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Characterization of endophytic bacteria isolated from wild rice plants in the Mekong Delta, Vietnam

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Abstract. *Thi QVC, Minh NLK, Thuy NP, Tat TQ, Tran T. 2024. Characterization of endophytic bacteria isolated from wild rice plants in the Mekong Delta, Vietnam. Biodiversitas 25: 2576-2585.* Endophytic bacteria bring many benefits to plants, such as stimulating plant growth and inhibiting many microbial pathogens that cause plant diseases. Hence, this investigation aimed to evaluate the association of endophytic bacteria with wild rice (*Oryza rufipogon*) that can fix nitrogen, solubilize phosphorus and produce indole-3-acetic acid (IAA). Twelve-five bacterial strains were recovered from wild rice stems and roots collected from Vinh Long and Tien Giang provinces of the Mekong Delta. Among these strains, six had phosphorus solubilizing and seven had nitrogen-fixing activity. Strain BR3.5 fixed the highest nitrogen, i.e. ammonia (NH_4^+) content of 0.109 ± 0.002 mg/L after eight-day incubation. However, the highest phosphorus solubilization was recorded in two isolates, RR3.7 and RR1.1, with clear zone diameters of 0.800 ± 0.020 mm and 0.800 ± 0.030 mm, respectively. In addition, bacterial strains could synthesize IAA, and BR2.5 produced the highest, i.e., 0.067 ± 0.002 µg/mL, IAA after eight-day incubation. Notably, strain BR3.5 exhibited multiple activities like nitrogen fixation (0.109 ± 0.002 mg/L), phosphorus solubilization (1.02 ± 0.002 mm), and IAA synthesis (0.056 ± 0.00 µg/mL). Strain BR3.5 was identified as *Pantoea* sp. based on 16S rRNA gene sequencing (92.69% similarity) together with morpho-physiological and biochemical characteristics. The findings indicate that strain BR3.5 may be used for the production of biofertilizers for rice farming.

Keywords: Endophytic bacteria, Mekong Delta, nitrogen fixation, phosphorus solubilization, wild rice

Abbreviations: IAA: indole-3-acetic acid, PSI: Phosphate Solubilization Index

INTRODUCTION

Rice (*Oryza sativa* L.) is the principal crop in many nations. The world population will reach 10 billion by 2050, which will concomitantly increase the demand for food (Ranganathan et al. 2018). In addition, the land area for rice cultivation is decreasing due to climate change (Kontgis et al. 2019; Saud et al. 2022). Therefore, ensuring food security is the most challenging issue in Vietnam and many other countries. In Vietnam, the Mekong Delta provides over 50% of the nation's overall output and over 90% of exported rice (Thuy 2021). However, excessive use of chemical pesticides and fertilizers in rice farming has resulted in soil degradation, hardening, and pollution (Baweja et al. 2020). Therefore, new and safer solutions, such as the application of biofertilizers using beneficial bacteria, algae, fungi, and higher plants, to reduce chemical fertilizers and pesticides in rice cultivation have attracted attention in many countries (Ammar et al. 2023). *Oryza rufipogon* (2n = 24, AA) is a wild rice species that commonly grows in the rivers and canals in some Mekong Delta provinces. This species is characterized by perennial growth, a clustered base, and sprawling stems that branch at the nodes. Its height (1-5 m) is influenced by water depth, and it produces open panicles with spikelets typically ranging from 4.5-10.6 mm long and 1.6-3.5 mm wide, adorned with awns of 4-10 cm in length. The anthers within these spikelets measure over 3 mm and can reach up to 7.4 mm long. Wild rice displays a remarkable capacity for adaptation to diverse environments, resistance to pests, diseases, and environmental stressors, cytoplasmic male sterility, and superior grain quality (Henry 2022). These traits make it a valuable genetic resource for enhancing cultivated rice varieties (Lam et al. 2019).

Endophytic bacteria are one of the beneficial groups of microorganisms for plants (Ali et al. 2021) and are currently receiving remarkable attention in many countries, including Vietnam (Anand et al. 2023). This group of microorganisms promotes plant growth by their ability to fix nitrogen (Hao et al. 2024), solubilize phosphorus (Mei et al. 2021), synthesize the growth stimulant IAA (indole-3-acetic acid) (Khianngam et al. 2023), increase mineral nutrient content (Verma et al. 2021), increase disease resistance (Parveen et al. 2023), and help eliminate environmental pollutants (Prodhan et al. 2023). Many studies have also found that endophytic bacteria can help plants withstand biotic and abiotic stress conditions (Chaudhary et al. 2022; Liu et al.

2022) and play a role in increasing crop productivity (Mbaye et al. 2023; Watts et al. 2023). Hernández et al. (2023) demonstrated that endophytic *Pseudomonas* spp. and *Pantoea* sp. strains of rice in Cuba and Chile increased root length, height, fresh weight, and the root and shoot dry weight after twenty-one days after inoculation.

Many endophytic bacteria are found in the stems, roots, and leaves of rice, such as *Azospirillium* sp. (Kaneko et al. 2010), *Pantoae* (Hernández et al. 2023), *Burkholderia* sp. (Kong and Hong 2020; Pal et al. 2022), *Enterobacter* sp. (Parveen et al. 2023; Prodhan et al. 2023), *Bacillus* (Bolivar-Anillo et al. 2021), and *Herbaspirillium* sp. (Walitang et al. 2023). In Vietnam, previous studies have also isolated and selected several beneficial endophytic bacterial strains in rice soil (Thuy et al. 2022). In the Mekong Delta, it has been shown that endophytic bacteria can decrease nitrogen and phosphate fertilizer application for rice plants while still ensuring rice yield and quality (Nhu et al. 2014). In particular, many studies have shown the presence and plant growth-stimulating role of endophytic bacteria in wild rice plants in many different regions of the world (Elbeltagy et al. 2000; Das et al. 2021; Peng et al. 2021). However, to date, reports of endophytic bacteria in wild rice plants in the Mekong Delta are limited. Therefore, it is essential to study and select of endophytic bacteria with beneficial traits such as nitrogen fixation, phosphorus solubilization, and IAA synthesis in wild rice plants in the Mekong Delta.

MATERIALS AND METHODS

Source of bacterial isolation

To isolate endophytic bacteria, wild rice stems and roots were collected from two provinces of the Mekong Delta: Vinh Long and Tien Giang (Figure 1). At each location, wild rice stem and root samples were collected at the flowering stage and at five locations on the same site. Rice samples were placed in sterile plastic bags and transported to the laboratory for the isolation of the endophytic bacteria.

Isolation of endophytic bacteria

Endophytic bacteria were isolated from the stems and roots of wild rice, according to Rangjaroen et al. (2015), with minor modifications. First, the samples (stem and root) were rinsed several times with tap water, then cut separately into about 1 cm pieces. The samples were soaked in 96% alcohol for 2-3 minutes, washed with sterile distilled water, and immersed in a 1% sodium hypochloride solution for 3 minutes. Finally, the samples were washed three times with sterile distilled water. Sterile rice stem and root samples (10 g) were grounded separately with sterile distilled water, and were then diluted to 10^{-6}. An aliquot (100 µL) of each dilution was spread onto TSA (Himedia, India) medium and placed in an incubator at 30°C for 2-3 days. Bacterial colonies grown on TSA medium were sub-cultured many times until pure colonies were obtained. As per Cappuccino and Welsh (2017), the features of colony morphology (shape, color, edge, elevation, and size), Gram stain, spore staining, catalase, and oxidase reactions of the isolated bacterial strains were investigated.

Nitrogen fixation ability of isolated bacterial strains

Purified bacterial strains were tested for their nitrogen fixation properties by culturing them many times on nitrogen-free Burk medium (Park et al. 2005). Bacteria grown on nitrogen-free Burk medium after 24-48 hours of incubation at 30°C are considered to be capable of nitrogen fixation. They were selected, sub-cultured and stored in glycerol 20% (v/v) at -80°C.

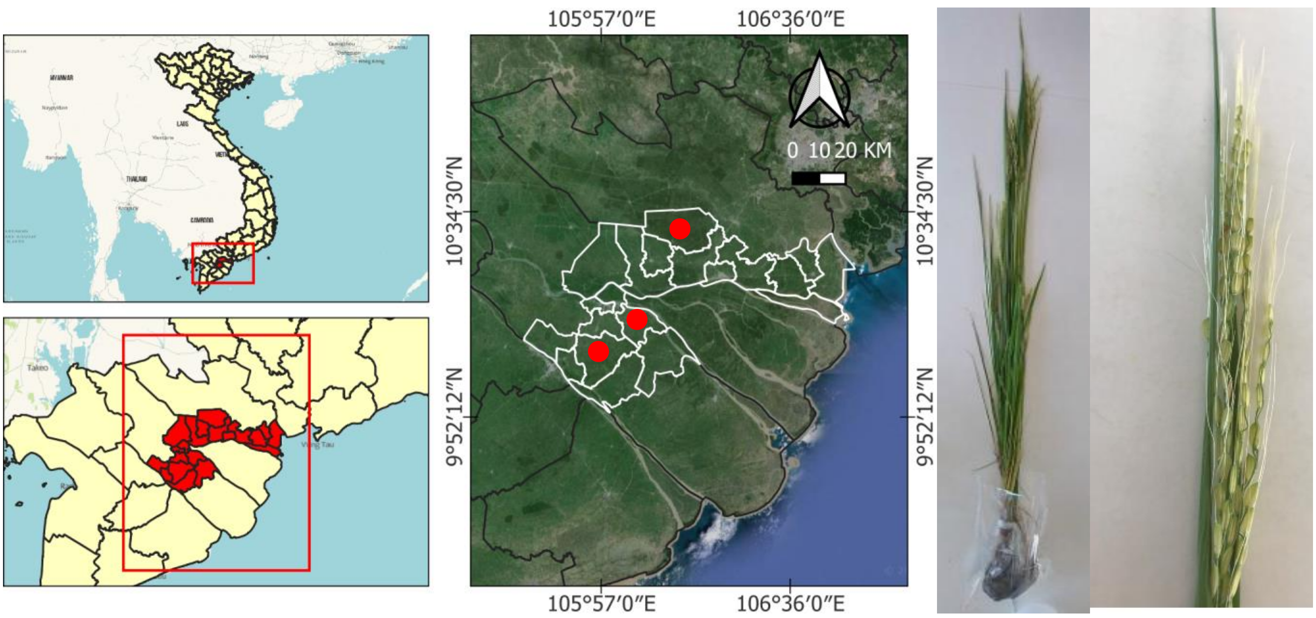

Figure 1. Location of the wild rice sample (blue circle) for isolation of endophytic bacteria in Vinh Long and Tien Giang, Vietnam

The indophenol method developed by Solarzano (1969) was used to assess bacterial strains for nitrogen fixation. First, bacteria were grown in nitrogen-free Burk medium on a shaker at 120 rpm. Then, the culture was centrifuged at 10,000 g to collect bacterial biomass. Bacterial suspensions were prepared at 10^8 CFU/mL (compared to MacFarland standard tubes) and added to nitrogen-free Burk medium. The culture after bacterial inoculation was placed on a shaker at 120 rpm. Finally, NH_4^+ content was determined at 640 nm on days 2, 4, 6, and 8 after bacterial inoculation.

Ability of phosphorus solubilization by isolated bacterial strains

Phosphorus solubilization properties of isolated bacterial strains were tested by culturing on NBRIP medium (Nautiyal 1999). The NBRIP plates were incubated for 24-48 hours at 30°C. The ability of the endophytic bacteria to dissolve phosphate is demonstrated by the formation of a clear zone. These bacterial strains were isolated on the same medium and stored in 20% (v/v) glycerol at -80°C.

Phosphorus solubilization by the isolated bacterial strain was quantified following Pathak et al. (2018). Bacteria were cultured on NBRIP medium, and halo diameter around the colony was measured on days 2, 4, and 6. The ability of the isolated bacterial strain to dissolve phosphorus was evaluated according to the formula: PSI (Phosphate Solubilization Index) = clear zone diameter (including colony diameter)/colony diameter (Pathak et al. 2018).

Indole-3-acetic acid (IAA) production of isolated bacterial strains

The ability of the isolated bacterial strain to synthesize IAA was determined based on the color reaction with the Salkowski reagent of Gordon and Weber (1951). Bacteria were grown in nitrogen-free Burk medium without tryptophan supplementation. The culture was incubated in the darkness at 28±2°C and 2, 4, 6, and 8 days after bacterial inoculation, cultures were centrifuged at 12,000 g for 5 minutes. Finally, the supernatant was reacted with a Salkowski reagent, and the optical absorbance of the sample was measured at 530 nm after incubation in the darkness for 15 minutes.

Identification and sequencing of the 16S rRNA gene fragment

Bacterial strains with high nitrogen fixation, phosphate solubilization, and IAA synthesis activities were selected to identify and sequence the 16S rRNA gene fragment. Bacterial DNA was extracted according to Sambrook et al. (1989). After extraction, DNA was checked for purity and concentration at wavelengths of 260 nm and 280 nm. They were amplified by PCR for the 16S rRNA gene segment. The 16S rRNA gene segments were amplified by the forward primer 27F and reverse primer 1492R (Weisburg et al. 1991). PCR reactions were performed in 25 µL, including 11.75 µL distilled water, 2.5 µL PCR buffer 1X, 2 µL $MgCl_2$ 2.0 mM, 4 µL dNTPs 150 µM, 1 µL primer 27F 10 pmol, 1 µL primer 1492R 10 pmol, 0.25 µL *Taq* DNA polymerase 2.0 UI, and 2 µL DNA sample (40 ng). The PCR reaction cycle was: initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. PCR products (1.500 bp) were electrophoresed on a 1.5% agarose gel at 5 v/cm. Following purification, the PCR product was delivered to Macrogen Company (Korea) for sequencing.

Data analysis

The data and graphs in the experiment were entered and processed using Microsoft Excel 2010 software. Duncan's tests and ANOVA (One-way analysis of variance) were used to examine the treatment differences with a 95% confidence level. The phylogenetic tree was built using MEGA X software, and the bootstrap value was 1,000 replications (Tamura et al. 2013).

RESULTS AND DISCUSSION

Isolation and morpho-physiological and biochemical characteristics of isolated endophytic bacterial isolates

Twenty-five bacterial strains from the roots (14/25 isolates) and stems (11/25 isolates) of wild rice plants were grown on TSA medium (Table 1). Most bacterial strains's colonies are opaque white (15/25 isolates) or clear white (10/25 isolates), regular (17/25 isolates) or irregular (8/25 isolates) round form, entire-edge (100%), raised elevation (100%), and wet surface. All bacterial strains do not produce spores, belong to the Gram-negative (10/25) or Gram-positive groups (15/25 isolates), are rod-shaped (15/25 isolates) or coccus (10/25 isolates), and are motile. In addition, most bacterial strains are found to have positive catalase (16/25 isolates) and positive oxidase (16/25 isolates) activity.

Selection of nitrogen-fixing bacteria

From twenty-five strains, six bacterial strains of stems (1/6 isolate) and roots (5/6 isolate) of wild rice could fix nitrogen in nitrogen-free Burk's medium (Table 2). The majority of bacterial strains have elevated, regular, round-shaped colonies that are either opaque or transparent white (Figures 2.A and 2.B). Bacterial strains are rod-shaped or coccus, motile, and positive or negative for oxidase and catalase activity. They do not produce spores and fall into the gram-positive or gram-negative (Figure 2.C) categories.

Screening of phosphorus-solubilizing bacteria

The isolated bacterial strains displayed various morphological traits. Most strains were regular, round in shape, small to large in size. Three different colors of the colony were found in seven phosphorus-solubilizing bacterial isolates, consisting of pale yellow (Figure 3.A), opaque white (Figure 3.B), and clear white. The results also demonstrated that the isolated bacterial strains had short-rods or coccus, were positive or negative for oxidase and catalase activity, and belonged to the gram-positive or gram-negative group (Figure 3.C).

Table 1. Morpho-physiological and biochemical characteristics of isolated endophytic bacterial isolates

Bacterial isolates	Source	Colony morphology					Cell shape	Gram staining	Catalase	Oxidase
		Form	Color	Edge	Elevation	Size				
BR1.1	Root, Vinh Long	Irregular, round	Opaque white	Entire	Raised	Small	Coccus	Gram-negative	+	+
BR1.2	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Coccus	Gram-negative	+	-
BR2.2	Stem, Vinh Long	Irregular, round	Clear white	Entire	Raised	Small	Short-rod	Gram-negative	-	-
BR2.3	Root, Vinh Long	Regular, round	Clear white	Entire	Raised	Small	Short-rod	Gram-posive	-	-
BR2.4	Stem, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Short-rod	Gram-negative	+	+
BR2.5	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small	Short-rod	Gram-negative	-	+
BR3.1	Root, Vinh Long	Regular, round	Opaque white	Entire	Raised	Small	Short-rod	Gram-posive	+	+
BR3.2	Stem, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Coccus	Gram-posive	+	+
BR3.4	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small	Short-rod	Gram-posive	+	+
BR3.5	Root, Vinh Long	Regular, round	Clear white	Entire	Raised	Large	Short-rod	Gram-posive	-	-
BT3.5	Stem, Vinh Long	Regular, round	Opaque white	Entire	Raised	Large	Coccus	Gram-posive	+	+
BT3.6	Stem, Tien Giang	Regular, round	Clear white	Entire	Raised	Small	Coccus	Gram-posive	+	+
RR1.1	Root, Vinh Long	Irregular, round	Clear white	Entire	Raised	Large	Short-rod	Gram-posive	-	+
RR1.2	Stem, Vinh Long	Irregular, round	Clear white	Entire	Raised	Large	Short-rod	Gram-negative	-	+
RR2.3	Stem, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Short-rod	Gram-negative	+	+
RR2.4	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Short-rod	Gram-posive	+	+
RR2.5	Stem, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large	Short-rod	Gram-posive	+	-
RR2.6	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small	Short-rod	Gram-posive	+	+
RR3.7	Root, Vinh Long	Regular, round	Opaque white	Entire	Raised	Small	Coccus	Gram-posive	+	+
RR3.8	Stem, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small	Coccus	Gram-negative	+	+
RR4.1	Root, Tien Giang	Irregular, round	Clear white	Entire	Raised	Small	Short-rod	Gram-negative	-	-
RR5.2	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small	Short-rod	Gram-negative	+	+
RT2.1	Stem, Tien Giang	Irregular, round	Clear white	Entire	Raised	Large	Coccus	Gram-posive	-	-
RT2.2	Stem, Tien Giang	Irregular, round	Clear white	Entire	Raised	Large	Coccus	Gram-posive	+	-
RT2.3	Root, Tien Giang	Irregular, round	Clear white	Entire	Raised	Large	Coccus	Gram-posive	-	-

Note: +: positive reaction; -: negative reaction

Table 2. Morphological characteristics of isolated endophytic nitrogen-fixing bacterial isolates observed on Burk medium

Bacterial isolates	Source	Colony morphology				
		Form	Color	Edge	Elevation	Size
BR1.1	Root, Vinh Long	Irregular, round	Opaque white	Entire	Raised	Small
BR2.3	Root, Vinh Long	Regular, round	Clear white	Entire	Raised	Small
BR2.5	Root, Vinh Long	Regular, round	Opaque white	Entire	Raised	Small
BR3.1	Root, Vinh Long	Regular, round	Opaque white	Entire	Raised	Small
BR3.5	Root, Vinh Long	Regular, round	Clear white	Entire	Raised	Large
BT3.6	Stem, Tien Giang	Regular, round	Clear white	Entire	Raised	Small

Table 3. Morphological characteristics of endophytic phosphorus-solubilizing bacterial isolates isolated on NBRIP medium

Bacterial isolates	Source	Colony morphology				
		Form	Color	Edge	Elevation	Size
BR2.3	Root, Vinh Long	Regular, round	Pale yellow	Entire	Raised	Small
BR3.5	Root, Vinh Long	Irregular, round	Opaque white	Entire	Raised	Large
RR1.1	Root, Vinh Long	Irregular, round	Clear white	Entire	Raised	Large
RR2.4	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Large
RR2.6	Root, Tien Giang	Regular, round	Opaque white	Entire	Raised	Small
RR3.7	Root, Vinh Long	Regular, round	Opaque white	Entire	Raised	Small
RT2.1	Stem, Tien Giang	Irregular, round	Clear white	Entire	Raised	Large

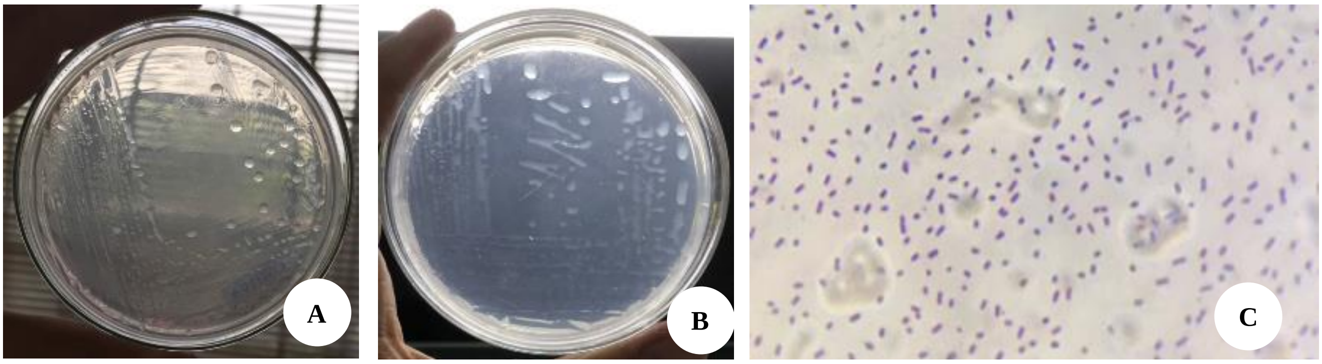

Figure 2. Nitrogen-fixing endophytic bacterial strains isolated on nitrogen-free Burk's medium: A. Clear white colonies, convex, and wet surface; B. Colonies are opaque white, round, raised, and have a wet surface; C. Gram staining (100X)

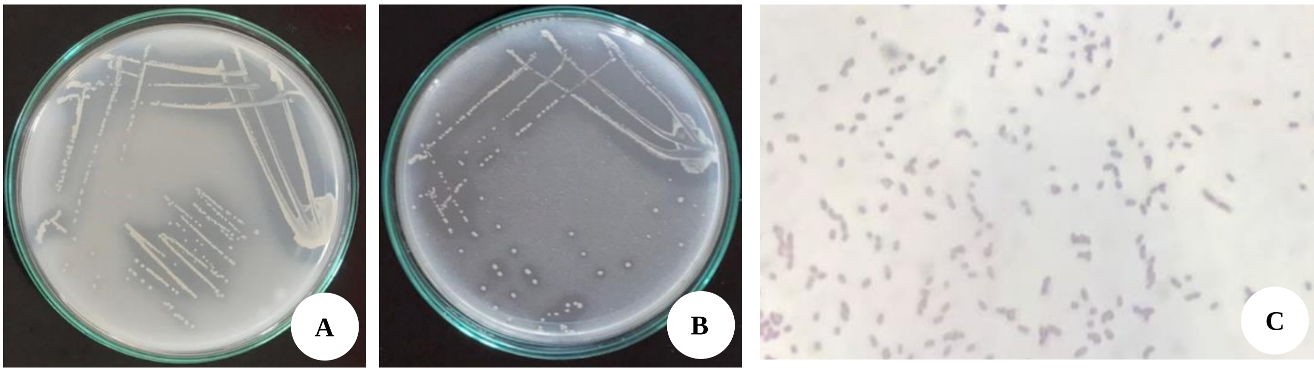

Figure 3. Phosphorus solubilizing endophytic bacterial isolates were isolated on NBRIP medium: A. Colonies are pale-yellow, round, and convex; B. Colonies are opaque white, small, and round; C. Gram staining (100X)

Nitrogen fixation ability of bacterial strains

Six of the bacterial strains were capable of fixing nitrogen (Table 4). After the eighth day of culture, the ammonium level in nitrogen-free Burk media ranges from 0.048 to 0.109 mg/L. Strain BR3.5 exhibited maximum nitrogen fixation activity with an ammonium content of 0.109 ± 0.002 mg/L, and strain BR2.3 had the lowest at 0.048 ± 0.000 mg/L.

Phosphorus solubilizing ability of bacterial strains

The isolated bacteria produced a phosphate-solubilizing clear zone on day two, expanded rapidly on day four, and the phosphate-solubilizing diameter shrank on day six (Table 5). After the sixth day of culture, the diameter of the phosphorus dissolving zone varied between 0.4 and 0.8 mm for the bacterial strains. The two studied bacterial strains, RR3.7 and RR1.1, exhibited maximum phosphorus-solubilizing activity, measuring 0.800 ± 0.020 mm and 0.800 ± 0.030 mm zone, respectively, and strain BR2.3 exhibited the lowest phosphate solubilizing activity (0.400 ± 0.000 mm zone).

IAA production from bacterial strains

The study identified five bacteria capable of synthesizing IAA. The IAA content of bacterial strains after the eighth day of culture ranged from 0.027-0.067 µg/mL (Table 6). Strain BR2.5 produced the highest IAA with a concentration of 0.067±0.002 µg/mL, and strain BT 3.6 had the lowest (0.027±0.000 µg/mL) production after the eighth day of bacterial inoculation.

Identification of bacterial strains by ribosomal gene sequence

The 16S rRNA gene sequencing result indicated that strain BR3.5 is 98.41% related to *Pantoea* sp. strain MBWS32 (OP990157.1) in the GenBank. According to the phylogenetic tree, strains BR3.5 and *Pantoea dispersa* strain A1 (MN8311963.1) belong to the same group (Figure 4).

Discussion

In the present research, nineteen endophytic bacteria, consisting of twelve strains of phosphate-solubilizing and six nitrogen-fixing bacteria, were obtained from the roots and stems of wild rice in the Mekong Delta. However, the number of endophytic bacterial strains isolated from wild rice roots was higher than that from the stems.

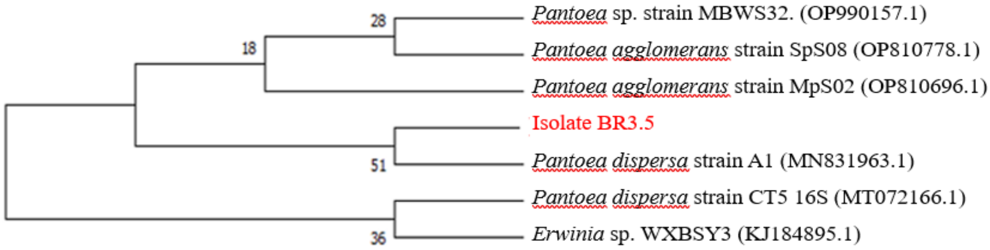

Figure 4. The genetic relationship between isolated bacterial isolate and reference strains on GenBank is shown in the phylogenetic tree

Table 4. Nitrogen fixation activity of endophytic bacterial strains from wild rice

Bacterial isolates	NH_4^+ content (mg/L)				
	Day 2	Day 4	Day 6	Day 8	Mean
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BR1.1	0.022[bc] ± 0.001	0.041[b] ± 0.001	0.066[ab] ± 0.001	0.083[c] ± 0.000	0.053 ± 0.001
BR3.5	0.031[a] ± 0.003	0.048[a] ± 0.000	0.069[a] ± 0.002	0.109[a] ± 0.002	0.064 ± 0.002
BR2.5	0.016[d] ± 0.001	0.025[cd] ± 0.001	0.034[d] ± 0.001	0.054[d] ± 0.002	0.032 ± 0.001
BR3.1	0.011[e] ± 0.002	0.024[d] ± 0.001	0.037[d] ± 0.001	0.059[d] ± 0.002	0.033 ± 0.002
BR2.3	0.018[cd] ± 0.001	0.027[c] ± 0.001	0.043[c] ± 0.002	0.048[e] ± 0.000	0.034 ± 0.001
BT3.6	0.025[b] ± 0.001	0.044[a] ± 0.001	0.064[b] ± 0.001	0.095[b] ± 0.002	0.057 ± 0.001

Note: Numbers with the same letter in the same column are not significantly different (p>0.05)

Table 5. Phosphate-solubilizing zone diameter of endophytic bacterial strains isolated from wild rice

Bacterial isolates	Phosphorus solubilization diameter (mm)			
	Day 2	Day 4	Day 6	Mean
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
RR2.6	0.423[a] ± 0.025	0.607[a] ± 0.011	0.693[b] ± 0.011	0.574 ± 0.016
RR2.2.4	0.200[c] ± 0.020	0.497[b] ± 0.025	0.507[c] ± 0.040	0.401 ± 0.028
RR3.7	0.303[b] ± 0.005	0.610[a] ± 0.017	0.800[a] ± 0.020	0.571 ± 0.014
RR1.1	0.307[b] ± 0.020	0.500[b] ± 0.020	0.800[a] ± 0.030	0.536 ± 0.023
BR2.3	0.123[d] ± 0.025	0.393[c] ± 0.011	0.400[d] ± 0.000	0.305 ± 0.012
RT2.2.1	0.283[b] ± 0.015	0.490[b] ± 0.010	0.643[b] ± 0.011	0.472 ± 0.012
BR3.5	0.113[d] ± 0.005	0.333[d] ± 0.015	0.407[d] ± 0.011	0.284 ± 0.010

Note: Numbers with the same letter in the same column are not significantly different (p>0.05)

Table 6. IAA synthesis ability of endophytic bacterial strains isolated from wild rice

Bacterial isolates	IAA content (µg/mL)				
	Day 2	Day 4	Day 6	Day 8	Mean
Control	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
BR1.1	0.006^{c} ± 0.000	0.011^{c} ± 0.001	0.023^{d} ± 0.000	0.033^{d} ± 0.001	0.018 ± 0.001
BR3.5	0.012^{b} ± 0.001	0.026^{ab} ± 0.001	0.039^{b} ± 0.002	0.056^{b} ± 0.002	0.033 ± 0.002
BR2.5	0.015^{a} ± 0.001	0.027^{a} ± 0.001	0.046^{a} ± 0.001	0.067^{a} ± 0.002	0.039 ± 0.001
BR3.1	0.016^{a} ± 0.001	0.023^{b} ± 0.001	0.033^{c} ± 0.000	0.041^{c} ± 0.001	0.028 ± 0.001
BR2.3	0.000^{d} ± 0.000	0.000^{d} ± 0.000	0.000^{e} ± 0.000	0.000^{f} ± 0.000	0.000 ± 0.000
BT3.6	0.004^{c} ± 0.001	0.012^{c} ± 0.001	0.020^{d} ± 0.002	0.027^{e} ± 0.000	0.016 ± 0.001

Note: Numbers with the same letter in the same column are not significantly different (p>0.05)

This result might be explained by the fact that endophytic bacteria thrive in the favorable aeration, water, and substance secretion conditions seen in rhizospheric soils. The strain BR3.5 was identified as *Pantoea* sp. based on 16S rRNA gene sequencing with 91.74% similarity in combination morphological and biochemical characteristics. This result is in line with a study by many previous studies showing that endophytic *Pantoea* has been detected in many staple crops, including maize (Gao et al. 2019), sugarcane (Singh et al. 2021), rice (Lu et al. 2021), sweet potato (Khan and Doty 2009), and citrus (Andreote et al. 2008). Our research has demonstrated that strain BR3.5 exhibits nitrogen fixation, phosphate solubilization, and IAA synthesis activities. This result is similar to many previous studies that have shown the potential of *Pantoea* spp. for solubilizing phosphorus (Suleimanova et al. 2023; Ma et al. 2023), nitrogen-fixing (Singh et al. 2021), and IAA production (Melini et al. 2023).

In the current finding, six bacterial strains from wild rice could fix nitrogen in nitrogen-free Burk's medium. The research shows that the fixed nitrogen content of bacterial strains is between 0.019 and 0.157 mg/L. The nitrogen content (NH_4^+) of the bacterial isolates in this investigation was lower than that of the finding by Parveen et al. (2023), which showed that the ammonia production of the endophytic bacterial isolates obtained from the stems, roots, and leaves of four well-known rice cultivars ranged from 83.42 µg/mL (b55) to 12.78 µg/mL (b18). Thuy et al. (2022) revealed that endophytic bacterial isolates from the roots and stems of rice variety HT1 in Thua Thien Hue province have the capability of nitrogen-fixing 1.42-23.80 mg/L. in which, three strains of TQP'1, TQP'3, and THC1 had the highest activity levels, with nitrogen fixation concentrations of 23.8, 22.55, and 10.43 mg/L NH_4^+, respectively. Lynh and Hiệp (2019) reported that bacterial endophytes in *Coffea canephora* grown in Dak Lak province were capable of synthesizing NH_4^+, with synthesis capacity ranging from 0.121-0.289 mg/L, of which strain L.R150-3 had the highest ability of N_2-fixing with 0.289 mg/L of NH_4^+concentration. Nhu and Diep (2014) showed that the concentration of nitrogen fixation (NH_4^+) in endophytic bacteria from rice plants in Phu Yen province of Vietnam ranged from 2.23 to 6.09 mg/L. Previous studies have shown that the ability of bacteria to fix nitrogen depends on many different factors, such as bacterial species, crops, and environmental, and soil conditions (Soumare et al. 2020).

In the present investigation, seven phosphorus-solubilizing bacterial isolates were found in wild rice. The results show that the average halo in bacterial strains ranges from 0.272-0.743 mm. The halozone demonstrates that the isolated bacterial strains can solubilize phosphate (Prihatiningsih et al. 2021). However, the clear zone diameter of endophytic bacterial strains in this finding is lower than in many previous studies. According to Sudewi et al. (2020), eight bacterial strains obtained in the local aromatic rice plant's rhizosphere in Indonesia formed a halozone with an area of 0.84-2.66 cm, and the PSI ranged from 2.17-2.33 at an acidic pH between 4.27-5.67. Similar results by Kirui et al. (2022) revealed that PSI isolated from semi-arid agroecosystems in eastern Kenya ranged between 1.143 and 5.883. In India, meanwhile, according to Pande et al. (2017), phosphorus-solubilizing bacteria were found to have a high PSI, ranging from 4.88 to 4.48, when they were collected from agricultural fields. The variation in the width of the halo zone produced by bacterial isolates is due to the differences in the ability of each isolate to secrete extracellular organic acids, the production of polysaccharides, or the activity of phosphatase enzymes in phosphate-solubilizing bacterial strains (Oteino et al. 2015). Whereas the changes in the medium around the colony from turbid to clear are due to the decreasing pH of the medium used (Paul and Sinha 2016). Because phosphate often occurs in the soil in a form that is unavailable to plants, bacteria's capacity to dissolve phosphate helps make nutrients available for plants (Olanrewaju et al. 2017). According to Mugiastuti et al. (2020), endophytic bacteria associated with maize can solubilize various types of phosphate, increasing its availability for plant uptake, growth, and yield.

Indole-3-acetic acid (IAA) is one hormone that can promote plant development by increasing the stem extension, elongation process, and cell differentiation (Zhang et al. 2022). The fitness of microbial-plant interactions can be enhanced by IAA production from bacterial isolates (Etesami et al. 2015). In the present research, the results show that the average amount of IAA concentration in bacterial strains ranges from 0.011-0.028 µg/mL. These findings are similar to many previous reports (Thanh et al. 2016; Das et al. 2021). However, the finding is lower than in a study by Khianngam et al. (2023), who revealed that the IAA synthesis of two endophytic bacterial strains, VR2 and MG9, derived from *Chrysopogon zizanioides* (L.), and *Bruguiera cylindrica* (L.) collected from a mangrove forest in Thailand, significantly increased the yield of IAA to 246.00 and 195.55 µg/mL in 1,000 µg/mL L-tryptophan over 48 hours at pH 6. Herlina et al. (2017) reported that sixteen endophytic bacterial isolates from peanut plants (*Arachis hypogaea*) could produce IAA. Following the fourth incubation day, the IAA concentrations that were highest were 69.68 mg/L, and the lowest were 8.50 mg/L. Hien et al. (2021) found that ten endophytic bacterial isolates collected from the roots of river mangrove (*Aegiceras corniculatum* L.) in Nam Dinh province were capable of producing IAA with a concentration of 3.01-47.20 µg/mL. In the report of Giang et al. (2016) showed that synthesized IAA content by endophytic bacterial strains from plant roots (*Aloe vera*) reaches 17.18 to 23.23 µg/mL. Trang et al. (2018) isolated four strains of endophytic bacteria from pepper roots that can synthesize IAA combinations from 24 to 68 µg/mL.

In conclusion, this study shows that endophytic bacteria exist in the Mekong Delta and can fix nitrogen, solubilize phosphorus, and synthesize IAA from the wild rice roots and stems. Based on 16S rRNA gene sequencing (92.69% similarity), colony shape, and biochemical traits, strain BR3.5 in the study was identified as *Pantoea* sp. It is noteworthy that strain BR3.5 is capable of numerous IAA synthesis, phosphate solubilization, and nitrogen fixation processes. The result demonstrates how bacterial strains may be used in biosafety rice farming.

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