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Identification of Phosphate Solubilizing Bacteria from Agricultural Soils of Modoji, Batagarawa, and Umaru Musa Yar'adua University of Katsina Metropolis

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Abstract

Phosphorus is the least available and mobile element to plants in most soil conditions despite being abundant in organic and inorganic forms. Phosphate solubilizing bacteria (PSB) play an important role in supplying phosphorus to plants in a more environmentally friendly and sustainable manner to circumvent phosphorus deficiency. This study aims to isolate and identify phosphate solubilizing bacteria from the agricultural soils of Modoji, Batagarawa, and Umaru Musa Yar'adua University Katsina. The rhizosphere soil samples were taken from 4 distinct locations and serially diluted, and the pour plate method was employed for isolation. Gram reaction and subsequent biochemical tests for the isolates were conducted. Rhizospheric soil bacterial isolates were isolated and screened for phosphate solubilization using the National Botanical Research Institute's (NBRI) Phosphate Growth Medium and Pikovskaya (PVK) medium. The results showed that total rhizospheric bacterial count ranged from $(6.84 \text{ to } 12.20 \times 10^7 \text{ CFU/g})$. All twelve (12) isolates were found to be gram-negative, with *Pseudomonas* sp. (91.7%) and *Bacillus* sp. (8.3%) observed to be positive for starch and gelatin hydrolysis test. The diameters of the phosphate solubilization clearance zones ranged from 14.5 ± 0.5 to 27.0 mm. It was concluded that these isolates can be used as plant growth-promoting agents and as biofertilizers in sustainable agriculture.

Keywords: Biofertilizer, *Bacillus*, *Pseudomonas*, Phosphate solubilizing bacteria (PSB), Rhizobacteria.

INTRODUCTION

The pressure on agriculture to produce food as the world's population increases is one of the largest problems facing the agricultural industry. Today's agriculture faces two challenges: the pressing need to feed a growing population and the necessity to do so in an environmentally friendly manner. The productivity and long-term sustainability of the soil are adversely affected by conventional agricultural practices, which degrade the quality of the soil by increasing compaction, water erosion, and salinization while decreasing soil organic matter, nutrient content, and biodiversity (Cárceles Rodríguez *et al.*, 2022). According to Ajayi (2015), yields have decreased due to soil variables, including erosion, which reduces the effective rooting depth, decreases available water capacity, and depletes soil organic carbon and other soil nutrients. The widespread and excessive use of chemicals has disturbed agricultural soil and is required to be

restored (Bisht and Singh, 2021). Worldwide, the decreased availability of Phosphorus (P) in plants impacts agricultural production. Monobasic (H_2PO_4) and dibasic (HPO_4^{2-}) ions are the forms of P that plants absorb (Gomes *et al.*, 2010).

Nevertheless, plants cannot use 95-99% of the P in the soil (Vassileva *et al.*, 2001). It is linked to the mineral fraction, which includes organic components in soils high in organic matter, iron and aluminum in acidic soils, and primarily calcium in calcareous soils (Gomes *et al.*, 2010; Rasul *et al.*, 2019). To overcome this challenge, a substantial amount of research focusing on the soil system and the agroecosystem is needed to better understand the complex interactions and processes that determine agricultural land stability (Sleeter *et al.*, 2018).

With the advancements in microbiology, molecular biology, and biotechnology, there has been a growing interest in utilizing the microbial phosphate-solubilizing ability to improve soil

phosphorus nutritional status (Li *et al.*, 2025). Using phosphate-solubilizing microorganisms (PSMs) is an environmentally friendly, low-cost, and biologically efficient way to achieve sustainable agriculture without posing environmental risks. As chemical fertilizers have been demonstrated to be hazardous to plants and pose substantial health risks to humans when used often (Richard and Ogunjobi, 2016), microorganisms play an important role in soil health, directly or indirectly. These helpful microbes are widely spread in the rhizosphere and may hydrolyze inorganic and organic insoluble P molecules into soluble P forms that plants directly absorb (Kaur *et al.*, 2024). These bacteria release organic acids, phosphatases, and other chemicals, increasing soil phosphorus content and improving soil conditions for plant growth and development (Kour *et al.*, 2021). Microorganisms that can solubilize phosphate increase soluble Phosphate availability, promoting plant growth (Lesueur *et al.*, 2016; Shrivastava *et al.*, 2018). They can convert insoluble Phosphate into a more accessible form (Hanif *et al.*, 2015). They help plants grow by fixing nitrogen, solubilizing Phosphate, and generating plant growth regulators (Satyaprakash *et al.*, 2017; Ali *et al.*, 2017). Using phosphate-solubilizing bacteria (PST) as an environmentally benign option to boost phosphorus uptake by plants appears promising (Teles *et al.*, 2024). Phosphate-solubilizing microorganisms play a vital role in the dynamics of P cycling in the soil (Alori *et al.*, 2017; Zhu *et al.*, 2018), making phosphate ions available to plants and promoting plant growth by up to 25% (Mihalache *et al.*, 2018; Goswami *et al.*, 2020). PSB strains from *Pseudomonas*, *Bacillus*, *Burkholderia*, *Mycobacterium*, and *Enterobacterium* are found naturally in plant roots and have been proposed as efficient P-solubilizers contributing to the cycling of inorganic P in soil (Hanif *et al.*, 2015; Li *et al.*, 2015; Adnan *et al.*, 2019; Elias *et al.*, 2016). The most effective PSBs are *Pseudomonas*, *Rhizobium*, and *Bacillus* (Abbasi *et al.*, 2023; Choudhary *et al.*, 2018). Adeleke *et al.* (2017) and Alori *et al.* (2017) found that phosphate solubilization is mostly achieved by producing and releasing organic acids or media acidification via proton extrusion. Several research have been conducted to isolate and identify phosphate-solubilizing bacteria as bioprospecting strains with the potential to develop sustainable options for P management in agriculture (Jiang *et al.*, 2018; Suleman *et al.*, 2018; Chen and Liu, 2019; Wan *et al.*, 2020; Jiang *et al.*, 2022). PSB-based biofertilizers are considered a crucial constituent to contribute

sustainable production in agro-ecosystems (Mitter *et al.*, 2021; Yahya *et al.*, 2022; Wang *et al.*, 2023) Ducouso-Détrez *et al.*, 2024). Studies have reported that PSB inoculation significantly increased the yield of wheat, maize, beans, millet, and rice, as well as increased effective phosphorus content, enzyme activity, and soil fertility in the crops (Yahya *et al.*, 2022; Salisu and Isiya, 2024). This study aims to isolate and identify phosphate-solubilizing bacteria from agricultural soil from Modoji, Batagarawa, Umaru Musa Yar'adua University of Katsina metropolis

MATERIALS AND METHODS

Sample collection and processing

The soil samples were taken from 4 distinct locations from rhizospheric soil in agricultural land: Modoji, Batagarawa, and Umaru Musa Yar'adua University Katsina, Nigeria, at 6 to 10 cm depth. The soil samples were placed inside a sterile polyethylene bag and transported to the Umaru Musa Yar'adua University, Katsina Microbiology Department. One gram (1g) of the soil sample was serially diluted from 10^{-1} to 10^{-12} using 9ml of distilled water. The experiment was conducted in a laminar airflow setting while following aseptic procedures.

Identification, characterization, and Screening of Phosphate solubilizing bacteria.

National Botanical Research Institute's phosphate growth medium NBRIP solid medium (glucose, 10 g/L; $(\text{NH}_4)_2\text{SO}_4$, 1 g/L; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 5 g/L; MgCl_2 , 5 g/L; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/L; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.002 g/L; NaCl, 0.2 g/L; KCl, 0.2 g/L; $\text{Ca}_3(\text{PO}_4)_2$, 5 g/L; agar, 15 g/L; pH 7) (Nautiyal, 1999) was prepared for the cultivation of the bacterial colonies. Pour plate methods were used, and the molten cool agar of NBRIP was applied to the sterile petri dishes (2 replicates for 10^{-5} and 10^{-6} dilutions each, respectively) containing 1 ml of diluent from the test tubes. The plates were incubated at 29°C for 7 days.

Gram staining of the bacterial species from the pure culture

The isolates were subjected to Gram staining using the standard procedure. The stained cells were observed under a compound microscope to determine their Gram reaction (Yulianti and Rakhmawati, 2017).

Bacterial strain screening for phosphate solubilization

The bacteria isolates from pure culture plates were recognized and described by morphological features, biochemical reactions, and gram reactions were examined under a microscope (Mengesha *et al.*, 2024).

The isolates were further screened on Pikovskaya solid medium (glucose, 10 g/L; (NH₄)₂SO₄, 0.5 g/L; NaCl, 0.3 g/L; MgSO₄, 0.3 g/L; K₂SO₄, 0.3 g/L; FeSO₄, 0.03 g/L; MnSO₄, 0.03 g/L; Ca₃(PO₄)₂, 5 g/L; agar, 15 g/L; pH 7.0) (Pikovskaya, 1948). To ascertain their capacity to solubilize Phosphate based on halo-zone formation (Liu *et al.*, 2016).

Starch hydrolysis

The isolates were streaked at the centre of the starch medium; the culture plates were incubated for 48hrs at 37C. After the incubation, the plate was flooded with Gram's iodine. The plate was observed for clear zone (Velmurugan *et al.*, 2021)

Gelatin hydrolysis

Nutrient gelatin stab method procedure was followed as described by previous work (Edison *et al.*, 2012). A heavy inoculum of an 18- to 24-hour-old test bacteria was stab-inoculated into the nutrient gelatin. Gelatin liquefaction indicates bacterial gelatin hydrolysis.

RESULTS

Mean value of total rhizospheric bacterial count

The mean value of the total rhizospheric bacterial count (TRBC) obtained from different samples of rhizospheric soil from agricultural land are presented in Table 1. The TRBC ranged from 6.84×10⁷ to 12.20×10⁷ colony-forming units (CFU) per gram of soil.

Table 1: Mean total rhizospheric bacterial count

Sample ID	CFU (× 10 ⁷ CFU/g)
UMYU 1	12.20
UMYU 2	8.67
MODJ	6.84
BTGW	8.83

Key: UMYU =Umaru Musa Yar'adua University, MDOJ =Modoji, BTGW =Batagarawa, CFU/g Colony forming unit per gram

Morphological characterization of Phosphate Solubilizing Bacteria (PSB)

The morphological characteristics of bacterial isolates are presented in Table 2. The isolates' morphological characteristics were recorded based on their shape, elevation, colour, pigmentation, and Gram's reaction. Isolates appeared to be rod-shaped, except PSB 6 appeared to be bacilli-shaped. All isolates

displayed white color except PSB6, PSB7, PSB8 and PSB8 appeared to be cream color, and all isolates appeared with raised colony elevation, except PSB5 and PSB12 appeared with flat elevation. All isolates imparted green pigmentation on nutrient agar Gram stain test revealed that *Pseudomonas* sp and *Bacillus* sp are gram-negative.

Table 2: Morphological characteristics of rhizospheric bacterial Isolates

Sample	Gram reaction	Shape	Pigmentation	Elevation	Colour
PSB1	-ve	Rods	Green	Raised	White
PSB2	-ve	Rods	Green	Raised	White
PSB3	-ve	Rods	Green	Raised	White
PSB4	-ve	Rods	Green	Raised	White
PSB5	-ve	Rods	Green	Flat	White
PSB6	-ve	Bacilli	Green	Raised	Cream
PSB7	-ve	Rods	Green	Raised	Cream
PSB8	-ve	Rods	Green	Raised	Cream
PSB9	-ve	Rods	Green	Raised	White
PSB10	-ve	Rods	Green	Raised	White
PSB11	-ve	Rods	Green	Raised	White
PSB12	-ve	Rods	Green	Flat	White

Key: PSB =Phosphate solubilizing bacteria, -ve =Negative, +ve =Positive

Biochemical characterization of Phosphate solubilizing bacteria (PSB)

The biochemical characteristics of bacterial isolates are presented in Table 3, which were obtained from the incubated National Botanical Research Institute's phosphate growth medium

(NBRIP). Biochemical reaction shows that all the isolated bacteria showed positive for citrate test and gelatin hydrolysis. However, all bacterial isolates responded differently to the Indole test, catalase test, oxidase test, and starch hydrolysis.

Table 3: Biochemical characteristics of rhizospheric bacterial isolates

Sample	Indole	Oxidase	Catalase	Citrate	Starch Hydrolysis	Gelatinase	Organism identified
PSB1	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB2	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB3	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB4	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB5	-	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB6	+	-	-	+	+	+	<i>Bacillus</i> sp
PSB7	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB8	-	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB9	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB10	+	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB11	-	+	+	+	+	+	<i>Pseudomonas</i> sp
PSB12	+	+	+	+	-	+	<i>Pseudomonas</i> sp

KEYS: + = Positive reaction, - = Negative reaction, PSB = Phosphate Solubilizing Bacteria

The phosphate solubilization activity from the bacteria isolated

The phosphate solubilization activity of bacterial isolates is presented in Figure 1, which was obtained from pikovskaya (PVK) media. The ability of the isolates to solubilize it produces a positive result which manifests as the zone of clearance by the 12 isolates screened. The production of organic acids by these solubilizers

was the reason for the zone of clearance. The genera of microbes were *Pseudomonas* sp. was 91.67% of the bacterial isolates, while *Bacillus* sp. (8.33%). The diameters of the phosphate solubilization clearance zones ranged from 14.5 ± 0.5 - 27.0 mm. Statistically, it shows a significance difference between the bacteria species (F-Crit: 12.17, P-value: 0.0068, P ≤ 0.05).

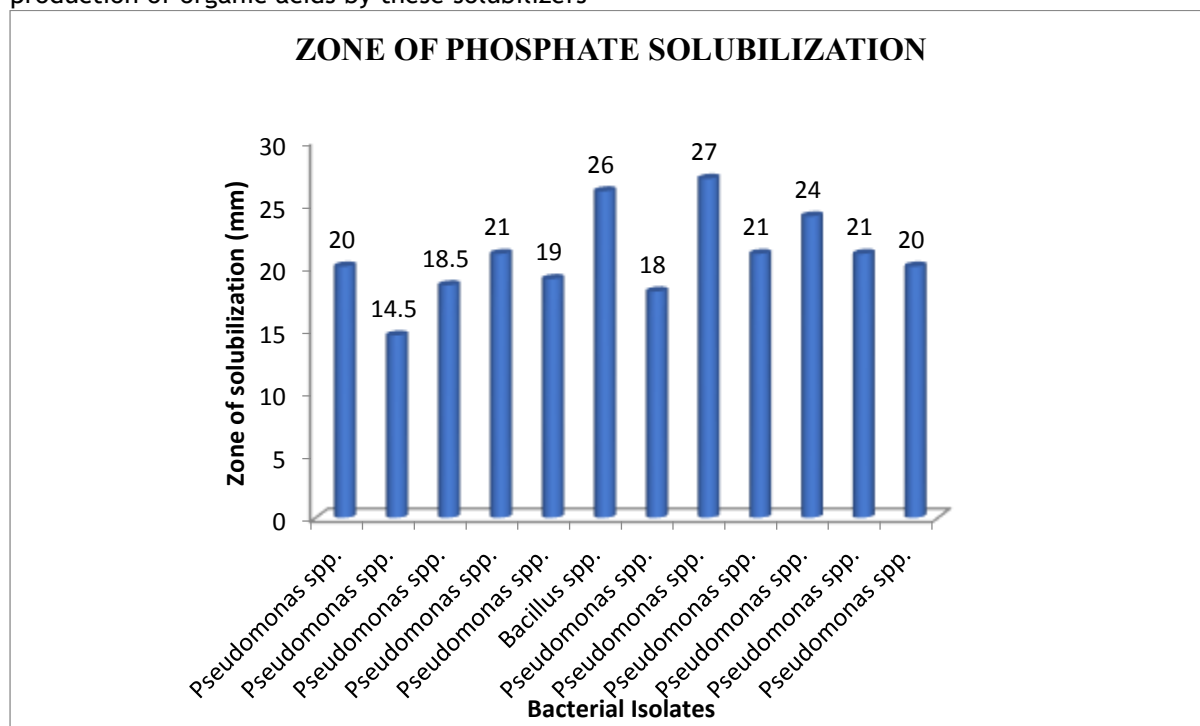


Fig. 1: The phosphate solubilization activity of the rhizospheric bacterial Isolates

DISCUSSION

The phosphate solubilizing bacteria strains identified in this study were *Pseudomonas* species and *Bacillus* species. Similar studies have been reported by Uyi *et al.* (2024) that *Pseudomonas* sp, *Bacillus* sp isolated from the rhizosphere could solubilize Phosphate in the Pikovskaya medium. Among the PSBs isolated in this study, *Pseudomonas* (91.7%) isolates were the predominant, and *Bacillus* (8.3%). Previous findings also showed that bacterial isolates frequently encountered in phosphate solubilization belong to *Pseudomonas* and *Bacillus* genus (Krishnaveni, 2010). Gram negative PSB strains predominate over gram positive bacteria (Khan *et al.* 2010; Mujahid *et al.* 2014). Common taxa that have been isolated from a variety of soils, including those in China, Morocco, and Japan, include *Bacillus*, *Pseudomonas*, and *Rhizobium* (Tao *et al.*, 2008; Damo *et al.*, 2022; Chen *et al.*, 2006) because of the distinctive growth of these bacteria, PSB may adapt to different types of soils, making them suitable for use in a variety of agroforestry regions (Khan *et al.*, 2007).

Microorganisms, particularly *Pseudomonads* and *Bacillus*, establish mechanisms for phosphate solubilization based on the secretion of organic acids and protons (Illmer and Schinner, 1995; Chen *et al.*, 2006; Yahya *et al.*, 2021; Ahmad *et al.*, 2022) or the production of phosphatases (Richardson, 2001). *Pseudomonas* and *Bacillus* were found to be effective phosphate solubilizers (Banerjee *et al.*, 2006; Bouizgarne, 2013; Bouizgarne *et al.*, 2023). *Pseudomonas* are gram-negative bacteria that live in a variety of settings and have a high metabolic diversity (Stanier *et al.*, 1966). According to researchers, *Bacillus* decomposes organic materials and transforms organic molecules in soil into plant-available nutrients (Sun *et al.*, 2020). Both species are well-known as plant growth-promoting rhizobacteria (PGPR) (Mohamed *et al.*, 2024). *Pseudomonas* is a P-solubilizing

microbe that considerably enhances soil P availability, boosts plant P absorption, and promotes plant growth (Ghorbanzadeh *et al.*, 2020; Farssi *et al.*, 2021). *Bacillus* ability to fix nitrogen (N) and solubilize unavailable Phosphorus (P) is critical to enhancing N and P availability in soil (Silva *et al.*, 2023). *Bacillus* has been shown to produce phytohormones essential for plant growth (Soni and Keharia, 2021). Recent research has shown that *Bacillus* produces exopolysaccharides (EPS) that help to improve soil porosity (Bhagat *et al.*, 2021). *Bacillus* improves soil quality, increases agricultural output, and promotes soil health (Radhakrishnan and Lee, 2016).

The use of phosphorus-solubilizing bacteria offers a novel technique to improving soil quality, which will aid in agricultural sustainability. The results of this study provide strong evidence that *Pseudomonas* and *Bacillus* isolates from rhizospheric agricultural soil have good potential as plant growth promoters. Using *Pseudomonas* and *Bacillus*-based biofertilizer is a sustainable strategy to boost plant output and improve soil management. Salisu and Isiya (2024), highlight the potential of indigenous *Pseudomonas* species from agricultural soil as excellent biofertilizers.

CONCLUSION

In this study, twelve (12) rhizospheric agricultural soil bacterial of Modoji, Batagarawa, and Umaru Musa Yar'adua University, Katsina, were isolated, with *Pseudomonas* sp (91.9%) being the predominant, followed by *Bacillus* sp (8.3%). The diameters of the phosphate solubilization clearance zones ranged from 14.5 ± 0.5 - 27.0 mm. This study provides preliminary baseline data on the occurrence of phosphate solubilising bacteria from agricultural soil. These isolates can potentially be used as plant growth-promoting agents and phosphate based-biofertilizers in sustainable agriculture.

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