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Isolation and Characterization of Phosphorus Solubilizing Bacteria from Millet Rhizosphere in Lafia Metropolis

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Abstract

Phosphorus is an essential, but often unavailable nutrient for plant growth due to its low solubility. Phosphorus solubilizing bacteria (PSB) convert insoluble forms of phosphorus into soluble forms that can be taken up by plants. The study aimed to isolate and characterize PSB from the rhizosphere of millet plants in Lafia, and to evaluate their potential for improving soil fertility and crop productivity. Soil samples were collected from four different locations and inoculated on Pikovskaya's agar medium. The PSB isolates were further characterized by morphological, biochemical, and molecular methods. The phosphate solubilizing activity and plant growth-promoting traits of the PSB isolates were also assessed. The PSB isolates were tested for their effect on the growth of millet seedlings in pots. The results showed that 16 PSB isolates belonging to the genera (*Bacillus*, *Pseudomonas*, *Enterobacter*, and *Klebsiella*) were obtained from the soil samples. The PSB isolates exhibited varying degrees of phosphate solubilizing activity, ranging from 11.2 to 81.6 mg/L. The PSB isolates also showed positive results for the production of indole acetic acid, ammonia, Voges-Proskauer and triple sugar iron tests. The PSB inoculation significantly increased the shoot and root lengths, and fresh and dry weight of the millet seedlings. The study concluded that the PSB isolates from the millet rhizosphere in Lafia could enhance soil phosphorus availability and millet growth. The study recommended further research on the field application and optimization of the PSB inoculation.

Keywords: Phosphorus, phosphorus solubilizing bacteria, plant growth promotion, rhizosphere, millet, soil

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Introduction

Phosphorus (P) is the second most essential macronutrient required for plant growth and development [1], and it is a component of biological molecules that affect root development, stalk and stem strength, crop maturity, and nitrogen fixation in legumes [2]. Phosphorus in soils exists both in organic (P_o) and inorganic (P_i) forms [3]. While about 30 μmol of phosphorus is required by plants for maximum development, only about 1 μmol is readily accessible for plant use in soils [4]. Fixation of the mineral with other soil minerals by adsorption and through precipitation makes it unavailable for plant use. Phosphorus is primarily taken up by plants in the form of orthophosphates (H_2PO^{4-} and HPO_4^{2-}) [4, 5].

The unavailability of phosphorus in many agricultural soils necessitated the application of soluble phosphorus in the form of phosphate fertilizers [6]. The use of phosphate fertilizers is accompanied by the disadvantages of it being readily fixed to insoluble forms on reacting with aluminum and iron minerals in the soil, and the depletion of phosphate rock from which the fertilizers are produced hence the need for securing alternative sources of phosphorus for agricultural use in plants. The presently adopted strategy for solving the challenge is the use of some bacteria of the genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* from soil known as Phosphate Solubilizing Bacteria (PSB) [7, 8]. PSBs can solubilize insoluble phosphorus into soluble form using phosphatase enzyme, organic acid and chelate which is readily released into soil solutions [2, 9, 10]. Many phosphate solubilizing bacteria are reported as plant growth promoters in many crops like tomato and rice with populations varying depending on soil characteristics like physical and chemical properties, organic matter, and P content and cultural activities [11].

As the world population continues to increase, demand is placed upon agriculture to supply more food which is one of the greatest challenges facing the agrarian community worldwide. To overcome this challenge, a great deal of effort focusing on the soil biological systems and the agroecosystem as a whole is needed to understand the complex interactions involved in soil systems. Phosphorus availability to plants could be increased either by managing indigenous microbial populations in soil with the purpose of optimizing their ability for the transformation of P or using specific bacteria as microbial inoculants [10, 12, 13]. Simultaneous application of PSB could lessen the use of chemical phosphorus fertilizer by up to 50% without causing a significant decrease in the grain yield of millet [14].

The study aimed to isolate and determine bacteria capable of solubilizing phosphorus in soil and promote millet growth.

Materials and Methods

Soil sample size and collection

The rhizosphere soil samples from plants were collected randomly from Shabu, Lafia, and Mararaba all different regions in Nasarawa State. Five replicate samples of dark, loamy soil weighing 20 g each were collected at a depth of 0-30 cm using auger from the three regions and samples were kept in polythene bags, transported to the laboratory and stored at 4ºC before analysis.

Isolation of phosphate-solubilizing bacteria

Isolation was carried out by inoculating soil samples on Pikovskaya (PVK) medium [15] and the plates were incubated for 5 days at 30°C and colonies with clear halos considered to be those of phosphate solubilizing bacteria [16]. Predominant colonies were further purified by re-streaking on fresh PVK agar plates and incubated at 30°C.

Characterization of isolates

Isolates were characterized based on morphological and biochemical tests which include oxidase, catalase, Methyl red, Voges Proskauer, indole, citrate, oxidase, triple iron sugar test while morphological characterization was based on Gram staining.

Pot experiment: Growth chamber trial

Pot experiments were carried out to investigate the activity of phosphate-solubilizing microorganisms with single inoculations. Seeds of millet were planted to evaluate the performance of PSB. Cultures (10^9 CFU ml^{-1}) of each strain were used as inoculants at a final concentration of 50, 25 and 12.5 $mlKg^{-1}$, respectively [17]. Loamy soil collected from Zee farm located in Lafia was profiled and used to grow the seed; the soil samples were sieved using a 2 mm sieve and clean polythene bags filled with the sieved soils. Seeds were first sterilized in 80% ethanol for 2 min and rinsed 10 times in sterile distilled water before bacterial treatment.

Prepared seeds inoculated with microbial suspension for 30 min were placed at a depth approximately 2.5 cm below the soil surface in all polythene bags and arranged in a completely randomized block design in the growth chamber. Three replicates (polythene bags) per microbial inoculums were made while the control treatment consisted of water-treated seeds. Plants were grown for 30 days under normal environmental conditions and the following parameters were recorded in the growing millet plants: height and number of leaves after every 3 days, root dry weight, root length and the soil available phosphorus content was recorded at harvest.

Statistical analysis

The experimental data was subjected to analysis of variance (ANOVA) using SPSS. Statistical analysis between groups was performed using one-way ANOVA. P<0.05 was considered statistically significant.

Results and Discussion

Isolation of PSB

Table 1 shows the phosphorus solubilizing bacteria isolated from the soil sample collected from the three different locations, they include, *Pseudomonas* spp, *Micrococcus* spp, *Bacillus subtilis*, *Enterobacter* spp, and *Bacillus megaterium*, gram reaction showed that all isolated organisms were rods and are also Catalase positive.

Table 1: List of isolated phosphorus solubilizing bacteria and their biochemical characteristics

Isolates	Gram reaction	Catalase test	Oxidase test	Citrate utilization test	Indole test	Methyl Red test	Voges Proskauer test	TSI	Gas
***Pseudomonas* spp**	- rods	+	-	+	-	-	-	-	-
***Micrococcus* spp**	+ rods	+	+	-	-	-	-	-	-
***Enterobacter* spp**	- rods	+	-	+	-	-	+	+	+
Bacillus subtilis	+ rods	+	-	+	-	-	+	+	-
Bacillus megaterium	+ rods	+	-	+	-	-	+	+	-

(+) positive, (-) negative, TSI- Tripple iron sugar

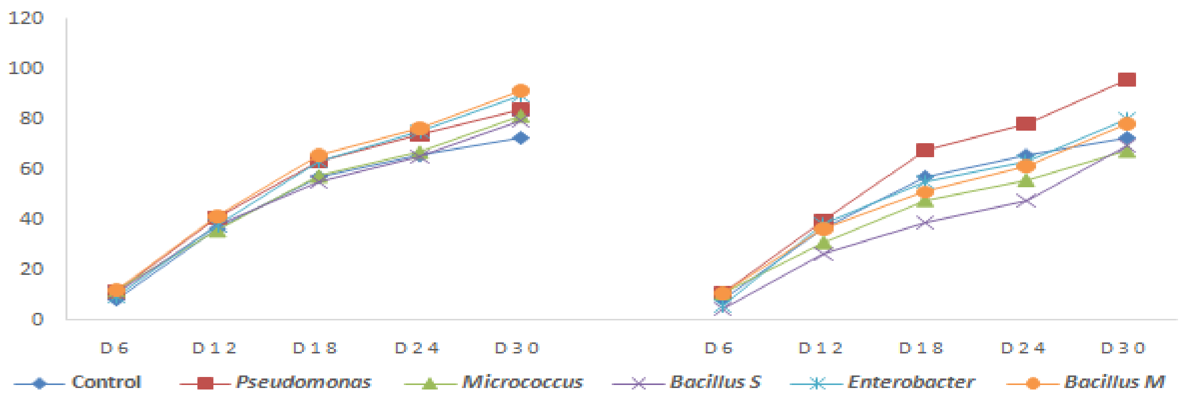

Figure 1: Height of millet plants after treatment with phosphorus solubilizing bacteria at different concentrations

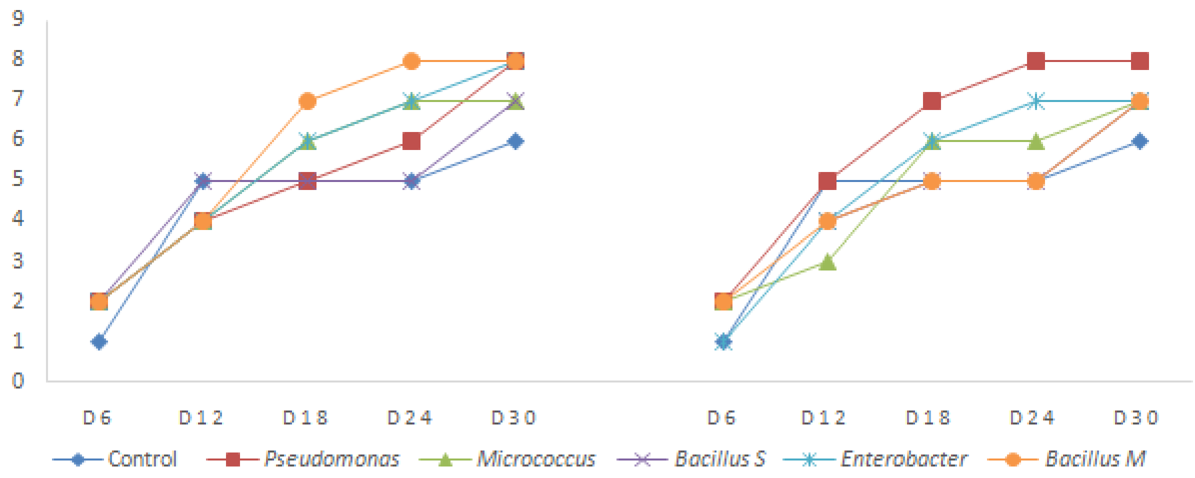

Figure 2: Number of leaves on millet plants after treatment with phosphorus solubilizing bacteria at different concentrations

Pot experiment

Figure 1 shows the effect of inoculation using PSB on plant height after 9 days. Inoculation using *Bacillus megaterium* at a concentration 25 mlkg^{-1} had the highest height of about 33 cm compared to other test organisms while *Bacillus subtilis* at a final concentration of 12.5 mlkg^{-1} had the lowest height of about 19 cm compared to other test organisms. Comparing treatments to control, all test organisms did better than the control except for treatments using *Pseudomonas* spp at a concentration 12.5 mlkg^{-1}. *Bacillus megaterium* at a concentration of 25 mlkg^{-1} had the highest height of 58.5 cm compared to other test organisms while *Bacillus subtilis* at a concentration of 50 mlkg^{-1} had the lowest height, which is 32.5 cm. At 30 days, inoculation using *Pseudomonas* spp at 50 mlkg^{-1} concentration had the highest height (95.4 cm), *Micrococcus* spp at a concentration of 50 mlkg^{-1} and *Bacillus megaterium* at a concentration of 12.5 mlkg^{-1} had lesser height compared to control.

Figure 2 shows the effect of inoculation using PSB on number of leaves after 30 days. Results show that *Pseudomonas* spp at 50 mlkg^{-1}, *Micrococcus* spp., *Enterobacter* spp., and *Bacillus megaterium* at a concentration of 25 mlkg^{-1} had the highest number of leaves (8). When compared to control all test organisms showed better effect on the number of leaves except for the two aforementioned concentration of inoculants. Fig. 3 shows the effect of inoculation using PSB on root length after 30 days. inoculation using *Pseudomonas* spp at a concentration of 50 mlkg^{-1} and *Bacillus megaterium* at a concentration of 25 mlkg^{-1} had the highest effect on root length (45 cm) compared to other test organisms, while *Pseudomonas* spp at a concentration of 12.5 mlkg^{-1} had the least effect on root length (21 cm). Compared to the control all test organisms had a better effect on root length except in cases where *Pseudomonas* spp at a concentration of 12.5 mlkg^{-1} and *Micrococcus* spp at a concentration of 50 mlkg^{-1} was used.

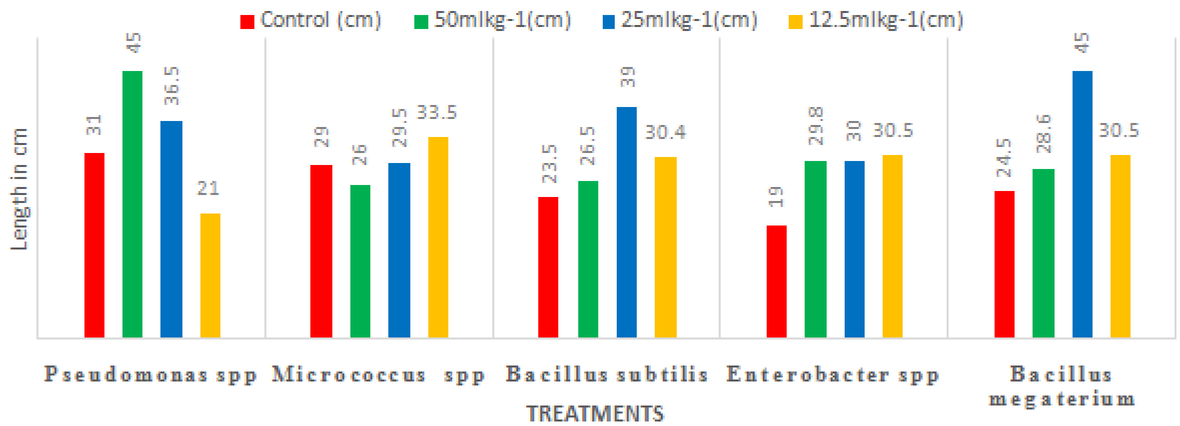

Figure 3: Effect of inoculation of millet seeds with phosphorus solubilizing bacteria on root length after 30 days

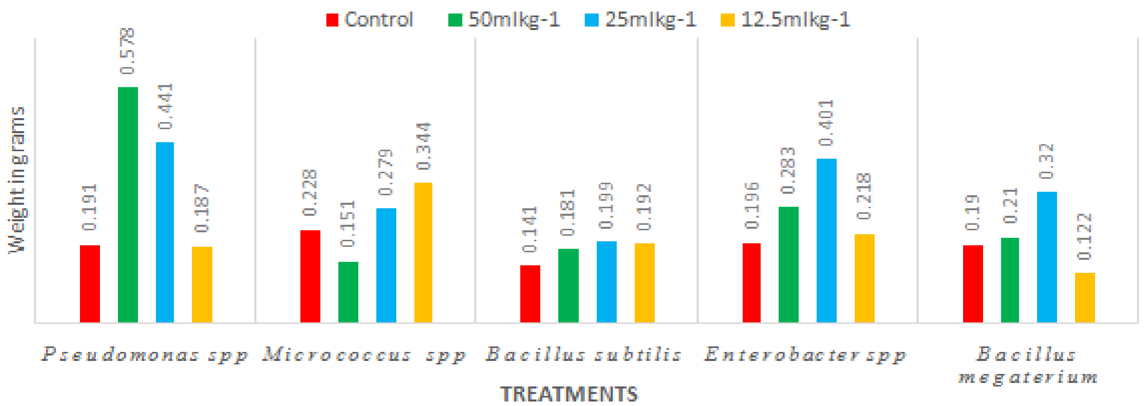

Figure 4: Effect of inoculation of millet seeds with phosphorus solubilizing bacteria on root dry weight after 30 days

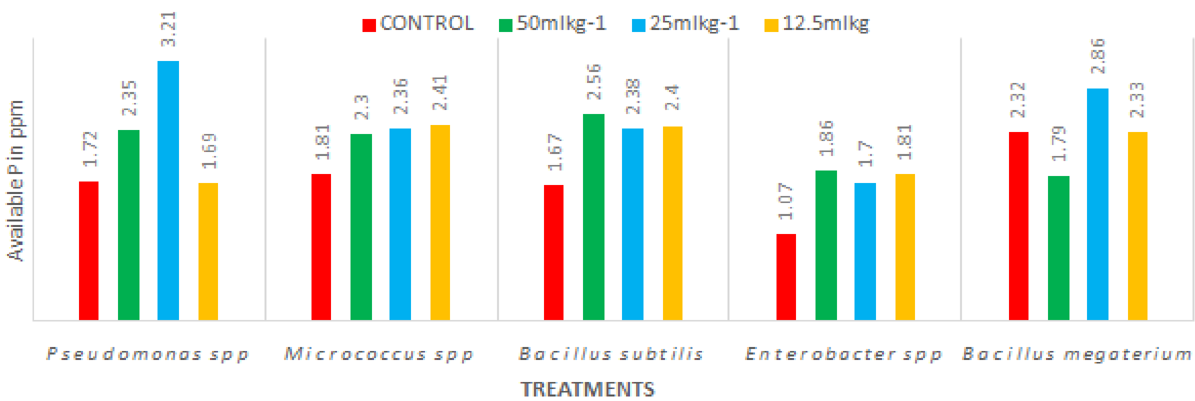

Figure 5: Effect of inoculation with phosphorus solubilizing bacteria on soil available phosphorus

Figure 4 shows the effect of inoculation using PSB on plant dry root weight after 30 days. Plants inoculated with *Pseudomonas* spp at a concentration 50 mlkg^{-1} had the best effect on plant roots dry weight (0.578 g) compared to other test organisms, while inoculation with *Bacillus megaterium* at a concentration of 12.5 mlkg^{-1} had the least effect (0.122 g). Compared to the control, all concentrations of test organisms showed a better effect on dry root weight except for *Pseudomonas* spp at a concentration 12.5 mlkg^{-1} (0.187 g), *Micrococcus* spp at a concentration of 50 mlkg^{-1} (0.151 g), and *Bacillus megaterium* at a concentration of 12.5 mlkg^{-1} (0.228 g).

Figure 5 shows the effect of inoculation using PSB on soil-available P. Results show the effect that inoculation using *Pseudomonas* spp at a concentration of 25 mlkg^{-1} had the best effect on P availability in the soil (3.21 ppm) whereas using the same organism at a concentration 12.5 mlkg^{-1} had the least effect on p availability (1.69 ppm) compared to other test organisms. Compared to the control, all concentrations of test organisms had a better effect on P availability compared to control except for *Pseudomonas* spp at a concentration 12.5 mlkg^{-1} (1.69 ppm) and *Bacillus megaterium* at a concentration of 50 mlkg^{-1} (1.79 ppm). At 25 mlkg^{-1} all test organisms showed a high prospect for increasing P availability in the soil.

Soil ecosystem comprises of a myriad of bacteria with the ability to make available phosphorus needed for plant growth by different transformation processes [18]. *Bacillus, Pseudomonas,* and *Enterobacter* are genera of bacteria that have been reported to be able to solubilize insoluble phosphate (P) in soil making it available for plant uptake [19]. In the present study, *Pseudomonas* spp, *Micrococcus* spp, *Enterobacter* spp, *Bacillus subtilis*, and *Bacillus megaterium* isolated from the rhizosphere were able to solubilize phosphate in Pikovskaya medium. All strains significantly increased soil available P and crop growth, the increase in growth may be attributed to auxin production [18], ACC-deaminase activity [20], production of organic acids [21], or phosphatase [22] which solubilize/mineralize insoluble phosphate thereby increasing the availability of phosphate in the soil for the inoculated plants [23].

In general, seeds inoculated with *Pseudomonas* spp were found to have the best effect on plant height, root length, root dry weight, and soil available P compared with other test organisms. This can be attributed to the fact that they have been found to effectively colonize the rhizosphere and internal tissues of the roots of crops and promote their growth [24]. Similar results of inoculation using strains of the genera *Pseudomonas* spp and *Bacillus* spp on several crops under controlled conditions were reported by Gupta *et al*. [18]. The other strains of bacteria isolated from the soil samples showed better yield compared with the control.

The result agreed with those carried out by Mohammad *et al*. [25] and Pande *et al*. [26]. Other studies showed that *Pseudomonas* spp and *Bacillus* spp enhanced the germination and seedling vigour of different crop plants [27].

This study also determined the effect of different concentrations of inoculums on the growth of millet plants. *Pseudomonas* spp at a final concentration of 50 $mlkg^{-1}$, *Micrococcus* spp at 12.5 $mlkg^{-1}$, *Bacillus subtilis* at 25 $mlkg^{-1}$, *Enterobacter* spp at 25 $mlkg^{-1}$ and *Bacillus megaterium* at concentration 25 mkg^{-1} were the most effective concentration for promoting plant growth. The concentration of inoculum has been shown to also be a key factor in the effectiveness of their activity [25]. At 25 $mlkg^{-1}$ all organisms had high prospect of increasing soil available P, this can be used as a reference for further research.

Conclusion

All tested strains tend to enhance the growth of millet (as measured by plant height, root length, root dry weight, number of leaves and soil available P), and also improved the uptake of available phosphorous content in soil compared with the control. These results indicate that bacterial inoculation into millet seeds might lead to higher grain yield as P-solubilizing *Pseudomonas* spp showed great potential for use as bioinoculants.

Competing interest: The authors declared no competing interest.

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