

Research Article

Isolation and characterization of plant growth-promoting rhizobacteria from rhizospheric soil of hill paddySiti Aisyah Rahman^a, Zakry Fitri Ab. Aziz^{b,*}, Tunung Robin^c, Nor'aini Abdul Rahman^d, Philip Lepun^e, Noorasmah Saupi^b, Patricia Jie Hung King^b, Shiamala Ramaiya^b, Ribka Alan^c, Adrian Daud^c, Anita Rosli^c, Zahora Ismail^a, Sivasangar Seenivasagam^f, Sui Sien Leong^g, Suziana Hassan^h^aInstitute of Ecosystem Science Borneo, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^bDepartment of Crop Science, Faculty of Agricultural and Forestry Science, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^cDepartment of Social Science, Faculty of Humanities, Management, and Science, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^dDepartment of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia Serdang Campus, Serdang, 43400, Selangor, Malaysia^eDepartment of Forestry Science, Faculty of Agricultural and Forestry Science, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^fDepartment of Science and Technology, Faculty of Humanities, Management, and Science, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^gDepartment of Animal Science and Fisheries, Faculty of Agricultural and Forestry Science, Universiti Putra Malaysia Sarawak, Nyabau Road, 97000 Bintulu, Sarawak, Malaysia^hCentre for Sustainable and Inclusive Development, Faculty of Economics and Management, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor, Malaysia

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) enhance plant growth through mechanisms such as nutrient solubilization, nitrogen fixation, phytohormone production, and pathogen suppression, yet species from uncommon environments like hill paddy rhizospheric soil remain underexplored. This study aimed to isolate and identify PGPR from hill paddy rhizospheric soil, assess their plant growth-promoting traits, and evaluate their effects on plant growth. Soil samples were processed to isolate rhizobacteria, which were screened for nutrient-solubilizing and nitrogen-fixing abilities, and tested for antifungal activity against *Fusarium solani*. Selected strains were evaluated using mustard (*Brassica juncea*) as a model plant, and the most promising isolate was identified via 16S rRNA gene sequencing. Eight isolates demonstrated nitrogen fixation and phosphate and potassium solubilization, with one strain, RRZ034, showing consistently higher mean plant growth values, a high phosphate solubilization index (3.42 ± 0.08), and positive antifungal activity. Molecular analysis identified RRZ034 as *Pseudomonas nicosulfuronedens*, a species with limited previous documentation. These findings highlight the potential of *P. nicosulfuronedens* RRZ034 as a nutrient-solubilizing and pathogen-suppressing PGPR for future application in sustainable agriculture.

1. Introduction

Rice (*Oryza sativa* L.) is an essential grain and often referred as one of the major staple foods for approximately half of the world's population. It is primarily cultivated in Asia, accounting for about 90% of global rice production (Muthayya *et al.*, 2014). It plays a significant economic, agricultural, and cultural role globally. In term of cultivation, rice cultivation can be divided into two types – lowland rice and upland rice. In Malaysia, both of the types of cultivation are widely practiced, with lowland rice cultivation majorly practiced in the Peninsular Malaysia, while in East Malaysia (Sabah and Sarawak) predominantly for upland rice (Che Omar *et al.*, 2022). In contrast to lowland rice, which the paddy will be submerged; the upland rice cultivation refers to the cultivation of rice on hilly or mountainous regions. The rice varieties cultivated as upland rice commonly termed as hill paddy (*padi huma/padi bukit*) (Che Omar *et al.*, 2022).

The productivity of hill paddy remains low, contributed by various factors, including poor soil fertility as reported by Department of

Agriculture (2018). The current practice of conventional fertilizer application might be ineffective, given that upland rice cultivation is prone to nutrient leaching and continuous fertilizer application can be counter-productive (Wang *et al.*, 2016). There has been a growing need and interest in identifying environmentally friendly approaches to improve productivity of hill paddy in Sarawak. Researchers have extensively explored the application of biofertilizer as a sustainable method to enhance soil health. It is known for its ability to increase nutrient availability by stimulating natural soil processes (Chatterjee *et al.*, 2019). This involve the employment of products or mixtures of living microorganisms that will improve nutrient supply to plants, promote growth, restore soil health, and suppress plant pathogens; thereby reducing reliance on conventional fertilizers to support sustainable agriculture and environmental conservation (Amoo *et al.*, 2019; Ferreira *et al.*, 2019).

One of the common types of biofertilizers is plant growth-promoting rhizobacteria (PGPR). It helps to support the well-being of crops through various direct and indirect mechanisms, such as

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nitrogen fixation, solubilization of phosphate and potassium, producing vital phytohormones like auxin and gibberellins, and indirectly by suppressing plant pathogens (He et al., 2019). Also, numerous studies have reported significant successes in the application of PGPR on rice, including improved tillering and grain formation and grain yield, highlighting its impacts on the growth of paddy and soil health (Xiao et al., 2020; Ghaffari et al., 2018; Mitra et al., 2018). Given their ability to stimulate plant growth and enhance nutrient efficiency, PGPR offer an eco-friendly alternative to chemical inputs, making them a promising approach for improving hill paddy productivity while supporting sustainable agriculture.

The main objective of this study is to isolate PGPR from the rhizospheric soil of hill paddy. The isolates were screened and characterized through a series of *in vitro* assays to evaluate their plant growth-promoting traits before being partially identified using 16S rRNA sequencing. Also, mustard (*Brassica juncea*) was selected as a model plant to evaluate the effectiveness of the isolates, due to its rapid growth cycle and adaptability to diverse climates, making it suitable for experiments requiring timely results (South Carolina Department of Agriculture, 2002). The findings from this study provide a foundation for the development of a biofertilizer based on the selected PGPR, contributing to the more fruitful cultivation of hill paddy. Further research, including field trials and mechanistic studies, is recommended to validate and optimize the use of these PGPR strains.

2. Materials and Methods

2.1 Collection site

Rhizospheric soil samples were collected from a field cultivated with hill paddy (Fig. 1), located at Universiti Putra Malaysia Bintulu Campus, Sarawak (3.2048° N, 113.0708° E). The sampling was conducted during the hill paddy reproductive phase (approximately 80 days after sowing) using an auger at a soil depth of 0–15 cm. Five replicates of the sample were collected and pooled to form a composite sample. Intact hill paddy plants were gently uprooted, and the loosely adhering soil was removed by gentle shaking. The soil firmly attached to the roots, referred to as rhizospheric soil, was collected in sterile containers and immediately transferred to the Laboratory of Microbiology, Faculty of Agricultural and Forestry Sciences, Universiti Putra Malaysia, Bintulu Campus. The sampling and isolation were conducted on the same day to prevent contamination.

2.2 Soil physiochemical analysis

For baseline characterization, a composite soil sample was collected from five random replicates around the hill paddy field at a depth of 0–15 cm prior to PGPR isolation. The soil samples were analyzed at the SP Laboratory, Sarawak Oil Palm Plantation, Kuching, Sarawak,

to assess the physicochemical properties of the soil. The analysis was conducted using an in-house method developed by the laboratory, in accordance with Malaysian Standard MS678. Organic matter content was determined using the Loss on Ignition (LOI) method (Heiri et al., 2001).

2.3 Isolation and morphological characterization of rhizobacteria

The rhizobacteria were isolated using the standard serial dilution method described by Cappucino and Sherman (2014). Approximately 10 g of rhizospheric soil was suspended and vortexed in 100 mL of sterile distilled water. For serial dilution, 1 mL of the suspension was transferred into another test tube containing 9 mL of sterile distilled water. A series of tenfold dilutions of the suspension was made up to 10^{-5} . Then, aliquots of 0.1 mL from each dilution factor were spread on nutrient agar (NA) media, and incubated at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After 24 h, the formation of colonies was observed. The colonies with distinct morphological characteristics were selected and purified by subculturing at least twice on NA media. Then, the colonies of the rhizobacteria isolates were examined morphologically for their shape, size, margin, elevation, appearance, texture, and pigmentation. In addition, Gram-staining was also conducted to examine the cell morphology and Gram reaction of the isolates using the standard four-step method: crystal violet staining, iodine treatment, decolorization with ethanol, and counterstaining with safranin (Beveridge, 2001). The bacterial isolates were maintained in 50% glycerol stock. Each isolate was given a label representing the resource, rhizosphere: RRZXXX.

2.4 In vitro assessment of plant growth-promoting traits

The plant growth-promoting characteristics of the rhizobacteria isolates were evaluated based on their nitrogen-fixing activity, production of indole-3-acetic acid (IAA), solubilization of phosphate and potassium, along with their ability to demonstrate antifungal activity against *Fusarium solani*. All assays were performed in triplicate to ensure the reliability and reproducibility of the results.

2.4.1 Nitrogen fixation assay

The isolated rhizobacteria isolates were qualitatively assessed for nitrogen fixation activity by using the method described by Park et al. (2005). The isolates were cultured in Burk's N-free semi solid medium containing the following component per liter: KH_2PO_4 (0.41 g), K_2HPO_4 (0.52 g), Na_2SO_4 (0.05 g), CaCl_2 (0.2 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.10 g), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.005 g), $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ (0.0025 g), and bacteriological agar (1.8 g), supplemented with 10 g of glucose. A loopful of each isolate was inoculated by stabbing it into the test tube containing the medium, before incubation at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for three days. Nitrogen-fixing activity was indicated by the formation of a pellicle in the medium.

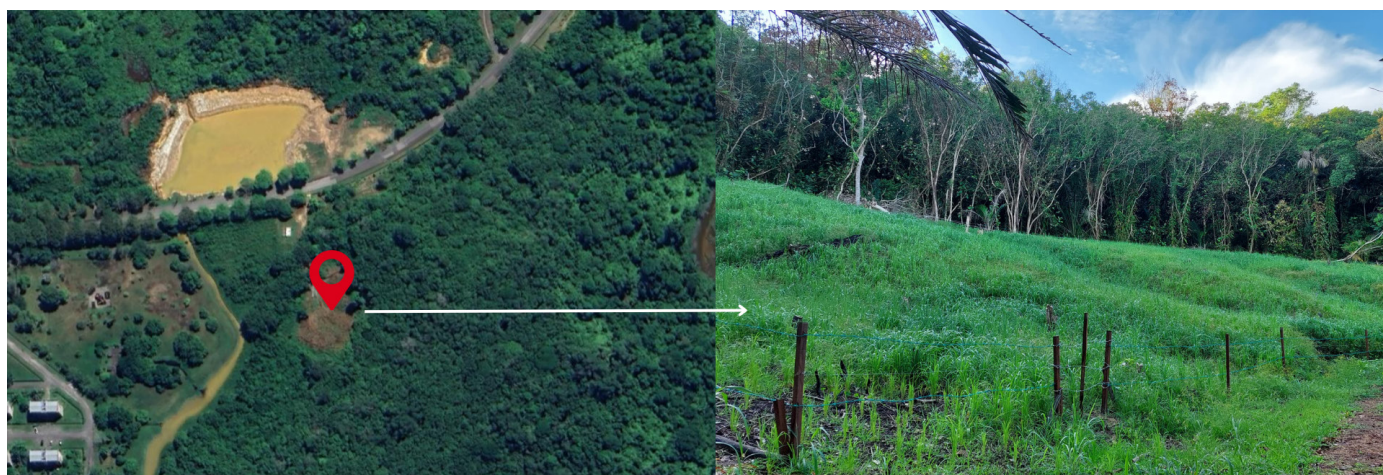


Fig. 1. Field cultivated with hill paddy as the sampling site.

2.4.2 Phosphate and potassium solubilization assay

The ability of the rhizobacteria isolates to solubilize phosphate and potassium was determined through spot inoculation of the isolates on Pikovskaya's agar and Aleksdandrow agar, respectively, following the method by Karuppusamy (2023). The plates were incubated at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 7 days. The formation of a transparent halo zone around the bacterial colony indicated positive solubilization activity of these two elements.

2.4.3 Indole-3-acetic acid (IAA) production

IAA production by the bacterial strains was detected using a colorimetric method (Mitchell & Brunstetter, 2004). The isolates were cultured in nutrient broth supplemented with 0.1 g/L of L-tryptophan, serving as IAA precursor, and incubated at room temperature in the dark for 5 days. After incubation, the cultures were centrifuged, and 1 mL of which was then mixed with 1 mL of Salkowski reagent (12 g/L FeCl_3 in 429 mL/L of H_2SO_4) and incubated in the dark at room temperature for 24 hours. The formation of pink color indicated IAA production.

2.4.4 Antifungal activity against *Fusarium solani*

The antifungal activity of the rhizobacteria isolates was tested against *Fusarium solani* by using a modified dual culture assay based on the method described by Hossain (2024). The mycelia plug of *Fusarium solani* was placed 2 cm from the edge of the NA media plate. On the opposite side, the rhizobacterial strains were streaked with a similar distance. The plates were incubated at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$, and observations were made after 7 days.

2.5 Plant test – Evaluation of plant growth-promoting ability in mustard plant

From the *in vitro* plant growth-promoting assays, potential isolates, which demonstrated multiple plant growth-promoting traits, were selected to evaluate the effect of their inoculation on the growth of the mustard plant. The plant growth-promoting effect of the bacterial isolates was primarily evaluated using mustard plants through a plant test in sterile soil, following the method adapted from Egamberdieva et al. (2008). Mustard seeds were surface-sterilized by immersion in 70% ethanol for 30 s, followed by 10% sodium hypochlorite for 30 min, and thoroughly rinsed with sterile distilled water. For inoculation, seeds were soaked in a bacterial suspension containing 10^9 colony-forming units per milliliter (CFU/mL) for 30 min. Each treatment consisted of 20 seeds sown in sterile soil-filled germination trays, with four replicates arranged in a completely randomized design. The trays were maintained at room temperature under natural light conditions and watered regularly. After seven days, germination percentage was recorded, and root length and shoot height of individual seedlings were measured to assess early growth performance. The vigor index was calculated using the following formula (Eq. 1):

$$\text{Vigor index} = (\text{Mean root length} + \text{Mean shoot height}) \times \text{Percentage of germination.} \quad (1)$$

Analysis of variance (ANOVA) was conducted to evaluate the effect of treatments (independent variable) on plant growth parameters such as germination percentage, root length, shoot height, and vigor index (dependent variables). Treatment means were compared using Tukey's Honestly Significant Difference (HSD) test at a 0.05 probability level using Statistical Analysis System (SAS) software version 9.4.

2.6 Molecular identification of the most promising rhizobacteria strain

After the evaluation of the plant growth-promoting traits of the selected rhizobacteria isolates, one promising isolate was selected for molecular identification. To identify the species of the isolated rhizobacteria, DNA barcoding was performed. The bacterial colony was selected and excised as a small agar plug. This agar plug was sent to Apical Scientific (Selangor, Malaysia) for further processing. The

bacterial DNA was extracted, and the 16S rRNA gene was amplified using universal primers 27F and 1492R at Apical Scientific, based on the method described by Weisburg et al. (1991). The amplified polymerase chain reaction (PCR) product was sequenced using Sanger sequencing. The resulting sequences were compared with reference sequences obtained from the National Center for Biotechnology Information (NCBI) GenBank database using Basic Local Alignment Search Tool (BLAST) to determine the species identity of the rhizobacterial isolate.

3. Results

3.1 Characteristics of soil

A preliminary analysis of the rhizospheric soil was conducted to characterize its baseline physicochemical properties (Table 1), providing context for the microbial environment from which the PGPR strains were isolated. The soil of this collection site was named Nyalau Series (*Typic Paleudults*). It was moderately acidic with low fertility, as indicated by its low cation exchange capacity, organic matter content, and limited availability of key nutrients. These characteristics are typical of hill paddy environments and may influence the presence and activity of native PGPR.

3.2 Isolation of rhizobacteria from the rhizospheric soil of hill paddy

In this study, a total of 44 rhizobacteria isolates with various colors and morphologies were obtained. The isolation resulted in a bacterial population with growth exceeding 100 CFU/mL after 24 h of incubation. From these, eight isolates were selected for further characterization based on their consistent growth and their demonstration of multiple plant growth-promoting traits during preliminary screening.

3.3 *In vitro* assessment of plant growth-promoting traits

From the *in vitro* bioassay evaluating plant growth-promoting traits, eight isolates, RRZ011, RRZ022, RRZ024, RRZ031, RRZ032, RRZ034, RRZ041, and RRZ044, demonstrated multiple beneficial characteristics, as summarized in Table 2. All isolates tested positive for nitrogen fixation and phosphate solubilization, both of which are crucial for enhancing plant growth. Among them, four isolates also exhibited potassium solubilization abilities. Furthermore, five isolates were capable of producing IAA, and three isolates displayed biocontrol activity against *Fusarium solani*. Notably, isolate RRZ034 exhibited the highest solubilization indices for both phosphate and potassium, alongside positive results for IAA production and biocontrol activity, underscoring its strong potential as a plant growth-promoting agent.

Following confirmation of their multiple PGPR traits, these isolates were further examined for their colony morphology and Gram reaction to provide a complete characterization profile. Morphological attributes, including colony shape, margin, elevation, texture, pigmentation, and Gram reaction, have been presented in Table 3.

Table 1.
Physicochemical properties of rhizospheric soil from hill paddy field.

Soil properties	Description and quantity
pH_{water}	5.20
pH_{KCl}	4.78
Total N (%)	0.07
Cation exchange capacity, CEC (meq/100g)	7.99
Organic matter (%)	3.16
Total organic carbon (%)	1.20
Available P (mg/kg)	4.00
Available K (mg/kg)	60.00
Available Mg (mg/kg)	144.00
Available Ca (mg/kg)	254.00

Table 2.*In vitro* plant growth-promoting traits of the PGPR isolates.

Isolate	<i>In vitro</i> plant growth-promoting traits assay				
	Nitrogen fixation	Phosphate solubilization	Potassium solubilization	IAA production	Biocontrol activity
RRZ011	+	+	-	+	-
RRZ022	+	+	+	-	-
RRZ024	+	+	-	+	+
RRZ031	+	+	-	+	-
RRZ032	+	+	+	+	-
RRZ034	+	+	+	+	+
RRZ041	+	+	-	-	+
RRZ044	+	+	+	-	-

+ indicates the presence of the trait; - indicates the absence of the trait.

Table 3.

Morphological characteristics of the PGPR isolates.

Isolate	Shape	Margin	Elevation	Texture	Pigmentation	Gram reaction
RRZ011	Circular	Entire	Raised	Smooth	White	Gram negative
RRZ022	Irregular	Undulate	Flat	Smooth	Yellowish	Gram positive
RRZ024	Circular	Entire	Raised	Mucoid	Creamy white	Gram positive
RRZ031	Circular	Entire	Raised	Smooth	White	Gram negative
RRZ032	Circular	Lobate	Flat	Mucoid	White	Gram negative
RRZ034	Circular	Entire	Raised	Smooth	Creamy white	Gram negative
RRZ041	Irregular	Entire	Convex	Rough	Creamy white	Gram positive
RRZ044	Circular	Undulate	Flat	Smooth	Yellowish	Gram negative

3.4 Plant test- Evaluation of plant growth-promoting ability in plants

An assessment of the overall performance of the plant growth-promoting activity by the rhizobacterial isolates was made based on the vigor index that incorporated the growth performance of the

mustard plant, including germination percentage, root length, and shoot height of the seedlings. The bar charts (Fig. 2) illustrate the germination rate, shoot height, root length, and vigor index across nine different rhizobacterial inoculation treatments, including the control. In general, most of the rhizobacteria isolates have the ability to improve seed germination percentage and the subsequent growth of seedlings within 7 days after sowing. Although none of the treatments exhibited statistically significant overall improvements in the plant test, strain RRZ034 (T3) consistently recorded the highest mean values across all evaluated parameters. Notably, the vigor index of T3 increased by at least 79% compared to the control, highlighting its potential as an effective PGPR.

3.5 Molecular identification of the most promising rhizobacteria strain

Based on the screening results, rhizobacterial strain RRZ034 was selected for further identification. The 16S rRNA gene was sequenced, and homologous gene segments were compared using pairwise alignment. The partial 16S rRNA nucleotide sequence of RRZ034 showed a high level of identity (99-100%) with *Pseudomonas nicosulfuronedens*. Phylogenetic analysis (Fig. 3) revealed that RRZ034 clustered closely with *Pseudomonas nicosulfuronedens* strain LAM1902, with <99% sequence similarity to this previously reported strain. A phylogenetic tree was constructed using the Neighbor-Joining method with 1000 bootstrap replicates to assess branch reliability, performed in MEGA11 software. The analysis also indicated a close evolutionary relationship between RRZ034 and related species such as *Pseudomonas nitroreducens*, *Pseudomonas multiresinivorans*, and *Pseudomonas nitritireducens*.

4. Discussion

In this study, 44 rhizobacterial isolates were successfully obtained from the rhizospheric soil of hill paddy. The presence of diverse PGPR in the environment associated with rice has been well-reported in previous studies. For instance, Basik et al. (2020) reported the isolation of over 300 bacterial strains from the rhizosphere of hill paddy in Kuching, highlighting a great diversity of genera and a significant proportion of

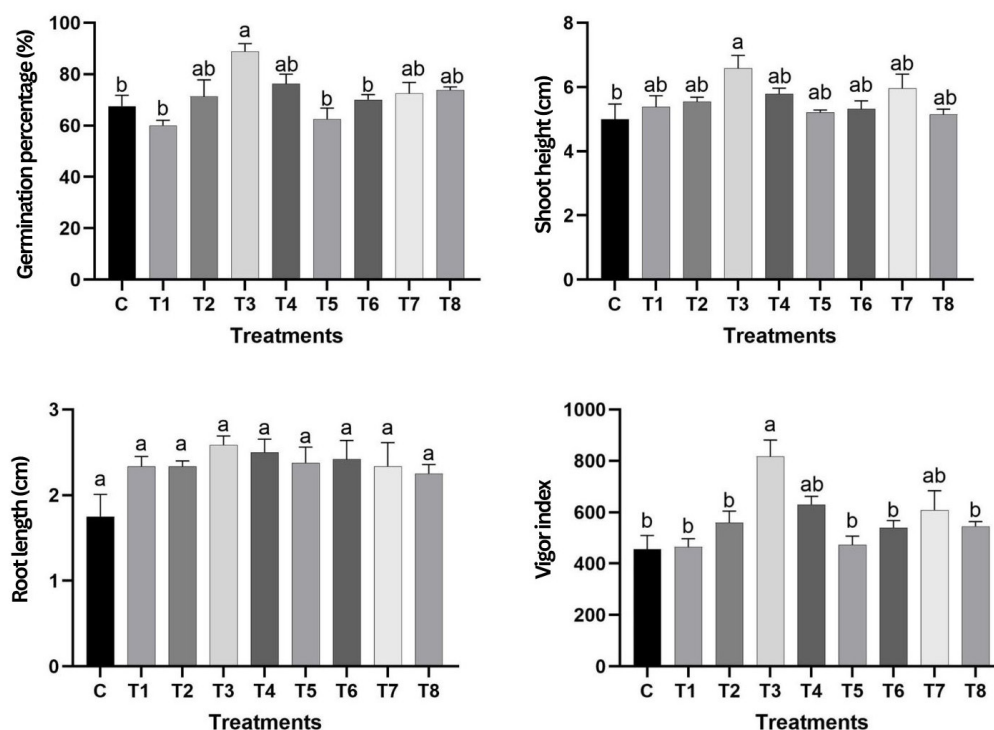


Fig. 2. Effects of different PGPR treatments (T1–T8) on germination percentage, shoot height, root length, and vigor index of hill paddy under laboratory conditions. The control (C) represents non-inoculated seeds. Each bar represents the mean ± standard error of triplicate measurements. Different letters (a, b, ab) above the bars indicate statistically significant differences among treatments according to Tukey's HSD test at $p < 0.05$. Bars sharing the same letter are not significantly different from each other, whereas bars with different letters are significantly different.

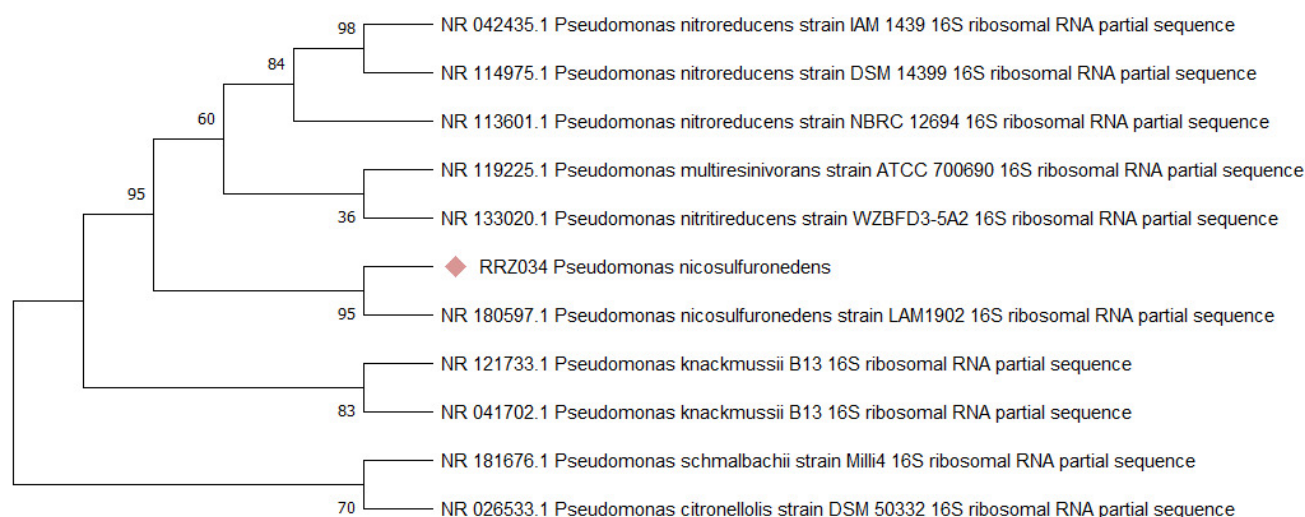


Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences shows the relationship between isolate RRZ034 and closely related *Pseudomonas* species.

novel isolates. Similarly, [Othman et al. \(2022\)](#) discovered rhizospheric bacteria from rice roots with zinc-solubilizing and biocontrol properties, consistent with the present finding of isolate RRZ034 that demonstrated antagonistic activity against pathogenic fungi. Furthermore, [Syaziana et al. \(2024\)](#) explained the potential of PGPR strains from rice irrigation systems, where a selected isolate from MR269 rice displayed multiple plant growth-promoting traits, including nitrogen fixation, phosphate and potassium solubilization, as well as production of siderophore and IAA, which promoted the overall growth performance of chili plants. These studies support the current findings and emphasize the rhizosphere as a potential reservoir for beneficial microbial strains.

The study revealed variability among treatments, with none showing statistically significant superiority. However, T3, which incorporated the PGPR strain RRZ034, consistently outperformed other treatments across all measured parameters. This performance is likely attributed to the strong plant growth-promoting traits of RRZ034 as demonstrated in the *in vitro* assays. The strain exhibited key functions such as nitrogen fixation and phosphate and potassium solubilization, which are essential for improving nutrient availability and uptake. These attributes are likely to include potassium solubilization, which is essential for improving nutrient availability and uptake. These attributes likely contributed to the enhanced seedling growth and vigor observed during the trials.

While the single-strain application of RRZ034 yielded promising results, it is important to consider the broader possibility of interactions among different PGPR strains. Its promising trend may become more pronounced with larger sample sizes or under real-world field conditions. The broader potential of co-inoculation strategies also deserves consideration. Previous studies have highlighted the synergistic effects of combining *Pseudomonas* spp. with other PGPR strains, particularly *Bacillus* spp. ([Singh et al., 2022](#)). Such combinations have been reported to improve nitrogen availability, enhance plant growth, and provide disease resistance ([Poveda et al., 2022](#)). Exploring similar co-inoculation approaches involving RRZ034 could enhance its efficacy and adaptability under diverse environmental conditions. However, further research is needed to confirm these findings across diverse agricultural settings and to better understand the mechanisms in which the PGPR strain RRZ034 promotes plant growth.

The 44 rhizobacterial isolates obtained in this study did not exhibit high functional diversity; many demonstrated consistent PGP traits, including phosphate and potassium solubilization, IAA production, and antifungal activity. The selected strain, RRZ034, was molecularly identified as *Pseudomonas nicosulfuronedens*, consistent with the strain LAM1902 previously described by [Li et al. \(2021\)](#). LAM1902 was isolated from a microbial consortium capable of degrading nicosulfuron, a herbicide, and used it as the sole nitrogen and energy source. Similarly, RRZ034 was isolated from a hill paddy farm established through

conventional land-clearing practices involving herbicide application. This suggests a potential adaptation of RRZ034 to chemically disturbed environments. In line with this, [Riddech et al. \(2024\)](#) reported that their *Pseudomonas nicosulfuronedens* strain AP01 exhibited high phosphate solubilization, strong pathogen suppression, and significantly enhanced the growth of roselle plants. RRZ034 showed comparable characteristics, with notable solubilization indices for both phosphate and potassium, further highlighting its potential as an effective PGPR.

Given that the soil properties can influence the microbial diversity and activity, it is possible that these properties may have supported the natural presence and functionality of *Pseudomonas nicosulfuronedens* RRZ034 in the hill paddy rhizosphere. The moderately acidic pH of the rhizospheric (pH 5.2 in water) aligned well with the growth preferences of *Pseudomonas* species, which are known to thrive in such conditions ([Wu et al., 2021](#)). Although the soil had low total nitrogen (0.07%) and a limited cation exchange capacity (7.99 mEq/100g), the *Pseudomonas nicosulfuronedens* was able to persist may be due to its ability to adapt and contribute to nutrient availability. Notably, the low available phosphorus level (4.00 mg/kg) in the soil may stimulate the phosphate-solubilizing activity of this bacterium, as *Pseudomonas* strains typically demonstrated efficient phosphate uptake ([Li et al., 2024](#)). These soil characteristics can collectively trigger the natural occurrence and functional role of *Pseudomonas nicosulfuronedens* in promoting plant growth under nutrient-limited environments.

Although *P. nicosulfuronedens* has been known for several years, studies on its role as a PGPR remain limited. However, the genus *Pseudomonas* is widely recognized for its plant growth-promoting capabilities. Numerous studies, including those by [Pandey et al. \(2020\)](#) and [Issifu et al. \(2022\)](#), have reported that *Pseudomonas* spp. frequently exhibit traits such as phosphate solubilization, IAA production, biocontrol activity, and stress alleviation. Among them, *P. aeruginosa* is often cited as an effective PGPR. For instance, [Ghamdamgahi et al. \(2022\)](#) and [Chandra et al. \(2020\)](#) reported its ability to promote plant growth through phosphate solubilization, siderophore and IAA production, and effective suppression of pathogens such as *Alternaria alternata*, *Aspergillus flavus*, and *Fusarium oxysporum*.

The identification of RRZ034 as a *Pseudomonas* sp. connects this study to a broader body of research emphasizing the importance of this genus in sustainable agriculture. *Pseudomonas* spp. are well-regarded for their contributions to biofertilization and biocontrol. According to [Okur et al. \(2018\)](#), microbial biofertilizers like *Pseudomonas* spp. enhance soil fertility, stimulate nutrient uptake, and improve crop yield by colonizing the rhizosphere or plant interior. These microbial inoculants, when applied to seeds, plant surfaces, or soil, not only support plant development but also protect against pests and diseases, making them a sustainable and cost-effective solution for modern agriculture ([Raghuwanshi, 2012](#); [Glick et al., 2020](#)).

5. Conclusions

This study identified several rhizobacterial isolates from the hill paddy rhizosphere, with eight strains demonstrating multiple plant growth-promoting traits *in vitro*. Among these, strain RRZ034 (*Pseudomonas nicosulfuronedens*) emerged as the most promising candidate. Although none of the treatments resulted in statistically significant differences in plant growth parameters, T3, which involved strain RRZ034, consistently recorded the highest mean values for germination percentage, root length, shoot height, and vigor index. The consistent performance across all parameters suggested potential biological relevance and highlighted RRZ034 as a promising strain for further investigations and trials. In the future, strain RRZ034 can be explored further for optimized application in crop production. Also, although *Pseudomonas nicosulfuronedens* is not a novel species, its plant growth-promoting traits remain largely underexplored. Future studies should focus on optimizing its formulation using compatible and effective carriers such as biochar or alginate beads to enhance its viability, stability, and shelf life. Additionally, field trials are essential to investigate its effectiveness in improving plant growth, yield, and soil health under natural environmental conditions. These efforts are critical to assess its potential as an effective and sustainable biofertilizer.

CRedit authorship contribution statement

Siti Aisyah Rahman: Writing—original draft, methodology, laboratory analysis, data analysis. **Zakry Fitri Ab Aziz:** Conceptualization, supervision, writing – review & editing, data curation and analysis, fieldwork. **Noorasmah Saupi:** Supervision, validation, writing – review & editing. **Tunung Robin:** Project leader, methodology, investigation, fieldwork. **Nor'aini Abdul Rahman:** Supervision, validation, advisory input. **Philip Lepun:** Conceptualization, field investigation and resources. **Patricia King Jie Hung:** Conceptualization, project support, funding acquisition, advisory input. **Shiamala Devi Ramaiya:** Conceptualization, project support. **Ribka Alan:** Fieldwork, project support, funding acquisition. **Adrian Daud:** Fieldwork, project support, funding acquisition. **Anita Rosli:** Project support, funding acquisition. **Zahora Ismail:** Project administration, funding acquisition. **Sivasangar Seenivasagam:** Conceptualization, project support. **Leong Sui Sien:** Project support, funding acquisition. **Suziana Hassan:** Project support, funding acquisition.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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