J. Trop. Plant Pests Dis.
Vol. 25, No. 2, September 2025
Pages: 169–178

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.225169-178

RESEARCH PAPER

Identification of plant growth promoting rhizobacteria around Pulang Pisang Food Estate, Central Kalimantan, Indonesia

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Manuscript received: 19 September 2024. Revision accepted: 30 January 2025. Available online: 2 May 2025.

ABSTRACT

Plant growth-promoting bacteria are well known as biostimulants, biofertilizers, bioprotectants, and biodegraders. The Pulang Pisau Food Estate is an Indonesian government program aimed at increasing rice crop production to achieve sustainable food self-sufficiency. Research on rhizobacteria in the Pulang Pisau Food Estate area is still relatively limited. In this study, we conducted in vitro assays to evaluate the potential of indigenous bacterial isolates from the Pulang Pisau Food Estate as plant growth-promoting rhizobacteria (PGPR). The study focused on four bacterial isolates, which were tested for plant growth-promoting traits including phosphate solubilization, indole-3-acetic acid (IAA) production, effects on rice seed germination, and detached leaf assays to assess the ability of rhizobacteria to inhibit bacterial pathogens. The results of 16S rRNA gene identification suggested that isolates UNS-P1, UNS-P3, and UNS-R1 were closely related to *Bacillus cereus*, while one previously identified isolate (UNS-R2) was confirmed as *Bacillus subtilis*. All bacterial strains were able to produce IAA, while only one isolate demonstrated the ability to solubilize phosphate. In the germination test, no significant differences were observed in root length, but a significant difference in shoot (plant) height was detected. *Bacillus subtilis* (UNS-R2), at a 10^3 dilution, resulted in significantly greater plant height compared to other treatments. Among the four bacterial isolates, only one showed the ability to inhibit the pathogen *Pantoea ananatis*. These results suggest that indigenous bacterial isolates from the Pulang Pisau Food Estate have potential as plant growth-promoting rhizobacteria (PGPR) and may contribute to enhancing plant growth and serve as biocontrol agents against *P. ananatis*.

Key words: *Bacillus cereus*, detached leaf assays, rhizobacteria

INTRODUCTION

Pulang Pisau Food Estate is an Indonesian government program aimed at increasing rice production to achieve sustainable food self-sufficiency. A food estate, envisioned as a foundation for modern national agriculture, is an integrated food development concept that encompasses agriculture, plantations, and livestock within a large area consisting of several farming and livestock clusters.

The soil in this food estate primarily consists of peat soils, which differ significantly from mineral soils in their chemical and physical characteristics due to the presence of undecomposed organic matter. Peat soils typically have low nutrient content and may contain harmful organic acids. Therefore, site-specific technologies are required to address land limitations and to support the goal of designating the area for food production development.

The use of plant growth-promoting rhizobacteria (PGPR) and the addition of organic fertilizers can enhance soil fertility, increasing the pH from very acidic to slightly alkaline and improving the C/N ratio from very low to high. Enhanced soil fertility positively impacts crop productivity on peatlands (Istikorini et al., 2022). Rhizobacteria that are resistant to acidic soil conditions have been used to promote rice growth. These rhizobacteria were isolated from various plant rhizospheres and roots, such as *Melastoma* sp., *Eleocharis dulcis*, *Stochlaena pacistris* sp., *Melaleuca leucadendra*, and local rice. As a result, rice was able to grow well in acidic soils with rhizobacteria serving as mediators (Yuliatin et al., 2023).

Rhizobacteria can also enhance the growth of the Inpara 2 rice seedling variety in acid-sulfate soil. Organic acids produced by rhizobacteria may help mitigate the toxicity of aluminum (Al) and iron (Fe) through chelation reaction. Additionally, the indole-3-acetic acid (IAA) phytohormone produced

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by rhizobacteria has been shown to promote swamp rice growth in acid-sulfate soil (Yuniarti et al., 2021). PGPR can also increase antioxidant compounds in medicinal plants grown in peat soils (Sakya et al., 2022). Moreover, PGPR enhances plant resilience to both acidity and alkalinity stress. In legume-rhizobia nitrogen-fixing symbioses, rhizobia isolated from acidic soils show a greater ability to colonize and support plant growth under such conditions. Several genes contributing to rhizobial survival in acidic environments have been identified, including those coding for stress tolerance proteins such as ActA (apolipoprotein N-acyl transferase) and ActR (response regulator (Msimbira & Smith, 2020).

PGPR can be isolated from intercellular spaces of various plant tissues (endophytic), the rhizoplane, rhizosphere, and plant surfaces. These bacteria offer numerous advantages as biostimulants, including the production of growth hormones and biofertilizers that improve nutrient availability (e.g., phosphate solubilization), act as biopesticides, support plant disease control, and assist in rhizoremediation (Stamenkovi´c et al, 2018). The biocontrol mechanisms of PGPR include the production of antibiotics, hydrogen cyanide (HCN), siderophores, and cell wall-degrading enzymes; competition for nutrients and space; reduction of ethylene levels; induction of systemic resistance; and the production of volatile compounds. These volatile organic compounds are secondary metabolites—low-molecular-weight chemicals that evaporate quickly at room temperature and pressure (Kai, 2020; Santoro et al., 2015; Wu et al, 2019).

Research on rhizobacteria in Kalimantan remains limited. Previous studies have isolated and selected PGPR from oil palm roots in Central Kalimantan based on their plant growth-promoting characteristics (Yuliatin et al., 2023). These include the production of indole acetic acid (IAA), ligninolytic activity, phosphate solubilization, nitrogen fixation, potassium solubilization, siderophore production, ACC deaminase activity, cellulolytic activity, and proteolytic activity (Ariyani et al., 2021). Another study isolated bacteria from the Kahayan River in Pahawan Village and identified *Pseudomonas* sp., which is capable of bioremediating mercury heavy metals (Neneng et al., 2020).

However, no research has been conducted specifically on plant growth-promoting rhizobacteria and their volatile compounds in the vicinity of the food estate program. Therefore, this study aims to elucidate the role of indigenous rhizobacteria from food estates as biostimulants, bioprotectants, and biofertilizers *in vitro*. To support sustainable agriculture within the food estate program, it is essential to increase the use of PGPRs that function as biostimulants, bioprotectants, and biofertilizers, particularly those resistant to acidic conditions and other abiotic stresses.

MATERIALS AND METHODS

Research Site. The research was conducted from April to May 2024 at the Plant Protection Installation of the International Center of Agricultural Training in Ketindan and the Integrated Laboratory of Darussalam Gontor University. Rhizospheric bamboo samples were collected in Siam Belanti Village, Pandih Batu, Pulang Pisau District, Central Kalimantan.

Sample Collection and Isolation of Rhizobacteria. Soil samples were taken from the root zone of yellow bamboo. Bacteria were isolated using the serial dilution method, and Nutrient Agar (NA) was used as the culture medium (Özdoğan et al., 2022). Media were sterilized by autoclaving at 121 ºC and 1 atm for 25 min. The sterile media were poured into Petri dishes. After serially diluting the rhizosphere extract to 10^{-6} and 10^{-7}, with a maximum of one drop, one drop was plated and incubated for approximately 24 hours. Bacterial colonies that grew were selected based on morphological differences and subcultured on fresh NA media for purification. Selected colonies were subjected to further testing to evaluate their plant growth-promoting abilities.

Plant Growth Promotion Assay

Hypersensitivity Reaction (HR) Test. All bacterial isolates were cultured in Nutrient Broth and incubated for 24 hours. The bacterial suspensions were infiltrated into tobacco leaves. Necrotic symptoms were observed 4–5 days after inoculation (Lestari et al., 2022).

Indole Acetic Acid (IAA) Production Assay. A total of 500 µL of bacterial suspension was inoculated into 10 mL of Nutrient Broth supplemented with L-Tryptophan. The cultures were incubated on a shaker for 72 hours at 150 rpm. After incubation, the cultures were centrifuged at 10,000 rpm for 10 min to obtain the supernatant. The supernatant was transferred to sterile tubes, followed by the addition of Salkowski reagent. After 30 min, a color change was observed to qualitatively determine IAA presence. The absorbance was measured at 530 nm to calculate IAA concentration using a standard curve (Ramadhani et al., 2020).

***Phosphate Solubilizing Activity*.** A loopful of bacterial culture was streaked onto the Pikovskaya's medium and incubated for 5 days. Phosphate solubilization was indicated by the formation of clear zones around the colonies (Rahma et al., 2019).

Biocontrol Assays Against *P. ananatis* Using Detached Leaf Assays. Fresh rice leaves were washed with sterile distilled water, swabbed with 70% ethanol, and rinsed again. Leaves were then swabbed with *P. ananatis* pathogenic bacteria and treated with rhizobacterial isolates (UNS-P1, UNS-P3, UNS-R1, and UNS-R2). For controls, leaves were either swabbed with sterile water (positive control) or *P. ananatis* only (negative control) (Aregbesola et al., 2020; Suresh et al., 2021; Wong et al., 2020).

GC-MS Analysis. Gas Chromatography-Mass Spectrometry (GC-MS) was used to analysis of secondary metabolites produced by the rhizobacterial antagonists. The analysis was conducted using a VF-5MS fused silica capillary column (30 m length; 0.25 mm ID, 0.25 µm film thickness) on an Agilent Technologies 7890B GC system with a 240 ION TRAP MS. The stationary phase was composed of 5% diphenyl and 95% dimethylpolysilane. The detector operated in electron impact mode at 70 eV. Helium gas (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. Samples were injected in a 2 µL volume with a 10:1 split ratio. The injector and ion source temperatures were maintained at 250 ºC and 200 ºC, respectively. The oven temperature was programmed from 110 ºC (held for 2 min), increased by 10 ºC/min to 200 ºC, then 5 ºC/min to 280 ºC, and held isothermally at 280 ºC for 9 min. The total run time was 36 min with a solvent delay of 2 min. Mass spectra (m/z 45–450) were compared with the NIST database for compound identification (Panigrahi & Rath, 2021).

Identification of Bacteria by 16S rRNA. Molecular identification involved DNA isolation, PCR amplification of the 16S rRNA gene, and sequencing. DNA was extracted using the Quick-DNA Bacterial Miniprep Kit (Zymo Research, D4082, D6005). Universal primers used for PCR were 27F (5'–AGAGTTGATCMTGGCTCAG–3') and 1492R (5'–GGTTACCTTGTTACGACTT–3'). PCR conditions included initial denaturation at 95 ºC for 1 min, followed by 35 cycles of denaturation at 96 ºC for 15 s, annealing at 52 ºC for 30 s, and extension at 72 ºC for 45 s. Amplified products were visualized using 0.8% agarose gel electrophoresis. Sequencing results were compared with 16S rRNA sequences in the GenBank database using the NCBI BLAST tool. Phylogenetic trees were constructed using the neighbor-joining method in MEGA 11 software (Sherpa et al., 2021; Vasseur-Coronado et al., 2021).

Effect of Rhizobacteria on Rice Seedling Growth. Rice seeds of the cultivar Ciherang were surface-sterilized with 70% alcohol for 2 min, rinsed with sterile distilled water, and soaked in rhizobacterial suspensions for 15 min. Ten seeds were placed on moistened merang paper and incubated for 10 days. Seedling height and root length were measured afterward (Rahma et al., 2019).

RESULTS AND DISCUSSION

Bacterial Isolate and Hypersensitivity Test (HR). Rhizosphere bacteria were isolated from bamboo roots in the former peatland project area around the Pulang Pisau Food Estate, Central Kalimantan. Four pure bacterial isolates were obtained and labeled UNS-P1, UNS-P3, UNS-R1, and UNS-R2. The UNS-R2 isolate had been previously identified as *Bacillus subtilis*. These four isolates were then tested for hypersensitivity on tobacco to identify non-pathogenic bacteria, indicated by the absence of necrosis on tobacco leaves after infiltration. The hypersensitivity test showed that all four isolates (UNS-P1, UNS-P3, UNS-R1, and UNS-R2) are non-pathogenic.

Indole Acetic Acid (IAA) Production Assay. Quantitative analysis of IAA production revealed that UNS-R2 produced the highest levels, followed by UNS-P1, UNS-P3, and UNS-R1 (Table 1). *Pseudomonas fluorescence* was used as positive control for IAA production, and NB medium without bacteria served as a negative control. A key trait of plant-growth-promoting rhizobacteria (PGPR) is their ability to produce indole acetic acid (IAA), a phytohormone that promotes root initiation, cell division, and elongation. The IAA production assay used L-tryptophan as a precursor. All isolates showed IAA production, evidenced by a color change to red in NB medium after reacting with Salkowski's reagent. The red coloration results from the interaction between IAA and Fe, forming the complex $[Fe_2(OH)_2(IA)_4]$. A deeper pink color correlates with higher IAA concentration (Figure 1) (Fallo et al., 2023; Sukmadewi et al, 2015).

Phosphate Solubilizing Activity. Among the tested

isolates, only UNS-P1 showed the ability to solubilize phosphate (Figure 2). The ability was determined using Pikovskaya agar medium, with clear zones observed after three days of incubation. Despite the molecular identification (16S rRNA) classifying isolates UNS-R1, UNS-P1, and UNS-P3 as *Bacillus cereus*, only UNS-P1 exhibited phosphate-solubilizing activity. This indicates that even within the same species, functional variability exists. Phosphate is essential for plant growth, and phosphate-solubilizing microorganisms (PSMs) play a key role in converting unavailable phosphorus into plant-accessible forms (Pang et al., 2024). Organic acids such as formic, acetic, propionic, lactic, and fumaric acids are involved in this process through the formation of stable phosphate complexes by reacting with metal ions like Ca^{2+}, Fe^{3+}, and Al^{3+} (Cahyaty, 2007; Zhang et al., 2020).

Antagonistic Activity Against *P. ananatis* Using Detached Leaf Assays. Antagonistic tests were conducted using detached rice leaves, co-inoculated with *P. ananatis* and the bacterial isolates. *P. ananatis* is known to cause leaf blight in crops such as rice (Mondal et al., 2011; Kini et al., 2017; Luna et al., 2018; Aksoy & Boluk, 2019; Toh et al., 2019; Reshma et al., 2022; Yu et al., 2022), garlic (Nurjanah et al., 2018), strawberries (Abdel-Gaied et al., 2022), and corn (Mamede et al., 2018). Treatments with UNS-P1 and UNS-P3 caused symptoms similar to *P. ananatis* infection, including leaf browning and drying. In contrast, leaves treated with UNS-R1 and UNS-R2, remained healthy and green, similar to those treated with sterile water (Figure 3). These results indicate that UNS-R1 and UNS-R2 may possess biocontrol potential. Rhizobacteria can suppress pathogen growth through antibiotic production, enzymatic degradation, hydrogen cyanide (HCN) production, and competition (Alawiye & Babalola, 2019).

Table 1. Indole acetic acid (IAA) content of rhizobacteria from around Pulang pisau food estate

Isolate code	IAA content (ppm)
UNS-P1	21.19
UNS-P3	18.73
UNS-R1	18.21
UNS-R2	72.59
PF	56.39

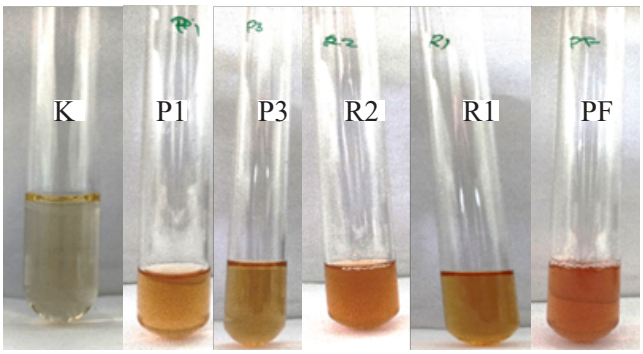

Figure 1. Indol Acetic Acid test of test of rhizobacteria from around Pulang Pisau Food Estate. K= control as a negative control reaction; PF= *Pseudomonas fluorescence* as a positive control reaction.

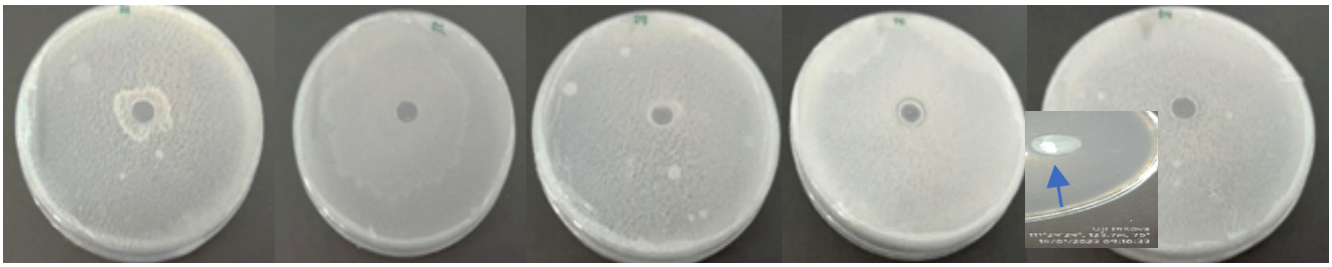

Figure 2. Phosphate solubilization test of rhizobacteria from around Pulang Pisau Food Estate. UNS-R1, UNS-R2, UNS-P3 showed no clear zone around bacterial colonies. UNS-P1 and PF showed clear zone indicates ability to solubilized phosphate. The blue arrow indicates a clear zone around bacteria UNS-P1.

Secondary Metabolites of Rhizobacteria. The antagonistic tests highlighted the potential of UNS-R2 (*Bacillus subtilis*) to inhibit *P. ananatis*, and also to promote root elongation and seedling growth. GC-MS analysis of secondary metabolites in UNS-R2 revealed the presence of several bioactive compounds, including 1-Pentanol, 2,3-dimethyl-; Ethyl Acetate; Geranyl Acetate; and Isopulegol (Table 2). *Bacillus* spp. are known for producing secondary metabolites such as antibiotics, VOCs, hydrolytic enzymes, and phytohormones that contribute to pathogen suppression and plant growth (Chowdhury et al., 2015).

Molecular Identification of Rhizobacteria (16S rRNA). Isolates UNS-P1, UNS-P3, and UNS-R1 were identified using 16S rRNAsequencing, while UNS-R2 had previously been identified as *Bacillus subtilis*. PCR amplification yielded amplicons of approximately 1423 bp for P1 and P3, and 1393 bp for R1 (Figure 4). Phylogenetic analysis showed that these isolates belong to *Bacillus cereus*, while UNS-R2 belongs to *Bacillus subtilis* (Table 3; Figure 5). Despite genetic similarities, differences were observed in IAA production and phosphate solubilization. These findings suggest low microbial diversity in the Pulang Pisau Food Estate area. Nonetheless, studying the bacterial community is crucial to assess soil health and support sustainable agricultural practices (Nditasari et al., 2023).

Effect on Rice Seedling Growth. The influence of rhizobacteria on seedling height and root length was evaluated using isolates UNS-P1, UNS-P3, UNS-R1, UNS-R2, and *Pseudomonas fluorescens* (positive control). Three dilution levels (10^1, 10^3, and 10^6) were tested. The longest roots were observed with *P. fluorescens*, while significant increases in seedling

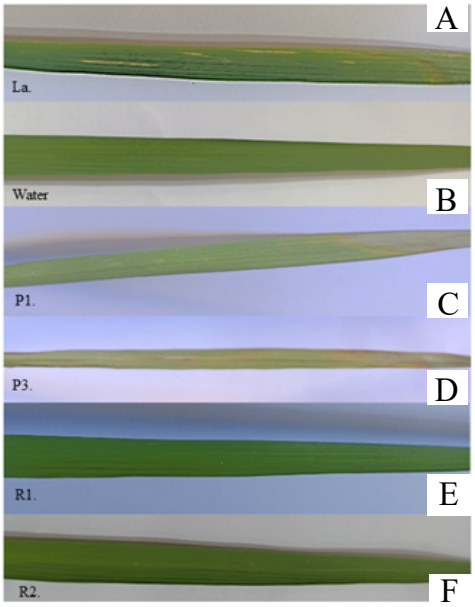

Figure 3. Detached leaf assay on rice 3 days after inoculation with *Pantoea ananatis*. Rhizobacteria inoculation treatment was able to inhibit the pathogen *P. ananatis* recognized by rice leaves stands out as green leaves, and the rhizobacteria inoculation treatment was unable to inhibit are recognized from yellowish-brownish leaves. A. Treatment with *Pantoea*; B. Control/water treatment; C. Treatment with UNS-P1 with *Pantoea*; D. Treatment with UNS-P3 rhizobacteria with *Pantoea*; E. Treatment with UNS-R1 rhizobacteria with *Pantoea*; F. Treatment with UNS-R2 rhizobacteria with *Pantoea*.

Tabel 2. Volatile organic compounds identified from GC-MS chromatograms of *B. subtilis* UNS-R2

Komponen	Retention time (min)	Height (%)
1-Pentanol, 2,3-dimethyl-	1.335	92.14
Ethyl Acetate	1.513	0.77
Geranyl acetate	11.100	0.67
6-Octen-1-ol, 3,7-dimethyl-, acetate	9.224	0.43
6-Octenal, 3,7-dimethyl-, (R)-	5.906	0.36

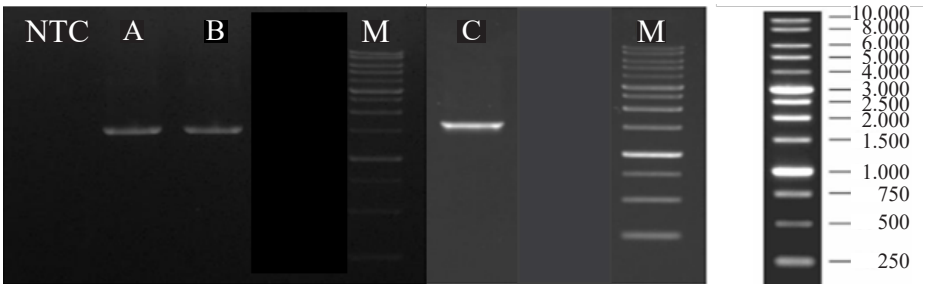

Figure 4. Gene sequence using PCR and electrophoresis using 0.8% gel agarose, Rhizobacteria UNS-P1. A. Rhizobacteria UNS-P3; B. Rhizobacteria UNS-R1; C. M: Marker, NTC: Non Template Control.

Table 3. Similarity index of rhizobacteria from around Pulang Pisau Food Estate

Isolate code	Nearest hist species	Accession number	Similarity (%)
UNS-P1	*Bacillus cereus*	MT337533.1	100
UNS-P3	*Bacillus cereus*	MT337533.1	100
UNS-R1	*Bacillus cereus*	MN543837.1	99.86

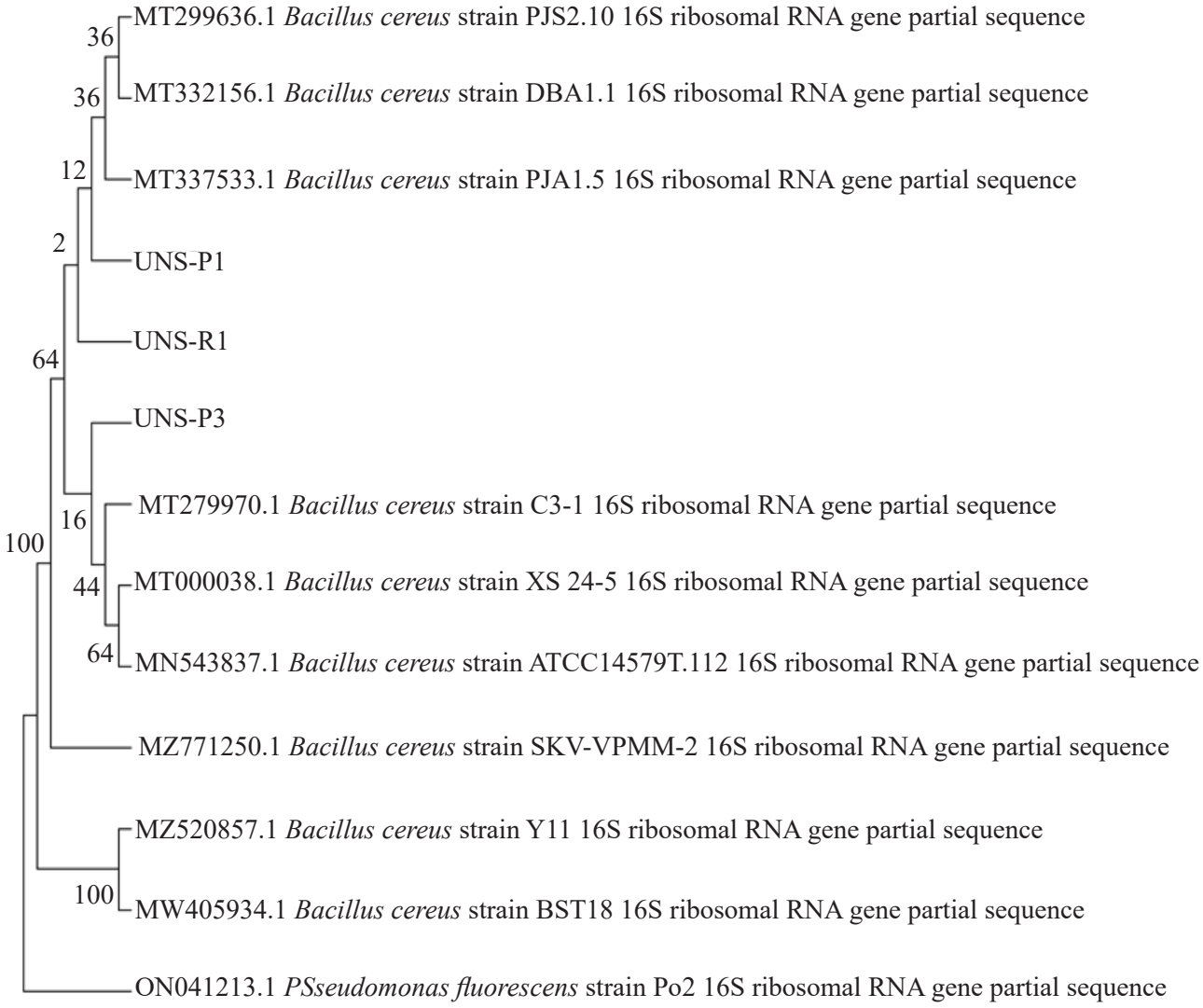

Figure 5. Phylogenetic Tree of rhizobacteria from around Pulang Pisau Food Estate (P1, P3, R1) among other bacteria *Bacillus* sp. and *Pseudomonas fluorescense.*

Table 4. The seedling height after application of rhizobacteria from around Pulang Pisau Food Estate

Treatment	Seedling height (cm)
Kontrol, Aquades steril	1.89 ab
Rhizobacteria UNS-P1, Dilution 10^{-1}	1.92 ab
Rhizobacteria UNS-P3, Dilution 10^{-1}	3.42 cd
Rhizobacteria UNS-R2, Dilution 10^{-1}	2.21 abc
Rhizobacteria UNS-R1, Dilution 10^{-1}	1.59 a
Rhizobacteria PF, Dilution 10^{-1}	2.41 abc
Rhizobacteria UNS-P1, Dilution 10^{-3}	1.74 a
Rhizobacteria UNS-P3, Dilution 10^{3}	3.57 cd
Rhizobacteria UNS-R2, Dilution 10^{-3}	4.06 d
Rhizobacteria UNS-R1, Dilution 10^{-3}	2.21 abc
Rhizobacteria PF, Dilution 10^{-3}	2.13 abc
Rhizobacteria UNS-P1, Dilution 10^{-6}	2.17 abc
Rhizobacteria UNS-P3, Dilution10^{-6}	3.25 bcd
Rhizobacteria UNS-R2, Dilution 10^{-6}	2.42 abc
Rhizobacteria UNS-R1, Dilution 10^{-6}	1.84 ab
Rhizobacteria PF, Dilution 10^{-6}	3.24 bcd

Mean values followed by the same letter are not significantly different according to LSD Follow-up Test at 0.05 level of significance.

height were observed with UNS-R2 and UNS-P3 (Table 3 and 4). Dilution had no significant effect on root or shoot length. The ability of *Bacillus cereus* and *Bacillus subtilis* to produce IAA and solubilize phosphate was positively correlated with seedling growth. IAA, a natural auxin, regulates various growth processes, including cell elongation and differentiation (Jeyanthi & Kanimozhi, 2018). High IAA levels observed in isolates from Pulang Pisau are consistent with enhanced root and shoot development.

Bacillus spp. are gram-positive bacteria capable of phosphate solubilization, and their thick peptidoglycan walls help them survive in acidic soils (Hidayati et al., 2022). In acid soils, *Bacillus* can also improve potassium availability (Setiawati et al., 2022). Considering the acidic nature of ex-peatland soils, the isolation and characterization of indigenous PGPR such as *Bacillus* spp. is essential for sustainable agricultural development. These bacteria can enhance soil fertility, produce growth hormones, and act as biocontrol agents. Previous studies have shown that soil fertility in the Pulang Pisau area increased with dolomite and manure application, especially when combined with commercial PGPR (Istikorini et al., 2022). Therefore, identifying indigenous PGPR is a critical step toward improving soil fertility and supporting productive agriculture in the region.

CONCLUSION

Four bacterial isolates (UNS-P1, UNS-P3, UNS-R1, UNS-R2) were obtained from the Pulang Pisau Food Estate area. Three were newly isolated *Bacillus cereus* strains, while UNS-R2 was a known *Bacillus subtilis* strain. All produced indole acetic acid (IAA), and only UNS-P1 could solubilize phosphate. Germination tests showed no difference in root length but a significant increase in shoot length, with *B. subtilis* (UNS-R2) at 10^3 dilution yielding the tallest plants. These isolates exhibited multiple plant growth-promoting traits, including IAA production, phosphate solubilization (UNS-P1), and inhibition of *Pantoea* spp. Their potential can be expanded through mass propagation using farmer-friendly, eco-friendly methods to boost plant growth and crop yield.

ACKNOWLEDGMENTS

The study was supported by the Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. We would like to thank our colleagues and all parties involved from the sampling stage to the publication of this article.

FUNDING

This research was financially supported by the research teams of all authors listed in this article.

AUTHORS' CONTRIBUTIONS

All authors contributed to the conceptualization, research design, implementation, and manuscript preparation. The authors also reviewed and provided input on the research workflow, data analysis, interpretation, and the final structure of the manuscript. All authors have read and approved the final version.

COMPETING INTEREST

The authors declared that there are no conflicts of interest or competing interests associated with this study.

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