

Enhancement Potential of Plant Growth-Promoting Rhizobacteria on White Beans (*Phaseolus vulgaris*) Seedlings

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ABSTRACT. The effect of inoculating single cultures of plant growth-promoting rhizobacteria (PGPR) on development of white beans seedling (*Phaseolus vulgaris*) was analyzed. Five PGPR were isolated from the rhizosphere of Okra plants and were assessed for abilities to solubilise phosphate and produce indole acetic acid (IAA). The phosphate solubilising index ranged from 6-10 while the concentration of IAA ranged from 17.48mg/l to 27.43mg/l. *Serratia* sp. produced the highest concentration of IAA (27.43mg/l) and had the highest solubilisation index (10mm). *Bacillus* sp. produced the least amount of IAA (17.48mg/l) while *Staphylococcus* sp. had the least solubilisation index (6mm). The highest percentage germination of 83.3% was observed in the seedling inoculated with *Bacillus* sp. The effect of PGPR on root and shoot elongation was studied hydroponically for 7 days. Significant increases ($P < 0.05$) in root elongation were observed.. The highest seedling root length (18.47cm) and shoot length (19.17cm) were observed with inoculation of *Staphylococcus* sp. and *Bacillus* sp. respectively. The use of these bacteria as bio-inoculants could be a sustainable practice to facilitate nutrient supply to white beans seedlings.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are soil bacteria that aggressively colonize the rhizosphere and enhance the growth and yield of plants when applied to seeds or crops [1]. These rhizosphere bacteria enhance plant growth by different mechanisms such as solubilisation of phosphate and production of plant hormones such as Indole acetic acid [2]. The use of these bacteria with the aim of improving nutrient availability for plants is an important practice which enhances agriculture. During the past couple of decades, the use of plant growth promoting rhizobacteria for sustainable agriculture has increased tremendously in various parts of the world and they are also the trend for the future [3, 2]. With increasing awareness about the possible negative effects of chemical fertilizer based agricultural practices, it is important to search for region specific microbial strains which can be used as growth promoting and enhancing inocula to achieve desired crop production [4]. Phosphorus (P) is one of the major macronutrients needed by plants and is available in soil in insoluble forms. They are often applied to soil as chemical fertilizers in soluble inorganic forms which could be immobilized by microorganisms and so are unavailable to plants. Apart from nitrogen, phosphorous is an essential plant nutrient whose deficiency restricts crop yield severely [5]. It is generally accepted that the mechanism of mineral phosphate solubilisation by phosphate solubilisation bacteria (PSB) strains is associated with the release of low molecular weight organic acids which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms [6,7]. Microorganisms with phosphate solubilising potentials increase the availability of soluble phosphates and can also enhance plant growth by increasing the availability of trace elements such as iron, zinc etc., by production of plant growth promoting regulators [8].

However, Phosphate solubilisation is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the microbial culture [9]. It has been observed by many investigators that a high proportion of phosphate solubilising microorganisms (PSMs) especially bacteria, fungi and actinomycetes reside in the rhizosphere of plants and play an

important role in plant nutrition as they enhance Phosphate availability to roots through converting the insoluble phosphates into soluble ions [10]. Inoculation of PSB has resulted in improving growth yield and phosphorus uptake in several crops. It is believed that production of plant growth promoting substances by PSB may contribute to their stimulatory effect on plant growth [11].

Seed or soil inoculation with PSBs is known to improve solubilisation of fixed soil phosphorous and applied phosphates, resulting in higher crop yields [12].

Increasing crop yield through the use of PGPR as microbial inoculants is preferable to the use of chemical fertilizers. This is pertinent given the increased international demand for food security and environmental sustainability.

MATERIALS AND METHODS

Location of study site

This present study was carried out at the Research Farmland of the School of Agriculture and Agricultural Technology (SAAT) of The Federal University of Technology, Owerri, Imo state, Nigeria between the month of June and July 2015.

Soil sampling

Soil samples from the rhizosphere of mature okra (*Abelmoschus esculentus*) growing in the research farmland of the Federal University of Technology, Owerri, Imo state, Nigeria were used. The soil samples attached to the roots of the plants were collected into sterile containers after uprooting the plants. The collected samples were homogenized and transferred to the laboratory for analysis.

Collection of White Bean Seedlings

Whole white bean seeds (*Phaseolus vulgaris*) free from weevil infestation and other deformities were purchased from the market.

Isolation of Rhizospheric bacteria from Rhizosphere of Okra seed (*Abelmoschus esculentus*)

Rhizospheric bacteria were isolated from one (1) grm of the Rhizosphere soil of mature Okra plant. The soil sample was serially diluted and further inoculated on Luria –Bertani (LB) agar.

Screening of isolates for phosphate solubilising ability

Isolates were screened for phosphate solubilizing ability by using modified National Botanical Research Institute Phosphate growth medium (NBRIP) [13]. Purified isolates were point inoculated on sterile NBRIP plates and incubated at room temperature for 4 days. Isolates which showed distinct zone of clearing (halozones) were selected and their solubilization indices (SI) determined.

Determination of Solubilisation Index, SI

The SI was calculated using the formula according:

$$SI = \frac{X + Y}{X} \quad \text{Equation 1}$$

Where X = Colony diameter

Y= Halozone diameter [14]

Estimation of Indole Acetic Acid (IAA) production by Phosphate solubilsing bacterial isolates

Phosphate solubilising bacteria were screened for ability to produce indole acetic acid using the method [15]. One (1) ml of each of the inoculum was added separately into 2mls of sterile Jeon's Medium contained in 15ml test tubes. The test tubes were incubated for 3 days at room temperature. After incubation the tubes were centrifuged at 3000rpm for 15mins. One (1) ml of each filtrate was pipetted into 15ml test tubes and 2ml of freshly prepared Salkowski reagent (2% 0.5M FeCl₃ in 35% perchloric acid) was added to each tube. This set up was incubated in a dark cupboard for 30mins. The appearance of a red discoloration indicated indole acetic acid (IAA) production. The amount of IAA was quantified spectroscopically at 530nm. The isolates which showed red discoloration were identified and used for further studies.

BIOASSAY OF SEEDLING

Estimation of the effects of inoculation of white beans seeds by bacteria isolates was done by monitoring germination, root elongation and shoot elongation.

Disinfection of White Beans Seeds:

The seeds were disinfected by soaking them in 1% sodium hypochlorite for 5 minutes. Thereafter the seeds were washed thoroughly with sterile distilled water to remove traces of sodium hypochlorite on them.

Effects of inoculants on germination of seeds:

Six (6) disinfected seeds were placed in 7 different sterile petri dishes containing sterile cotton wool. The seeds were watered with 1ml of distilled water in order to moisten the sterile cotton wool to stimulate germination and incubated on a laboratory bench at room temperature ($28 \pm 2^\circ\text{C}$). The sprouted seeds were inoculated with 100 μl of microbial inoculants using sterile 1ml micropipette. The seeds were allowed to germinate.

Percentage germination was calculated thus:

$$\frac{\text{Number of germinated seeds}}{\text{Number of seeds planted}} \times \frac{100}{1}$$

Effects of inoculation on seedling elongation:

The influence of microbial inoculants on root and shoot elongation of the White Bean was determined daily for a period of 7 days. This was done hydroponically by growing the germinated seeds in the absence of soil as support [16]. The best sprouted seeds were suspended via a sterile millipore net over a 40ml beaker containing sterile water in such a manner as to allow the root gravitate towards the water (hydrotropism) and the emerging shoot gravitates towards natural sunlight (Lithotropism). This set up was placed on a laboratory bench at room temperature. The primary root and shoot elongations were measured in centimeter.

Statistical Analysis:

The data were subjected to two-way analysis of variance (two-way ANOVA) using SPSS 16.0 statistical program followed by post hoc testing. Mean values were separated using the Duncan and Student-Newman Keuls method at $P=0.05$ respectively.

RESULTS

A total of five PGPR isolates were obtained. The isolates were identified as *Klebsiella* sp., *Micrococcus* sp., *Staphylococcus* sp., *Serratia* sp., and *Bacillus* sp.. Among PSB isolates from the rhizosphere soil samples, *Serratia* sp. showed best utilization of phosphate with solubilisation index of 10mm and *Staphylococcus* sp. showed the least solubilisation of phosphate with solubilisation index of 6mm.

Estimation of Solubilisation Index (SI)

The ranking order of the results of the estimation of solubilisation indices by the bacterial isolates was *Serratia* sp. (10) > *Klebsiella* sp. (8.5) > *Micrococcus* sp. (8) > *Bacillus* sp. (7.5) > *Staphylococcus* sp. (6). All the isolates showed SI greater than 5. After 3 days of incubation, *Serratia* sp. showed the highest SI of 10mm.

Table 1: Solubilisation indices of bacterial isolates

Isolates	Solubilisation index (mm)
<i>Klebsiella</i> sp.	8.5
<i>Micrococcus</i> sp.	8
<i>Staphylococcus</i> sp.	6
<i>Serratia</i> sp.	10
<i>Bacillus</i> sp.	7.5

Production of Indole Acetic Acid (IAA)

Serratia sp. produced highest amount of IAA (27.43mg/l) while least amount of IAA (17.48mg/l) was produced by *Bacillus* sp. as seen in Table 2 below. The ranking order of IAA production by the species from rhizosphere soil bacteria was *Serratia* sp. > *Staphylococcus* sp. > *Micrococcus* sp. > *Klebsiella* sp. > *Bacillus* sp.

Table 2: Estimation of Indole acetic acid production by isolates

Isolate	IAA (mg/l)
<i>Klebsiella</i> sp.	17.52
<i>Micrococcus</i> sp.	17.57
<i>Staphylococcus</i> sp.	17.90
<i>Serratia</i> sp.	27.43
<i>Bacillus</i> sp.	17.48

Effects of inoculants on germination of seeds

Germination was monitored for two days.

Table 3: Percentage germination of seedling treatments:

Inoculants Seedling Treatment	% Germination
BS + <i>Serratia</i> sp.	66.7
BS + <i>Staphylococcus</i> sp.	66.7
BS + <i>Micrococcus</i> sp.	50
BS + <i>Bacillus</i> sp.	83.3
BS + <i>Klebsiella</i> sp.	50

KEY: BS= Bean Seed

Effect of single microbial inoculation on Root length:

Inoculation with *Staphylococcus* sp. and *Serratia* sp. significantly increased the root length as compared to the uninoculated control. Inoculation with *Klebsiella* sp. and *Micrococcus* sp. did not result in any significant increase in root length of white beans as compared to uninoculated control.

Table 4: Root elongation of Bean seedling inoculated with single Bacterial isolates

		TREATMENT					
Root length (cm)	Day	Control	<i>Klebsiella</i> sp.	<i>Micrococcus</i> sp.	<i>Staphylococcus</i> sp.	<i>Serratia</i> sp.	<i>Bacillus</i> sp.
	1	0.5	4	2	4	4	2.5
	2	1.5	5	2.5	6.5	7	6
	3	3	5.5	4	7	7.5	6.5
	4	4	6	5.5	8.5	9.5	8
	5	4.5	7	7	9.5	11	9.5
	6	6.5	8	7.5	11	12	12
	7	8	10	9.5	18.5	18	13.5

Table 5: Shoot elongation of Bean seedling inoculated with single Bacterial isolates

		TREATMENT					
Shoot length (cm)	Day	Control	<i>Klebsiella</i> sp.	<i>Micrococcus</i> sp.	<i>Staphylococcus</i> sp.	<i>Serratia</i> sp.	<i>Bacillus</i> sp.
	1	0	0	0	1	0	0
	2	0	1.5	1	1.5	2	3
	3	2	3.5	3.5	2	3	5
	4	3	4	4.5	3	3	9
	5	4.5	7.5	6.5	5.5	5.5	13
	6	7.5	11	10.5	9.5	7	14.5
	7	9.2	14.5	12.5	13	11.5	20

7th day response of single inoculation of PGPR isolates on beans seedlings:

Root length

The PGPR isolates significantly ($P=0.05$) affected the root length of beans seedlings. Results reveal that root length increased in PGPR treated plants over uninoculated control. The highest root length (18.47 cm) was recorded in *Staphylococcus* sp. which was statistically similar to *Serratia* sp. (18.17 cm).

Shoot length

A significant increase in shoot length of beans seedlings was observed in response to PGPR isolates. The highest effect on shoot length was recorded in *Bacillus* sp. (19.17cm) followed by *Klebsiella* sp. and *Staphylococcus* sp. (13.83cm and 13.07cm) and also *Micrococcus* sp. (12.37 cm). The lowest effect on shoot length was noted in uninoculated control (9.27cm).

Table 6: Estimation of Indole acetic acid production by isolates

Treatment	N	Root length (cm)	Shoot length (cm)
Single inoculation treatment			
Control	3	7.5000	9.2667
<i>Klebsiella</i> sp.	3	9.5000	13.8333
<i>Micrococcus</i> sp.	3	9.2000	12.3667
<i>Staphylococcus</i> sp.	3	18.4667	13.0667
<i>Serratia</i> sp.	3	18.1667	11.2667
<i>Bacillus</i> sp.	3	13.0000	19.1667

DISCUSSION

In this study, plant growth-promoting rhizobacteria, *Serratia* sp., *Bacillus* sp., *Staphylococcus* sp., *Klebsiella* sp. and *Micrococcus* sp. were isolated from the rhizospheric soil of okra and their influence on root and shoot elongation of white beans seedlings was monitored hydroponically for seven days. *Serratia* sp. showed the highest solubilisation index of 10mm within 72hrs compared to other bacterial isolates obtained. All other bacterial isolates obtained from rhizospheric soil samples were however capable of solubilizing phosphate forming clear halos. This finding is in agreement with [9] who reported that all species of phosphate solubilizing bacteria produce regulating substances and possess the ability to solubilize inorganic phosphate. In addition to phosphate solubilisation ability, another important trait of plant growth promoting rhizobacteria is production of indole acetic acid. *Serratia* sp. produced the highest quantity of indole acetic acid of 27.43mg/l. The hydroponic culture results showed that *Serratia* sp. achieved a percentage germination of 66.7% and achieved root elongation of 18.17cm. In hydroponic culture performed with *Serratia* sp. SY 5 [17], its inoculation had a favourable influence on the shoot length of *Zea mays* and significantly increased the plant's growth. They also observed that *Serratia* sp. showed capacities for indole acetic acid production. *Bacillus* sp. showed best percentage germination ability of 83.3% and recorded a solubilisation index of 7.5mm and indole acetic acid production of 17.48mg/l.

In a study conducted by [18] it was noted that high percentage germination observed in certain inoculated treatments could be due to plant growth activities of the bacterial species and the fact that the germinating seeds receive most of the nutrients from seed reserves and plant growth hormones such as indole acetic acid, auxins and gibberellic acid produced by the rhizobacteria which may act as stimulants.

Bacillus sp. showed highest shoot elongation measurement of 19.17cm. This significant influence by *Bacillus* sp. on germination and shoot elongation can be attributed to the fact that *Bacillus* sp. is relatively more versatile than others as a PGPR because of its ability to form endospores, which make them retain viability for long periods either in storage or in the soil. *Bacillus subtilis* resulted in emergence of crop establishment and development of seedling vigour and also provided increase in plant growth [19]. While investigating the effect of plant growth promoting rhizobacteria on seed

germination and plant growth of chickpea plant, it was observed that *Bacillus* sp., amongst others, showed significant increase in shoot and root lengths of chickpea [20].

Staphylococcus sp. recorded an indole acetic acid production of 17.90mg/l when used as single treatment it achieved highest root length of 18.47cm and highest shoot length of 12.67cm when used in combination with *Bacillus* sp.

Klebsiella sp. which produced 17.52mg/l of indole acetic acid showed significant effect on shoot elongation of 13.83cm when applied alone.

Six strains of *Klebsiella* were isolated and tested for their plant growth promoting effects on moth bean seeds. The strains demonstrated favourable increase in root length of inoculated moth beans over the control [21].

Conclusion and Recommendation

This study has provided evidence to prove the capacity of certain phosphate solubilising bacterial species to enhance elongation of White Beans seedling (*Phaseolus vulgaris*) as well as increase its seed germination rate. Taken together, these results suggest that application of plant growth promoting rhizobacteria can induce seed germination and improve plant growth.

It is therefore recommended that these microbial inoculants especially *Serratia* sp., *Bacillus* sp. and *Staphylococcus* sp. be used in the production of bio-fertilizers to replace or supplement chemical fertilizers and for crop yield improvement by farmers and researchers in areas where phosphorus availability is limited or unavailable.

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