Int.J.Curr.Microbiol.App.Sci (2022) *11*(07): 222-236

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 11 Number 07 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1107.027

Microbial Activity of Phosphate Solubilizing Organisms Isolated from Rhizosphere Soil on the Growth of Sorghum Plant (*Bicolor sorghum*)

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Keywords

Phosphate-solubilizing microorganisms, rhizosphere, phosphate, qualitative, quantitative, sorghum

Article Info

Received:
08 June 2022
Accepted:
04 July 2022
Available Online:
10 July 2022

A B S T R A C T

Phosphorus (P) is one of the most vital macronutrient required for the growth and development of plant. Although P may be present in high concentration in most soils. It is mostly not plant available form. Phosphorus Solubilizing Microorganism (PSM) present in the rhizosphere play a role in increasing the bioavailability of soil phosphate for plant through solubilzation and mineralization. The present study aimed at isolating and analysing PSM from different rhizospheres and testing isolates on growth of sorghum plant at different concentration levels with the future prospect of formulating the potential biofertilizers. A total number of 35 samples in five replicate were randomly collected from rhizosphere soil of of wamba, Doma, Awe, Lafia, Akwanga, Obi and Nasarawa-Eggon in Nasarawa State. PSM were isolated by spread plate technique using Pikovskaya's medium (PVK) and further identified using appropriate biochemical test. Quantitative and qualitative methods was used to evaluate the phosphate solubilization potency of isolates by employing the PVK with supplementation of bromophenol blue and liquid assay by chlorostannous reduced molybdophosphoric acid method. A pot trial experiment using polyethene bags was conducted to determine the activity of the isolated PSM on growth of sorghum plant. A total number of 22 microbial isolates with halo zone of phosphate solubilization more than 5mm were isolated from the various rhizosphere soils samples. Among the isolates, 5 showed relative potency to solubilize the inorganic phosphate and were selected for further studies. All of the 5 isolates showed variation in the quality and quantity efficiency to solubilize phosphate ranged from 1.15cm to 2.25cm qualitatively and from 0.517 mg/l to 0.764 mg/l quantitatively. The isolates *Pseudomonas* spp, and *Bacillus* spp, were most potent for their qualitative and quantitative activities to solubilize the inorganic phosphate, ranged from 2.25cm and 2.17cm and 0.764 mg/l and 0.626 mg/l. Further analyses for physiological optimization of PSM indicates maximum growth and P solubilization activity on PVK broth at temperature range of 30-35°C for bacteria and 25-30°C for fungi and pH at 6-7 for all PSM. The pot experiment showed that application of PSM on sorghum plant significantly improved plant height, numbers of leaves, root length and dry root weight. Inoculation using *Pseudomonas* spp increased plant growth the best when compared to other inoculants. *Aspergillus* spp had the least effect on the growth of sorghum in all measured parameters. All inoculants showed a high phosphate solubilizing abilities at a concentration of 50mLkg-1 and 25mLkg-1. The result showed that there was significant difference at (0.05) confidence limit in all treatment levels (<0.05). The present study indicated that the use of PSM is considered a sound eco-friendly and cost effective strategy in improving the productivity of sorghum and other crops.

Introduction

Sorghum (*Sorghum bicolor*) is among the major cereal crop in the world grown for human consumption and some in pastures for animals. The composition of Sorghum grain is similar to maize in many respects. Sorghum grains are rich source of Starch (68 - 80%), Protein (10 - 15%), Fat (3%), Fibre (2%), Vitamin B1 (26%) and Riboflavin (7%). It is relatively rich in micronutrients (mg/kg) iron (41 - 127), zinc (14 - 35), phosphorus (1150 - 2569), sodium (12 - 54) and magnesium (750 - 1506) (Shegro *et al.*, 2012). It is also used in the production of alcoholic beverages. This various uses of sorghum makes it an important crop for both domestic and foreign trade in developing country like Nigeria.

In either to provide sufficient edible starch, it is very important to increase the total production of Sorghum which depends on many factors such as fertilizer management and other management practices for better yield. After Nitrogen, Phosphorus (P) is the second important key nutrient. Adequate supply of phosphorus is therefore required for proper functioning and various metabolic processes of plants (Khan *et al.*, 2010). It is a component of biological molecules, such as Deoxyribonucleic acid (DNA), Ribonucleic acid (RNA), Adenosine triphosphate (ATP) and phospholipids. On a macro level, it affects root development, stalk and stem strength, crop maturity, and nitrogen fixation in legumes (Hutchins *et al.*, 2019). Phosphorus in soils can exist in both organic (Po) and inorganic (Pi) forms; the organic phosphorus constitutes more than 25% of total phosphorus in soil, being derived from biological processes like microbial plant and animal residues. The inorganic forms of phosphorus have been calculated to account for 35 – 70% of total P in soil (Khan *et al.*, 2009). Inorganic phosphate phosphate in the soil comprises phosphate bound to minerals like calcium phosphate, aluminum phosphate and iron phosphate which are the predominant source of phosphate for young plants (Boitt *et al.*, 2018). Majority of P in soils is fixed, and hence, plant available P is scarcely available despite the abundance of both inorganic and organic P forms in soils. When phosphate fertilizers are applied to the soil, they often become insoluble (more than 90 %) and are converted into complexes such as calcium phosphate, aluminum phosphate and iron phosphate in the soil (Mittal *et al.*, 2008). Crop plants can therefore utilize only a fraction of applied phosphorus, which ultimately results in poor crop performance.

In nature, a group of soil microorganisms capable of transforming insoluble P into soluble and plant accessible forms across different genera, collectively called Phosphate Solubilizing Microorganisms (PSM), have been found as best ecofriendly option for providing inexpensive P to plants (Khan *et al.*, 2009). Several mechanisms are adopted by PSMs for the dissolution of phosphorus, although the dominant one was found to be the acidification of the surrounding by the production of organic acids. These mechanism need to be explored in greater details for better understanding of the microbial action for phosphate dissolution (Ameen *et al.*, 2019). These organisms in addition to supplying soluble P to plants also facilitate the growth of plants by several other mechanisms, For instance, improving the agricultural yield in harmony with ecological concerns, improving the uptake of nutrients and stimulating the production of some phytohormones (Rajwar *et al.*, 2018). PSM are likely to serve as an efficient bio-fertilizer especially in areas deficient in P to increase the overall performance of crops by playing a vital roles in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in plants.

Phosphorus in top soil generally accounts for 50 to 3000 mg kg-1 of soil, yet only 0.1% of total phosphorus is available for plants uptake (Zhu *et al.*, 2018). Plants require approximately 30µmol of phosphorus for maximum productivity, but only about 1 µmol is available in many soils. This unavailability is due to phosphorus fixation, either it is adsorbed on the soil minerals or get precipitated

by free Aluminum ion (Al3+) Ferric ion (Fe3+) in the soil solution (Kishore *et al.,* 2015). The orthophosphates H_2PO_4 and HPO_2- are the primary forms of phosphorus taken up by plants (Vyas *et al.,* 2009).

The unavailability of phosphorus in many soils has been recognized as a major growth limiting factor in agricultural and horticultural systems (Cordell *et al.,* 2009). This necessitates the application of soluble forms of phosphorus in the form of phosphate fertilizers, which in itself has constraints in that it too is rapidly immobilized (fixed) to insoluble forms upon its application in the soil due to its reaction with calcium, aluminum and iron minerals. Phosphate fertilizers are dependent on phosphorus derived from phosphate rock, which is a non-renewable resource and current global reserves may be depleted in 50–100 years (Cordell *et al.,* 2009). Therefore, exploring alternative forms of agriculture, where nutrient conservation is key is of vital importance.

The use of organic residues, crop rotation and inclusion of nitrogen fixing fast growing trees are strategies known to conserver nutrient (Ezawa *et al.,* 2002). Among the use of these strategies are specific soil microflora known as Phosphate Solubilizing Microorganisms which are bio- inoculants that are promising substitute for agrochemicals and considered as one of the most efficient means to solubilize insoluble soil phosphorus and can reduce the phosphate fertilizer input in agricultural land (Hussain *et al.,* 2019). Phosphate solubilizing Microorganisms excrete organic acids or Phosphatase enzymes that dissolve phosphatic materials and chelate cationic partners of the phosphorus ions that is, PO43- directly, releasing phosphorus into solution (He *et al.,* 2004). Phosphate solubilizing Microorganisms have being used as bio fertilizer since the 1950s. There is also some evidence of naturally occurring rhizospheric Phosphorus Solubilizing Bacteria (PSB) since 1903 (Khan *et al.,* 2009). Among the whole microbial population in soil, Phosphate Solubilizing Bacteria constitute 1 to 50 %, while Phosphorus Solubilizing Fungi (PSF) constitute only 0.1 to 0.5 % in P solubilization potential (Chen *et al.,* 2006). More over PSMs may also possess the other plant growth promoting activities such as indole acetic (IAA), gibberellic acid, cytokinins, ethylene production, hydrogen cyanide (HCN) production, asymbiotic nitrogen fixation and resistance to soil borne pathogens (Puri *et al.,* 2020). Many PSM are reported as plant growth promoter in many crops like tomato, rice etc (Kumar *et al.,* 2016).

There are a number of Phosphate Solubilizing Microorganisms present in the soil and their potential for P solubiliztion can be analyzed through qualitative and quantitative methods (Mehta and Nautiyal 2001). Some of the most common examples are the species of *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Penicillium and Aspergillus*. Strains from bacterial genera *Pseudomonas, Bacillus, Rhizobium* and *Enterobacter* are the most powerful phosphorus solubilizers (Biswas *et al.,* 2018). *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, and *Enterobacter* are referred as the most important strains (Kumar *et al.,* 2012). Commonly there are more bacilli in soil whereas spirilli are very rare in natural environments (Baudoin *et al.,* 2002)**.**

The Phosphate Solubilizing Bacteria are ubiquitous with variation in forms and population in different soils. The population of Phosphate Solubilizing Microorganisms depends on different soil properties such as physical and chemical properties, organic matter, and P content) and cultural activities**.** Larger populations of Phosphate Solubilizing Microorganisms are found in agricultural and rangeland soils (Kumar *et al.,* 2012).

A few investigation have been carried out on the occurrence and distribution of PSMs from various soil rhizospheres around the world. Therefore the present investigation was designed to examine the activity of PSMs using the conventional method of identification from different rhizosphere soil on the growth of sorghum plant in Nasarawa State.

Materials and Methods

Isolation and Screening for Phosphate Solubilizing Microorganisms

Seven randomized samples of 10g rhizosphere soil was collected from seven (7) different farms located in the city of Lafia, Nasarawa State in five replicate each. All samples were carefully collected and transported in sterile polythene bags to the laboratory and stored at 4ºC prior to analysis (Sharon *et al.,* 2016). For the isolation of PSMs, soil samples were prepared by the inoculation of approximately 1g of each rhizoshere soil sample into sterile test tubes containing 9 mL of sterile water. Each test tube was mixed thoroughly on shaker (NYC HY-2) for 30 minutes at 120 rpm at 27oC temperature and a series of 10-fold dilutions was prepared down to 10-9. A calibrated pipette was used to measure 0.1 mL from each 10-3 to 10-6 dilution and plated on Pikovskaya (PVK) media agar plates (Pikovskaya, 1948). PVK's medium contains (g/l); Glucose, 10.0 g; Ca3 $(PO_4)_2$, 5.0 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4$ 7 H_2O, 0.1 g, pH 7.3 ± 2. The plates were incubated for 7 days at 30°C. Colonies with clear halos were considered positive for phosphate solubilization (Ravindra Naik *et al.,* 2008). Predominant colonies were sub cultured by re-streaking on the fresh PVK agar plates and incubated at 30°C.

Characterization of Isolates

For identification of fungi, Potato Dextrose Agar (PDA) was used to hasten the growth rate and formation of enough conidia as reported by Diba *et al.,* (2014). The characteristics of fresh cultures were compared with mycological identification keys and taxonomic description to identify the isolated fungi to the genus level.

Identification was based on microscopic features and colony characteristics such as surface appearance, texture and colour of the colonies both from upper and lower side. The isolated bacteria were subjected to Gram stains and microscopy analysis for cell type. Isolation by spread plating reveals colony morphologies (color, shape, margins, diameter, opacity, and texture). In addition, all bacteria isolates were characterized biochemically by carrying out appropriate biochemical tests: Citrate utilization test, Indole, Coagulase, Oxidase, Starch hydrolysis, Motility, Lactose fermentation and Catalase (Sharon *et al.,* 2016).

Analysis of Phosphate Solubilization Activity Quantitative Method of Analysis

Pikovskaya's broth medium (100 mL) with 0.5g/100mL Tricalcium phosphate was prepared and sterilized. A calibrated pipette was used to measure 1mL of each of the 24 hrs old culture isolate and inoculated into the broth in the 250 mL Erlenmeyer flasks. The inoculated sample were incubated at 30o C for 10days on rotatory shaker, after which the culture broth was centrifuge at 1000rpm for 30min. Uninoculated broth served as control and the whole experiment were performed in triplicate.

The estimated amount of phosphorus was determined using chlorostannous reduced molybdophosphoric acid blue method (Jackson. 1973) spectrophotometrically. The absorbance of the resultant blue colour was measured at 660nm using KH_2PO_4 as standard and the amount of soluble phosphate were expressed as mg/l over control.

Qualitative Method of Analysis

Pikovskaya's medium was used to screen all the suspected colonies for phosphate solubilization. Isolate showing phosphate solubilizing ability were spot inoculated at the center of Pikovskaya's plate and incubated at 30o C. The diameter of clearance zones was measured successively after 24 hours, up to 7 days. The Phosphate Solubilization Efficiency which is the ratio of total diameter.i.e. clearance zone including bacterial growth and the colony diameter was determine.

PSI = Colony diameter + Halozone diameter/ Colony diameter

Strains with clear zones around their colonies could easily be identify as Phosphorus Solubilizing Microorganisms with all the observations recorded in triplicate.

Production of Enzyme Phosphatase

Diphosphate as phosphate source in Phenolphthalein phosphate agar media (Harrigan and Mc Cance, 1976) laboratory method were used for identification of phosphatase positive PSMs. The suspected organisms were inoculated and incubated at room temperature for 48 hours.

After incubation period, isolates with growth were expose to ammonia vapors. Positive isolates for phosphatase production will turn into pink colour colonies after exposure due to the shift in pH (Barber and Kuper. 1951).

Optimization of Physiological conditions (Temperature and pH)

The Phosphate Solubilization potentials of the isolates was also studied on pikovskaya medium adjusted at different pH values 5, 6, 7, 8 and 9 and incubation temperature 25°C, 30°C, 35°C and 40°C, to determine the optimal growth conditions and the level of phosphate solubilizing activities of the various isolated PSMs.

Growth Chamber Trial

A pot experiment was carried out to determine the effects of phosphate solubilizing microorganisms (PSMs) with single inoculations in the presence or absence of phosphate sources using different microbial strains (Viruel *et al.*, 2011).

All strains were maintained at -4°C in Nutrient broth. PSM cultures (10^9 CFU ml-1) of each strain were used as bioinoculants at a final concentration of 50 mlKg-1, 25 mlKg-1and 12.5 mLKg-1 on seeds. Seeds of local sorghum (*Bicolor sorghum)* were used as host plants to evaluate the effect of PSM.

Treatment of Soil and Seed Samples

Loamy soils from Glow's farm located in Lafia was profiled and used to grow the seed; soil was sieved (2 mm) and sterilized. Clean polythene bags were filled with the sieved (< 2 mm) and sterile soil. Seeds were first sterilized in 80% ethanol for 2 minutes and rinsed 10 times in sterile distilled water before bacterial treatment.

Inoculation of Seed Samples

For inoculation assays, seeds were soaked for 30 min in the microbial suspension and placed at a depth (approximately 2.5 cm below the soil surface) in all polythene bags. The polythene bags were arranged in a completely randomized factorial design in the growth chamber. Three replicates (polythene bags) per microbial inoculum were made. The control treatment consist of water-treated seeds (without inoculation) for the purpose of comparison. Plants were grown for 45 days under normal environmental condition and the following parameters were recorded after every 15 days in sorghum plants: height, number of leaves, root lenght and root dry weight, (Viruel *et al.*, 2011).

Statistical analysis

The experimental data were subjected to analysis of variance (ANOVA) using the SPSS. Statistical analysis between groups were performed using 1-way ANOVA using the Sigma Stat software. The analysis was based on three replications of every experiment that produced quantitative data. $P<0.05$ was considered statistically significant.

Results and Discussion

A total of 22 Phosphate Solubilizing Microbial colonies were isolated on the Pikovskaya's agar, containing insoluble tri-calcium phosphate (TCP) from the different selected cultivating agricultural fields in Lafia, Nigeria. Out of the 22 microbial isolates, few bacterial and fungi isolates showing clear zone around their colonies in Pikovskaya agar

medium were considered as phosphate solubilization and were further sub-cultured on nutrient agar media and maintained at 4°C for further studies.

The bacterial isolates were further characterized by series of biochemical reaction and identified as *Pseudomanas* spp, *Bacillus* spp, *Rhizobium* spp, *Enterobacter* and *Micrococcus* spp, gram reaction showed that all isolated organisms were rods and are also catalase positive, while fungal isolates were identified as *Penicillium* and *Aspergillus* spp. The biochemical and morphological characteristics of these isolates were assigned a particular code presented in table 1 and 2 and further analyzed for their phosphate solubilization potential by quantitative and qualitative methods. Primary screening from rhizosphere soil samples on PVK agar is shown in figure 1.

All phosphate solubilizers showing clear zone around their colonies were selected. Out of the 22 microbial isolates, 5 (Five) isolates showed highest Phosphate Solubilization Index (PSI) ranging from 1.10cm – 2.25cm. Among these 5 potent isolates are strains of the genus *Pseudomonas* spp showing the highest PSI of 2.25cm, followed by *Bacillus* spp (2.17cm), *Rhizobium* spp (2.09cm), *Penicillium* spp (1.91cm) and *Aspergillus* spp (1.76cm). The measurement is shown in table 3.

Phosphorus is one of the vital macronutrient for biological growth and development. Numerous microorganisms have been known to possess the characteristics of phosphate solubilization among rhizosphere microbes are higher in number with more potency than non rhizosphere (Rodriguez and Fragas, 1999).

Bacillus, Pseudomonas, Enterobacter, Rhizobium, Micrococcus, Flavobacterium, Aspergillus and *Penicillium* are genera of bacterial and fungal isolates that have been reported to be able to soloubilize insoluble phosphate (p) in soil making it available for plant uptake (Hameeda *et al.*, 2008). In the present study, various rhizosphere samples were evaluated and analysed based on quality, quantity and optimal physiological condition for P solubilizing capability in PKV medium containing insoluble tri-calcium phosphate (TCP).

In terms of quality, 5 selected isolates showed the larger, clear and yellow coloured halo zone around their colony indicated as Phosphate Solubilzing Index (PSI). *Pseudomonas* spp and *Bacillus* spp were the most efficient for their quality trial to solubilize the phosphate having PSI value of 2.25cm and 2.17cm. Whereas the strain *Aspergillus* spp (1.76cm) was least efficient. This findings is similar to the earlier studies of (Hameeda *et al.*, 2008) who recorded *Pseudomonas* spp and *Bacillus* spp as a potent phosphorus solubilizer.

However, as the reliability of this halo-based techniques is not clear as many isolates which did not produce any visible halo/zone on agar plates could solubilize various types of insoluble inorganic phosphate in liquid medium (Gupta *et al.*, 2002). Certain investigation have also reported that the zone formation could be due to the activity of phosphatase enzyme in bacterial isolates, therefore after analyzing the qualitative ability to solubilize the phosphate on solid medium, it is necessary to also analyze quantitatively in liqud medium (PVK).

Base on quantity, the amount of phosphate solubilized by all PSMs was showed to be significantly ($p < 0.05$) higher over uninoculated control and all selected isolates also have good potential to solubilize the inorganic phosphate, indicated by rapid increase in amount of P after 10 days of incubation. Amount of phosphate solubilized was ranging from 0.500 mg P/l to 0.764 mg P/l. The isolates *Pseudomonas* spp showed the highest potential for phosphate solubilization with amount 0.764 mg P/l after 10 days of incubation followed by *Bacillus* spp with amount of phosphate solubilized 0.626 mg P/l whereas strain *Aspergillus* spp (0.517 mg P/l) was found to be with least potential. These results are in conformity of Gupa *et al.*, (2002), who observed maximum solubilization of phosphorus occurring at day 10 of incubation for TCP under control conditions.

Table.1 Biochemical characterization of Phosphate Solubilizing Bacteria

Characteristics	SPM 1	SPM 4	SPM 8	SPM 7	SPM 5
Gram reaction	–	+	–	–	+
Cell shape	Rods	Rods	Rods	Rods	Rods
H_2S production	+	–	+	–	–
Oxidase	–	–	–	–	+
Catalase	+	+	+	+	+
Citrate Utilization	+	+	–	+	–
Indole	–	–	+	–	–
Methyl red	–	–	+	–	–
Vogues proskauer	–	+	+	+	+
Sucrose fermentation	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Starch Hydrolysis	+	+	+	+	+

Key: SPM 1-*Pseudomonas* spp, SPM 4- *Bacillus* spp, SPM 8- *Rhizobium* spp, SPM 5- *Enterobacter* spp, SPM 7- *Micrococcus* spp. + = Positive, - = Negative

Table.2 Colony morphology and microscopic characteristics of fungal and bacterial isolates

Microorganism	Colony morphology	Microscopic observation
(SPM 6)	Colony appeared initially White and become dark green On PVK and PDA medium. Reverse appeared white in colour.	**Conidia were greenish, smooth and brush-like, with septate hyphae.**
(SPM 9)	were initially white and turned yellowish green to light green with production of black spores which appears as white to pale yellow at the back.	**Conidia were small, brownish black, green in colour with rough and distinctive conidia head (flask shape)**
(SPM 5)	Small, irregular, low convex, translucent and pale cream colour.	
(SPM 1)	Large, irregular, flat, opaque and grayish coloured.	
(SPM 4)	Medium, irregular, round, opaque, flat and gray-white.	
(SPM 8)	Medium-sized, low convex, opaque and cream coloured.	

Key- SPM 6- *Penicillium* spp, SPM 9- *Aspergillus* spp

Table.3 Phosphate Solubilization index of selected P- Solubilizing Microorganisms on Pikovskaya Agar Medium at 10th Day of Incubation and Production of Enzyme Phosphatase

Isolate	S.I (cm)	Phosphates production
SPM 1	2.25	++
SPM 4	2.17	++
SPM 8	2.09	++
SPM 6	1.91	+
SPM 9	1.76	+
SPM 5	1.10	+
SPM 7	**0.99**	+

Solubilization Index = (Colony diameter + Halo zone) / colony diameter
+ Weak production, ++ good production – negative.

Table.4 Quantitative estimation of Phosphate Solubilization Exhibited by the selected Phosphate Solubilizing Microorganism on Pikovskaya's broth with Tricalcium phosphate after 10 days of incubation at 30°C

Isolate of PSM	Soluble P concentration mg/l
SPM 1	**0.764**
SPM 4	**0.626**
SPM 8	**0.618**
SPM 6	**0.592**
SPM 9	**0.517**
SPM 5	**0.499**
SPM 7	**0.478**

Table.5 Optimization of pH on phosphate solubilization at different pH range after 10 days of incubation at 30°C in Pikovskaya's Broth

S/N	pH Range	Phosphate solubilization (mg/l) (Mean of triplicate values)				
		SPM 9	SPM 4	SPM 6	SPM 8	SPM 9
1.	5	0.30	0.31	0.29	0.29	0.28
2.	6	0.35	0.36	0.30	0.31	0.30
3.	7	0.42	0.39	0.40	0.32	0.30
4.	8	0.34	0.35	0.30	0.30	0.29
5.	9	0.31	0.30	0.29	0.28	0.28

Fig.1 PVK medium showing halo zones by PSMs

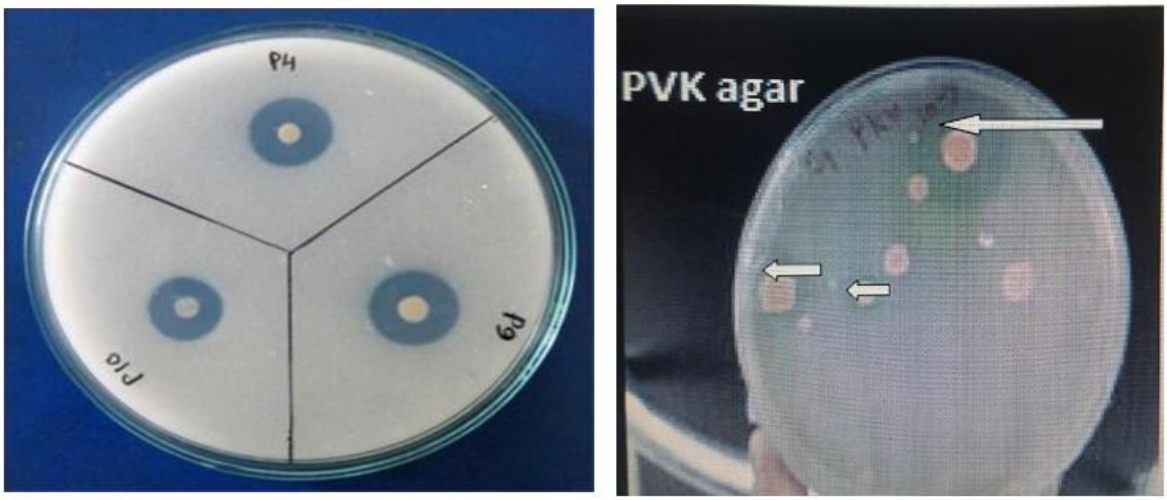


Table.6 Optimization of Phosphate Solubilization at different Temperature after 10 days of incubation at 30°C in Pikovskaya's Broth

S/N	Temperature (°C)	Phosphate solubilization (mg/l) (Mean of triplicate values)				
		SPM 1	SPM 4	SPM 6	SPM 8	SPM 9
1.	25	0.37	0.34	0.34	0.33	0.32
2.	30	0.37	0.36	0.33	0.32	0.31
3.	35	0.42	0.39	0.40	0.32	0.30
4.	40	0.34	0.35	0.32	0.30	0.29
5.	45	0.31	0.31	0.29	0.28	0.28

Fig.2 Effect of inoculation of sorghum seeds with Phosphorus solubilizing Microorganisms on Plant's height after 45 days.

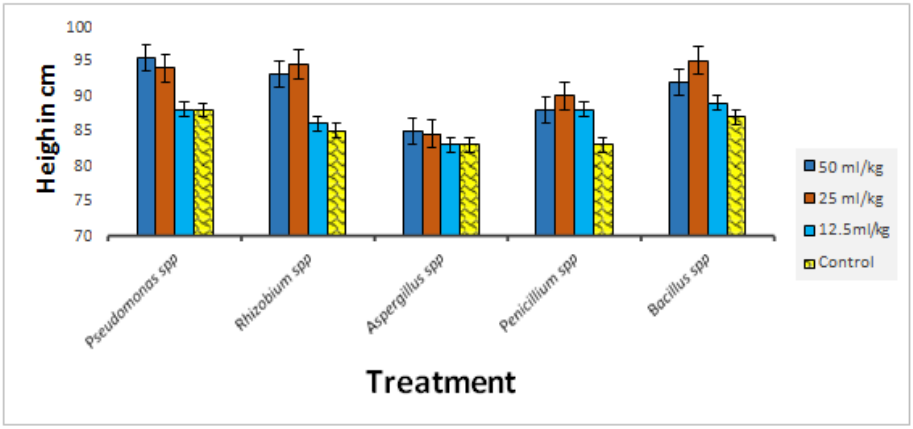


Fig.3 Effect of inoculation of sorghum seeds with Phosphorus solubilizing Microorganisms on number of leaves after 45 days.

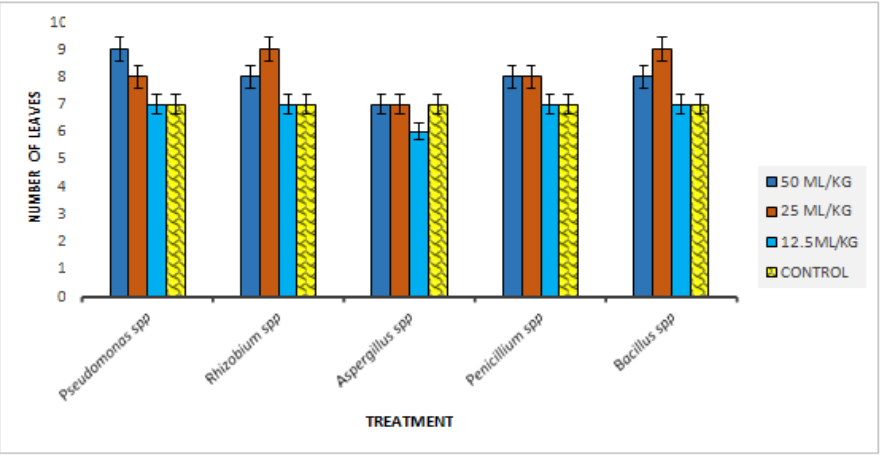


Fig.4 Effect of inoculation of sorghum seeds with Phosphorus solubilizing Microorganisms on root length after 45 days

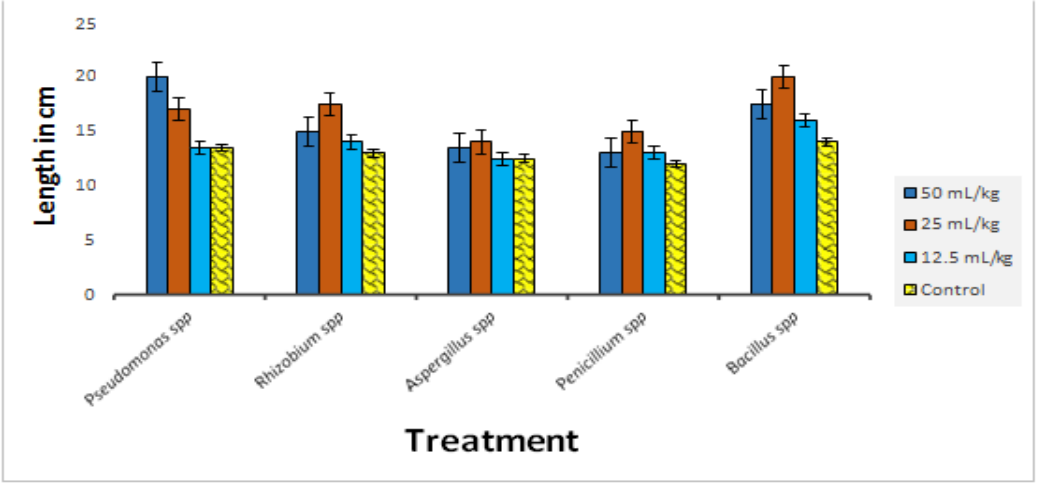
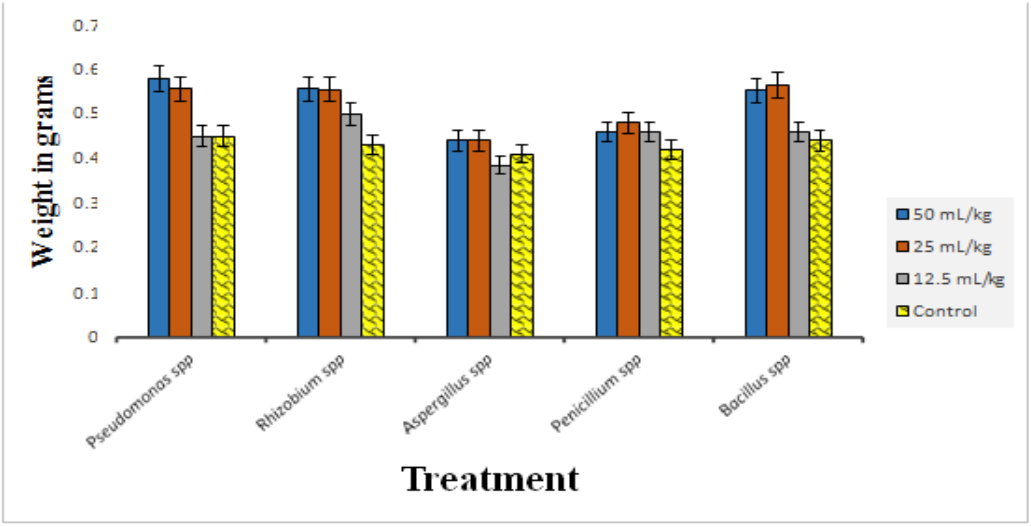


Fig.5 Effect of inoculation of sorghum seeds with Phosphorus solubilizing Microorganisms on dry weight after 45 days



All the selected PSM strains were inoculated separately in PKV broth at pH range of 5-9 and temperature range of 30oC – 40oC and the optical density of culture medium revealed the presence of rapid growth after 7 days at pH 6, 7 and 8, with the maximum growth at pH 6 - 7. The phosphate solubilization of all potent strain were optimized for temperature (25oC – 45oC), in which maximum growth were observed between 30oC – 40oC for most bacteria species and 25oC – 30oC for Fungal, other findings were similar to Uma Maheswar and Sathiyavani, (2012). Out of 22 selected phosphate solubilizers, 7 were found positive for the production of enzyme phosphatase, as shown in table 3. Out of the 7 phosphatase producer, 3 isolates were the good producers of enzyme predicted from the intensity of pink colour after exposure to ammonia vapours whereas the other 4 isolates were

the weak producers. In this investigation, *Pseudomonas* spp, *Aspergillus* Spp, *Rhizobium* spp, *Penicillium* spp, *Enterobacter* spp, *Bacillus* spp and *Micrococcus* spp isolated from rhizosphere soil were able to solubilize phosphate in pikovskaya's medium. All strains significantly increased soil available P and crop growth. The increase in growth may be attributed to auxin production (Gyaneshwar *et al.,* 2002), ACC- deminase activity (Naik *et al.,* 2008), production of organic acids (Frankem *et al.,* 2006), or phosphatase (Chabot *et al.,* 1996) which solubilize/mineralize insoluble phosphate thereby increasing the availability of phosphate in soil for inoculated plant (Hameeda *et al.,* 2008). In general, seed inoculated with *Pseudomonas* spp and *Bacillus* spp was found to have the best effect on plant height, root length, root dry weight, and soil available P compared to other test organisms. This can be attributed to the fact that they have been found to effectively colonize the rhizosphere and internal tissues of roots of sorghum to promote its growth (Fang *et al.,* 2009). Similar result of inoculation using strains of the genera *Pseudomonas* spp and *Bacillus* spp on several crops under controlled condition has been reported (Hameeda *et al.,* 2008).

All other genera of isolated bacteria showed better yield compared to control which revealed that deficiency of available phosphate retard plant growth in different parameters such as plant height and root length of sorghum as compared to test which are in agreement with many authors who reported phytohormones production by PSMs Sreedevi *et al.,* (2015). Previous studies have shown that *Pseudomonas* and *Bacillus* spp enhanced germination and seed vigor of different crop plants (Abbaszadeh Dahaji *et al.,* 2020). Seed inoculation using *Bacillus* spp was found to be effective compared to other test organisms other than *Pseudomonas* spp in terms of growth performance; this is true according to work carried out by (Mohan *et al.,* 2012).

In this study, a comparative experiment were done to determine the effect of PSM on growing sorghum by comparing the vegetative and reproductive plant growth patterns of sorghum seeds from germination to maturation of plant in polythene bags containing soil with treated seeds of the test organisms

Pseudomonas spp, *Bacillus* spp, *Rhizobium* spp, *Penicillium* and *Aspergillus* spp at different concentration level and untreated seed with soil as control. Crop development data were collected after 45days from the sowing of seeds. Different parameters related to vegetative growth pattern such as plant number of leaves, plant height, root length and dry root weight were recorded for comparative evaluation. The best growth was recorded for plant treated with *Pseudomonas* spp at the concentration of 50 mLKg-1 and *Bacillus* spp at concentration level of 25 mLKg-1 followed by *Rhizobium* spp at concentration of 25 mLKg-1 compared to their other concentration and control. At 25 mLkg-1 all organisms had high prospect of increasing soil available P, this can be used as reference for further research.

The result showed that there was significant difference at (0.05) confidence limit in all treatment levels ($p<0.05$) except in the case where there was no significant difference when comparing available phosphate irrespective of concentration of inoculum.

At present, the use of chemical fertilizers and manures cannot be eliminated without avoiding a consequent drastic decrease in food production. Hence, there is an urgent need for integrated management of nutrients that are incorporated into the soil as agricultural inputs to reduce the adverse environmental impacts of chemical fertilizers. All tested strains tend to enhance growth of maize (as measure by plant height, root length, root dry weight, numbers of leaves and soil available P). The strain also improves the uptake of the available phosphorus content in the soil compared to the control. These results also indicate that bacterial inoculation in sorghum seeds may lead to a higher grain yield potential. In particular, P-solubilizing *Pseudomonas* SPP showed great potential for use as bioinoculants. PSM are abundant in many of the

soil, isolation, identification and selection of PSB have not as yet been successfully commercialized, and thus application is still found to be limited. Investigations on the subject are often designed to confirm a specific response of PSM to a particular environment, thus large- scale application in field level is still limited.

This research must be proactive and the field trials must be established across a broad range of soil and environmental conditions and must be conducted within the context of current and future farming practices.

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How to cite this article:

Onyemaechi Adibe, Esther Eneyi Ebah, Benjamin Vandelun Ado, Stanislaus Onyeberechiya Osuagwu and Victor Kolawole Fadayomi. 2022. Microbial Activity of Phosphate Solubilizing Organisms Isolated from Rhizosphere Soil on the Growth of Sorghum Plant (*Bicolor sorghum*). *Int.J.Curr.Microbiol.App.Sci.* 11(07): 222-236. doi: https://doi.org/10.20546/ijcmas.2022.1107.027