UMYU Scientifica, Vol. 5 NO. 1, March 2026, Pp 359 – 369
https://doi.org/10.56919/usci.2651.030
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Isolation and Molecular Identification of Plant Growth-Promoting *Bacillus subtilis* from Rice Rhizosphere in North-Eastern Nigeria

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ABSTRACT

Background: The use of plant growth-promoting rhizobacteria (PGPR) as biofertilizers offers a sustainable alternative to synthetic inputs in rice cultivation. However, there is limited data on the diversity and functional potential of indigenous *Bacillus* species from rice rhizospheres in semi-arid regions of North-Eastern Nigeria. This study aimed to isolate and characterize native *Bacillus* species from the rice rhizosphere in Damaturu and evaluate their plant growth-promoting (PGP) potential for biofertilizer development. Methods: Ten rhizosphere soil samples (0–15 cm depth) were randomly collected from irrigated rice fields in Damaturu, Yobe State. Soil physicochemical properties were analyzed using standard methods. Bacterial isolation was performed by serial dilution (10^{-1} to 10^{-7}) and pour plating on nutrient agar. Isolates were preliminarily identified based on cultural, morphological, and biochemical characteristics. PGP traits, including indole-3-acetic acid (IAA) production, ammonia synthesis, and phosphate solubilization, were assessed *in vitro*. A representative *Bacillus* sp. isolate was subjected to molecular identification through partial 16S rRNA gene sequencing and phylogenetic analysis. Results: The rhizosphere soil was characterized as clay-loamy, slightly acidic to neutral (pH 6.1–7.1), and moderately fertile. Total heterotrophic bacterial counts ranged from 1.02×10^{11} to 3.18×10^{11} CFU/g. Seven rhizobacterial isolates were obtained, comprising *Bacillus* sp. (50%), *Pseudomonas* sp. (30%), and *Azotobacter* sp. (20%). All isolates demonstrated PGP traits, with *Bacillus* sp. strain S9 showing the highest colony count (3.18×10^{11} CFU/g). Partial 16S rRNA gene sequencing revealed that strain *Bacillus* sp. S9 shared 93% sequence similarity with reference strains of *Bacillus subtilis* in the GenBank database. While this level of homology supports genus-level identification as *Bacillus* sp. Conclusion: Indigenous rhizobacteria from the rice rhizosphere in Damaturu, particularly *Bacillus* sp. strain S9, exhibit promising PGP traits and potential for biofertilizer development. However, further molecular characterization using full-length 16S rRNA sequencing is recommended to resolve species-level identity. Harnessing these native microbial resources could reduce dependency on chemical fertilizers and support sustainable rice (*Oryza sativa* L. FARO 44) cultivation in semi-arid agroecosystems.

ARTICLE HISTORY

Received December 06, 2025
Accepted March 14, 2026
Published March 27, 2026

KEYWORDS

Bacillus spp., rice rhizosphere, plant growth-promoting rhizobacteria, 16S rRNA, biofertilizer, semi-arid region, North-Eastern Nigeria

INTRODUCTION

The rhizosphere microbiome serves as a critical determinant of plant health and soil fertility, particularly in intensive agricultural systems such as irrigated rice farming (Ukwa *et al.*, 2024). Within this complex microbial community, rhizosphere bacteria play pivotal roles in nutrient cycling, phosphate solubilization, nitrogen fixation, phytohormone production, and pathogen suppression, thereby enhancing plant growth and stress tolerance (Glick, 2005; Fahad *et al.*, 2021). Genera such as *Bacillus*, *Pseudomonas,* and *Azotobacter* are well-documented plant growth-promoting rhizobacteria (PGPR) that contribute to sustainable agriculture through multiple mechanisms (Adesemoye and Kloepper, 2009; Hayat *et al.*, 2010). These microorganisms improve soil structure, supply essential nutrients to crops, suppress phytopathogens, and can be harnessed for biofertilizer and bioremediation applications. Their small size and rapid growth rates enable them to colonize soil microhabitats efficiently, with populations capable of doubling within 30 minutes under optimal conditions,

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How to cite: Wakil, S., Haruna, I. Y., Ibrahim, A. A., Adam, L. N., & Abbator, A. (2026). Isolation and Molecular Identification of Plant Growth-Promoting *Bacillus subtilis* from Rice Rhizosphere in North-Eastern Nigeria. *UMYU Scientifica*, 5(1), 359 – 369. https://doi.org/10.56919/usci.2651.030

thereby driving soil productivity (Lowenfels and Lewis, 2006). The activity of soil bacteria is largely governed by oxygen availability, with aerobic bacteria dominating well-oxygenated soils where they decompose organic carbon compounds (Adesemoye and Kloepper, 2009).

The escalating use of chemical fertilizers in modern agriculture has raised significant environmental concerns, including soil degradation, water contamination, and greenhouse gas emissions (Bar-On *et al.*, 2018). The microbial-based fertilizers (biofertilizers) have gained prominence as sustainable alternatives that enhance soil fertility and crop productivity while minimizing ecological harm (Sakariyawo *et al.*, 2013; Serri *et al.*, 2022; Ibrahim et al., 2024; Grema et al., 2022; Isiya and Salisu, 2024; Ibrahim et al., 2024). Biofertilizers function by mobilizing nutritionally important elements from non-usable to plant-available forms through biological processes such as nitrogen fixation, phosphate solubilization, and the production of plant growth-promoting substances (Agu *et al.*, 2021). These products enhance root proliferation through phytohormone release and contribute to the integrated nutrient supply system essential to sustainable farming.

Rice (*Oryza sativa* L.) is a staple cereal crop consumed by nearly 250 million people globally, with demand steadily increasing due to population growth (Nikmatul *et al.*, 2020; Somchit *et al.*, 2017). In Nigeria, rice (particularly the FARO 44 variety) is a dietary staple, and its production must be intensified to meet rising consumption without expanding agricultural land into forested areas (Rahmah *et al.*, 2017; Grema *et al.*, 2022). High-yielding varieties have contributed to increased production; however, sustaining productivity under resource-limited conditions requires complementary strategies, such as the application of PGPR-based biofertilizers (Long-ping, 2014; Sakariyawo *et al.*, 2013). Rhizobacteria accelerate the mineralization of organic residues, enhancing nutrient availability and supporting crop growth (Agu *et al.*, 2021). Despite the recognized potential of PGPR for sustainable rice production, there is a paucity of information on the diversity, functional capabilities, and molecular characterization of indigenous rhizobacteria in semi-arid rice-growing regions of North-Eastern Nigeria. Previous studies in the region have largely relied on phenotypic characterization alone, with limited application of gene-level validation for key plant growth-promoting (PGP) traits. This gap is particularly significant given that semi-arid agroecosystems impose unique environmental stresses, such as high temperatures, low soil moisture, and nutrient limitation, that may select for locally adapted microbial strains with superior biofertilizer potential. This study addresses the knowledge gap by providing a combined phenotypic screening for PGP traits **(**indole-3-acetic acid (IAA) production, ammonia synthesis, and phosphate solubilization**)** and molecular characterization (through partial 16S rRNA gene sequencing and phylogenetic analysis) of indigenous rhizobacteria, *Bacillus* sp. S9A isolated from rice rhizosphere soils in Damaturu, Yobe State, Nigeria. Therefore, this study aimed to isolate and identify rhizobacteria from irrigated rice fields in Damaturu, evaluate their PGP traits (IAA production, ammonia production, and phosphate solubilization), and molecularly identify a promising isolate to explore their biofertilizer potential for sustainable rice production in semi-arid agroecosystems.

MATERIAL AND METHOD

Study area and sample collection

The study was conducted in the Waziri Ibrahim Estate Extension Irrigation area of rice fields in Damaturu, Yobe State, Nigeria (Geographic coordinates: 11.7644549°00'N, 11.9965083°00'N). Ten (10) rhizosphere soil samples (approximately 10g each) were aseptically collected from the root zone (0-15 cm depth) of rice plants using sterile polyethylene bags between January and April 2025. Samples were transported to the Chemistry and Microbiology Laboratories of Yobe State University for physicochemical analysis and isolation of rhizobacteria isolates using standard methods.

Soil physicochemical analysis

The soil texture, pH, organic carbon, soil nitrogen, water-holding capacity, exchangeable bases, and cation exchange capacity were determined using standard laboratory procedures (Machido, 2010; Ukwa et al., 2024; Ofori, 2016) at the Chemistry Laboratory, Yobe State University, Damaturu, Nigeria.

Microbiological analysis of bacterial isolate and plant growth-promoting traits

One (1) gram of each soil sample was serially diluted (10^{-1} to 10^{-7}) in sterile distilled water. 0.1 mL aliquot from appropriate dilutions was spread-plated onto yeast extract mannitol agar (YEMA) and nutrient agar for total rhizobial bacteria. The YEMA plates were incubated at 37°C for three days, while the nutrient agar plates were incubated at 37°C for 24 hours. The resulting colonies were counted using a colony counter. The isolated rhizobacteria were sub-cultured and incubated at 37°C for 24 hours; the morphological characteristics of the isolates, Gram stain, and biochemical characteristics were observed (Cheesbrough, 2006; Vimala *et al.,* 2018; Oyeleke and Manga, 2008).

The rhizobacterial isolates were screened for their plant growth-promoting (PGP) potential by assessing key functional traits. The specific characteristics evaluated included the production of indole-3-acetic acid (IAA) and ammonia, as well as the ability to solubilize phosphate, which were determined using the procedure below.

The ability of the rhizobacterial isolates to produce indole-3-acetic acid (IAA) was assessed using the method described by Sarker *et al.* (2014). Each isolate was inoculated into tryptophan broth and incubated at 30–32 °C for 24-72 hours. Following incubation, the cultures were transferred into labeled sterile test tubes and centrifuged at 1500 rpm for 15 minutes. Subsequently, 1 mL of the supernatant was carefully transferred into fresh sterile test tubes, and 4 mL of Salkowski's reagent was added to each tube. The mixtures were gently agitated and incubated at 37 °C for 30 minutes. The development of pink to light red coloration was considered a positive indication of IAA production, while the absence of color change was considered a negative result.

The ability of the rhizobacterial isolates to produce ammonia was assessed. The isolate was inoculated into 5 mL of nutrient broth and incubated at 30–37 °C for 48-72 hours. 1 mL of Nessler's reagent was added to each culture. The development of a yellow to light brown coloration was recorded as a positive result, indicating ammonia production. The absence of color change was recorded as a negative result (Justin *et al*., 2020; Joseph *et al*., 2007).

The phosphate solubilization ability of the rhizobacterial isolates was assessed; each isolate was spot-inoculated onto Pikovskaya's agar plates and incubated at 30–37 °C for 48-72 hours. The plates were examined for clear halo zones surrounding the colonies. The formation of a distinct, transparent zone indicated positive phosphate solubilization activity, whereas the absence of a clear zone indicated a negative result (Mahbouba et al., 2013; Zaghloul *et al*., 2016).

Molecular identification of Rhizobacteria with multiple PGPT

The plant growth-promoting trait genes of rhizobacterial isolates capable of producing biofertilizer were further detected, and the 16S rRNA of *Bacillus* sp. S9A was sequenced using molecular analysis, which involved extracting DNA, PCR amplification, and gel electrophoresis of the rhizobacterial isolates at the Molecular Laboratory, Nigerian Institute for Trypanosomiasis Research, Kaduna State, Nigeria, and sequencing at Inqaba Biotechnology West Africa Ltd., Africa's Genomic Company, South Africa.

Molecular detection of PGPB genes in rhizobacterial isolates capable of producing biofertilizer was carried out using a DNA template and primers, and PCR amplification was performed by picking a colony of the rhizobacterial isolate *Bacillus* sp. S9A. The PCR premix (Hot Start) from Bioneers Company contained all the PCR components, except primers; deionized water was used for amplification. Using simplex PCR, 17 µL of PCR premix was mixed with 1 µL of DNA template and 1 µL each of the reverse and forward primers for the PGPB genes (ipdC, ureC, and gcd) to make a 20 µL mixed reaction. The mixed samples were placed in a PCR machine (Gene Amp 9700 thermal cycler; Applied Biosystems, USA). The PCR conditions for the PGBT genes were set as follows:

Indole acetic acid gene *ipdC:* initial step of predenaturation at 95 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, then annealing at 52 °C for 45 seconds, extension at 72 °C for 45 seconds, and then final extension at 72 °C for 7 minutes.

Ammonia production gene *ureC*: initial step of predenaturation at 95 °C for 5minutes followed by 35 cycles of denaturation at 94 °C for 1minute, then annealing at 55°C for 1 minute, extension at 72 °C for 2 minute, and then final extension at 72 °C for 5 minutes.

Phosphate solubilization gene *gcd*: initial step of predenaturation at 95 °C for 5minutes followed by 35 cycles of denaturation at 94 °C for 1 minute, then annealing at 58 °C for 2 minutes, extension at 72 °C for 2 minutes, and then final extension at 72 °C for 7 minutes

The amplified PCR products were separated by electrophoresis using a 1.5% agarose gel and stained with ethidium bromide, with a 1000 base pair DNA marker, at 100 volts for 30-35 minutes—the molecular detection of (3) PGPB genes of *Bacillus* sp. S9A, indole-3-acetic acid (ipdC), ammonia production (ureC), and phosphate solubilization (gcd) were identified by comparing the separated PCR products with the DNA ladder (Neamat et al., 2013; Uzah et al., 2020; Guardiola-Márquez *et al*., 2023).

The 16S rRNA genes were polymerase chain reaction (PCR) amplified by using universal bacterial primer F: 5-AGAGTTTGATCCTGGCTCAG-3 R: 5 CGGTTACCTTGTTACGACTT-3 (Kumar *et al*., 2018). The PCR products were sequenced by ABI Applied Biosystems TM 3500 DNA Analyzer. The sequences were assembled and aligned using the Codon-Code Aligner software. The sequencing was conducted at Inqaba Biotech West Africa Ltd, Africa's Genomic Company, South Africa. The sequences were identified using the nucleotide blast tool at National Center for Biotechnology Information (NCBI) search tool, and the phylogenetic tree was created by using the neighbor-joining method with the Jukes–Cantor evolutionary distance measurement using MEGA v.10. After the 16S rRNA gene sequences were obtained, they were matched with the GenBank database using the NCBI Basic Local Alignment Search Tool (BLAST) (Erickson, 2019, Najar *et al*., 2018, Uzah *et al*. 2020, Guardiola-Márquez *et al*., 2023).

RESULTS

The physicochemical analysis revealed a clay-loamy soil texture, known for good water and nutrient retention. The soil pH ranged from slightly acidic to neutral (6.1-7.1). Other parameters (Table 1) indicated moderately fertile conditions conducive to microbial activity and rice growth.

The bacterial population count varied across samples, with the highest count observed in sample S9 (3.18×10^{11} CFU/g) and the lowest in S3 (1.02×10^{11} CFU/g) (Table 2).

Based on morphological, Gram staining, and biochemical characteristics (Table 3), the isolates were identified as Bacillus spp. (50%), *Pseudomonas* spp. (30%), and *Azotobacter* spp. (20%). All isolates were positive for catalase and oxidase tests.

Potential plant growth-promoting (PGP) traits, such as indole-3-acetic acid, ammonia production, and phosphate solubilization, were observed in *Bacillus sp.* (Table 4). S9A had the multiple PGP trait with string activities, indicating potential to serve as biofertilizers, contributing to sustainable rice (*Oryza sativa L.* FARO) growth and the cultivation of other crops, as well as reducing the use of chemical fertilizer.

Table 1: Physicochemical properties of rhizosphere soil samples from irrigated rice fields in Damaturu, Yobe State, Nigeria.

Parameter	Range	Mean ± SD
Texture	Clay-loam	
pH	6.1 - 7.1	6.6 ± 0.3
Electrical conductivity (mS/cm)	0.12 - 0.35	0.24 ± 0.07
Organic carbon (%)	0.62 - 0.89	0.75 ± 0.08
Total nitrogen (mg/kg)	800 - 1200	1020 ± 120
Water holding capacity (%)	42.5 – 58.3	50.4 ± 4.8
Sodium (cmol/kg)	0.18 – 0.45	0.32 ± 0.09
Cation exchange capacity (cmol/kg)	8.5 – 14.2	11.3 ± 1.6

SD = Standard Deviation

Table 2: Population of bacteria in rhizosphere soil samples

Sample Code	CFU/g ($\times 10^{11}$)
S1	1.04
S2	1.16
S3	1.02
S4	1.08
S5	2.18
S6	1.18
S7	1.96
S 8	1.64
S 9	3.18
S 10	1.86

Rhizosphere soil sample = S1-S10, CFU/g = Colony Forming Unit

Table 3: Gram stain, morphological characteristics, and biochemical test results of rhizobacterial isolates.

			Biochemical Test						
Isolate code	**GS**	**MPA**	**Ind**	**MR**	**Ox**	**Cat**	**Cit**	**VP**	**Suspected isolate**
S1	+	Milky white, irregular shape	-	-	-	+	+	-	*Bacillus* sp.
S2	+	Milky white, irregular shape	-	+	+	+	+	-	*Bacillus* sp.
S3	-	Pale yellow/green	-	-	+	+	+	-	*Pseudomonas* sp.
S4	-	Greenish	-	-	-	-	+	+	*Pseudomonas* sp.
S5	+	Creamy white, round	-	+	+	+	+	-	*Bacillus* sp.
S6	-	Brownish mucoid	-	-	+	-	+	-	*Azotobacter* sp.
S7	-	Brownish mucoid	-	-	+	+	+	-	*Azotobacter* sp.
S8	+	Creamy white	-	+	+	+	+	-	*Bacillus* sp.
S9	+	Milky white	-	+	+	+	+	+	*Bacillus* sp.
S10	-	Greenish	-	-	+	-	+	+	*Pseudomonas* sp.

S= Soil samples, GS= Gram stain, MPA= Morphological appearance, Ind= Indole test, MR= Methyl red, Ox= Oxidase, Cat= Catalase, Cit= Citrate, VP= Voges-Proskauer, -= negative, += positive

Table 4: Plant growth-promoting traits of rhizobacterial isolates: IAA production, ammonia production, and phosphate solubilization.

Isolate	Indole Acetic Acid (IAA)	Ammonia Production	Phosphate Solubilization
Bacillus sp. S2A	+	0	++
Pseudomonas sp. S3B	0	0	0
Bacillus sp. S5A	+	0	+
Azotobacter sp. S7A	0	0	0
Azotobacter sp. S8A	0	++	0
Bacillus sp. S9A	+++	+++	+++
Pseudomonas sp. S10C	0	0	0

+ = weak plant growth promoting activity, ++ = moderate plant growth promoting activity, +++ = strong plant growth promoting activity, 0 = no activity

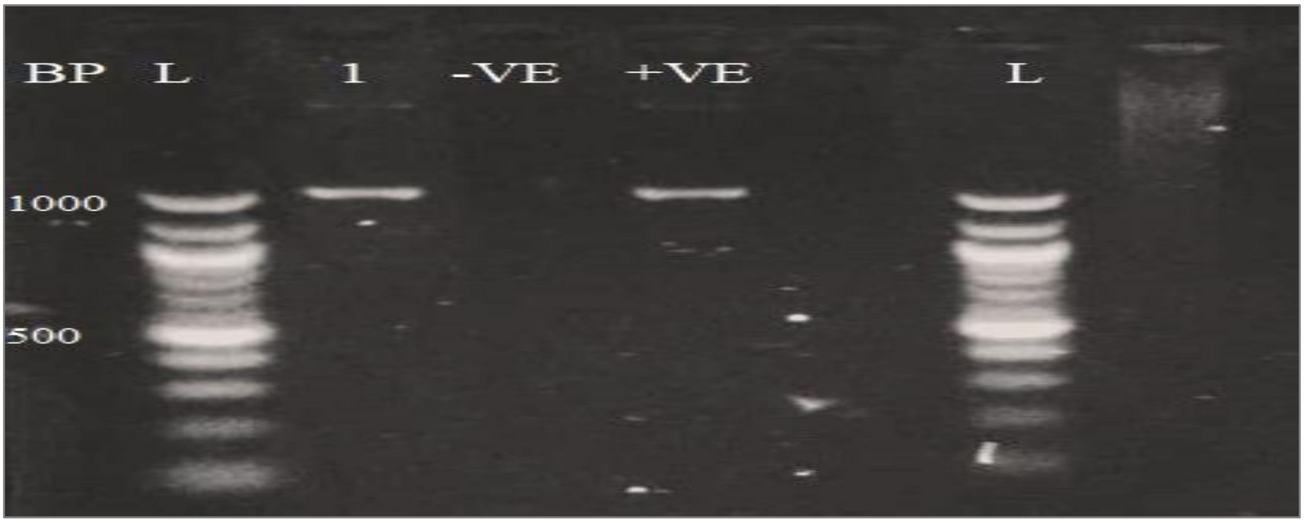

Figure 1: PCR representative gel for the detection of the 16S rRNA gene for *Bacillus* sp. S9A. A 1000 base pair DNA ladder was used in lane L, lane 1, representing *Bacillus* sp. S9A, negative control lane -ve, positive control lane +ve, produced the expected band.

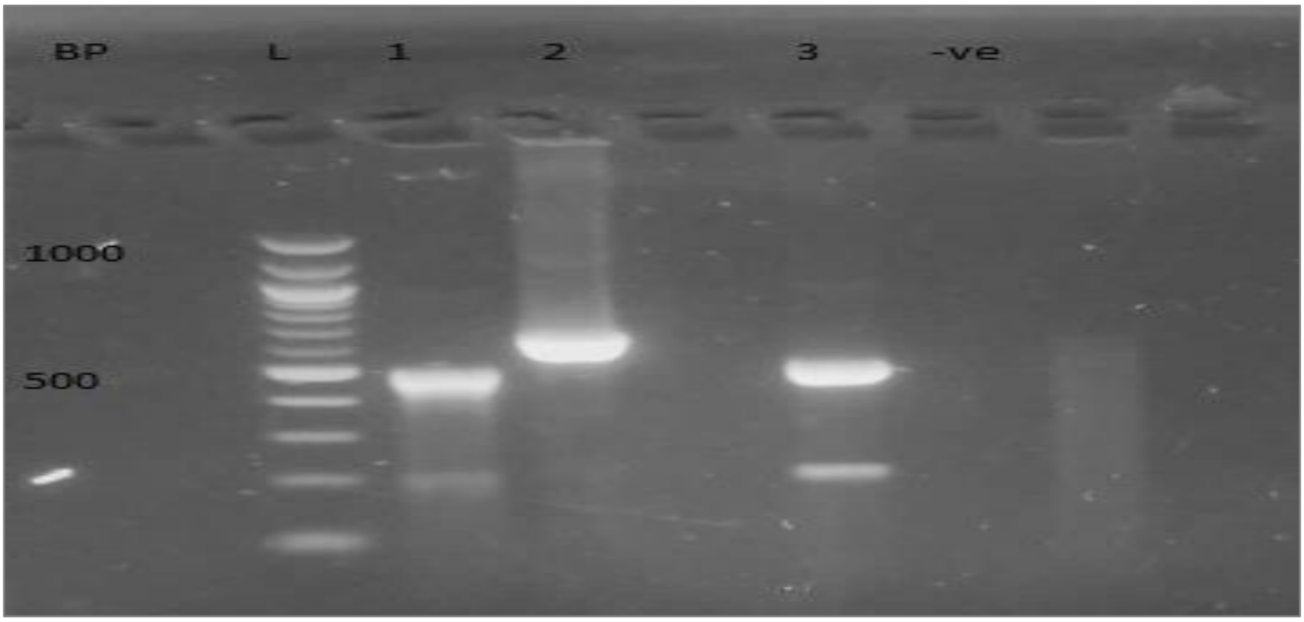

Figure. 2: PCR representative agarose gel showing PCR-Amplified Plant Growth-Promoting Genes from *Bacillus* sp. S9A. A 1000 base pair DNA ladder was used, PGPB genes: indole acetic acid gene (*ipdC*) labeled 1 shows at 500bp, ammonia production gene (*ureC*) labeled 2 shows at 700bp, phosphate solubilization gene (*gcd*) labeled 3 shows at 600bp, and no amplification was observed in the negative control labeled -ve.

The identity of the rhizobacterial isolate was confirmed through molecular characterization by amplification of the 16S rRNA gene. Genomic DNA extracted from *Bacillus* sp. S9A was used as a template for PCR, and universal primers were used. The amplified products were observed by electrophoresis, and a 1.5% agarose gel was used to stain with a nucleic acid dye and visualize under UV transillumination. 1000 bp DNA ladder (Lane L) was used to estimate the size of the amplified fragments—lane 1, representing *Bacillus* sp. S9A revealed a single, distinct band of approximately 1000 base pairs (bp), corresponding to the expected size of the 16S rRNA gene fragment. The negative control (Lane N), which lacked a DNA template, showed no band, confirming that the reagents were free of contaminating DNA. The positive control (Lane P) produced the expected band, confirming the efficacy of the PCR reagents and conditions—the successful amplification of the 16S rRNA gene from *Bacillus* sp. S9A provided the basis for subsequent sequencing and phylogenetic analysis to confirm the isolate's taxonomic identity.

Figure 2 shows the rhizobacterial isolate *Bacillus* sp. S9A was screened for the presence of multiple plant growth-promoting (PGP) genes using polymerase chain reaction (PCR) with specific primers. The genes targeted included *ipdC*, indole-3-acetic acid (IAA); ureC, ammonia production; and gcd, phosphate solubilization. 1000 bp DNA ladder (Lane L) was used as a molecular weight marker to estimate the size of the amplified fragments. Lane 1 shows the amplification product obtained with *ipdC*-specific primers, revealing a distinct band at approximately 500 bp, which corresponds to the expected amplicon size for the IAA biosynthesis gene. Lane 2 shows the product amplified with *ureC*-specific primers, displaying a band at approximately 700 bp, confirming the presence of the urease gene associated with ammonia production. Lane 3 shows the product obtained with *gcd*-specific primers, exhibiting a band at approximately 600 bp, indicating the presence of the glucose dehydrogenase gene involved in phosphate solubilization. No amplification was observed in the negative control (Lane N), confirming the absence of contamination. The successful amplification of these PGP genes confirms the genetic potential of biofertilizer production using the *Bacillus* sp. S9A, which produces IAA, generates ammonia, and solubilizes phosphate.

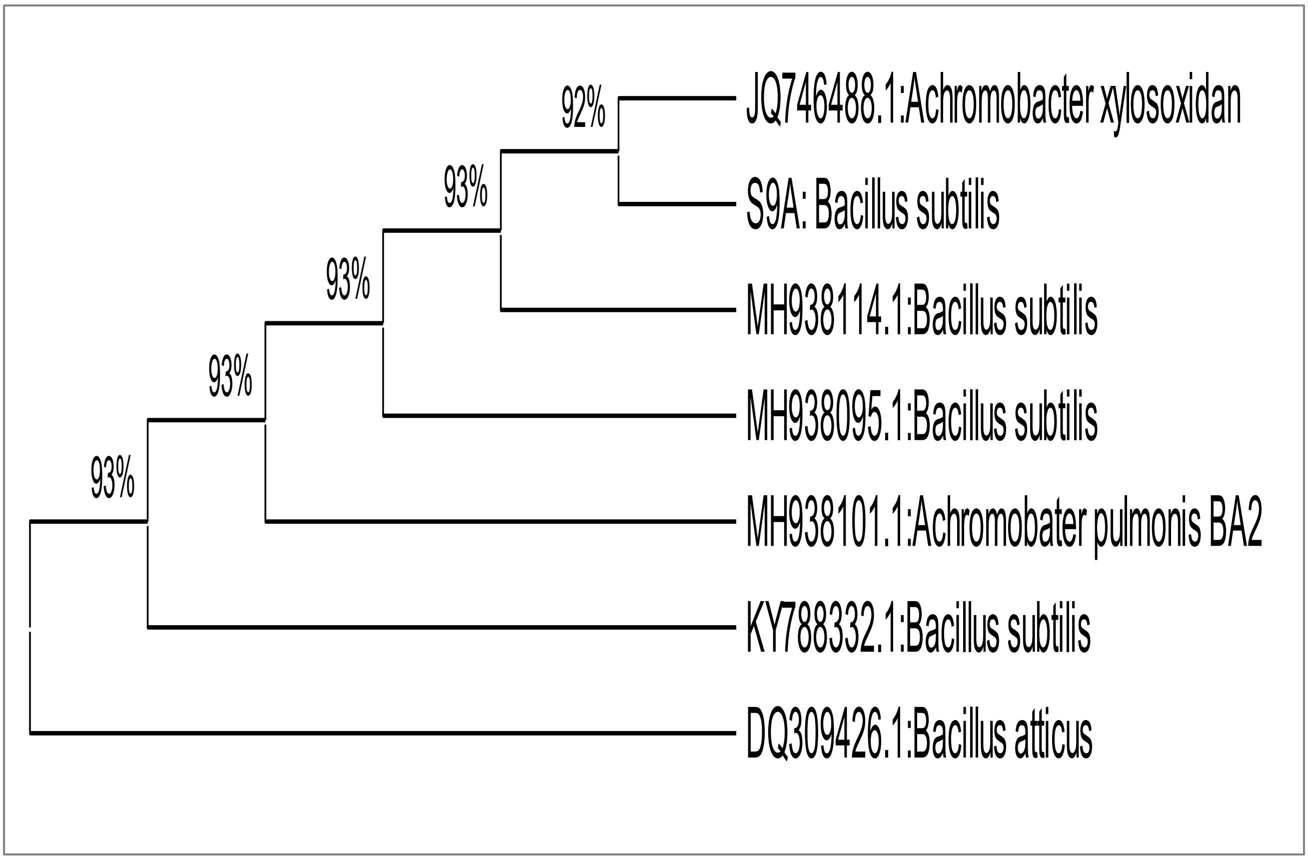

Figure 3: Presents the phylogenetic tree showing the relationship between *Bacillus* sp. S9A and other related *Bacillus* species

Figure 3 shows the molecular identity and evolutionary relationship of the rhizobacterial isolate *Bacillus* sp. S9A were further detected by sequencing the amplified 16S rRNA gene fragment and constructing a phylogenetic tree. The sequences were assembled and aligned using the Codon-Code Aligner software. The sequences were identified using the nucleotide blast tool (National Center for Biotechnology Information NCBI search tool), and the phylogenetic tree was created by using the neighbor-joining method with the Jukes–Cantor evolutionary distance measurement using MEGA v.10. After the 16S rRNA gene sequences were obtained, they were matched with the GenBank database using the NCBI Basic Local Alignment Search Tool (BLAST). Identified sequences were submitted to NCBI GenBank, awaiting accession numbers for the selected isolates.

The phylogenetic analysis revealed that *Bacillus* sp. S9A clustered closely with reference strains of *Bacillus subtilis*, forming a distinct clade with high bootstrap support (93%). This confirms the taxonomic affiliation of the isolate within the genus *Bacillus* and its close relationship to *B. subtilis* group.

DISCUSSION

This study provides a foundational characterization of the cultivable rhizobacterial community in the irrigated rice fields of Damaturu, Yobe State, Nigeria. The predominance of *Bacillus* spp. Is consistent with global studies on rice rhizospheres (Smalla *et al.*, 2001; Agu *et al.*, 2021; Xun *et al.*, 2022; Oledele *et al.*, 2014) and can be attributed to their ability to form endospores, allowing them to withstand the fluctuating moisture and temperature conditions typical of irrigated soils in semi-arid climates.

The observed soil properties create a favorable environment for these bacteria. The near-neutral pH (6.1-7.1) is optimal for the activity of many PGPR, including *Bacillus, Pseudomonas,* and *Azotobacter spp.* (Kumar *et al.*, 2020). The moderate levels of organic carbon (1.15-1.85%) provide a critical energy source for microbial metabolism. The correlation between higher bacterial counts (*Bacillus* sp. S9A) and specific soil parameters, such as organic carbon, while preliminary, suggests that soil fertility management could directly influence the size of the beneficial microbial pool.

The physicochemical properties of the rice rhizosphere soil in Damaturu revealed a clay-loam texture, with a near-neutral pH (6.1–7.1) and a moderate organic carbon content (0.62–0.89%) (Table 1). These conditions are consistent with previous reports on rice-growing soils in semi-arid regions of Nigeria (Yusuf *et al.*, 2018; Adegbite *et al.*, 2020) and are considered optimal for the proliferation of plant growth-promoting rhizobacteria (PGPR), particularly *Bacillus*, *Pseudomonas*, and *Azotobacter* species (Kumar *et al.*, 2020). The near-neutral pH enhances nutrient availability and enzymatic activity, which are critical for microbial metabolism, while organic carbon serves as a primary energy source for heterotrophic bacteria (Fierer, 2017).

The total rhizobacterial counts ranged from 1.02 × 10^{11} to 3.18 × 10^{11} CFU/g, with the highest count recorded in sample S9, which also yielded the *Bacillus* sp. isolate with the multiple PGP traits in Table 2. This observation aligns with findings by Agu *et al.* (2021), who reported higher bacterial densities in rhizosphere soils with elevated organic carbon content. However, unlike the study by Pérez-Jaramillo *et al.* (2018), which demonstrated that soil type more strongly influences rhizosphere community structure than plant genotype, the present study did not conduct multivariate analysis to partition these effects, representing a limitation that should be addressed in future investigations.

The phenotypic characterization identified three dominant genera: *Bacillus* spp. (50%), *Pseudomonas* spp. (30%), and *Azotobacter* spp. (20%) In Table 3. The predominance of *Bacillus* spp. is consistent with global studies on rice rhizospheres (Smalla *et al.*, 2001; Xun *et al.*, 2022) and can be attributed to their endospore-forming ability, which confers resilience to the fluctuating moisture and temperature conditions characteristic of semi-arid irrigated systems (Radhakrishnan *et al.*, 2017). The relative abundance of *Bacillus* spp. (50%) observed in this study is higher than the 32.29% reported by Ismail *et al.* (2023) in rice rhizospheres in Northern Nigeria, but lower than the 68% reported by Shao *et al.* (2021) in temperate rice systems. This variation likely reflects differences in climatic conditions, soil types, and agricultural management practices, as well as the proportion of *Pseudomonas* spp. (30%) in our study exceeds the 17.64% reported by Ismail *et al.* (2023), suggesting that the semi-arid conditions of Damaturu may favor pseudomonads to a greater extent than previously recognized.

The rhizobacteria isolates in this study exhibited at least one PGP trait, with *Bacillus* sp. S9A demonstrated or showed strong multiple plant growth-promoting traits compared to other rhizobacterial isolates. The simultaneous expression of multiple PGP mechanisms in a single isolate is particularly significant, as previous study which have indicated that the most effective PGPR inoculants possess multiple growth-promoting mechanisms (Backer *et al.*, 2018; Kumar *et al.*, 2021).

The potential plant growth-promoting (PGP) traits, such as indole-3-acetic acid, ammonia production, and phosphate solubilization, were shown in Table 4 for *Bacillus* sp. S9A had multiple PGP trait indicate potential to serve as biofertilizers, contributing to sustainable rice (*Oryza sativa* L. FARO 44) growth and other crops cultivation, as well as reducing the use of chemical fertilizer which agrees with a study by Ajmal *et al.* (2021) showing that diverse groups of bacteria thrive in environments that may have PGPB ability even under myriad stress conditions. Another study by Agu *et al.* (2021) shows the potential of rhizobacteria isolates from Agricultural farms in Ibadan to produce most PGP traits, including phosphate solubilization, IAA production, and ammonia production. Similarly, Farah Ahmad *et al.* (2008) isolated 19 rhizobacteria from different rhizosphere soils, determined IAA production, and observed that more than 70% of the Bacillus spp. isolates. produced IAA, whereas only 20% of *Pseudomonas* and 10% *Azotobacter* isolates were IAA producers. Studies by Tang *et al.* (2020) have shown that bacteria can produce IAA, and a diverse group of bacterial species has been reported by Khatoon et al. (2020) to produce IAA, including some species reported in this study, thereby supporting the findings of this study. The findings of this study are consistent with global studies identifying these genera as dominant and highly effective PGPR (Backer *et al.*, 2018). Systematically reviewed that the most successful PGPR inoculants often possess multiple growth-promoting mechanisms. The combination of plant growth-promoting traits in this study, obtained from the rhizobacterium Bacillus sp. (S9A), includes IAA, which promotes root growth and is directly linked to the observed increase in root length. Ammonia production provides a readily available nitrogen source, and phosphate solubilization is crucial in neutral to alkaline soils where P is mostly insoluble. This finding aligned study by Sharma *et al.* (2011) and Kumar *et al.* (2021).

Figure 1 shows a representative PCR gel for the detection of the 16S rRNA gene in isolated rhizobacteria, *Bacillus* sp. S9A. Gel electrophoresis was performed to visualize the amplified DNA segment from the isolates. Fig. 1 shows the PCR-amplified products from the extracted DNA template of *Bacillus* sp. S9A for 16S rRNA and fig. 2 shows the PGPB genes: Indole Acetic Acid (IAA) ipdC, Ammonia Production ureC, and Phosphate Solubilization gcd. A 1000 bp DNA ladder was used, labeled as L, followed by lane 1: indole-3-acetic acid (IAA) ipdC gene; lane 2: ammonia production ureC gene; and lane 3: phosphate solubilization gcd gene. Fig. 3 shows the neighbor-joining (NJ) phylogenetic tree after sequencing the 16S rRNA of rhizobacterial isolates. Bacillus sp. (S9A) was used as a biofertilizer; the 16S rRNA gene sequences of the bacteria isolated were compared with the GenBank database using the Blast Server at NCBI. Blast analysis of 16S rRNA sequences of the strain *Bacillus* sp. S9A revealed homology to *Bacillus subtilis* (92%). The molecular phylogenetic studies identified in this study using the neighbour joining method linked the identity of the obtained bacteria sequences in existing database of National Center of Bioinformatics, respectively (NCBI) were MH938114.1: *Bacillus subtilis* (93%), MH938095.1: *Bacillus subtilis* (93%), KY788332.1: *Bacillus subtilis* (93%)

and DQ309428.1: *Bacillus atticus* (93%). The 16S rRNA, PGPB genes, and phylogenetic tree obtained from this study were similar to various studies (Basobi *et al.*, 2017; Oluwale *et al.*, 2023; Guardiola-Márquez et al., 2023; Uzah *et al.*, 2024).

CONCLUSION

This study successfully achieved its primary objective of isolating and molecularly characterizing indigenous *Bacillus* species from the rice rhizosphere in Damaturu, North-Eastern Nigeria, and evaluating their plant growth-promoting (PGP) potential for biofertilizer development. All three genera demonstrated functional PGP traits in vitro, including indole acetic acid (IAA) production, ammonia synthesis, and phosphate solubilization, confirming their potential to enhance plant growth through multiple mechanisms. Notably, *Bacillus* sp. strain S9A, which exhibited the highest colony count (3.18×10^{11} CFU/g) and superior PGP traits, was selected for molecular characterization. Partial sequencing of the 16S rRNA gene and phylogenetic analysis confirmed that the isolate is Bacillus subtilis , with 93% homology to reference strains in the GenBank database, validating its taxonomic identity and evolutionary relationships within the *Bacillus* genus.

The findings of this research demonstrated that indigenous rhizobacteria, particularly the Bacillus subtilis strain S9, possess significant plant growth-promoting attributes and hold considerable promise as effective biofertilizers for sustainable rice (*Oryza sativa* L. FARO 44) cultivation in semi-arid agroecosystems. The successful isolation and molecular confirmation of this strain from local soils underscores the value of prospecting for native microbial resources that are already adapted to the prevailing environmental conditions.

This study contributes original data to the growing body of knowledge on plant growth-promoting rhizobacteria in understudied regions of sub-Saharan Africa. Harnessing these indigenous microbial resources offers a viable pathway to reduce dependency on synthetic chemical fertilizers, enhance soil health and fertility, improve crop productivity, and promote environmentally sustainable farming practices in Damaturu, North-Eastern Nigeria, and similar agroecological zones. Furthermore, the use of locally adapted *Bacillus* strains as biofertilizers aligns with global efforts to achieve food security through sustainable agricultural intensification while mitigating the environmental footprint of conventional farming practices.

There should be greater awareness among all stakeholders and farmers of the need to use rhizobacterial isolates (*Bacillus subtilis*) as biofertilizers to reduce reliance on chemical fertilizers and mitigate environmental hazards. The primary contribution of this work is the provision of a characterized, locally sourced pool of rhizobacterial isolates (*Bacillus subtilis*) from a rice irrigation site in Damaturu, Yobe State, Nigeria.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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