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Original scientific article

HARNESSING INDIGENOUS PGPR TO ENHANCE SOIL PHOSPHORUS AVAILABILITY AND MAIZE GROWTH

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This study investigated indigenous plant growth-promoting rhizobacteria (PGPR) isolated from Nigerian soils for their potential to enhance phosphorus availability and promote maize (*Zea mays* L.) growth. Five bacterial isolates obtained from soil collected at the Federal University Oye-Ekiti field were screened for key plant growth-promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, ammonia production, and hydrolytic enzyme activities (α-amylase and pectinase). Two promising isolates, IS-4 and IS-5, were selected for molecular identification. Among them, IS-5 showed the strongest overall plant growth-promoting performance, exhibiting moderate

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phosphate solubilization (PSI = 2.90), the highest IAA production, ammonia production, and pectinase activity. IS-4 was selected as a representative isolate from the preliminary screening. Whole-genome sequencing identified IS-5 as *Enterobacter hormaechei* OYAS29 and IS-4 as *Acinetobacter* sp. OYAS30.A pot experiment was conducted to evaluate the effect of *Enterobacter hormaechei* OYAS29 on soil phosphorus availability and maize growth. Treatment with OYAS29 slightly increased available phosphorus in soil from 0.4905 ppm in the control to 0.4934 ppm. Inoculated maize plants also showed improved growth compared with the control, including increases in plant height (26.12%), stem girth (30.03%), leaf length (24.78%), root length (39.88%), and total biomass (58.69%). Overall, the results indicate that *E. hormaechei* OYAS29 has strong potential as an indigenous PGPR for sustainable maize production and improved phosphorus availability in soil.

Keywords: biofertilizer, Indole-3-acetic acid, soil microbiome, sustainable agriculture, whole-genome sequencing

INTRODUCTION

The global population is projected to reach 10 billion by 2050, intensifying the need for sustainable food production to address hunger and malnutrition, particularly in developing regions like sub-Saharan Africa (GODFRAY *et al*., 2010; AJIBADE *et al*., 2022). Maize (*Zea mays* L.), a staple crop in Nigeria, plays a critical role in food security but faces challenges due to nutrient deficiencies in soils (FAO, 2013; IFPRI, 2014; MONTOYA-MARTÍNEZ *et al*., 2022). The Sustainable Development Goals (SDGs), especially SDG-2, aim to end hunger and promote sustainable agriculture, highlighting the need for innovative solutions to improve crop yields in regions with persistent micronutrient deficiencies (BROOKS, 2016; FAN *et al*., 2016). Among the essential nutrients required for plant growth, including nitrogen, potassium, and phosphorus, phosphorus (P) is a major limiting factor because its availability to plants is often restricted in agricultural soil (ARIF *et al.*, 2017)

Phosphorus is abundant in many soils but frequently exists in insoluble forms due to binding with iron, calcium, or aluminum ions, reducing its uptake by plants (TIMOFEEVA *et al*., 2022). Chemical fertilizers are commonly used to address phosphorus deficiency, but their continuous application can lead to environmental issues, such as soil acidity and trace metal accumulation, and their high cost makes them inaccessible to many Nigerian farmers (USMAN *et al.*, 2015; RICHARD *et al*., 2018). Traditional practices, such as leaving land fallow to restore fertility, are less viable due to increasing food demands, and organic amendments alone may not meet productivity needs (ASADU and UNAGWU, 2012).

Plant growth-promoting rhizobacteria (PGPR), which inhabit the root zones of plants, offer a sustainable alternative by solubilizing phosphorus, fixing nitrogen, producing growth hormones, and protecting plants from stress (ADEOTI and USMAN, 2021). Despite their potential, the use of PGPR in Nigerian agriculture remains underexplored, particularly with indigenous strains that are adapted to local soil and climate conditions (ABIALA *et al*., 2015). Many studies focus on imported PGPR, which often perform poorly in Nigeria due to environmental differences (MONTOYA-MARTÍNEZ *et al.*, 2022). This gap underscores the need to identify and characterize native PGPR that can enhance maize production. Preliminary characterization of

rhizosphere bacteria commonly involves colony morphology, Gram reaction, and biochemical tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, citrate utilization, sugar fermentation, starch hydrolysis, urease, coagulase, and ammonia production, while molecular methods such as 16S rRNA gene sequencing or whole-genome sequencing provide more reliable taxonomic confirmation (DINESH *et al*., 2015; SINGH *et al*., 2022). Accordingly, this study aimed to isolate and characterize PGPR from Nigerian rhizosphere soils and assess their impact on maize growth and soil phosphorus availability. It was hypothesized that indigenous PGPR, through mechanisms such as auxin synthesis and phosphate solubilization, could improve phosphorus availability and maize growth, providing a cost-effective and eco-friendly alternative to chemical fertilizers.

MATERIALS AND METHODS

Soil Sample Collection

Rhizosphere soil was collected from an undisturbed maize field at the Federal University Oye-Ekiti, Nigeria (7°46'22.5"N, 5°18'57.4"E). Samples were obtained at depths of 5 mm and 20 mm using a sterilized hand trowel and shovel to capture the root-soil interface, which is rich in microbial activity (ROBINSON *et al.*, 2021). A total of 3 samples were collected at 5 mm depth, while 2 samples were collected at 20 mm depth within a sampling plot measuring 15.24 m × 30.48 m. The soil samples were processed fresh, sieved to remove stones, plant debris, and roots, and mixed thoroughly for uniformity. The samples were then placed in sterile polyethylene bags and transported in an icebox to the microbiology laboratory for processing.

Bacterial Isolation and Quantification

Fresh soil samples (1.0g) were suspended in 9.0 mL of sterile distilled water and mixed thoroughly by shaking. Serial dilutions up to 10^{-7} were prepared, and 1.0 mL of aliquots from the 10^{-4} and 10^{-7} dilutions were transferred into sterile labelled Petri dishes. About 15 mL of molten nutrient agar and Tryptic Soy Agar, cooled to about 45°C, was added using the pour plate method. Plates were gently swirled for even distribution, allowed to solidify, and incubated at 37°C for 48 hours to isolate diverse rhizosphere bacteria (DINESH *et al*., 2015). Colonies with distinct morphologies were subcultured to obtain pure cultures. Colony-forming units (CFU) were counted and expressed as CFU/g to quantify bacterial populations.

Phenotypic Characterization

Isolates were characterized by morphological traits (cell shape), Gram staining, and biochemical tests, including oxidase, catalase, starch hydrolysis, methyl red, Voges-Proskauer, indole, citrate utilization, urease, coagulase, sugar fermentation, and ammonia production. Gram staining was used to determine the Gram reaction and cell shape of the isolates. Catalase activity was tested using 3% hydrogen peroxide, while oxidase activity was tested using oxidase reagent. The indole test was carried out in peptone water using Kovac's reagent, while methyl red and Voges-Proskauer tests were carried out using peptone water and MR-VP broth, respectively. Citrate utilization was tested on Simmons citrate agar, and sugar fermentation was tested using glucose, lactose, sucrose, fructose, mannitol, and galactose. Starch hydrolysis was tested on starch agar, urease activity was tested on urea agar, coagulase activity was tested using plasma,

and ammonia production was tested in peptone broth using Nessler's reagent. These tests, described by CHEESBROUGH (2006) and TINDALL *et al*. (2007) enabled preliminary classification of isolates into suspected genera.

Screening for Plant Growth-Promoting Traits

Isolates were tested for plant growth-promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, ammonia production, and hydrolytic enzyme synthesis.

Phosphate Solubilization

A loopful of 24-hour broth culture of each isolate was spot-inoculated on Pikovskaya's agar (10 g glucose, 5.0 g calcium phosphate, 0.5 g ammonium sulfate, 0.2 g potassium chloride, 0.1 g magnesium sulfate, 0.5 g yeast extract, 15 g agar, and 1000 mL distilled water) and incubated at 28°C for 96 hours. Clear zones around colonies indicated phosphate solubilization. The solubilization index (PSI) was calculated as PSI = Dh/Dc, where Dh is the halo diameter and Dc is the colony diameter, with levels classified as low (PSI < 2), medium (2 ≤ PSI < 3), or high (PSI ≥ 3) (BATISTA *et al*., 2021). Calcium phosphate was used as a standard substrate.

IAA Production

A 50 µL aliquot of each bacterial slurry was inoculated into 5 mL of peptone-yeast extract broth supplemented with L-tryptophan and incubated at 28°C for 72 hours, following the IAA screening approach used for rhizosphere bacteria (DAS *et al*., 2019). After incubation, 1.5 mL of the broth culture was transferred into Eppendorf tubes and centrifuged at 13,000 rpm for 10 minutes. 1 mL of the resulting supernatant was mixed with 1 mL of Salkowski reagent for colorimetric detection of bacterial IAA production (SARWAR and KREMER, 1995). The Salkowski reagent was prepared by mixing 150 mL of H_2SO_4, 250 mL of distilled water, and 7.5 mL of 0.5 M $FeCl_3$. A red colour confirmed IAA production, and absorbance was measured at 530 nm.

Ammonia Production

A 50 µL aliquot of each bacterial slurry was inoculated into 15 mL 4% peptone broth and incubated at 25–37°C for 72 hours. After incubation, 1 mL of Nessler's reagent was added to the broth. The formation of a yellow to brown colour or precipitate indicated ammonia production (CAPPUCCINO and SHERMAN, 1992).

Hydrolytic Enzyme Production

Isolates were screened for hydrolytic enzyme production using starch agar and skim milk agar, as described by DINESH *et al*. (2015). The starch agar was prepared using 5 g peptone, 3 g beef extract, 10 g soluble starch, 15 g agar, and 1000 mL distilled water, while the skim milk agar was prepared using 100 g skim milk, 5 g peptone, 15 g agar, and 1000 mL distilled water. About 15 mL of each prepared medium was poured into sterile Petri dishes and allowed to solidify. One loopful of bacterial suspension was streaked on each agar plate, and the plates were incubated at 28°C for 48 hours. Clear zones around the streaked area indicated enzymatic activity.

Molecular Characterization of the Selected Isolates

Molecular identification was performed on two promising bacterial isolates, P-IAA 4 (*Acinetobacter* sp. OYAS30) and P-IAA 5 (*Enterobacter hormaechei* OYAS29). Genomic DNA was extracted using the ZymoBIOMICS™ DNA Miniprep Kit, and concentrations were measured via Qubit. Libraries were prepared using 10 bp unique dual indices (UDI) with a target insert size of 320 bp and the Illumina DNA Prep kit, followed by sequencing on an Illumina NovaSeq 6000 (2 × 151 bp). Demultiplexing and quality control were performed with Bcl-convert (v4.1.5). Reads were assembled with Unicycler (v0.4.8) and polished with Trim Galore and Pilon (v1.23). Genome annotation was conducted using RAST tk and PGAP, while Mash/MinHash identified closest reference genomes. Phylogenetic relationships were determined using PATRIC global protein families (PGFams) aligned with MUSCLE. A concatenated alignment of nucleotide and protein sequences was analyzed in RaxML, with rapid bootstrapping to assess tree support values. Genomic analyses were submitted to the BV-BRC for functional insights.

Metagenomics Study

Phased primers targeting the V3/V4 regions of the 16S rRNA gene were used in a metagenomics study with Zymo Research's Quick-16S kit, using DNA extracted from three 10 g portions of the mixed rhizosphere soil sample labelled SA, SB, and SC. The forward primer sequences were V3-CCTACGGGDGGCWGCAG and V4-CCTAYGGGGYGCWGCAG, while the reverse primer was GACTACHVGGGTATCTAATCC. The amplicon libraries were cleaned and normalized before sequencing on a P1 600-cycle NextSeq2000 flow cell to generate 2 × 301 bp paired-end reads. Quality control steps, including adapter trimming, were applied to ensure data accuracy. Sequence analysis was conducted using QIIME2, with the DADA2 plugin for denoising and Cutadapt for primer removal. Taxonomic classification was performed with VSEARCH against the SILVA 138 99% OTUs database. Operational taxonomic units (OTUs) were refined and normalized for relative abundance analysis, and a "Microbial Composition.tsv" file was generated alongside HTML visualization. Alpha diversity metrics, including evenness, Faith's phylogenetic diversity, observed features, and Shannon's diversity index, were calculated to evaluate community richness and evenness. These results were compiled into an "Alpha Diversity Metrics.tsv" file for comprehensive assessment.

Plant Pot Experiment

A pot experiment assessed the effect of *Enterobacter hormaechei* OYAS29 on maize growth and available phosphorus in soil. Soil was sieved using a 2–10 mm mesh, autoclaved to eliminate contaminants, and filled into nylon pots at 2 kg soil per pot and 14 cm depth. Maize seeds were surface-sterilized with 70% ethanol for 1 minute and 1% sodium hypochlorite for 3 minutes, and then rinsed with sterile water to remove pre-existing microbes. Control seeds were separated from the treated seeds, while the remaining seeds were used for seed bacterization (AJIBADE *et al*., 2022). OYAS29 was cultured in nutrient broth to 10^8 CFU/mL. Seeds were soaked in the bacterial suspension for 30 minutes, and 10 mL of the suspension was applied to the soil at planting as a single dose to mimic practical agricultural conditions. The experiment consisted of two treatments: OYAS29-treated maize seeds and uninoculated control seeds.

Thirteen plants were included in each treatment. Pots were placed in an open-air greenhouse setup behind the laboratory and exposed to natural rainfall and sunlight (25–30°C, variable rainfall) for 43 days. Seeds germinated within 15 days, after which growth parameters were measured over 4 weeks (AZIZ *et al*., 2015).

Agronomical and Soil Measurements

After 15 days of germination, maize growth parameters (plant height, stem girth and leaf length) were measured over 4 weeks at 7-day intervals, on days 15, 22, 29, 36, and 43 of the experiment. At the end of the 43-day experiment, root length, shoot biomass, root biomass, and total biomass were measured after harvesting.. Soil pH was measured using a digital pH meter (1:2.5 soil-to-water ratio), and available phosphorus was determined according to the method described by Olsen (Olsen, 1954). Data were recorded as mean ± standard deviation (N=13).

Statistical Analysis

The IBM Corp of Armonk, New York, USA provided the SPSS version 27 software, which was used for all data analysis. Statistically significant differences between the variables were evaluated using t-test at significance level of $p < 0.05$.

RESULTS

Bacterial Isolation and Phenotypic Characterization

Serial dilution and pour plating of rhizosphere soil yielded 75 morphologically distinct bacterial colonies. Five isolates (IS-1 to IS-5) were selected for further study based on Gram staining and biochemical tests, followed by screening for plant growth-promoting traits. The biochemical and morphological characteristics of the five isolates are presented in Table 1.

Table 1. Gram Rxn and biochemical test for isolates identity

ISOLATES	GRAM RXN	CATALASE	OXIDASE	METHYL RED	VOGES-PROSKAUER	SUGARS	STARCH HYDROLYSIS	INDOLE	AMMONIA	CITRATE	INFERENCE (SUSPECTED ORGANISM)
IS-1	+ve Short rods	+	−	+	−	+	+	+	+	−	*Bacillus* spp
IS-2	-ve Short rods	+	+	+	−	+	−	+	+	+	*Pseudomonas* spp
IS-3	-ve Rods	+	−	+	−	+	+	+	+	+	*Pseudomonas* spp
IS-4	-ve Rods	+	−	+	−	+	−	+	+	+	*Acinetobacter* spp
IS-5	-ve Short rods	+	−	+	−	+	−	+	+	+	*Enterobacter* spp

KEY: + = Positive, − (ve) = Negative. Sugars tested were glucose, mannitol, galactose, and fructose.

All isolates were negative for coagulase and urease which is why these results are not presented in the table. IS-1 was Gram-positive with short rods, while IS-2 to IS-5 were Gram-negative with short rods (IS-2, IS-5) or rods (IS-3, IS-4). All isolates were catalase-positive, while only IS-2 was oxidase-positive. IS-1 and IS-3 showed starch hydrolysis. Ammonia

production and citrate utilization were observed in IS-2 to IS-5, while IS-1 was negative for citrate utilization. Based on these traits, isolates were tentatively identified as *Bacillus* spp. (IS-1), *Pseudomonas* spp. (IS-2, IS-3), *Acinetobacter* spp. (IS-4), and *Enterobacter* spp. (IS-5), with "spp." indicating genus-level identification pending molecular confirmation.

Plant Growth-Promoting Traits

Screening results for plant growth-promoting traits are shown in Table 2. IS-2 exhibited high phosphate solubilization (PSI = 3.0), IS-5 showed medium phosphate solubilization (PSI = 2.9), while IS-1, IS-3, and IS-4 showed low phosphate solubilization (PSI = 1.8, 1.44, and 1.66, respectively). IS-5 showed the highest IAA production, followed by IS-3, IS-1, IS-2, and IS-4, as shown in Figure 1. All isolates produced ammonia. Enzyme production varied: IS-1, and IS-3 synthesized both α-amylase and pectinase, while IS-2, IS-4 and IS-5 produced only pectinase**.** IS-5 was selected for molecular identification because of its stronger overall plant growth-promoting traits, while IS-4 was also selected for molecular identification as a representative isolate from the preliminary screening.

Table 2. Shortlisted rhizobacteria that exhibit multiple traits that promote plant growth in vitro.

Isolates	Auxin Synthesis	P-Solubilization		NH_3 Production	Enzymes Production	
		Index	Inference		α- Amylase	Pectinase
IS-1	+	1.8	+	+	+	+
IS-2	+	3.0	+	+	_	+
IS-3	+	1.44	+	+	+	+
IS-4	+	1.66	+	+	_	+
IS-5	+	2.9	+	+	_	+

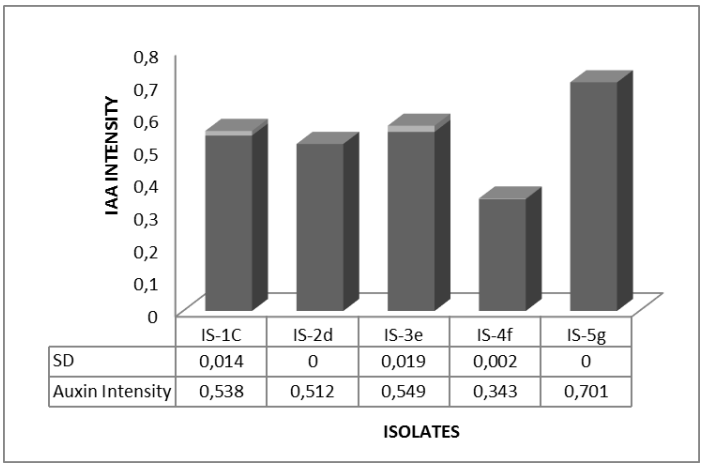

Figure 1. The auxin concentration produced by each PGPR isolate.

The Whole Genome Result of PGPR Isolates

Whole-genome sequencing identified IS-4 as *Acinetobacter* sp. OYAS30 and IS-5 as *Enterobacter hormaechei* OYAS29 (Supplementary Tables S1 and S2). Genomic features included 4240 genes for OYAS29 (4068 coding, 97 RNA) and 3941 genes for OYAS30 (3812 coding, 59 RNA), with high-quality assemblies. Sequences were deposited in GenBank under accession numbers JAWVAV000000000 (OYAS30) and JAXCLF000000000 (OYAS29). Figure 2 illustrates the phylogenetic tree for both isolates, showing their taxonomic placement based on the available reference sequences.

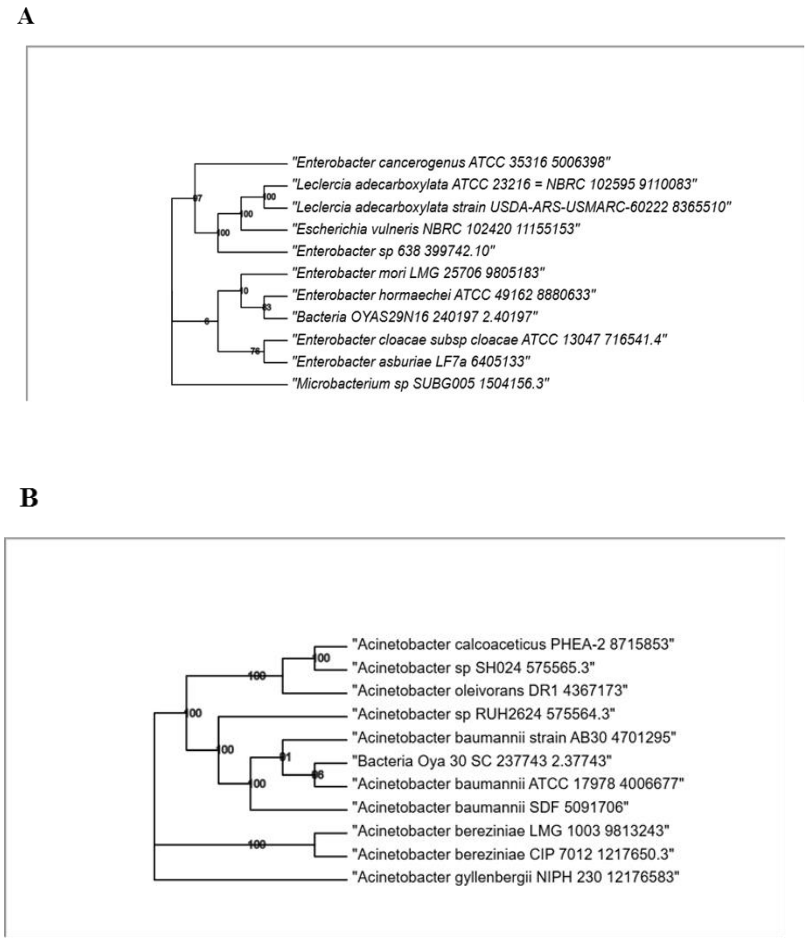

Figure 2. Phylogenetic trees of selected PGPR isolates. (A) *Enterobacter hormaechei* OYAS29. (B) *Acinetobacter* sp. OYAS30.

Metagenomics Result

Metagenomic analysis revealed a bacteria-dominated soil microbiome, with 99.758% d_Bacteria and 0.242% d_Archaea. Proteobacteria were most abundant (66.661%), followed by *Actinobacteriota* (10.970%), *Bacteroidota* (4.980%), *Acidobacteriota* (4.859%), *Gemmatimonadota* (4.473%), and others. *Firmicutes* comprised 0.516%. Figure 3 illustrates the phylum-level taxonomic composition across samples SA, SB, and SC, indicating a diverse

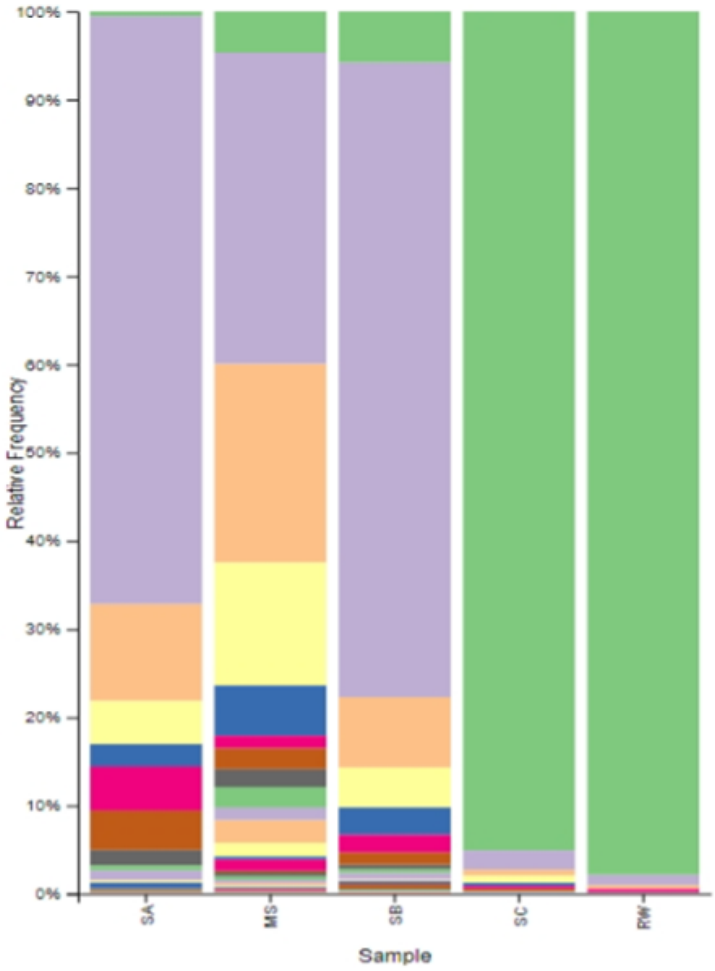

Figure 3. The taxonomic composition of the soil microbiome (Samples: SA, SB, and SC) at the phylum level.

SA, SB, and SC represent the three portions of the mixed rhizosphere soil sample used for metagenomic analysis. MS represents the mock community control used during sequencing, while RW represents blank control.

Measurement of Agronomical Parameters of Bacterized Seeds with Enterobacter hormaechei OYAS29

Maize plants inoculated with *Enterobacter hormaechei* OYAS29 showed enhanced growth compared to the control across multiple parameters, as shown in Table 3. Plant height was 45.72 ± 12.56 cm in the OYAS29-treated plants compared to 36.25 ± 13.05 cm in the control, although the difference was not statistically significant ($p = 0.270$). Stem girth, which refers to the thickness of the maize stem, was 4.20 ± 0.47 cm in the OYAS29-treated plants compared to 3.23 ± 0.49 cm in the control ($p = 0.030$). Root length was 23.43 ± 5.87 cm in the OYAS29-treated plants compared to 16.75 ± 8.21 cm in the control ($p = 0.020$). Leaf length was 35.50 ± 8.79 cm in the OYAS29-treated plants compared to 28.45 ± 11.98 cm in the control ($p = 0.040$). Shoot biomass, root biomass, and total biomass were also higher in the OYAS29-treated plants compared to the control, but the difference was statistically significant only for total biomass. Soil available phosphorus increased slightly from 0.4905 ppm in the control to 0.4934 ppm in the OYAS29-treated soil, although the difference was not statistically significant ($p = 0.140$). For soil pH difference between treatments ($p = 0.147$) was no statistically significant.

Table 3. Effect of Enterobacter hormaechei OYAS29 on maize growth, biomass, and soil properties

Parameter	OYAS29-treated plants	Control	p-value	Significance
Plant height (cm)	45.72 ± 12.56	36.25 ± 13.05	0.270	Not significant
Stem girth (cm)	4.20 ± 0.47	3.23 ± 0.49	0.030	Significant
Root length (cm)	23.43 ± 5.87	16.75 ± 8.21	0.020	Significant
Leaf length (cm)	35.50 ± 8.79	28.45 ± 11.98	0.040	Significant
Shoot biomass (g)	4.68 ± 2.95	3.04 ± 2.75	0.367	Not significant
Root biomass (g)	2.81 ± 1.59	1.69 ± 1.40	0.246	Not significant
Total biomass (g)	7.49 ± 4.48	4.72 ± 4.15	0.010	Significant
Soil pH	7.29 ± 0.16	7.49 ± 0.20	0.147	Not significant
Available soil phosphorus (ppm)	0.4934 ± 0.360	0.4905 ± 0.290	0.140	Not significant

Key: Values are presented as mean ± standard deviation, N = 13 per treatment. Control = maize plants from sterile water-treated seeds. Significant difference was accepted at $p \leq 0.05$.

DISCUSSION

This study isolated and characterized multi-trait plant growth-promoting rhizobacteria that enhance soil phosphorus availability and maize growth, offering a sustainable solution for agriculture in Ekiti State, Nigeria. The isolation of five PGPR isolates (IS-1 to IS-5) from local rhizosphere soil, tentatively identified as *Bacillus* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Enterobacter* spp. through biochemical tests, confirmed the presence of diverse bacteria in the soil microbiome, consistent with global reports (MALGIOGLIO *et al.*, 2022; KUMAR *et al.*, 2022; DINESH *et al.*, 2015). Molecular analysis identified IS-5 as *Enterobacter hormaechei*

OYAS29, an indigenous isolate from Ekiti State, exhibiting medium phosphate solubilization (PSI = 2.90) and strong indole-3-acetic acid (IAA) production, making it a prime candidate for in vivo testing. All isolates displayed multiple traits in vitro, including ammonia production, and pectinase synthesis, supporting their growth-promoting potential through diverse mechanisms such as improved phosphorus availability, root stimulation, nitrogen contribution, and enzyme-mediated nutrient release (ANTOUN and PRÉVOST, 2005). However, the qualitative IAA assessment lacked a standard curve, which limited precise quantification and suggests the need for chromatographic methods in future studies (BACKER *et al.*, 2018; LYU *et al.*, 2019). Similarly, screening with calcium phosphate may not fully mimic local soil conditions, warranting broader phosphate sources in future assays (TIMOFEEVA *et al.*, 2022).

43-day open-air pot experiment, conducted under Ekiti's natural conditions, demonstrated OYAS29's significant improvement of maize growth compared with the control. Total biomass increased by 58.69%, stem girth by 30.03%, leaf length by 24.78%, and root length by 39.88% compared with the control. These improvements, likely driven by IAA and ammonia enhancing root development and nutrient uptake, align with PGPR effects in crops like chili and tomato (OEDJIJONO and PROKLAMASININGSIH, 2020; ISWATI, 2012). The non-significant increase in plant may reflect variability in rainfall and sunlight under open-air conditions, compounded by a limited sample size (N=13), which reduced statistical power (GUPTA *et al.*, 2021). Controlled greenhouse trials with larger samples could clarify effects that affect height. The increase in biomass, a critical indicator of productivity, supports the potential of OYAS29 as a locally adapted biofertilizer, consistent with reports that other PGPR can improve maize growth and biomass (SILVA *et al.*, 2021).

Furthermore, soil phosphorus content showed a modest, non-significant increase of 0.59%, while soil pH decreased slightly by 2.67% compared with the control, despite OYAS29's medium in vitro phosphate solubilization. This may result from phosphorus leaching due to rainfall or autoclaved soil limiting microbial interactions, as autoclaving alters soil properties (WANG *et al.*, 2022; BAJPAI *et al.*, 2006). The 43-day duration and N=13 likely restricted detectable changes, as soil properties require longer periods to shift (GUPTA *et al.*, 2021). Field trials in Ekiti soils with extended durations could better evaluate OYAS29's phosphate-solubilizing capacity under natural soil conditions. Additional limitations, such as the absence of a phylogenetic out-group and use of qualitative enzyme detection, suggest that including outgroups and conducting quantitative assays would enhance taxonomic and functional insights.

This study is the first in Ekiti State, and potentially in Nigeria, to characterize an indigenous multi-trait PGPR with potential for maize growth improvement, thereby addressing a regional research gap. Unlike consortia-based approaches (GRAY and SMITH, 2005; GONZALEZ *et al.*, 2021), OYAS29's single-isolate efficacy encourages significant maize growth under open-air conditions, offering a cost-effective biofertilizer for local farmers. The metagenomic profile which revealed a bacteria-rich soil (99.758% d_Bacteria) underscores OYAS29's ecological relevance in Ekiti's microbiome. Constraints like single-isolate testing and phylum-level metagenomics indicate needs for future multi-isolate trials and species-level analyses. This work lays the foundation for field-scale validation in Nigeria's agricultural systems, using an indigenous PGPR for sustainable maize production.

CONCLUSIONS

This study supports sustainable agriculture in Ekiti State, Nigeria, by demonstrating the potential of *Enterobacter hormaechei* OYAS29, an indigenous plant growth-promoting rhizobacterium, to enhance maize production. The pot experiment showed that OYAS29 improved maize growth with total biomass increasing by 58.69%, alongside notable increases in stem girth, leaf length, and root length. These improvements may be linked to its medium phosphate solubilization, IAA production, ammonia production, and pectinase activity. The slight, non-significant increase in available soil phosphorus may be due to environmental variability in the open-air greenhouse setup and the limited sample size, which may have reduced the ability to detect significant changes.

OYAS29's single-isolate efficacy, unlike consortia-based approaches, suggests its potential as a cost-effective biofertilizer suited to local soils because it was isolated from the Ekiti rhizosphere environment. Future studies should include field trials on diverse soils in Ekiti State, development of low-cost microbial formulations for smallholder farmers, and evaluation of multi-isolate synergies to improve soil health. These steps will build on the findings, strengthen the role of indigenous PGPR in transforming maize production and supporting sustainable agriculture in Nigeria.

List of Abbreviations
PGPR: Plant Growth-Promoting Rhizobacteria
IAA: Indole-3-Acetic Acid
CFU: Colony-Forming Units
PSI: Phosphate Solubilization Index
DNA: Deoxyribonucleic Acid
RNA: Ribonucleic Acid
CDS: Coding Sequence

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ISKORIŠTAVANJE AUTOHTONIH PGPR ZA POBOLJŠANJE DOSTUPNOSTI FOSFORA U ZEMLJIŠTU I RASTA KUKURUZA

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Izvod

Ova studija je istraživala autohtone rizobakterije koje podstiču rast biljaka (PGPR) izolovane iz nigerijskog zemljišta zbog njihovog potencijala da poboljšaju dostupnost fosfora i podstaknu rast kukuruza (*Zea mays* L.). Pet bakterijskih izolata dobijenih iz zemljišta sakupljenog na polju Oje-Ekiti Federalnog univerziteta ispitano je na ključne osobine koje podstiču rast biljaka, uključujući rastvorljivost fosfata, proizvodnju indol-3-sirćetne kiseline (IAA), proizvodnju amonijaka i aktivnost hidrolitičkih enzima (a-amilaza i pektinaza). Dva obećavajuća izolata, IS-4 i IS-5, odabrana su za molekularnu identifikaciju. Među njima, IS-5 je pokazao najjače ukupne performanse u podsticanju rasta biljaka, pokazujući umerenu rastvorljivost fosfata (PSI = 2,90), najveću proizvodnju IAA, proizvodnju amonijaka i aktivnost pektinaze. IS-4 je izabran kao reprezentativni izolat iz preliminarnog skrininga. Sekvenciranje celog genoma identifikovalo je IS-5 kao *Enterobacter hormaechei* OYAS29, a IS-4 kao *Acinetobacter* sp. OYAS30. Eksperiment u saksiji je sproveden kako bi se procenio efekat *Enterobacter hormaechei* OYAS29 na dostupnost fosfora u zemljištu i rast kukuruza. Tretman sa OYAS29 je blago povećao dostupni fosfor u zemljištu sa 0,4905 ppm u kontroli na 0,4934 ppm. Inokulirane biljke kukuruza su takođe pokazale poboljšan rast u poređenju sa kontrolom, uključujući povećanje visine biljke (26,12%), obima stabljike (30,03%), dužine lista (24,78%), dužine korena (39,88%) i ukupne biomase (58,69%). Generalno, rezultati ukazuju da *E. hormaechei* OYAS29 ima snažan potencijal kao autohtoni PGPR za održivu proizvodnju kukuruza i poboljšanu dostupnost fosfora u zemljištu.

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