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Characterization of Rhizosphere Bacteria Associated with *Vitellaria paradoxa* and Their Potential Applications in Sustainable Agriculture

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ABSTRACT. The rhizosphere, a biologically active soil region encircling plant roots, serves as a crucial locus for plant-microbe interactions that affect plant development, nutrient cycling, and overall ecosystem vitality. The bacterial communities residing in the rhizosphere of *Vitellaria paradoxa* (shea tree) in Nigeria are inadequately characterized. This study aimed to examine the bacterial communities in the rhizosphere of *V. paradoxa* naturally occurring in the Botanical Garden at the University of Ilorin, Nigeria. Bacterial isolates were procured via serial dilution plating and identified according to colony morphology and standard biochemical assays. The isolated species comprised *Bacillus subtilis*, *Proteus vulgaris*, *Micrococcus luteus*, and *Lactobacillus brevis*. These organisms, identified as Plant Growth-Promoting Rhizobacteria (PGPR), enhance plant health by increasing nutrient availability, fostering plant growth, and inhibiting soil-borne pathogens. The results underscore the prospective use of PGPR as natural, environmentally friendly agents for sustainable agriculture, enhancing soil fertility and crop yield while diminishing reliance on synthetic agrochemicals.

Keywords: Lactobacillus brevis, micrococcus luteus, proteus vulgaris, soil fertility, soil pathogens

1. INTRODUCTION

Vitellaria paradoxa, or the shea butter tree, is a keystone species in the West African savanna, significantly influencing both ecological and economic systems (Choungo Nguekeng et al., 2021). This perennial tree can reach a height of 25 meters and is distinguished by its robustness, drought resistance, and ability to flourish in nutrient-deficient soils. The fruits and seeds are collected to manufacture shea butter, a substance widely utilized in cosmetics, pharmaceuticals, and the food industry. The production of shea butter substantially bolsters local economies, generating income for millions, particularly rural women in West Africa, who frequently engage in the labor-intensive processing and commerce of this valuable commodity. In addition to its economic significance, *V. paradoxa* plays an ecological role by stabilizing soils, mitigating erosion, and providing habitat for diverse organisms (Jepsen et al., 2024). Nonetheless, akin to numerous indigenous African tree species, it confronts challenges from agricultural encroachment, climate change, and excessive exploitation. Understanding the factors that enhance the growth and resilience of *V. paradoxa* is essential due to its critical role in local economies and ecosystems (Aleza et al., 2018).

The rhizosphere, a narrow soil zone directly affected by plant roots, is a dynamic environment characterized by interactions among plant roots, soil, and microorganisms (Thepbandit & Athinuwat, 2024; Olahan & Ajadi, 2024). This region is a nexus of microbial diversity and activity, primarily influenced by the exudates emitted by plant roots. Root exudates supply carbon-dense compounds that facilitate microbial proliferation, fostering a community of bacteria, fungi, and other microorganisms that engage intimately with the host plant (Pantigoso et al., 2022). Microorganisms in the rhizosphere are essential for nutrient cycling,

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organic matter decomposition, and the suppression of soilborne pathogens. Rhizosphere bacteria provide significant advantages to plants in adverse conditions, particularly in nutrient-deficient and drought-prone soils where *V. paradoxa* typically thrives (Afridi et al., 2022; Saeed et al., 2021). These bacteria facilitate nutrient availability, improve soil structure, and, in certain instances, generate bioactive compounds that support plant defense.

Bacteria, including *Bacillus* and *Lactobacillus* species, are recognized for their roles in nitrogen cycling, phosphorus solubilization, and organic matter decomposition (Gupta et al., 2016). Their metabolic processes enhance the availability of essential nutrients to plants, potentially diminishing the reliance on external fertilizers and advancing sustainable agricultural practices. Numerous rhizosphere bacteria demonstrate antagonistic effects against pathogens by producing enzymes or metabolites that inhibit detrimental microbes. *Bacillus subtilis* synthesizes antibiotics that inhibit soilborne diseases, thereby fostering a more favorable rhizosphere for the host plant (Maslennikova et al., 2023). These advantageous interactions contribute to the enhancement of soil structure by facilitating the formation of soil aggregates, improving water retention, and mitigating soil erosion. Considering the environmental pressures and restricted nutrient accessibility frequently present in West African soils, the rhizosphere bacteria linked to *V. paradoxa* may play a crucial role in the tree's survival and overall soil health.

Although extensive research has been undertaken on the rhizosphere microbiomes of prominent crop plants, there is a paucity of knowledge regarding the bacterial communities linked to indigenous African trees such as *V. paradoxa*. The majority of research on *V. paradoxa* has concentrated on its economic significance, fatty acid profile, and applications in traditional medicine (Ojo et al., 2021; Bairy et al., 2023). Conversely, limited research has investigated the function of rhizosphere bacteria, despite their potential significance in nutrient cycling, soil fertility, and resilience to environmental stressors. Addressing this knowledge deficiency could yield significant insights into the ecological interactions that sustain *V. paradoxa*, potentially guiding conservation strategies and sustainable agricultural practices in West Africa. This study sought to fill these gaps by isolating and characterizing bacteria from the rhizosphere of *V. paradoxa* in the Botanical Garden of the University of Ilorin, Nigeria.

2. METHODOLOGY

2.1. Study Site and Sample Collection

This research was performed at the Botanical Garden of the University of Ilorin in Ilorin, Nigeria, situated in an area defined by a primarily savanna climate featuring distinct wet and dry seasons (Coordinates: 8°20'N, 4°35'E). Rhizosphere soil samples were obtained from a solitary mature *Vitellaria paradoxa* tree, commonly referred to as the shea tree, which is naturally cultivated in the garden and exemplifies the typical species without recognized varietal distinctions. The chosen tree measures approximately 10–12 meters in height, with a trunk diameter of 40–50 cm at breast height, suggesting an estimated age exceeding 25 years and categorizing it as a mature specimen capable of sustaining a well-developed rhizosphere (Figure 1). Sampling occurred on January 15, 2024, during the dry season to mitigate the potential confounding effects of soil saturation on microbial activity and composition, thus yielding a more stable and representative profile of the microbial community structure.

Rhizosphere soil samples were methodically obtained from four equidistant locations surrounding the tree's root zone at a depth of 10–15 cm to focus on the microbe-rich area affected by root exudates. Approximately 10 grams of soil were collected from each location using sterile instruments to avoid contamination, combined into a composite sample, and promptly placed into sterile containers. The samples were subsequently transported to the laboratory under refrigerated conditions and stored at 4°C until further analysis to maintain microbial viability.

Figure 1. Pictures of *Vitellaria paradoxa* (shea tree) from Botanical Garden of the University of Ilorin.

2.2. Determination of physicochemical properties of soil

Fresh soil samples were subjected to determine physicochemical properties. Soil pH was determined according to the procedure described by Zhou et al. (2019). The moisture content of the sample was determined in a hot air oven at 105 °C until a constant weight was achieved (Ma et al., 2016). The temperature and humidity were measured using a thermometer and a hydrometer (Bhadani & Vashisht, 2019).

2.3. Isolation and Identification of Bacterial Isolates

The serial dilution technique was utilized to isolate bacterial species from rhizosphere soil samples. One gram of soil was suspended in 9 mL of sterile distilled water and subsequently serially diluted to 10^{-6}. A 0.1 mL aliquot from each dilution was inoculated onto nutrient agar plates to facilitate colony growth. Plates were incubated at 37 °C for a duration of 24 to 48 hours. The study examined colony morphology, including shape, edge, pigmentation, consistency, evaluation, surface characteristics, and optical properties. The cellular morphology and biochemical assays of the isolated bacteria were conducted, including Gram staining, oxidase test, motility assessment via wet mount, oxygen relationship evaluation, MRVP test, starch hydrolysis test, and sugar fermentation tests.

3. RESULTS AND DISCUSSION

3.1. Physicochemical properties of soil

The soil sample was examined for its physicochemical characteristics, indicating a pH of 6.7, favorable for nutrient availability. The balanced pH, coupled with optimal temperature (32 °C) and moisture content (48.5%), facilitates nutrient absorption by plants and enhances microbial activity, thereby promoting plant growth (Xia et al., 2024).

3.2. Isolation and Identification of Rhizosphere Bacteria

This study acquired four bacterial strains from the soil adjacent to the roots of a *Vitellaria paradoxa* tree, designated as isolates A_1, A_2, A_3, and A_4. Isolates A_1, A_2, and A_3 exhibited traits including a transparent appearance, a smooth colony surface, flat elevation, and a lobate margin. Isolate A_3 exhibited an anomalous morphology, blue-green coloration, and a gelatinous consistency. Isolate A_1 exhibited a swarm-like morphology and a buttery texture, whereas A_2 displayed an irregular form and a creamy hue. Isolate A4 exhibited a circular morphology, buttery consistency, elevated structure, smooth colony surface, opaque aspect, and a yellow or pale-yellow coloration (Table 1).

Table 1. Colonial Morphology of the bacteria isolated from the soil sample.

Bacterial isolates	Shape	Edge	Pigmentation	Consistency	Evaluation	Colony surface	Optics
A_1	Swarmy	Lobate	Creamy	Boutyrous	Flat	Smooth	Transparent
A_2	Irregular	Lobate	Creamy	Viscid	Flat	Smooth	Transparent
A_3	Irregular	Lobate	Bluish green	Viscid	Flat	Smooth	Transparent
A_4	Circular	Entire	Yellow	Boutyrous	Raised	Smooth	Opaque

The cellular morphology of the bacterial isolates is illustrated in Table 2. Among the four isolates, two (A_1 and A_4) were Gram-positive, whereas the remaining two (A_2 and A_3) were Gram-negative. All isolates exhibited positive outcomes in the motility assessment, with three classified as facultative anaerobes and only one (A_2) identified as strictly aerobic. All isolates yielded negative results in the oxidase test. Furthermore, two isolates (A_3 and A_4) tested negative for the MRVP assay, while only A_1 and A_4 exhibited starch hydrolysis capability.

Biochemical analysis indicated that two isolates (A_2 and A_4) were positive for the indole test, while all isolates exhibited catalase positivity (Table 3). Only one isolate, A_4, exhibited a positive result in the coagulase test, while the other isolates yielded negative results. Among the isolates, only A_2 exhibited positive results for hydrogen sulfide production. Urease was detected in A_2 and A_3, whereas only A_1 and A_3 demonstrated the capacity to utilize citrate in this study.

Table 2. Cellular Morphology of the Bacteria Isolated from the Soil sample.

	Gram staining	Motility	Oxygen relationship	MRVP test	Oxidase test	Starch hydrolysis
A1	+	+	FA	+	-	+
A2	-	+	AE	+	-	-
A3	+	+	FA	-	-	-
A4	+	+	FA	-	-	+

Key: FA = Facultatively Anaerobic, AE = Aerobic, + = Positive, - = Negative

Table 3. Biochemical tests on the bacteria isolated from the soil sample.

	Indole test	Catalase test	Coagulase test	Hydrogen sulphide test	Urease	Citrate utilization
A_1	-	+	-	-	-	+
A_2	+	+	-	+	+	-
A_3	-	+	-	-	+	+
A_4	+	+	-	-	-	+

The sugar fermentation test indicated that three bacterial isolates fermented sugars, leading to acid production, whereas isolate A_2 produced both acid and gas. Isolate A_3 did not ferment any carbohydrates in this study (Table 4). The glucose fermentation patterns resembled those seen in the sugar fermentation test, except that isolate A_4 generated both acid and gas, unlike the sugar fermentation test, which produced acid only (Table 4). Only isolates A_1 and A_4 fermented lactose, maltose, and fructose, whereas isolates A_2 and A_3 did not ferment any of these carbohydrates (Table 4).

Table 4. Sugar fermentation of bacteria isolated from the soil sample.

	Sugar	Glucose	Lactose	Maltose	Fructose
A_1	A	A	A	A	A
A_2	AG	AG	-	-	-
A_3	-	-	-	-	-
A_4	A	AG	A	A	A

Key: - = Negative, A = Acid production, AG = Acid and Gas production
Based on the above results, the isolates were identified as follows:
A_1 = *Proteus vulgaris*
A_2 = *Bacillus subtilis*
A_3 = *Micrococcus luteus*
A_4 = *Lactobaccilus brevis*

These strains belong to a group of beneficial microbes known as Plant Growth-Promoting Rhizobacteria (PGPR), which contribute positively to plant development by supplying essential nutrients, producing growth-stimulating hormones, and suppressing or inhibiting plant pathogens (Vejan et al., 2016; Hashem et al., 2023; Kumar et al., 2021). In particular, PGPR have been explored as biocontrol agents and resistance inducers in plants (Jiao et al., 2021), often thriving in response to nutrients secreted by plant roots. As a result, PGPR have been employed to enhance soil productivity (Zhang et al., 2024).

The results of this study align with findings from previous studies. Ahmad et al. (2017) isolated *Bacillus subtilis* from the rhizosphere of rapeseed and observed its antagonistic effects against phytopathogenic fungi. Similarly, Edkie et al. (2014) found that *B. subtilis* isolated from the sugarcane rhizosphere acted as a bioinoculant to mitigate salinity stress in sugarcane. Lafi et al. (2017) also reported isolating *Micrococcus luteus* from the rhizosphere of *Cyperus conglomeratus*. Oyeyiola et al. (2013) further documented the presence of *B. subtilis*, *L. brevis*, and other beneficial bacteria in the rhizosphere and rhizoplane of okra plants. Blake et al. (2021) highlighted *B. subtilis* ability to control plant pathogens while simultaneously promoting plant growth. Cazorla et al. (2007) also noted that *B. subtilis* strains isolated from avocado displayed biocontrol properties. Specifically, volatile organic compounds like acetoin and 2,3-butanediol, synthesized by *B. subtilis*, have been associated with significant improvements in plant growth (Yi et al., 2016). Song et al. (2023) demonstrated that *B. subtilis* inoculation in maize roots improved water absorption under saline conditions.

Tizabi & Hill (2023) highlighted the importance of *Micrococcus luteus* in sustainable agriculture, noting its antimicrobial activity against several plant pathogens. *M. luteus* was also noted for its hydrogen cyanide and siderophore production, along with antifungal activity. Swarnalakshmi et al. (2020) observed that *Proteus vulgaris* and certain *Bacillus* species produce growth hormones like auxins (indole-3-acetic acid), gibberellins, cytokinins, and abscisic acid, which regulate plant growth. Furthermore, Visser et al. (1986) showed that *Lactobacillus* strains associated with plants exhibit antagonistic effects against phytopathogens such as *Xanthomonas campestris*, *Erwinia carotovora*, and *Pseudomonas syringae* (Garmasheva et al., 2024).

4. CONCLUSION

The rhizosphere of *Vitellaria paradoxa* (shea tree) at the Botanical Garden of the University of Ilorin was observed to contain a diverse assemblage of bacterial species, including *Proteus vulgaris*, *Bacillus subtilis*, *Micrococcus luteus*, and *Lactobacillus brevis*. These isolates, identified for their capacity as plant growth-promoting rhizobacteria, highlight the essential function of microbial communities in improving plant health and soil quality. The results establish a basis for additional investigation of these bacterial species in the advancement of sustainable, environmentally friendly agricultural methods.

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AUTHOR CONTRIBUTIONS

Ganiyu Shittu Olahan: Conceived the project. **Ganiyu Shittu Olahan and Ibrahim Ajadi:** Conceptualization, Investigation, performed experiments, analyzed the data and drafted the manuscript.

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COMPETING INTEREST

The authors declare no competing interest.

COMPLIANCE OF ETHICAL STANDARDS

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