JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2026, 27(5), 128–143
https://doi.org/10.12911/22998993/215937
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.11.27
Accepted: 2026.03.16
Published: 2026.04.01

Indigenous plant growth-promoting rhizobacteria as biofertilizer candidates for hybrid maize in acidic soils

Akhmad Syakur[1,2], Baharuddin Patandjengi[3*], Fahruddin[4], Muhammad Fuad Anshori[5], Sofyan[1], Suharman[1], Darmawan Risal[1]

[1] Doctoral Program of Agricultural Science, Postgraduate School, Hasanuddin University, Makassar, Indonesia
[2] Department of Science Education, Faculty of Mathematics and Natural Sciences, State University of Makassar, Makassar, Indonesia
[3] Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia
[4] Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar, Indonesia
[5] Department of Agronomy, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia
* Corresponding author's e-mail: baharuddin@agri.unhas.ac.id

ABSTRACT

Maize (*Zea mays* L.) is a major staple crop, but its cultivation on acidic soils is constrained by low pH, nutrient deficiencies, and aluminum toxicity, which together limit soil fertility and plant growth. Exploiting indigenous plant growth-promoting rhizobacteria (PGPR) offers a sustainable approach to improving nutrient availability and reducing reliance on synthetic fertilizers. This study aimed to isolate and characterize rhizosphere bacteria associated with hybrid maize grown on acidic ultisol in the Moncongloe District (South Sulawesi, Indonesia) and to evaluate their biofertilizer potential. Soil samples from five locations yielded ten representative bacterial isolates that were screened in vitro for indole-3-acetic acid (IAA) production, biological nitrogen fixation, and phosphate solubilization. Two superior isolates, TJMS19 and TJMTE54, exhibited high nitrogen-fixation capacity (up to 49.07 mg L^{-1}) and strong phosphate-solubilizing ability (up to 14.54 mg L^{-1}), while TJMTE54 also produced the highest IAA level (6.55 mg L^{-1}). Inoculation of hybrid maize seedlings with these isolates significantly increased plant height, root length, and fresh biomass compared with the uninoculated control. Molecular identification based on 16S rRNA gene sequences showed that TJMS19 is closely related to *Bacillus paramycoides*, whereas TJMTE54 clusters within the genus *Pseudomonas.* The combined physiological traits and growth-promotion effects indicate that these indigenous PGPR strains are promising candidates for development as eco-friendly biofertilizers to enhance hybrid maize productivity on acidic, marginal soils.

Keywords: Rhizosphere bacteria, nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA), ultisol.

INTRODUCTION

Maize (*Zea mays* L.) represents one of the most essential cereal crops worldwide, contributing profoundly to global food security systems. Increasing production remains a priority as demands from food, feed, and industrial sectors continue to intensify (He et al., 2025; Ríos-Ruiz et al., 2024). Rapid population growth and the expansion of industrial activities have further escalated the need for maize (Rozi et al., 2023). Consequently, this crop now occupies a strategic role comparable to that of wheat and rice within international food supply chains (Khubna et al., 2021).

Hybrid maize cultivation in Indonesia shows strong potential, reflected in an estimated annual market expansion of around 5%. However, the scarcity of highly fertile land has encouraged the shift toward marginal areas dominated by acidic soils. Intensified and prolonged use of synthetic fertilizers in such landscapes can hasten soil deterioration and diminish crop yields (Bisht et al., 2025; Y. Yuan et al., 2025). Acidic soils typically exhibit low pH, high concentrations of phytotoxic ions such as Al^{3+} and Fe^{2+} (Hayet et al., 2021), and limited levels of key nutrients including nitrogen, phosphorus, and potassium (Li et al., 2024; Regasa et al., 2025). These constraints collectively exert substantial negative impacts on maize vigor and productivity.

The application of plant growth-promoting rhizobacteria (PGPR) offers a promising ecological approach to overcoming these limitations (Liu et al., 2021). These beneficial microorganisms support plant development by fixing atmospheric nitrogen, solubilizing phosphate, producing phytohormones such as gibberellins, abscisic acid, and indole-3-acetic acid (IAA), and strengthening plant tolerance to environmental stresses (Orozco-Mosqueda et al., 2023; Subair et al., 2015). PGPR inoculation has been shown to enhance maize growth while enabling reductions of up to 50% in chemical fertilizer requirements (Chen et al., 2021). Moreover, integrating PGPR with organic amendments can restructure rhizosphere microbial communities, elevate soil organic matter, and promote bacterial diversity (Dincă et al., 2022; Zhao, Mao, et al., 2021). For instance, *Bacillus* sp. SYM-4 was reported to improve antioxidant enzyme activities and support maize performance when combined with mineral fertilizers (Song et al., 2025). Under acidic soil conditions, genera including *Azospirillum*, *Bacillus*, and *Pseudomonas* exhibit natural tolerance to low pH and effectively solubilize phosphate and fix nitrogen (Fanai et al., 2024; Khoso et al., 2024), emphasizing their suitability as localized biofertilizer resources.

Biofertilizers consist of beneficial microbial taxa – such as fungi, algae, and bacteria – that stimulate plant growth by colonizing the rhizosphere and improving nutrient availability (Risanti et al., 2024). These biological inputs also modify soil physicochemical properties and reorganize soil microbial communities by influencing microbial carbon dynamics and diversity (Aponte et al., 2022). Species like *Chlorella vulgaris* and *Spirulina platensis* have been shown to enhance soil nitrogen and phosphorus availability (Dineshkumar et al., 2022), whereas supplementing chemical fertilizers with microbial consortia can reduce fertilizer requirements by up to 25% while increasing yields (Jain et al., 2021). Soil microbial assemblages are sensitive to site-specific edaphic conditions and agroecosystem characteristics (Ding et al., 2024; Kiprotich et al., 2025). While numerous PGPR investigations have been conducted in neutral soils, studies on hybrid maize grown in acidic environments remain limited. Indigenous microorganisms adapted to such soils therefore hold strong potential as effective biofertilizer candidates (Asghar et al., 2023; Rasul et al., 2024).

This research addresses these gaps by focusing on indigenous PGPR with multiple growth-promoting capabilities associated with hybrid maize cultivated on acidic soils in Indonesia. Specifically, it examines rhizosphere bacteria from maize grown in acidic soils in the Moncongloe District of South Sulawesi, a region where integrated assessments of IAA synthesis, nitrogen fixation, phosphate solubilization, and plant growth responses remain scarce. The study aimed to (i) identify indigenous bacterial isolates exhibiting key plant growth-promoting functions – namely IAA production, biological nitrogen fixation, and phosphate solubilization; (ii) assess the effects of selected isolates on maize seedling performance under acidic soil conditions; and (iii) determine promising strains through 16S rRNA gene characterization for future development of locally adapted, environmentally sound biofertilizers suitable for marginal acidic lands.

MATERIAL AND METHODS

Study site and soil sampling

The study was carried out from June 2024 to March 2025. Rhizosphere soils were obtained from hybrid maize cultivation areas located in the Moncongloe District, Maros Regency, South Sulawesi, Indonesia. The research site spans roughly 17 ha at coordinates 5°02′ S and 119°36′ E, positioned at an altitude of 25–50 m above sea level (Figure 1). The soil type is classified as Ultisol and exhibits acidic conditions (pH 5.3), characterized by a sandy clay texture, low organic matter, and restricted levels of essential macronutrients (N, P, and K). The Moncongloe area experiences a humid tropical climate with annual rainfall ranging from 2.500 to 3.000 mm, average daily temperatures between 26 and 28 °C, and relative humidity of 75–85%. Soil sampling was performed at five distinct points within the field. Subsequent isolation and characterization of rhizosphere-associated bacteria were conducted at the Plant Pest

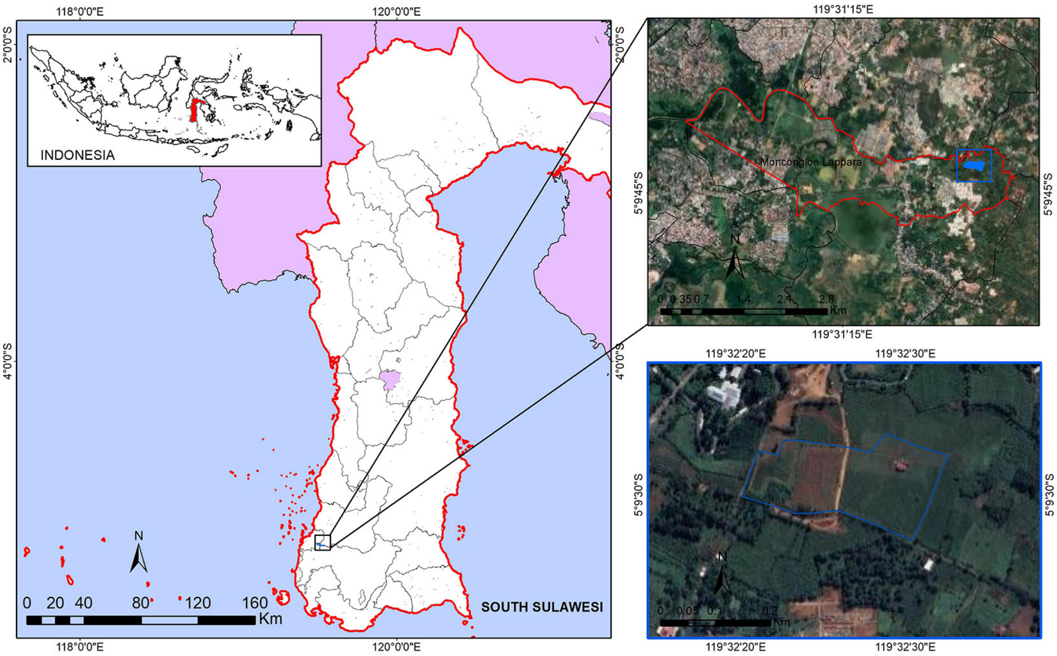

Figure 1. Location of the hybrid maize study site in Moncongloe District, Maros Regency, South Sulawesi, Indonesia

and Disease Microbiology Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar.

Characterization of the study site

Chemical properties of the soil at the five sampling locations were determined to characterize the rhizosphere environment of the bacterial isolates. The parameters measured included pH, organic carbon (C-organic), total nitrogen (total N), and the C/N ratio (Table 1). Soil pH was measured potentiometrically using a 1:2.5 soil-to-distilled-water ratio (pH H_2O), organic carbon content was determined using the Walkley–Black dichromate oxidation method, and total nitrogen was analyzed using the Kjeldahl digestion–distillation procedure. Soil samples from sites coded TJMU, TJMB, TJMS, TJMT, and TJMTE had pH values ranging from 5.2 to 5.4. The lowest pH (5.2) was recorded at the TJMB site, whereas the highest pH (5.4) was recorded at the TJMTE site. Organic C content ranged from 0.85 to 1.92%, total N from 0.11 to 0.31%, and the C/N ratio from 5.9 to 8.1.

Isolation of rhizosphere bacteria

Rhizosphere soil from each sampling point was homogenized and serially diluted in sterile distilled water up to 10^{-7}. Aliquots (0.1 mL) from the 10^{-5}–10^{-7} dilutions were spread onto nutrient agar (NA) plates using a sterile glass spreader. Plates were incubated at 28–30 °C for 48 h. Colonies displaying distinct morphological characteristics were selected and purified by repeated streaking on fresh NA plates until homogeneous colonies were obtained (Sherpa et al., 2021).

Morphological characterization of rhizosphere bacterial colonies

Pure isolates obtained from the isolation process were characterized based on macroscopic colony morphology on NA, including colony shape, size, elevation, margin, and color. Morphological observations were carried out using an image-processing-based approach to aid in the characterization of colony traits (Yadu et al., 2023).

Biochemical and physiological characterization of bacterial isolates

Gram reaction and catalase test

Biochemical characterization of the bacterial isolates included Gram reaction and catalase activity. Gram reaction was assessed using the 3% KOH method, in which the formation of a viscous mucoid thread indicates Gram-negative bacteria, whereas the absence of mucus indicates Gram-positive bacteria. Catalase activity was evaluated by adding a drop of 3% hydrogen peroxide (H_2O_2) to a fresh bacterial colony on a glass slide;

Table 1. Soil characteristics at the research site

Isolate Code	pH	C Organic (%)	N Total (%)	C/N ratio
TJMU	5.3	0.85	0.11	6.5
TJMB	5.2	1.60	0.29	6.6
TJMS	5.3	1.82	0.27	7
TJMT	5.3	1.10	0.31	5.9
TJMTE	5.4	1.92	0.27	8.1

the formation of gas bubbles indicated a positive reaction, whereas the absence of bubbles indicated a negative result (Sudewi et al., 2020).

Hypersensitive reaction assay

A hypersensitive reaction assay was performed to assess the pathogenic potential of the isolates in a non-host plant. Bacterial suspensions were adjusted to approximately 10^8 CFU mL^{-1} and infiltrated into tobacco (*Nicotiana tabacum*) leaves using a needleless syringe. A *Xanthomonas* sp. suspension was used as a positive control, and sterile distilled water served as a negative control. Leaves were observed for up to 72 h post-inoculation. The appearance of necrotic lesions around the infiltration site was interpreted as a hypersensitive response, indicating potential pathogenicity (Amaria et al., 2023).

Hemolysis assay

Hemolytic activity was examined using blood agar formulated from sterile tryptic soy agar (TSA) enriched with 5% goat blood. The bacterial isolates were streaked onto the agar surface and incubated at 28 °C for 24–48 h. Hemolytic responses were classified according to the zones formed around the colonies: α-hemolysis (partial) was recognized by a greenish discoloration, β-hemolysis (complete) by a transparent clearing, and γ-hemolysis (non-hemolytic) by the absence of visible alterations surrounding the colonies (Amaria et al., 2023; Mogrovejo et al., 2020).

Determination of plant growth-promoting traits

Indole-3-acetic acid (IAA) production

IAA synthesis was assessed in vitro using a colorimetric approach based on the Salkowski reagent. Bacterial cultures (approximately 10^7 CFU mL^{-1}) were inoculated into Minimal Salt medium amended with L-tryptophan (1 mg mL^{-1}) and incubated at 28 °C on a rotary shaker operating at 100 rpm for up to 6 days. Aliquots were obtained every 2 days, centrifuged at 5.500 rpm for 10 min, and the resulting supernatant was combined with Salkowski reagent at a 4:1 ratio (supernatant:reagent). Following a 20-min reaction period at ambient temperature, the intensity of the pink to reddish coloration was determined at 535 nm using a UV–Vis spectrophotometer (Sudewi et al., 2020; Das et al., 2019). IAA concentration (y) was calculated from a standard calibration curve using the following regression equation:

$$y = 0.064x + 0.009 \quad (1)$$

where: x – absorbance value at 535 nm, y – IAA concentration (mg L^{-1}). All assays were performed in triplicate.

Nitrogen-fixation potential

The potential of rhizosphere bacterial isolates to fix nitrogen was evaluated in vitro following Meng et al., (2023) with modifications. Isolates were inoculated into nitrogen-free Biological N_2 Fixation (BNF) medium containing (g L^{-1}): K_2HPO_4, $MgSO_4$, KH_2PO_4, $FeCl_3 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$, and dextrose (5.0), and trace elements (µg L^{-1}): $Na_2MoO_4 \cdot 2H_2O$ (240), H_3BO_4 (3000), $MnSO_4 \cdot H_2O$, $CoCl_2 \cdot 6H_2O$, $CuSO_4 \cdot 5H_2O$, and $ZnSO_4 \cdot 7H_2O$ (Córdova-Rodríguez et al., 2022). Cultures were incubated at 28 °C with shaking. Fixed nitrogen in the culture supernatant was determined colorimetrically, and results were expressed as mg L^{-1}. All tests were conducted in triplicate for each isolate.

Phosphate solubilization assay

Phosphate-solubilizing ability was assessed in liquid Pikovskaya medium using the phosphomolybdate method (Pande et al., 2017). Bacterial cultures were incubated at 28 °C for 7 days and then centrifuged at 10.000

rpm for 20 min. The supernatant was reacted with a mixture of ammonium molybdate, sulfuric acid, and ferrous sulfate, and absorbance was measured at 693 nm. The concentration of dissolved phosphate (y) was calculated from a standard calibration curve using the following regression equation:

$$y = 0.191x + 0.048 \qquad (2)$$

where: x –absorbance value at 693 nm, y – concentration of dissolved phosphate (mg L^{-1}). Each treatment was performed in triplicate.

In vitro maize seedling assay

The influence of bacterial inoculation on the growth of hybrid maize seedlings was assessed under controlled experimental conditions. The bacterial isolates were cultured on NA for 24 h, and the resulting cell suspensions were standardized to approximately 10^8 CFU mL^{-1} using optical density readings at 660 nm. Maize seeds underwent surface sterilization using 70% ethanol followed by treatment with 2.5% NaOCl, after which they were thoroughly rinsed with sterile distilled water. The disinfected seeds were immersed in bacterial suspensions for 24 h, whereas the uninoculated control group was soaked in sterile distilled water.

Seed germination was carried out in sterile petri dishes lined with moist filter paper. Once the radicle and plumule emerged, seedlings of uniform size were transferred into glass bottles containing a nutrient solution and maintained under controlled environmental settings. Seedlings were maintained at 27 ± 1 °C, with a 12-hour light / 12-hour dark photoperiod, and a light intensity of approximately 200 µmol m^{-2} s^{-1}. Relative humidity was controlled at 70–75%. All other procedures followed the previously described protocol. The experiment followed a completely randomized design consisting of one control (without inoculation) and ten inoculation treatments (isolates TJMU6, TJMB10, TJMB12, TJMS18, TJMS19, TJMS21, TJMT29, TJMT39, TJMTE53, and TJMTE54), each with three replicates. After 14 days of growth, seedling height, root length, and fresh biomass were recorded for all treatments.

Genomic DNA extraction, PCR amplification, and sequencing

Genomic DNA from selected bacterial isolates was extracted using the gSYNC™ DNA Extraction Kit (Geneaid, Taiwan), following the procedures recommended by the manufacturer. Amplification of the 16S rRNA gene was conducted in a 50 µL PCR mixture containing GoTaq® Green Master Mix (Promega, USA), the primers 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and 1387R (5′-GGG CGG WGT GTA CAA GGC-3′) (Mai et al., 2023), template DNA, and nuclease-free water. The PCR cycling protocol consisted of an initial denaturation at 95 °C, followed by 35 cycles comprising denaturation at 95 °C, annealing at 50 °C, and extension at 72 °C, with a final extension step at 72 °C for 5 min.

PCR amplicons were resolved by electrophoresis on 2% agarose gels prepared in 1× Tris–acetate–EDTA (TAE) buffer containing ethidium bromide. Approximately 8 µL of each PCR product was loaded onto the gel, and electrophoresis was run at 100 V for 50 min. DNA fragments were visualized under UV illumination and photographed for documentation (Noer, 2021). The remaining PCR products were purified and submitted to First BASE Laboratories (Singapore) for Sanger sequencing. The resulting 16S rRNA gene sequences were then compared with reference sequences in GenBank using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the closest phylogenetic matches.

Statistical analysis

All quantitative data (IAA concentration, fixed nitrogen, dissolved phosphate, and seedling growth parameters) were analyzed using one-way analysis of variance (ANOVA). When ANOVA indicated significant treatment effects, means were separated using Tukey's test at $P \leq 0.05$. Data are presented as mean ± standard deviation (SD).

RESULTS

Morphological characteristics of rhizosphere bacterial isolates

A total of 59 bacterial colonies were initially isolated from rhizosphere soil, and 10 representative isolates were selected for further characterization based on distinct colony

Table 2. Morphological characteristics of rhizosphere bacterial isolates

Isolate code	Morphology				
	Size	Colony color	Colony form	Height	Colony edge
TJMU06	Moderate	Cream	Circular	Convex	Entire
TJMB10	Small	Cream	Circular	Convex	Lobate
TJMB12	Punctiform	Yellow	Circular	Raised	Undulate
TJMS18	Punctiform	Yellow	Circular	Flat	Entire
TJMS19	Punctiform	Cream	Circular	Flat	Entire
TJMS21	Small	Cream	Circular	Flat	Entire
TJMT29	Moderate	White	Circular	Convex	Undulate
TJMT39	Punctiform	Cream	Circular	Convex	Undulate
TJMTE53	Punctiform	Yellow	Circular	Convex	Undulate
TJMTE54	Punctiform	Cream	Circular	Convex	Undulate

morphology and consistent growth on nutrient agar (Table 2). The morphological characteristics of the rhizosphere bacterial colonies indicated clear phenotypic diversity.

As shown in Table 2, the isolates varied in colony size, elevation, margin, and color. Colony sizes ranged from punctiform to moderate, and all isolates exhibited circular colony forms. Colony margins were entire in TJMU06, TJMS18, TJMS19, and TJMS21; lobate in TJMB10; and undulate in TJMB12, TJMT29, TJMT39, TJMTE53, and TJMTE54. Elevations included convex, raised, and flat types, with convex being the most common. Colony colors were cream, yellow, and white, with cream predominating, yellow occurring in TJMB12, TJMS18, and TJMTE53, and white in TJMT29. These characteristics reflect considerable phenotypic diversity among the isolates, suggesting underlying taxonomic differences.

Biochemical and physiological characteristics of rhizosphere bacterial isolates

Physiological characterization of the rhizosphere bacterial isolates was carried out using Gram reaction and catalase tests, a hypersensitive reaction assay, and a hemolysis test (Table 3). These assays revealed variability in biochemical and physiological traits among the isolates.

Based on the catalase test, five isolates (TJMU6, TJMB12, TJMS21, TJMT39, and TJMTE53) showed positive catalase activity, indicating their ability to decompose H_2O_2 into water and oxygen. Gram reaction analysis indicated that isolates TJMS18, TJMS19, and TJMT29 were Gram positive, whereas the remaining isolates were Gram negative. In the hypersensitive reaction assay, none of the isolates induced necrotic lesions on tobacco leaves within 72 h, indicating that they did not elicit a hypersensitive response under the conditions tested.

Hemolysis tests on goat blood agar showed that most isolates exhibited γ-hemolysis (no hemolysis), whereas TJMB12 showed α-hemolysis (partial hemolysis). These results indicate that the majority of isolates are non-hemolytic on goat blood agar; however, further testing would be required to comprehensively assess their pathogenicity toward plants or animals.

Indole-3-acetic acid (IAA) production

One-way analysis of variance (ANOVA) showed that IAA production differed significantly among the rhizosphere bacterial isolates tested ($P < 0.05$; Figure 2). Mean IAA concentrations ranged from 0.36 to 6.55 mg L^{-1}. Isolate TJMTE54 produced the highest IAA level (6.55 mg L^{-1}) and formed a distinct statistical group, indicating a very high auxin-producing capacity. The next highest IAA producers were TJMS21 (2.00 mg L^{-1}), followed by TJMB10 (1.57 mg L^{-1}), TJMS19 (1.53 mg L^{-1}), TJMT39 (1.23 mg L^{-1}), and TJMS18 (1.11 mg L^{-1}), which showed intermediate IAA production. In contrast, TJMU6, TJMB12, TJMT29, and TJMTE53 produced low IAA levels (< 0.5 mg L^{-1}). These results indicate that TJMTE54, TJMS21, and TJMS19 have the greatest IAA-producing capacity among the isolates evaluated.

Table 3. Biochemical and physiological characteristics of bacteria

Isolate code	Catalase (+/-)	Gram (+/-)	Hypersensitivity reaction (+/-)	Hemolysis
TJMU6	+	-	-	Γ
TJMB10	-	-	-	Γ
TJMB12	+	-	-	A
TJMS18	-	+	-	Γ
TJMS19	-	+	-	Γ
TJMS21	+	-	-	Γ
TJMT29	-	+	-	Γ
TJMT39	+	-	-	Γ
TJMTE53	+	-	-	Γ
TJMTE54	-	-	-	Γ

Note: +, positive reaction; –, negative reaction; γ, non-hemolytic; α, partial hemolysis.

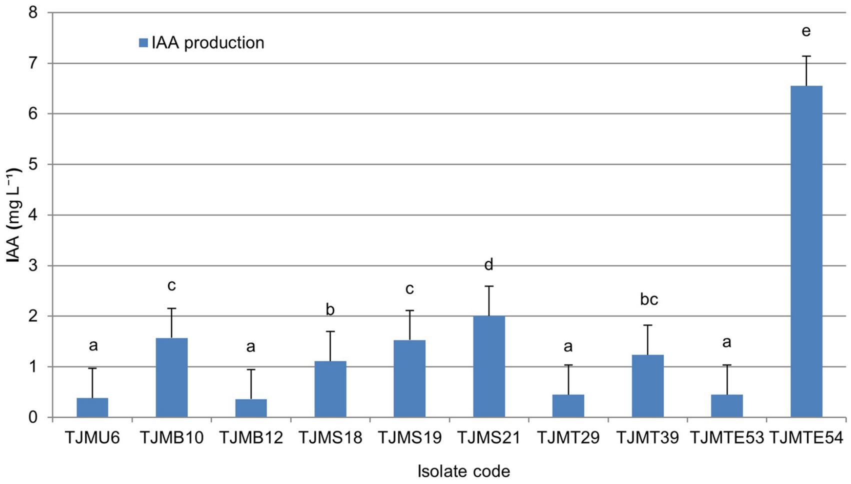

Figure 2. Indole-3-acetic acid (IAA) production by rhizosphere bacterial isolates. Bars represent the mean IAA concentration produced by each rhizosphere bacterial isolate, with error bars indicating standard deviation (n = 3). Different letters (a–e) above the bars indicate significant differences among treatments according to Tukey's test at $P \leq 0.05$

Nitrogen fixation capacity of bacterial isolates

Based on the results of statistical analysis, it was showed that nitrogen-fixation capacity differed significantly among the rhizosphere bacterial isolates tested ($P < 0.05$; Figure 3). Mean concentrations of fixed nitrogen ranged from 26.82 to 49.07 mg L^{-1}. Isolates TJMS19, and TJMTE54 produced the highest levels of fixed nitrogen, 49.07 and 47.30 mg L^{-1}, respectively, indicating very strong nitrogen-fixation activity. Intermediate nitrogen-fixation values were recorded for isolates TJMU6, TJMB12, TJMS18, TJMS 21 and TJMTE53, with concentrations ranging from approximately 32.65 to 42.63 mg L^{-1}. In contrast, TJMB10, TJMT29 and TJMT 39 produced the low concentration of fixed nitrogen (30.14 mg L^{-1}, 26.82 mg L^{-1}, and 29.73 mg L^{-1}). Overall, these results indicate that TJMS19, and TJMTE54 have the greatest nitrogen-fixation capacity among the isolates evaluated and therefore have strong potential to improve nitrogen availability in acidic soils.

Bars represent the mean concentration of fixed nitrogen produced by each rhizosphere bacterial isolate, with error bars indicating standard deviation (n = 3). Different letters (a-d) above the bars indicate significant differences among treatments according to Tukey's test at $P \leq 0.05$

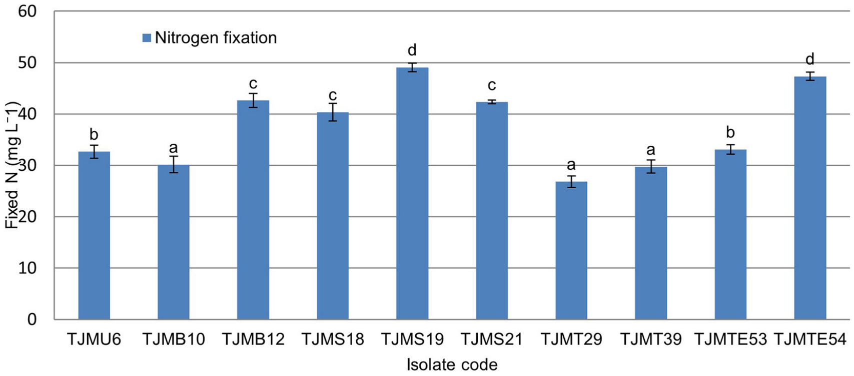

Figure 3. Nitrogen fixation by rhizosphere bacterial isolates

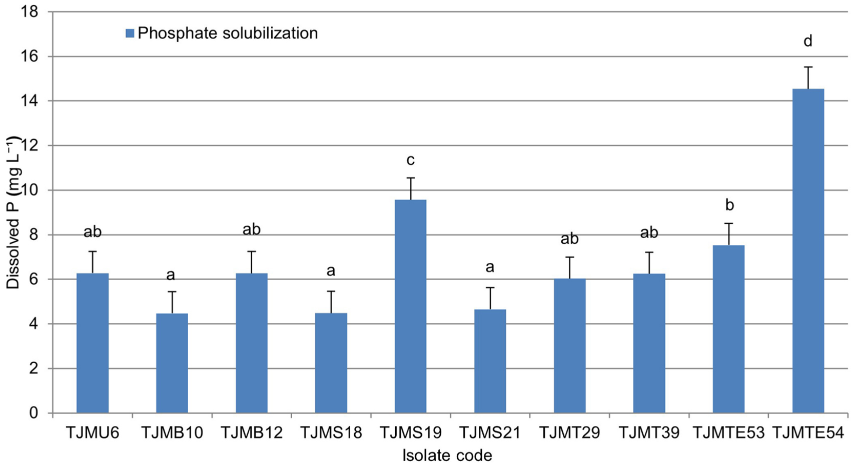

Figure 4. Phosphate solubilization by rhizosphere bacterial isolates. Bars represent the mean concentration of dissolved phosphate produced by each rhizosphere bacterial isolate, with error bars indicating standard deviation (n = 3). Different letters (a-d) above the bars indicate significant differences among treatments according to Tukey's test at $P \leq 0.05$

Phosphate-solubilizing capacity of bacterial isolates

One-way analysis of variance (ANOVA) showed that phosphate-solubilizing capacity differed significantly among the rhizosphere bacterial isolates tested ($P < 0.05$; Figure 4). The concentrations of dissolved phosphate produced by the isolates ranged from 4.47 to 14.54 mg L^{-1}. Isolate TJMTE54 exhibited the highest phosphate-solubilizing activity (14.54 mg L^{-1}) and formed a distinct statistical group, indicating a very strong ability to solubilize phosphate. TJMS19 ranked second with a dissolved phosphate concentration of 9.57 mg L^{-1}, while TJMTE53 also showed relatively high activity (7.53 mg L^{-1}). Isolates TJMU6, TJMB12, TJMT29, and TJMT39 produced intermediate levels of dissolved phosphate (approximately 6.03–6.28 mg L^{-1}), whereas TJMB10, TJMS18, and TJMS21 showed the lowest phosphate-solubilizing activity (4.47–4.65 mg L^{-1}). Overall, these results indicate that TJMTE54, followed by TJMS19 and TJMTE53, are the most effective phosphate-solubilizing isolates among those evaluated.

Molecular identification and phylogenetic relationships of superior bacterial isolates

Agarose gel electrophoresis of the 16S rRNA gene PCR products produced clear, well-resolved DNA bands for the selected isolates (Figure 5). The strong band intensity and absence of smearing indicated that the extracted DNA was of good quality and suitable for further molecular analysis. The two rhizosphere bacterial isolates with the highest physiological activity, TJMS19 and TJMTE54, were subsequently analyzed by 16S rRNA gene sequencing and phylogenetic analysis (Figures 6 and 7). The

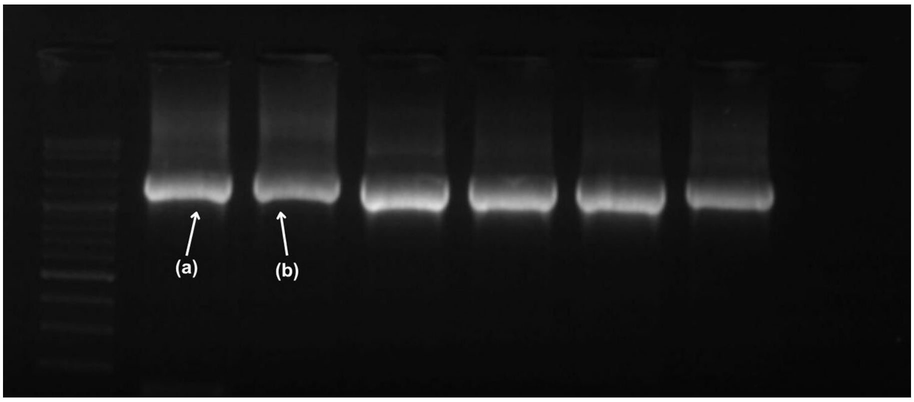

Figure 5. Visualization of 16S rRNA gene PCR products from selected rhizosphere bacterial isolates (a) TJMS19, (b) TJMTE54

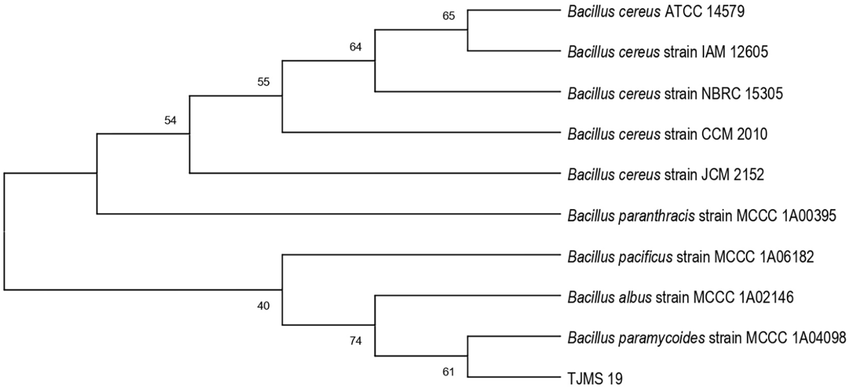

Figure 6. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of isolate TJMS19 with *Bacillus paramycoides* strains

phylogenetic tree for TJMS19 showed that this isolate is most closely related to *Bacillus paramycoides* strain MCCC 1A04098, with a bootstrap value of 61%, providing moderate support for this relationship. TJMS19 clustered within a major group containing other species of the genus *Bacillus,* such as *B. cereus*, *B. paranthracis*, *B. pacificus*, and *B. albus*, confirming that TJMS19 belongs to a group of Gram-positive, endospore-forming bacteria.

The phylogenetic tree for TJMTE54 indicated that this isolate is most closely related to *Pseudomonas* sp. XY3, with a bootstrap value of 100%, reflecting very high sequence similarity. TJMTE54 clustered together with *Pseudomonas* strains previously reported as PGPR, including *P. protegens*, *P. putida*, and *P. lundensis*. The high bootstrap values (≥97%) supporting the clustering of TJMTE54 with these strains indicate a close phylogenetic relationship and support the placement of TJMTE54 within the genus *Pseudomonas*.

Effect of bacterial isolates on hybrid maize seedling growth

Inoculation with rhizosphere bacterial isolates had a significant effect on hybrid maize seedling growth compared with the uninoculated control, as reflected in plant height, root length, and fresh weight ($P \leq 0.05$; Table 4).

For plant height, seedlings inoculated with TJMTE54 were the tallest (33.97 cm), and this value was significantly higher than all other treatments, including the control (22.50 cm). Isolate TJMS19 also produced a marked increase in plant height (31.21 cm). Intermediate plant heights were observed in seedlings inoculated with TJMB10 (27.93 cm), TJMT29 (27.77 cm), TJMS18 (26.07 cm), and TJMT39 (25.90 cm), which were generally higher than or comparable to the control. In contrast, TJMS21 (22.74 cm), TJMU6 (23.47 cm), TJMB12 (25.04 cm), and TJMTE53 (23.33 cm) did not differ significantly from the control, as indicated by the same significance letters.

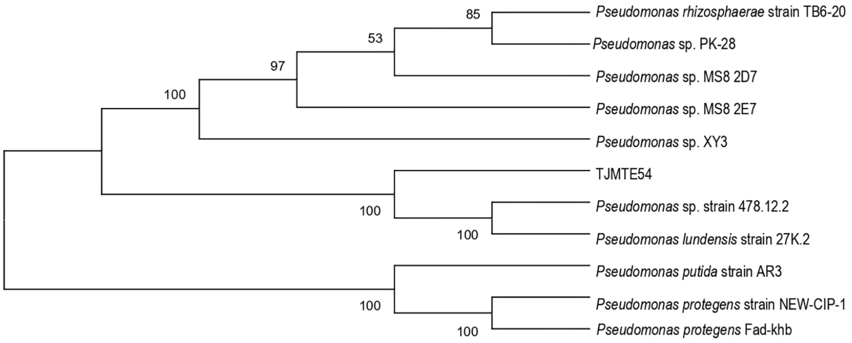

Figure 7. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of isolate TJMTE54 with *Pseudomonas* sp. strains

Table 4. Effect of rhizosphere bacterial isolates on hybrid maize seedling growth

Isolate code	Plant height (cm)	Root length (cm)	Wet weight (g)
Control	22.50 ± 0.50 a	10.27 ± 0.71 a	0.96 ± 0.02 a
TJMU6	23.47 ± 0.46 abc	10.30 ± 0.90 a	1.05 ± 0.10 a
TJMB10	27.93 ± 0.83 d	13.97 ± 0.15 bc	1.34 ± 0.04 b
TJMB12	25.04 ± 0.93 abc	13.70 ± 0.70 bc	1.23 ± 0.08 b
TJMS18	26.07 ± 1.03 cd	15.30 ± 0.36 c	1.25 ± 0.05 b
TJMS19	31.21 ± 1.01 e	20.13 ± 1.25 d	1.78 ± 0.05 c
TJMS21	22.74 ± 0.96 a	10.10 ± 1.05 a	0.93 ± 0.03 a
TJMT29	27.77 ± 0.32 d	15.80 ± 0.80 c	1.34 ± 0.04 b
TJMT39	25.90 ± 1.28 bcd	14.60 ± 0.78 c	1.30 ± 0.04 b
TJMTE53	23.33 ± 1.53 ab	11.80 ± 1.11 abc	0.99 ± 0.04 a
TJMTE54	33.97 ± 0.65 f	22.10 ± 0.46 d	1.87 ± 0.05 c

Note: Values are means ± standard deviation (n = 3). Means within the same column followed by the same letter are not significantly different according to Tukey's test at $P \leq 0.05$.

A similar pattern was observed for root length. TJMTE54 and TJMS19 produced the longest roots (22.10 and 20.13 cm, respectively), both significantly greater than the control (10.27 cm). Isolates TJMS18 (15.30 cm), TJMT29 (15.80 cm), and TJMT39 (14.60 cm) also increased root length relative to the control, whereas TJMU6 (10.30 cm) and TJMS21 (10.10 cm) did not differ significantly from the control. Fresh weight followed the same trend. The highest fresh weights were obtained in seedlings inoculated with TJMTE54 (1.87 g) and TJMS19 (1.78 g), which were significantly greater than the control (0.96 g). Seedlings inoculated with TJMB10, TJMB12, TJMS18, TJMT29, and TJMT39 showed intermediate fresh weights (1.23–1.34 g), whereas TJMU6 (1.05 g), TJMTE53 (0.99 g), and TJMS21 (0.93 g) did not differ significantly from the control. Overall, these results show that TJMTE54 and TJMS19 consistently enhanced plant height, root development, and biomass accumulation in hybrid maize seedlings and were the most effective growth-promoting isolates under the conditions of this study.

DISCUSSION

Morphological and physiological diversity as an adaptation to acidic soils

Isolation from the rhizosphere of hybrid maize grown on acidic tropical soils showed that, from 59 initial colonies, 10 selected isolates exhibited clear diversity in colony morphology, including variations in size, color, elevation, and colony margin. This phenotypic diversity reflects a bacterial community structured by local edaphic conditions such as low pH, limited organic matter content, and macronutrient deficiencies. These findings are consistent with the reports of (Bano et al., 2021) and Ren et al., (2025), who stated that soil factors and site-specific environmental conditions play a crucial role in shaping soil microbial community structure.

The presence of multiple colony types in the hybrid maize rhizosphere indicates that several bacterial groups are able to adapt to environmental stresses and potentially contribute to rhizosphere functioning. This supports the view that a diverse bacterial community in the root zone can serve as a "functional bank" that supports plant growth, directly or indirectly, through the production and release of regulatory compounds in the rhizosphere (Imade and Babalola, 2021). From a functional perspective, this morphological and physiological diversity provides a genetic and metabolic basis for the development of biofertilization functions in acidic soils (Shu and Huang, 2021; Zhao, Sun, et al., 2021).

Biochemical characterization showed that most isolates were catalase-positive and Gram-negative, with several Gram-positive isolates grouped within the genus *Bacillus*. Catalase activity in the isolates indicates the presence of antioxidative defense mechanisms against oxidative stress caused by H_2O_2 accumulation (Yuan et al., 2021), a condition frequently encountered in acidic soils. The hypersensitive reaction assay showed that none of the isolates induced necrotic symptoms on tobacco leaves up to 72 h after inoculation, indicating the absence of a hypersensitive response and suggesting a non-pathogenic nature toward plants (Balint-Kurti, 2019; Junaid et al., 2023).

The hemolysis assay on goat blood agar further showed that most isolates exhibited γ-hemolysis (no hemolysis), and only one isolate exhibited partial hemolysis (α). This pattern suggests that the majority of isolates are relatively safe in terms of potential pathogenicity to animals (Sudewi et al., 2020; Amaria et al., 2023). Overall, the combination of non-pathogenic characteristics toward plants and animals and the ability to adapt to acidic environments supports the use of these isolates as safe PGPR candidates in sustainable agricultural systems.

Key physiological traits: IAA production, nitrogen fixation, and phosphate solubilization

The marked differences in IAA production, nitrogen-fixation capacity, and phosphate solubilization among the isolates underline the need for a multi-trait selection strategy when identifying superior PGPR candidates. Among all isolates, TJMTE54 was clearly outstanding in IAA production (6.55 mg L^{-1}) and phosphate-solubilizing capacity (14.54 mg L^{-1}). In contrast, the highest fixed-nitrogen concentration was recorded for isolate TJMS19 (49.07 mg L^{-1}), followed closely by TJMTE54 (47.30 mg L^{-1}). The IAA concentrations recorded for these isolates fall within the range reported for multi-trait *Bacillus* and *Pseudomonas* strains (approximately 3–12 mg L^{-1} IAA) that have been developed as biofertilizers in other crops, and their phosphate-solubilization values are likewise comparable to those of efficient phosphate-solubilizing PGPR (80–460 mg L^{-1} dissolved P) described in recent studies. (Aliyat et al., 2022; Panichikkal and Krishnankutty, 2022; Sun et al., 2023). This pattern indicates that both TJMTE54 and TJMS19 are not only well adapted to acidic ultisol conditions but also possess metabolic capacities that meet or exceed those of internationally recognized PGPR strains.

Rhizosphere bacteria enhance plant performance under environmental stress primarily by improving nutrient acquisition (N and P), producing phytohormones such as IAA, and synthesizing siderophores and other metabolites that modulate plant physiology. In our study, the high IAA production by TJMTE54 and TJMS19 is consistent with the pronounced increases in root length and fresh biomass observed in inoculated maize seedlings. Similar relationships between bacterial IAA production and stimulation of root elongation, expansion of the root exploration zone, and biomass accumulation have been documented for various PGPR associated with cereals and other crops. These findings support earlier reports by (Lata et al. (2024) and Yamini P & R (2021), who showed that IAA-producing rhizobacteria can substantially improve early root growth and nutrient uptake, thereby enhancing overall plant vigor.

The high nitrogen-fixation capacities of TJMTE54 and TJMS19 suggest that these isolates can significantly contribute to nitrogen inputs in acidic, nutrient-poor soils. Although methods and units used to quantify fixed nitrogen differ among studies, our values are in line with those reported for effective free-living and associative N_2-fixing PGPR that improve N status and growth of maize and other cereals under low-input conditions (Kaya et al., 2022). Similarly, Djuuna et al., (2022) likewise emphasized that *Bacillus* and *Pseudomonas* strains adapted to low pH can remain metabolically active and enhance

N availability in acidic soils, which is consistent with the strong N-fixation performance of the indigenous isolates characterized here.

The phosphate-solubilizing ability of TJMTE54 (14.54 mg L^{-1} in our assay) confirms its role as an efficient phosphate-solubilizing bacterium. Conversion of insoluble P into plant-available forms is regarded as a key mechanism by which PGPR sustain crop production on acidic soils, where P is commonly immobilized by Al and Fe oxides. Several recent studies have shown that *Pseudomonas* and *Bacillus* strains with strong P-solubilizing activity can simultaneously increase plant P uptake, growth, and resistance to pathogens in various crops. The performance of TJMTE54 and TJMS19 fits well within this body of evidence and supports the view of Bai et al., (2024), N et al. (2021) and Sah et al. (2021) that *Pseudomonas*-based biofertilizers are particularly suitable for improving P nutrition in acidic soils. Taken together, the combination of robust IAA production, biologically meaningful nitrogen fixation, and effective phosphate solubilization in TJMTE54 and TJMS19 provides a strong physiological basis for proposing these two indigenous isolates as multifunctional biofertilizer candidates for hybrid maize cultivation on acidic soils.

Significance of indigenous *Bacillus paramycoides* and *Pseudomonas* sp.

Molecular analysis based on the 16S rRNA gene confirmed that TJMS19 is closely related to *Bacillus paramycoides*, whereas TJMTE54 is closely related to *Pseudomonas* sp. XY3. Both isolates clustered with other *Bacillus* and *Pseudomonas* strains known as PGPR and biocontrol agents. *Bacillus* is a Gram-positive, endospore-forming bacterium with broad metabolic potential, including phosphate solubilization, nitrogen fixation, phytohormone production, and high tolerance to fluctuating environmental conditions. *Pseudomonas* is recognized as a metabolically active rhizosphere inhabitant, with the ability to produce IAA, siderophores, and antimicrobial compounds (Mitter et al., 2021).

The moderate bootstrap value in the *Bacillus paramycoides* cluster and the high bootstrap values in the *Pseudomonas* cluster indicate that both isolates have sufficiently strong phylogenetic affinities with previously documented PGPR groups. This is important because it links the observed physiological traits (IAA production, N fixation, P solubilization, and potential biocontrol activity) with a well-established evolutionary framework. These findings complement earlier reports on the roles of *Bacillus* and *Pseudomonas* as PGPR in various agroecosystems (Mitter et al., 2021), while adding new evidence that indigenous strains from the rhizosphere of hybrid maize in acidic tropical soils possess similar potential.

From a novelty perspective, this study not only reconfirms the roles of these two genera as PGPR but also identifies indigenous strains that are specifically adapted to acidic ultisol in the Moncongloe region. Such local adaptation is important for the success of biofertilizer formulations based on local microbes, whose performance in the field is generally more stable than that of exotic inoculants.

Growth response of hybrid maize and agronomic implications

The in planta assay on hybrid maize seedlings showed that inoculation with TJMTE54 and TJMS19 significantly increased plant height, root length, and fresh biomass compared with the uninoculated control. Other isolates produced intermediate responses or did not differ significantly from the control, underscoring the importance of combining physiological screening with plant-based assays to identify truly effective PGPR candidates. The observed improvements in vegetative growth are likely the result of synergistic PGPR mechanisms, including phytohormone production such as IAA, enhanced nitrogen availability through biological fixation, and increased phosphorus availability through phosphate solubilization. These findings are in line with Yang et al., (2024), who reported that PGPR application in maize can improve vegetative growth while reducing dependence on synthetic chemical fertilizers. Thus, the in planta data in this study provide functional evidence that the physiological traits quantified under laboratory conditions are effectively translated into enhanced plant growth in an acidic soil system.

From an agronomic standpoint, increases in early plant height, root length, and biomass may contribute to improved nutrient uptake and greater tolerance to abiotic stress in subsequent growth stages. Although this study was limited to the seedling stage and controlled conditions, the results provide strong indications that inoculation with TJMTE54 and TJMS19 could form part of a more efficient and environmentally friendly fertilization strategy for maize cultivation on acidic tropical soils.

Overall, the present findings demonstrate that TJMTE54 (*Pseudomonas* sp.) and TJMS19 (*Bacillus paramycoides*.) are promising candidates for the development of locally adapted biofertilizers for acidic tropical soils. These isolates combine several desirable traits: adaptation to acidic soil conditions, non-pathogenic behavior toward plants and animals, high IAA production, strong nitrogen-fixation capacity, efficient phosphate solubilization, and proven functional effects on hybrid maize seedlings. This study contributes to sustainable agriculture by (i) providing indigenous isolates that are physiologically and molecularly characterized and can help reduce reliance on synthetic fertilizers, (ii) showing that acidic ultisol, often considered marginal, still harbors rhizosphere microbes with high biofertilizer potential, and (iii) reinforcing the concept that ecological engineering of soil fertility can be achieved by exploiting local PGPR that are compatible with native soils and cropping systems. Nevertheless, this work has limitations, as it is restricted to the seedling stage and controlled conditions and does not yet address long-term interactions with the broader soil microbiome. Follow-up studies under greenhouse and field conditions, including optimization of inoculum dose and formulation and evaluation of performance stability across seasons and locations, are required before TJMTE54 and TJMS19 can be proposed as commercial biofertilizer products.

CONCLUSIONS

This study demonstrates that the rhizosphere of hybrid maize grown on acidic ultisol in Moncongloe harbors a diverse community of indigenous bacteria with substantial morphological, biochemical, and physiological variability, indicating strong potential to support soil fertility and plant growth. Among the ten isolates characterized, TJMTE54 and TJMS19 emerged as superior strains because they produced high levels of IAA, fixed nitrogen, and solubilized phosphate effectively, while exhibiting non-pathogenic behavior toward plants and being relatively safe for animals. Molecular analysis of the 16S rRNA gene identified TJMTE54 as belonging to the genus *Pseudomonas* and TJMS19 to the genus *Bacillus*, two key PGPR groups known to promote plant growth and enhance nutrient availability. The in planta assay with hybrid maize seedlings confirmed that inoculation with TJMTE54 and TJMS19 consistently increased plant height, root length, and fresh biomass compared with the uninoculated control, thereby validating their functional roles as growth promoters under acidic soil conditions. Overall, these findings indicate that TJMTE54 and TJMS19 are prime indigenous biofertilizer candidates with the potential to support the sustainable use of acidic lands and reduce dependence on synthetic chemical fertilizers, although further greenhouse and field studies are required to evaluate performance stability, inoculum formulation, and long-term impacts on maize productivity and soil ecosystem health.

Acknowledgements

The authors extend their deepest appreciation to the Center for Higher Education Funding and Assessment (PPAPT) through the Indonesia Endowment Fund for Education (LPDP) under the Indonesian Educational Scholarship (BPI), as well as to the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia for their invaluable support.

REFERENCES

1. Aliyat, F. Z., Maldani, M., Guilli, M. El, Nassiri, L., Ibijbijen, J. (2022). Phosphate-solubilizing bacteria isolated from phosphate solid sludge and their ability to solubilize three inorganic phosphate forms: calcium, iron, and aluminum phosphates. *Microorganisms 10*(5), 980. https://doi.org/10.3390/microorganisms10050980
2. Amaria, W., Suradji Sinaga, M., Hamzah Mutaqin, K. (2023). Plant pests dis. *J. Trop. Plant Pests Dis*, *23*(2), 24–30.
3. Aponte, H., Galindo-Castañeda, T., Yáñez, C., Hartmann, M., Rojas, C. (2022). Microbial community-level physiological profiles and genetic prokaryotic structure of burned soils under mediterranean sclerophyll forests in Central Chile. *Frontiers in Microbiology*, *13*(April), 1–16. https://doi.org/10.3389/fmicb.2022.824813
4. Asghar, I., Ahmed, M., Farooq, M. A., Ishtiaq, M., Arshad, M., Akram, M., Umair, A., Alrefaei, A. F., Jat Baloch, M. Y., Naeem, A. (2023). Characterizing indigenous plant growth promoting bacteria and their synergistic effects with organic and chemical fertilizers on wheat (*Triticum aestivum*). *Frontiers*

in Plant Science, *14*(August), 1–17. https://doi.org/10.3389/fpls.2023.1232271

5. Bai, K., Wang, W., Zhang, J., Yao, P., Cai, C., Xie, Z., Luo, L., Li, T., Wang, Z. (2024). Effects of phosphorus-solubilizing bacteria and biochar application on phosphorus availability and tomato growth under phosphorus stress. *BMC Biology*, *22*(1). https://doi.org/10.1186/s12915-024-02011-y
6. Balint-Kurti, P. (2019). The plant hypersensitive response: concepts, control and consequences. *Molecular Plant Pathology*, *20*(8), 1163–1178. https://doi.org/10.1111/mpp.12821
7. Bano, S., WU, X., Zhang, X. (2021). Towards sustainable agriculture: rhizosphere microbiome engineering. *Applied Microbiology and Biotechnology*, *105*, 7141–7160. https://doi.org/https://doi.org/10.1007/s00253-021-11555-w
8. Bisht, N., Singh, T., Ansari, M. M., Chauhan, P. S. (2025). Harnessing plant-rhizobacteria alliances for nutrient homeostasis in depleting soilscape: Shifting paradigms beyond chemical fertilizers. *Pedosphere*, 1–33. https://doi.org/10.1016/j.pedsph.2025.07.015
9. Chen, L., Li, K., Shang, J., Wu, Y., Chen, T., Wanyan, Y., Wang, E., Tian, C., Chen, W., Chen, W., Mi, G., Sui, X. (2021). Plant growth–promoting bacteria improve maize growth through reshaping the rhizobacterial community in low-nitrogen and low-phosphorus soil. *Biology and Fertility of Soils*, *57*(8), 1075–1088. https://doi.org/10.1007/s00374-021-01598-6
10. Das, S., Nurunnabi, T. R., Parveen, R., Nur Mou, A., Emdadul Islam, M., Didarul Islam, K. M., Rahman, S. M. M. (2019). Isolation and characterization of indole acetic acid producing bacteria from rhizosphere soil and their effect on seed germination. *International Journal of Current Microbiology and Applied Sciences*, *8*(3), 1237–1245. https://doi.org/10.20546/ijcmas.2019.803.146
11. Dincă, L. C., Grenni, P., Onet, C., Onet, A. (2022). Fertilization and Soil Microbial Community: A Review. *Applied Sciences (Switzerland)*, *12*(3), 1–20. https://doi.org/10.3390/app12031198
12. Dineshkumar, R., Duraimurugan, M., Sharmiladevi, N., Lakshmi, L. P., Rasheeq, A. A., Arumugam, A., Sampathkumar, P. (2022). Microalgal liquid biofertilizer and biostimulant effect on green gram (*Vigna radiata* L) an experimental cultivation. *Biomass Conversion and Biorefinery*, *12*(8), 3007–3027. https://doi.org/10.1007/s13399-020-00857-0
13. Ding, M., Dai, H., He, Y., Liang, T., Zhai, Z., Zhang, S., Hu, B., Cai, H., Dai, B., Xu, Y., Zhang, Y. (2024). Continuous cropping system altered soil microbial communities and nutrient cycles. *Frontiers in Microbiology*, *15*(April), 1–12. https://doi.org/10.3389/fmicb.2024.1374550
14. Djuuna, I. A. F., Prabawardani, S., Massora, M. (2022). Population distribution of phosphate-solubilizing microorganisms in agricultural soil. *Microbes and Environments*, *37*(1), 1–8. https://doi.org/10.1264/jsme2.ME21041
15. Fanai, A., Bohia, B., Lalremruati, F., Lalhriatpuii, N., Lalrokimi, Lalmuanpuii, R., Singh, P. K., & Zothanpuia. (2024). Plant growth promoting bacteria (PGPB) induced plant adaptations to stresses: an updated review. *PeerJ*, *12*(8), 1–37. https://doi.org/10.7717/peerj.17882
16. Hayet, S., Sujan, K. M., Mustari, A., Miah, M. A. (2021). Hemato-biochemical profile of turkey birds selected from Sherpur district of Bangladesh. *Int. J. Adv. Res. Biol. Sci*, *8*(6), 1–5. https://doi.org/10.22192/ijarbs
17. He, X., Zhu, J., Gong, X., Zhang, D., Li, Y., Zhang, X., Zhao, X., Zhou, C. (2025). Advances in deciphering the mechanisms of salt tolerance in Maize. *Plant Signaling and Behavior*, *20*(1). https://doi.org/10.1080/15592324.2025.2479513
18. Imade, E. E., Babalola, O. O. (2021). Biotechnological utilization: the role of *Zea mays* rhizospheric bacteria in ecosystem sustainability. *Applied Microbiology and Biotechnology*, *105*, 4487–4500. https://doi.org/https://link.springer.com/article/10.1007/s00253-021-11351-6
19. Jain, D., Sharma, J., Kaur, G., Bhojiya, A. A., Chauhan, S., Sharma, V., Suman, A., Mohanty, S. R., Maharjan, E. (2021). Phenetic and molecular diversity of nitrogen fixating plant growth promoting *Azotobacter* isolated from semiarid regions of India. *BioMed Research International*, *2021*. https://doi.org/10.1155/2021/6686283
20. Junaid, M., Gassing, S., Muslimah, A., Saharuddin, Y., Nuralisah, S., Kuswinanti, T., Baharuddin, Tara, A. (2023). Quantitative analysis of rhizosphere microbial population obtained from main-croplands in South Sulawesi. *IOP Conference Series: Earth and Environmental Science*, *1230*(1). https://doi.org/10.1088/1755-1315/1230/1/012082
21. Kaya, D., Nefise, Ö., Mustafa, A. (2022). Genetic diversity and characterization of plant growth - promoting effects of bacteria isolated from rhizospheric soils. *Current Microbiology*, *79*(5), 1–11. https://doi.org/10.1007/s00284-022-02827-3
22. Khoso, M. A., Wagan, S., Alam, I., Hussain, A., Ali, Q., Saha, S., Poudel, T. R., Manghwar, H., Liu, F. (2024). Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. *Plant Stress*, *11*(December 2023), 100341. https://doi.org/10.1016/j.stress.2023.100341
23. Khubna, M., Supriyono, Nyoto, S., Budiastuti, M. T. S. (2021). The growth and yield of hybrid corn on different plant spacing. *IOP Conference Series: Earth and Environmental Science*, *637*(1). https://doi.org/10.1088/1755-1315/637/1/012064

24. Kiprotich, K., Muema, E., Wekesa, C., Ndombi, T., Muoma, J., Omayio, D., Ochieno, D., Motsi, H., Mncedi, S., Tarus, J. (2025). Unveiling the roles, mechanisms and prospects of soil microbial communities in sustainable agriculture. *Discover Soil*, *2*(1). https://doi.org/10.1007/s44378-025-00037-4

25. Lata, D. L., Abdie, O., Rezene, Y. (2024). IAA-producing bacteria from the rhizosphere of chickpea (*Cicer arietinum* L.): Isolation, characterization, and their effects on plant growth performance. *Heliyon*, *10*(21), e39702. https://doi.org/10.1016/j.heliyon.2024.e39702

26. Li, K., Xu, R., Cai, Z. (2024). Aluminum mobilization characteristics in four typical soils from different climate zones during their acidification. *Plant and Soil*, *504*, 29–46. https://doi.org/https://doi.org/10.1007/s11104-024-06476-2

27. Liu, Y., Gao, J., Bai, Z., Wu, S., Li, X., Wang, N., Du, X., Fan, H., Zhuang, G., Bohu, T., Zhuang, X. (2021). Unraveling mechanisms and impact of microbial recruitment on oilseed rape (*Brassica napus* L.) and the rhizosphere mediated by plant growth-promoting rhizobacteria. *Microorganisms*, *9*(1), 1–21. https://doi.org/10.3390/microorganisms9010161

28. Mai, Z., Wang, H., Wang, Y., Chen, Q., Lyu, L., Wei, X., Zhou, W., Cheng, H. (2023). Radial oxygen loss from the roots of mangrove seedlings enhances the removal of polycyclic aromatic hydrocarbons. *Plants*, *12*(21), 3711. https://doi.org/10.3390/plants12213711

29. Meng, X., Zhu, Z., Xue, J., Wang, C., Sun, X. (2023). Methane and nitrous oxide emissions from a temperate peatland under simulated enhanced nitrogen deposition. *Sustainability (Switzerland)*, *15*(2). https://doi.org/10.3390/su15021010

30. Mitter, E. K., Tosi, M., Obregón, D., Dunfield, K. E., Germida, J. J. (2021). Rethinking Crop nutrition in times of modern microbiology: Innovative biofertilizer technologies. *Frontiers in Sustainable Food Systems*, *5*(February), 1–23. https://doi.org/10.3389/fsufs.2021.606815

31. Mogrovejo, D. C., Perini, L., Gostinčar, C., Sepčić, K., Turk, M., Ambrožič-Avguštin, J., Brill, F. H. H., Gunde-Cimerman, N. (2020). Prevalence of antimicrobial resistance and hemolytic phenotypes in culturable arctic bacteria. *Frontiers in Microbiology*, *11*(April), 1–13. https://doi.org/10.3389/fmicb.2020.00570

32. N, A., Ram, A. B., P, R., R, A., DJ, B. (2021). *Pseudomonas* species in soil as a natural resource for plant growth promotion and biocontrol characteristics – an overview. *Madras Agricultural Journal*, *108*, 10–12. https://doi.org/10.29321/maj.10.000571

33. Noer, S. (2021). Identifikasi bakteri secara molekular menggunakan 16S rRNA. *EduBiologia Biological Science and Education Journal*, *1*(1), 1–6. https://doi.org/http://dx.doi.org/10.30998/edubiologia.v1i1.8596

34. Orozco-Mosqueda, M. del C., Santoyo, G., Glick, B. R. (2023). Recent advances in the bacterial phytohormone modulation of plant growth. *Plants*, *12*(3). https://doi.org/10.3390/plants12030606

35. Pande, A., Pandey, P., Mehra, S., Singh, M., Kaushik, S. (2017). Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. *Journal of Genetic Engineering and Biotechnology*, *15*(2), 379–391. https://doi.org/10.1016/j.jgeb.2017.06.005

36. Panichikkal, J., Krishnankutty, R. E. (2022). Chitosan and gold nanoparticles supplementation for augmentation of Indole - 3 - Acetic acid production by rhizospheric *Pseudomonas aeruginosa* and plant growth enhancement. *Current Microbiology*, *79*(6), 1–8. https://doi.org/10.1007/s00284-022-02850-4

37. Rasul, M., Yahya, M., Suleman, M., Hakim, S., Mirza, B. S., Mirza, M. S., Reitz, T., Tarkka, M. T., Yasmin, S. (2024). Diversity and functional traits based indigenous rhizosphere associated phosphate solubilizing bacteria for sustainable production of rice. *Frontiers in Microbiology*, *15*(December), 1–14. https://doi.org/10.3389/fmicb.2024.1470019

38. Regasa, A., Haile, W., Abera, G. (2025). Effects of lime and vermicompost application on soil physicochemical properties and phosphorus availability in acidic soils. *Scientific Reports*, *15*(1), 1–17. https://doi.org/10.1038/s41598-025-02053-4

39. Ren, H., Hong, H., Zha, B., Lamlom, S. F., Qiu, H., Cao, Y., Sun, R., Wang, H., Ma, J., Zhang, H., Sun, L., Yang, Q., Zhou, C., Liu, X., Wang, X., Zhang, C., Zhang, F., Zhao, K., Yuan, R., … Lu, W. (2025). Soybean productivity can be enhanced by understanding rhizosphere microbiota: evidence from metagenomics analysis from diverse agroecosystems. *Microbiome*, *13*(1). https://doi.org/10.1186/s40168-025-02104-y

40. Ríos-Ruiz, W. F., Casique-Huamanguli, R. D., Valdez-Nuñez, R. A., Rojas-García, J. C., Calixto-García, A. R., Ríos-Reátegui, F., Pompa-Vásquez, D. F., Padilla-Santa-Cruz, E. (2024). Rhizospheric bacteria of cover legumes from acidic soils are capable of solubilizing different inorganic phosphates. *Microorganisms*, *12*(6), 1–15. https://doi.org/10.3390/microorganisms12061101

41. Risanti, R. R., Hindersah, R., Fitriatin, B. N., Suryatmana, P., Maksum, I. P., Setiawati, M. R., Hanindipto, F. A., Nugraha, G. B. (2024). Exploring the *Bacillus* from vegetable rhizosphere for plant growth. *Journal of Ecological Engineering*, *26*(1), 109–120. https://doi.org/10.12911/22998993/195286

42. Rozi, F., Santoso, A. B., Mahendri, I. G. A. P., Hutapea, R. T. P., Wamaer, D., Siagian, V., Elisabeth, D. A. A., Sugiono, S., Handoko, H., Subagio, H., Syam,

A. (2023). Indonesian market demand patterns for food commodity sources of carbohydrates in facing the global food crisis. *Heliyon*, *9*(6), e16809. https://doi.org/10.1016/j.heliyon.2023.e16809

43. Sah, S., Krishnani, S., Singh, R. (2021). *Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security. *Current Research in Microbial Sciences*, *2*, 100084. https://doi.org/10.1016/j.crmicr.2021.100084
44. Sherpa, M. T., Bag, N., Das, S., Haokip, P., Sharma, L. (2021). Isolation and characterization of plant growth promoting rhizobacteria isolated from organically grown high yielding pole type native pea (*Pisum sativum* L.) variety Dentami of Sikkim, India. *Current Research in Microbial Sciences*, *2*, 100068. https://doi.org/10.1016/j.crmicr.2021.100068
45. Shu, W.-S., Huang, L.-N. (2021). Microbial diversity in extreme environments. *Nature Reviews Microbiology*, *20*, 219–235.
46. Song, Y., Chen, Q., Hua, J., Zhang, S., Luo, S. (2025). The IAA-producing rhizobacterium *Bacillus* sp. SYM-4 promotes maize growth and yield. *Plants*, *14*(11). https://doi.org/10.3390/plants14111587
47. Subair, H., Mu'minah, Baharuddin, Darwisah, B., Fahruddin. (2015). Isolation and screening of exopolysaccharide producing bacterial (EPS) from potato rhizosphere for soil aggregation. *International Journal of Current Microbiology and Applied Sciences*, *4*(6), 341–349. http://www.ijcmas.com
48. Sudewi, S., Ala, A., Baharuddin, Farid, M. (2020). The isolation, characterization endophytic bacteria from roots of local rice plant kamba in, central sulawesi, indonesia. *Biodiversitas*, *21*(4), 1614–1624. https://doi.org/10.13057/biodiv/d210442
49. Sun, X., Men, X., Huang, W., Yi, S., Wang, W., Zheng, F. (2023). Effects of *Exiguobacterium* sp. DYS212, a saline-alkaline-tolerant P-solubilizing bacterium, on suaeda salsa germination and growth. 1–14. *Sustainability 15*(7), 6259; https://doi.org/10.3390/su15076259
50. Wan, L. J., Tian, Y., He, M., Zheng, Y. Q., Lyu, Q., Xie, R. J., Ma, Y. Y., Deng, L., Yi, S. L. (2021). Effects of chemical fertilizer combined with organic fertilizer application on soil properties, citrus growth physiology, and yield. *Agriculture (Switzerland)*, *11*(12). https://doi.org/10.3390/agriculture11121207
51. Yadu, S., Kerketta, A., Thakur, K., Singh, A., Pujari, D. K., Yadav, N., Netam, R. S. (2023). Isolation and morphological characterization of indigenous rhizobacteria of Bastar Plateau. *The Pharma Innovation Journal*, *12*(10), 4–9.
52. Yamini P., Reshmi G., Sanjay P. S., Karkuvelraja R (2021). Isolation and identification of indole acetic acid (IAA) producing bacteria from organic soil: Investigating its efficacy on plant growth. *Journal of University of Shanghai for Science and Technology*, *23*(6), 1294–1312. https://doi.org/https://doi.org/10.51201/jusst/21/06443
53. Yang, P., Condrich, A., Scranton, S., Hebner, C., Lu, L., Ali, M. A. (2024). Utilizing plant growth-promoting rhizobacteria (PGPR) to advance sustainable agriculture. *Bacteria*, *3*(4), 434–451. https://doi.org/10.3390/bacteria3040030
54. Yuan, F., Yin, S., Xu, Y., Xiang, L., Wang, H., Li, Z., Fan, K., Pan, G. (2021). The richness and diversity of catalases in bacteria. *Frontiers in Microbiology*, *12*(March), 1–11. https://doi.org/10.3389/fmicb.2021.645477
55. Yuan, Y., Feng, Z., Yan, S., Zhang, J., Song, H., Zou, Y., Jin, D. (2025). The effect of the application of chemical fertilizer and arbuscular MyCorrhizal fungi on maize yield and soil microbiota in saline agricultural soil. *Journal of Fungi*, *11*(4). https://doi.org/10.3390/jof11040319
56. Zhao, Y., Mao, X., Zhang, M., Yang, W., Di, H. J., Ma, L., Liu, W., Li, B. (2021). The application of *Bacillus megaterium* alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. *Agriculture, Ecosystems and Environment*, *307*(July 2020). https://doi.org/10.1016/j.agee.2020.107236
57. Zhao, Y., Sun, Y., Pei, M., Fu, J., Ji, H., Zhao, L., Xiao, X. (2021). Enhanced rice yields are related to pronounced shifts in soil resident bacterial community structures in response to *Rhodopseudomonas palustris* and *Bacillus subtilis* inoculation. *Journal of Soils and Sediments*, *21*, 2369–2380. https://doi.org/https://doi.org/10.1007/s11368-021-02929-8