, beans and groundnut were surface sterilized with 0.02% sodium hypochlorite for 2 min and rinsed thoroughly in sterile deionised water. Thereafter, the seeds were coated with 15% gum Arabic, an adhesive, for better adhesion of cells and rolled into the suspension of the bacteria strains (10^8cell/ml) for 30 min to ensure uniform coating with the bacterial strains. The treated seeds were carefully sown (placed 2.0 cm deep) in the corresponding labeled pots. Uninoculated seeds given the same treatment were also set up as control and were placed in the corresponding control pots (Salantur *et al.*, 2006) after which the pots were kept in the open. After one week of germination, plants were thinned out and two plants per pot were left. The pots were irrigated at regular intervals of seven days to maintain a proper moisture level (1.45 %) and no artificial fertilizer was applied. The seedlings were allowed to grow within a 30 day period after which the effects of promoting bacterial growth treated seedling plants were assessed by measuring shoot height, plant height and leaf area (Ruget *et al.*, 1996).

Statistical analysis:

Data obtained were statistically analyzed using Student's t-test at $P < 0.05$ level. All tests were conducted in triplicates.

Results and discussion

A total of 41 bacterial isolates that exhibited clear zones on the agar plates (results not shown) were selected as phosphate solubilizing bacteria. Three of these bacterial isolates that showed larger clear zones (> 2) were selected as efficient phosphate solubilizing organisms (PSBA2, PSBN1 and PSBC1). The three selected had a marked insoluble phosphate solubilizing ability as indicated by the clear zone development around the colonies after incubation. The organisms had a solubilization index > 2 (Table 2) after five days of incubation and were identified as *Pseudomonas* sp. PSBA2, *Pseudomonas* sp. PSBN1 and *Bacillus* sp. PSBC1 respectively. This was greater than the solubilization index (SI) of 2.5 obtained after fourteen days incubation recorded for phosphate solubilizing *Pseudomonas* sp. BRS-2 isolated from field-grown rice Rhizosphere soil sample but within the range of solubilization indices of 1.4 – 6.7 reported for fifteen phosphate solubilizing isolates from rice rhizosphere (Alam *et al.*,2002). The ability of the test organisms to grow and

solubilize phosphate on NBRIP-BPB medium suggested that uncultivated farmlands are also rich in such phosphate-solubilizers that effectively utilize unavailable phosphate. *Pseudomonas* and *Bacillus* species are known to play important roles in phosphate solubilization activities. This property has attracted researchers to exploit the strains potential to utilize phosphate reserves in semi arid regions and other sites in order to enhance the crop yields (Khan *et al.*, 2006).

Increased plant height, leaf area and shoot length of the plants were recorded from the seedlings (beans, groundnut and maize) treated with the phosphate solubilizing bacteria (Figure 1). The overall best growth performances (31.2, 38.4, and 2.93 cm for plant height, leaf area and shoot length respectively) were observed in maize inoculated with *Pseudomonas* sp. PSBN1. The next best growth performance was recorded from groundnut inoculated with *Pseudomonas* sp. PSBA2 with enhanced values of 7.0, 3.1 and 5.2 cm for plant heights, leave size and shoot lengths respectively. Same value of 3.1 and 2.8 cm on the enhancement of leave size (area) was

Table 2: Phosphate solubilization properties of the test isolates

Organism	Phosphate solubilization index (PSI)
Pseudomonas sp. PSBA2	2.8
Pseudomonas sp. PSBN1	3.1
Bacillus sp. PSBC1	2.9

observed on beans and groundnut inoculated with test organisms of *Pseudomonas* sp. PSBN1 and *Bacillus* sp. PBSC1. The increase in plant height and shoot length could be associated with cell elongation and multiplication induced by greater absorption of nutrients, particularly phosphorous while the least overall performances with values of 6.4, 2.8, and 2.9 cm for plant height, leaf area and shoot length respectively was observed in beans inoculated with *Bacillus* sp. PBSC1. These values differed significantly compared to the control. The present results are quite similar to the results obtained by Walpola and Yoon (2013) and Farooq and Bano (2013) who investigated phosphorous uptake and growth promotion of tomato plant and maize plant (*Zea mays* L.) though not the plants investigated in growth chamber and green house experiments. Moreso, Yu *et al.*, (2011) reported that *Pseudomonas chlororaphis* and *Pseudomonas fluorescens* remarkably enhanced plant height, shoot and phosphorous uptake of walnut seedlings.

The results of obtained from the present study suggested that bacteria strains isolated from uncultived farmland for the enhancement of plant growth is an efficient tool to select effective plant growth promoting bacteria in the development of bio-inoculants for crop plants. Nonetheless, further studies are needed under field conditions to confirm the present

findings and their eventual commercial applications.

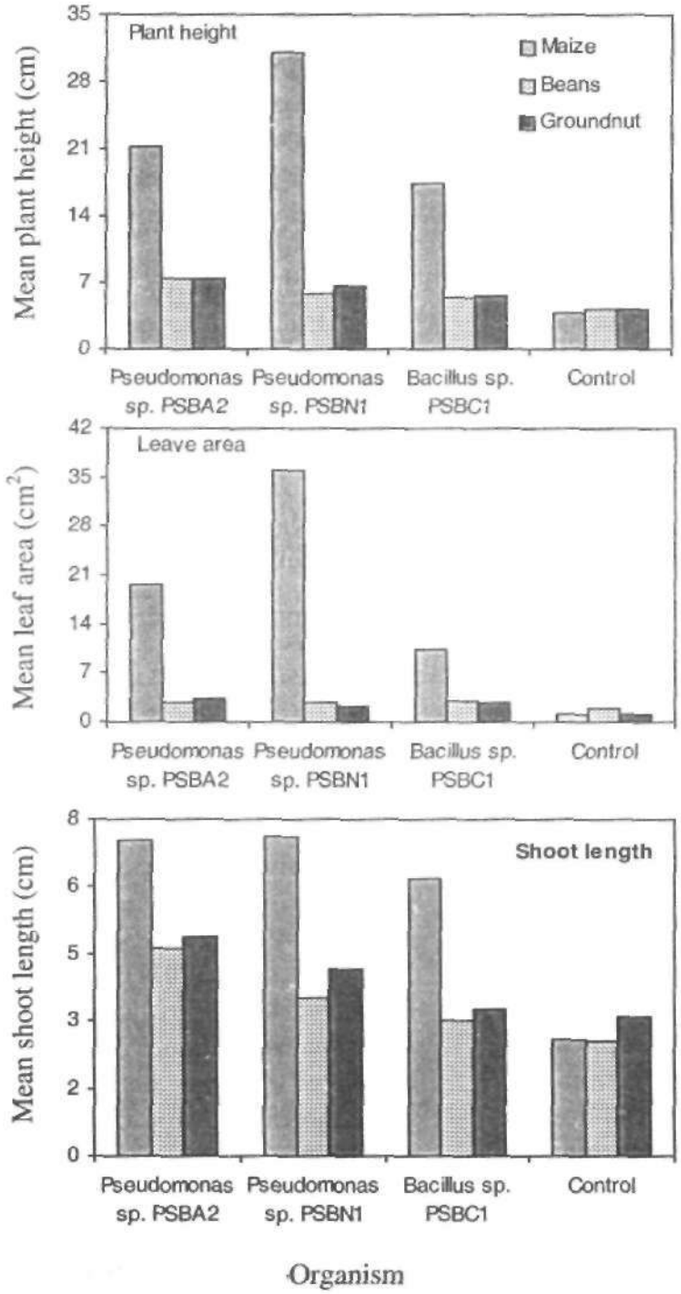

Figure 1: Effect of the organisms on the height, leaf area and shoot length of maize beans and groundnut.

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