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Isolation and characterization of plant growth-promoting rhizobacteria from wild rice (*Oryza rufipogon*) in the Mekong Delta, Vietnam

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Abstract. *Chi NTY, Minh NLK, Thi QVC. 2025. Isolation and characterization of plant growth-promoting rhizobacteria from wild rice* (Oryza rufipogon) *in the Mekong Delta, Vietnam. Biodiversitas 26: 1221-1228.* Rhizospheric bacteria play a very crucial role in crops, especially rice. In contrast to cultivated varieties, wild rice (*Oryza rufipogon*), which is abundantly found in Mekong Delta canals, may have distinct rhizobacterial populations. Therefore, the objective of this work was to isolate and characterize Plant Growth-Promoting Rhizobacteria (PGPR) from wild rice with the characteristics of nitrogen fixation, phosphate solubilization, and IAA synthesis. A total of 12 bacterial strains, including seven bacterial strains of nitrogen-fixation and five strains of phosphate-solubilizing microbes, from four rhizospheric soil samples of wild rice in Tien Giang and Vinh Long provinces of the Mekong Delta, Vietnam. The results showed that strain BĐ1.2 had the highest nitrogen fixation activity with NH_4^+ content of 0.281 ± 0.007 mg/L. In contrast, strain BĐ1.3, had the highest phosphorus solubilizing ability with a halo diameter of 1.123 ± 0.025 mm. Also, the finding showed that six out of twelve bacterial strains were capable of synthesizing IAA (Indole-3-Acetic Acid), of which strain BĐ2.3.1 produced the highest IAA with a concentration of 0.053 ± 0.001 mg/L after 8 days of bacterial inoculation. In particular, BĐ2.3.2, BĐ1.3, and BĐ2.4 simultaneously exhibited the ability of nitrogen fixation, phosphate solubilization, and IAA synthesis. The strain BĐ1.3 was identified as *Stenotrophomonas* sp. based on colony morphology, biochemical characteristics, and 16S rRNA gene sequencing with 91.74% similarity. To our knowledge, this is the first report of isolation of rhizospheric bacteria from *O. rufipogon* with the characteristics of nitrogen fixation, phosphorus solubilization, and IAA production in the Mekong Delta, Vietnam.

Keywords: Indole acetic acid, nitrogen fixation, *Oryza rufipogon*, phosphorus solubilization, *Stenotrophomonas* sp.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the essential food crops not only in Vietnam but also in many other countries in Asia, such as India, Thailand, China, and Cambodia. In 2023, Vietnam was the country with the third largest rice export output worldwide, with an output of 8.1 million tons (reaching a turnover of 4.68 billion USD) and was forecasted to have a lot of potential to increase output and prices of rice export value due to increased global rice demand (Chi 2024). During rice cultivation, farmers often employ a lot of pesticides and chemical fertilizers to ensure output for domestic supply and export (Nguyen 2017). However, the use of chemical fertilizers and pesticides has affected human health, polluted the environment, and negatively impacted on the natural ecosystem (Pathak et al. 2022). Currently, environmentally and friendly organic rice farming is being focused on by many countries, and this trend is increasing (Uphoff and Dazzo 2016; Tu et al. 2018). The production of microbial fertilizers from bacteria capable of fixing nitrogen and phosphorus solubilization as well as synthesizing plant growth-stimulating hormones such as IAA and gibberellin is more popular for rice cultivation (Timofeeva et al. 2023). The advantage of microbial fertilizers is that they do not pollute the environment, restore soil fertility, and help save costs while still ensuring productivity and quality of agricultural products (Timofeeva et al. 2023; Wei et al. 2024).

Wild rice (*Oryza rufipogon* Griff.) is considered the ancestor of today's cultivated rice (*O. sativa*) (Thanh and Hirata 2002). This species grows wild in swamps or along canals in many different regions of the Mekong Delta. Many studies have shown that wild rice species contain many resistance genes that are used to improve cultivated rice varieties, such as insect resistance (Rahman et al. 2009) and disease resistance genes (Angeles-Shim et al. 2020; Chen et al. 2022). In addition, wild rice also has genes that are highly resistant to salinity, alum, drought, flooding, and high and low temperatures (Quan et al. 2018; Yichie et al. 2018; Kairam and Sran 2022). In particular, many recent studies around the world have shown that the presence of plant growth-promoting rhizobacteria on wild rice plants has many properties that can be exploited in rice cultivation (Zhang et al. 2021; Chang et al. 2022; Tian et al. 2023).

Plant Growth-Promoting Rhizobacteria (PGPR) are a class of bacteria that influence on the growth and development of plants in many ways, including biological nitrogen fixation and release of growth hormones (cytokinin, IAA, and gibberellin), enhanced absorption of nutrients (solubilized phosphorus, potassium), and enhanced resistance to soil-borne pathogens through siderophore production (Ahemad and Kibret 2014; Verma et al. 2016; Olanrewaju et al. 2017; Riddech et al. 2022; Yao et al. 2022; Ahbar et al. 2024; Ramly et al. 2024). Additionally, they are crucial in controlling how plants react to both biotic and abiotic stressors (Shameer and Prasad 2018; Kumar et al. 2022). Many studies in the world and in Vietnam have demonstrated that PGPR bacteria have promoted the growth of rice stems, leaves, and roots, increased the plant's resistance to adverse conditions, and increased rice productivity (Sharma et al. 2014; Abd El Mageed et al. 2022; Cẩm et al. 2023; Chen et al. 2023). Up to now, however, there have not been many studies on PGPR bacteria from wild rice in Vietnam. Therefore, the aim of this study was to isolate indigenous PGPR bacteria with the plant growth stimulating characteristics, such as nitrogen fixation, phosphorus solubilization, and IAA production from wild rice. Bacterial strains with potential plant growth promoting properties can be used to produce microbial fertilizers to reduce to reduce chemical fertilizers and pesticides in agricultural production.

MATERIALS AND METHODS

Isolation of bacteria

To isolate rhizobacteria, soil was collected from the rhizosphere of wild rice in the Mekong Delta's Tien Giang and Vinh Long Province, Vietnam (Figure 1). At each location, soil samples (0.5-1 kg) from the wild rice rhizosphere (flowering stage, Figure 1) were collected at 5 locations with a depth of 0-10 cm. The soil sample was then mixed and left to dry naturally in the laboratory.

Isolation of nitrogen-fixing bacteria

After drying, the soil sample was ground finely and stored in the refrigerator. A 10 g sample of soil was mixed with 90 mL of sterile distilled water and placed overnight on a shaker at 110 rpm. The sample was then serially diluted to 10^6. From each dilution, 50 µL of sample was spread onto an agar plate containing nitrogen-free Burk's medium, including (g/L): sucrose 10, KH_2PO_4 0.41, K_2HPO_4 0.52, Na_2SO_4 0.05, $CaCl_2$ 0.2, $MgSO_4.7H_2O$ 0.1, $FeSO_4.7H_2O$ 0.005, $Na_2MoO_4.2H_2O$ 0.0025, and pH 7.0 (Park et al. 2005) to isolate bacteria. Bacterial isolates grown on nitrogen-free Burk's medium after 24-48 hours at 30°C were selected for purity and stored in 20% (v/v) glycerol at -80°C for further studies.

Isolation of phosphorus-solubilizing bacteria

The samples after dilution as above were spread onto NBRIP (National Botanical Research Institute's Phosphate) medium (g/L), including: glucose 10, $Ca_3(PO_4)_2$ 2, $MgCl_2.6H_2O$ 5, $MgSO_4.7H_2O$ 0.25, KCl 0.2, $(NH_4)_2SO_4$ 0.2, NaCl 0.2, yeast extract 0.2, and pH 7.0 (Nautiyal 1999) to isolate phosphorus-solubilizing bacteria. After spreading the samples, plates were incubated at 30°C for 24-48 hours. The strains formed clear zones around the colony (halo) showed that bacterial strains were capable of solubilizing phosphate. They were sub-cultured on the same medium for purity and stored in 20% (v/v) glycerol at -80°C for further studies.

Nitrogen fixation ability of bacterial strains

The nitrogen fixation ability of bacterial strains was tested by the indophenol method of Solórzano (1969). First, bacteria were cultured in broth nitrogen-free Burk's medium on a shaker at 120 rpm. The bacterial solution was then centrifuged at 10,000 rpm to collect bacterial biomass. Bacterial suspensions were prepared at 10^8 CFU/mL (compared to standard MacFarland tubes) and inoculated onto broth nitrogen-free Burk's medium. The solution after bacterial inoculation was placed on a shaker at a speed of 120 rpm. Finally, ammonia (NH_4^+) content was determined at 640 nm wavelength on days 2, 4, 6, and 8 after bacterial inoculation. The control treatment was performed similarly but broth in nitrogen-free Burk's medium without bacteria.

Phosphate solubilizing capacity of bacterial strains

The phosphorus solubilization ability of bacterial strains was checked using the method of Qingwei et al. (2023). Bacteria were cultured on NBRIP medium, and the halo diameter around the colony was measured on days 2, 4, and 6. The capacity of isolated bacterial strain to dissolve phosphorus was calculated according to the formula: R = I (inhibition diameter)/C (colony diameter) (Qingwei et al. 2023).

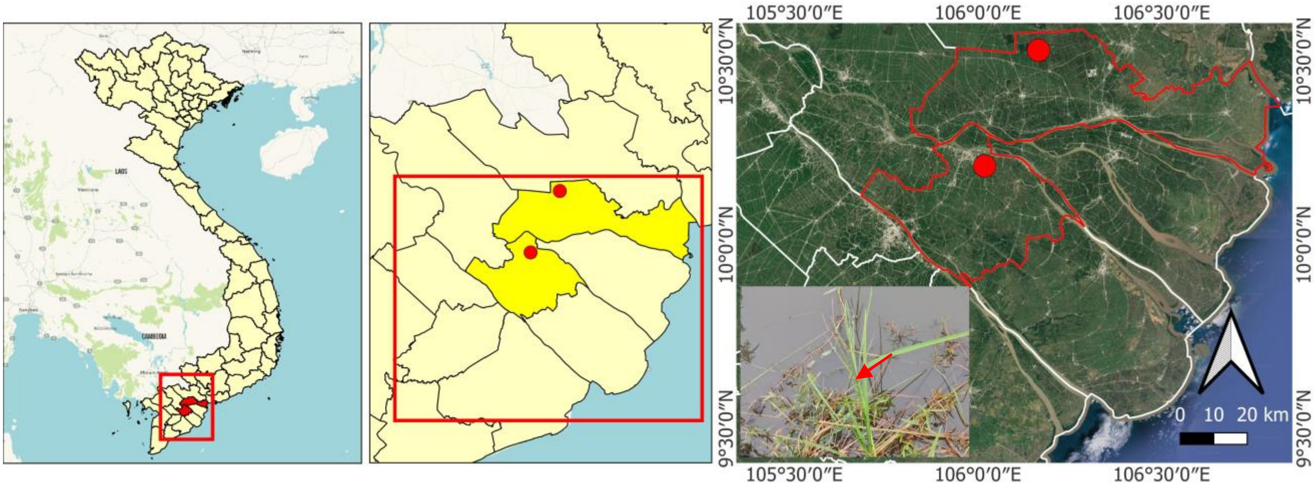

Figure 1. Locations of the soil samples (red dots) and wild rice samples (arrow) used for bacterial isolation

IAA production of bacterial strains

The capacity of isolated bacterial strain to synthesize IAA was determined based on the color reaction with the Salkowski reagent of Gordon and Weber (1951). Bacteria were cultured in broth nitrogen-free Burk's medium under conditions without tryptophan supplementation. The broth was incubated in the dark at a temperature of 28 ± 2°C for 8 days. Then, cultures on days 2, 4, 6, and 8 after bacterial inoculation were centrifuged at 12,000 rpm for 5 minutes. Finally, the supernatant was reacted with Salkowski reagent, and the optical absorbance of the sample was measured at 530 nm after being stored in the dark for 15 minutes. Using broth in nitrogen-free Burk's medium devoid of microorganisms, the control treatment was carried out in a similar manner.

Identification and sequencing of the 16S rRNA gene fragment

Bacterial strains with high nitrogen fixation, phosphate solubilization, and IAA synthesis activities were selected to test colony morphological characteristics, Gram staining, spore staining, oxidase, and catalase activities. They were identified by PCR and sequencing of the 16S rRNA gene segment. The method of Sambrook et al. (1989) was adopted to extract bacterial DNA. Following extraction, DNA was examined at 260 and 280 nm wavelengths to determine its content and purity. The bacterial DNA samples required a 16S rRNA gene segment amplified with the primer pair 27F: 5'-GAGTTTGATCMTGGCTCAG-3' and reverse primer 1492R: 5'-TACGGYTACCTTGTTACGACTT-3' (Weisburg et al. 1991). PCR reactions were performed in a volume of 50 µL, including: distilled water, PCR buffer 1X, $MgCl_2$ 2.0 mM, dNTPs 150 µM, primer 27F 20 pmol, primer 1492R 20 pmol, Taq DNA polymerase 2.0 UI, and a DNA sample (30 ng). The PCR reaction cycle was performed as follows: initial denaturation at 95°C for 4 minutes, then 35 cycles including denaturation at 94°C for 1 minute, primer annealing at 60°C for 1 minute, extension at 72°C for 2 minutes, and final extension at 72°C for 5 minutes. In TBE buffer 1X, PCR products were electrophoresed on a 1.5% agarose gel. Following purification, the PCR product was delivered to Macrogen Company (Korea) for sequencing.

Data analysis

Microsoft Excel 2016 was used to analyze data and graphic presentation. ANOVA (One-way Analysis of Variance) and Turkey's tests were employed to test treatment differences at a 95% confidence level. The bootstrap value was 1,000 replications, and the phylogenetic tree was constructed with MEGA X software (Tamura et al. 2013).

RESULTS AND DISCUSSION

Isolation of nitrogen-fixing bacteria

A total of seven bacterial strains capable of fixing nitrogen were isolated on nitrogen-free Burk's medium from rhizospheric soils of wild rice in the two provinces of Vinh Long and Tien Giang. After 3 days of culturing on nitrogen-free Burk's medium, the bacteria were observed to grow quite well. Colonies of most bacterial strains were clear white (Figure 2.A) or opaque white (Figure 2.B), round or irregular in shape, raised, and with a wet surface. Not all bacterial strains produce spores, belong to Gram-negative or Gram-positive (Figure 2.C) groups, were rod-shaped or spherical, motile, and positive for oxidase and catalase activity.

Isolation of phosphorus-solubilizing bacteria

Total five bacterial strains capable of solubilizing phosphorus on NBRIP medium were isolated from rhizospheric soil samples of wild rice in the two provinces of Vinh Long and Tien Giang. The results showed that the bacteria isolates grew well after 3 days of culture. Most strains produced halo after two days of culture, while some strains formed halo after three days on NBRIP medium. The isolated bacterial strains were pale yellow (Figure 3.A) or small, opaque white colonies (Figure 3.B), round shape, convex, and dry or wet surfaces. The findings also revealed that the isolated bacterial strains belonged to the Gram-positive (Figure 3.C) group, were short rods, and positive for oxidase and catalase activity.

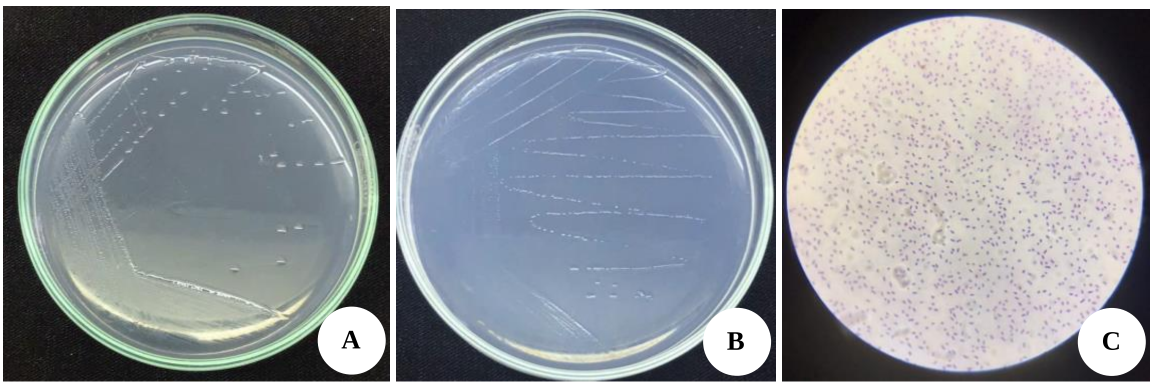

Figure 2. Nitrogen-fixing bacterial strains isolated on nitrogen-free Burk's medium. A. Clear white colonies, convex, and wet surface; B. Colonies opaque white, round, raised, and wet surface; C. Gram staining (100×)

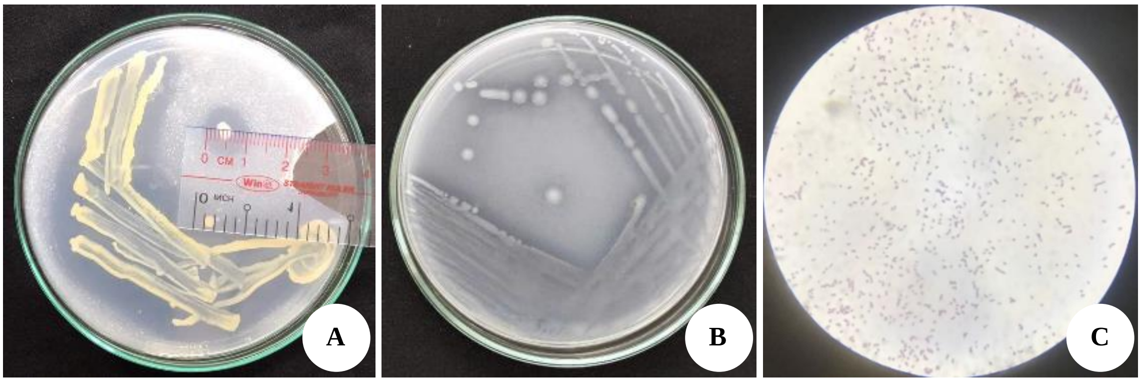

Figure 3. Phosphorus-solubilizing bacterial strain isolated on NBRIP media. A. Colonies pale yellow, round, and convex; B. Colonies opaque white, small, and round; C. Gram staining (100×)

Nitrogen fixation ability of bacterial strains

The results showed that seven bacterial strains isolated had the ability to fix nitrogen (Table 1). Ammonium content in nitrogen-free Burk medium after 8 days of culture ranged from 0.019-0.157 mg/L. Among the seven isolated bacterial strains, strain BĐ1.2 showed the highest nitrogen fixation activity, with an ammonium content of 0.157 ± 0.003 mg/L after 8 days of culture. Meanwhile, bacterial strain BĐ2.4 had the lowest nitrogen fixation activity (0.019 ± 0.00 mg/L).

Phosphorus solubilizing ability of bacterial strains

The results showed that the isolated bacteria formed phosphate-solubilizing rings on day 2 and grew strongly on day 4, and the phosphate-solubilizing diameter decreased on day 6 (Table 2). The diameter of phosphorus dissolution circle of bacterial strains after 6 days of culture ranged from 0.272 to 0.743 mm. Among the tested bacterial strains, BĐ1.3 strain showed the highest phosphorus-solubilizing activity, with a phosphorus-solubilizing diameter of 0.743 ± 0.025 mm. Whereas, the phosphate solubilizing activity of strain RĐ2.3.2 was the lowest at 0.272 ± 0.017 mm.

IAA production from bacterial strains

In this study six of twelve strains of nitrogen-fixing and phosphate-solubilizing bacteria were capable of synthesizing IAA. The IAA content of bacterial strains after 6 days of culture ranged from 0.011-0.028 µg/mL (Table 3). Among the strains capable of synthesizing IAA, strain BĐ2.3.1 produced the highest IAA with a concentration of 0.053 ± 0.001 µg/mL after 8 days of bacterial inoculation. However, the ability to synthesize IAA from two bacterial strains, BĐ2.3.2 and BĐ2.2, was the lowest (0.023 µg/mL).

Identification of bacterial strains by PCR technique

Sequencing result showed that strain BĐ1.3 (exhibiting three characteristics: nitrogen fixation, phosphate solubilization, and IAA production) showed 91.74% similarity with *Stenotrophomonas* sp. on GenBank database. The phylogenetic tree shows that strain BĐ1.3 was in the same group with *Stenotrophomonas* sp. EnB-bsy9 (KM596516.1) (Figure 4).

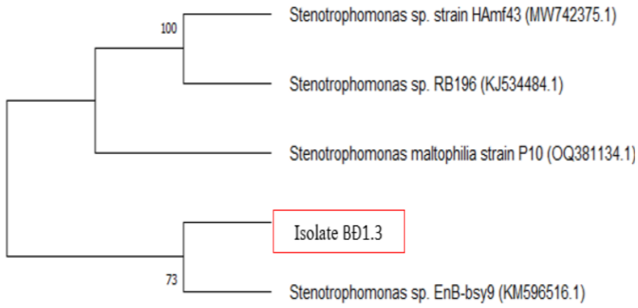

Figure 4. The phylogenetic tree analysis of bacterial isolate

Table 1. The ability of rhizospheric bacterial strains from wild rice to fix nitrogen

Bacterial isolates	NH_4^+ concentration (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
BĐ2.3.2	0.025^{b} ± 0.000	0.048^{c} ± 0.002	0.063^{c} ± 0.001	0.040^{c} ± 0.002	0.044 ± 0.001
BĐ1.2	0.034^{a} ± 0.001	0.088^{a} ± 0.003	0.227^{a} ± 0.002	0.281^{a} ± 0.007	0.157 ± 0.003
BĐ1.3	0.018^{c} ± 0.001	0.023^{d} ± 0.001	0.052^{c} ± 0.002	0.013^{e} ± 0.002	0.027 ± 0.002
BĐ2.2	0.009^{d} ± 0.001	0.015^{e} ± 0.001	0.025^{d} ± 0.003	0.033^{cd} ± 0.003	0.020 ± 0.002
BĐ2.4	0.007^{d} ± 0.000	0.015^{e} ± 0.001	0.024^{d} ± 0.001	0.029^{d} ± 0.002	0.019 ± 0.001
BĐ2.3.3	0.009^{d} ± 0.001	0.027^{d} ± 0.001	0.034^{d} ± 0.002	0.038^{cd} ± 0.001	0.027 ± 0.001
BĐ2.3.1	0.018^{c} ± 0.000	0.063^{b} ± 0.001	0.110^{b} ± 0.011	0.123^{b} ± 0.001	0.079 ± 0.003

Note: No significant difference (p>0.05) was found between numbers with the same letter in the same column

Table 2. Phosphate solubilization diameter of bacterial strains derived from wild rice rhizosphere soil

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
RĐ2.3.2	0.217^{cd} ± 0.028	0.293^{e} ± 0.011	0.307^{e} ± 0.011	0.272 ± 0.017
RĐ2.3	0.333^{a} ± 0.015	0.413^{cd} ± 0.015	0.447^{d} ± 0.005	0.398 ± 0.012
RĐ2.6	0.300^{ab} ± 0.000	0.430^{cd} ± 0.026	0.677^{d} ± 0.025	0.469 ± 0.017
RĐ2.2	0.260^{bc} ± 0.017	0.367^{de} ± 0.028	0.437^{d} ± 0.015	0.355 ± 0.020
BĐ1.3	0.330^{a} ± 0.026	0.777^{a} ± 0.025	1.123^{a} ± 0.025	0.743 ± 0.025
BĐ2.4	0.207^{d} ± 0.011	0.627^{b} ± 0.030	0.710^{b} ± 0.017	0.515 ± 0.019
RĐ2.2.1	0.300^{ab} ± 0.000	0.433^{c} ± 0.011	0.590^{c} ± 0.017	0.441 ± 0.009

Note: No significant difference ($p>0.05$) was found between numbers with the same letter in the same column

Table 3. IAA synthesis ability of bacterial strains isolated from wild rice rhizospheric soil

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
BĐ2.3.2	0.000^{d} ± 0.000	0.006^{c} ± 0.001	0.014^{d} ± 0.001	0.023^{d} ± 0.000	0.011 ± 0.001
BĐ1.3	0.004^{c} ± 0.001	0.010^{c} ± 0.001	0.022^{c} ± 0.001	0.033^{c} ± 0.001	0.017 ± 0.001
BĐ2.2	0.005^{c} ± 0.001	0.009^{c} ± 0.001	0.016^{d} ± 0.001	0.023^{d} ± 0.001	0.014 ± 0.001
BĐ2.4	0.008^{b} ± 0.000	0.015^{b} ± 0.001	0.024^{bc} ± 0.001	0.033^{c} ± 0.001	0.020 ± 0.001
BĐ2.3.3	0.014^{a} ± 0.001	0.023^{a} ± 0.001	0.031^{a} ± 0.001	0.043^{b} ± 0.001	0.028 ± 0.001
BĐ2.3.1	0.007^{bc} ± 0.001	0.019^{ab} ± 0.002	0.025^{b} ± 0.002	0.053^{a} ± 0.001	0.026 ± 0.002

Note: No significant difference ($p>0.05$) was found between numbers with the same letter in the same column

Discussion

Previous studies have shown the diversity and richness of rhizospheric bacteria in many different crops (Alawiye et al. 2019). Commonly present bacterial genera include: *Bacillus*, *Azospirillum*, *Acetobacter*, *Burkholderia*, *Pantoea*, *Enterobacter*, *Azotobacter*, *Klebsiella*, *Gluconacetobacter*, *Herbaspirillum*, *Stenotrophomonas*, *Pseudomonas* and *Serratia* (Glick 2012; Olanrewaju et al. 2017; Gómez-Godínez et al. 2023). In the present study, a total of 12 strains of phosphate-solubilizing and nitrogen-fixing bacteria were isolated from wild rice in the Mekong Delta. The strain BĐ1.3 was identified as *Stenotrophomonas* sp. based on colony morphology, biochemical characteristics, and 16S rRNA gene sequencing with 91.74% similarity. This result is in line with previous research that also reported the presence of *Stenotrophomonas* in rice rhizosphere soil in the Mekong Delta (Pha et al. 2015). Tian et al. (2023) isolated *Stenotrophomonas* from different tissues (the root, stem, and leaf) of wild rice (*O. officinalis*) in China. Results showed that strain BĐ1.3 had the abilities of nitrogen fixation, phosphate solubilization, and IAA synthesis activities. This result is consistent with a study by Fariman et al. (2022), which found that by giving the plant a consistent supply of nitrogen, the *S. maltophilia* strain UPMKH2 can raise rice output and productivity. Similarly, *S. maltophilia* bacteria was found by to be capable of solubilize phosphate, originated from peat soil in Indonesia (Suliasih and Widawati 2021). In Tunisia, according to Amri et al. (2023), *S. maltophilia* exhibited phosphate solubilization in the PVK medium between 374.20 and 544.28 µg/mL and the NBRIP medium between 535.70 and 618.57 µg/mL. *S. maltophilia* also produced IAA in higher amounts of 50.4 ± 0.9 g/mL (Amri et al. 2023). Othman et al. (2013) reported that diazotrophic *S. maltophilia* produces 2.6 mg/mL of IAA. In medium enriched with L-tryptophan, isolate EB-40 (*Bacillus* sp.) produced IAA in the maximum amount of 47.88 µg/mL and was able to synthesis IAA without L-tryptophan (Andrade et al. 2014).

Nitrogen is an essential ingredient for plant growth (Moreau et al. 2019). It is a significant component of amino acids, proteins, and precursors to nucleic acids in plant systems (Ohyama 2010). It promotes the growth of leaves on plants by strengthening the structural integrity of proteins and chlorophyll (Mager and Ludewig 2018). Biological nitrogen fertilizers fixed by bacteria account for up to 70% of the total nitrogen on earth (Vitousek et al. 2002). The result of current research showed that the average amount of fixed nitrogen in bacterial strains ranged from 0.019-0.157 mg/L. The bacterial isolates (BĐ2.3.2, BĐ1.2, BĐ1.3, BĐ2.2, BĐ2.4, BĐ2.3.3, and BĐ2.3.1) in this study exhibited a lower nitrogen fixing ability (NH_4^+) than those found in earlier studies (Xa and Nghia 2019; Zhang et al. 2022). Oanh et al. (2013) isolated fifteen isolates obtained from rhizospheric soils of four rice varieties collected from Tra Vinh and Can Tho with high nitrogen fixation capacity. The NH_4^+ content of isolates TV2C2, TV1A4, TV3A4, TV2B3, and TV3B4a was 4.6720 mg/L, 4.1762 mg/L, 4.1380 mg/L, 3.7948 mg/L, and 3.7498 mg/L, respectively. Similarly, the study by Xa and Nghia (2019) showed that indigenous bacterial isolates derived from different cropping systems in Soc Trang province of Vietnam had highly biological nitrogen fixation, with NH_4^+ concentrations ranging from 1.00 to 4.23 mg NH_4^+/L.

Phosphorus is an indispensable macronutrient for plant growth because the easily digestible phosphorus content in the soil is low (Meng et al. 2021). Therefore, providing enough phosphorus to plants is an important requirement to achieve optimal productivity (Lucero et al. 2021). The results of present study showed that the average halo diameter in bacterial strains ranged from 0.272-0.743 mm. However, the ability of phosphorus solubilization in the study was lower than that of the research by Qingwei et al. (2023), who indicated that seven strains derived from the rhizosphere of road verge in China displayed high phosphorus solubilizing abilities for $Ca_3(PO_4)_2$ with a concentration of 150-453 mg/L. In another study by Zhang et al. (2021), thirty-seven bacterial strains could mobilize calcium phosphate, and *Methylobacterium* sp. isolate Fse5 had the highest activity of phosphate solubilization with a concentration of 576.34 ± 24.63 µg/mL. Suliasih and Widawati (2021) recorded the maximum phosphate solubilization (52.26 µg/mL) of *S. maltophilia* at the 72-hour incubation period. In Vietnam, Nghĩa and Oanh (2017) found that twenty out of ninety-five isolates derived from fifteen paddy rice soil samples displayed a high capacity for phosphorus solubilization with concentration greater than 1,000 mg/L. In particular, isolate BL1-10 showed that the maximum phosphate solubilization after five incubation days was 2,044 mg/L. Acidification of the media and release of organic acids, such as fumaric, citric, oxalic acids, malic, and ketoglutaric by phosphate solubilizing bacteria are the mechanism solubility of insoluble inorganic phosphate (Zuluaga et al. 2023). Tricalcium phosphate can be converted into mono- and di-basic phosphate that plants can use by the organic acid that phosphate solubilizing bacteria release (Awais et al. 2015). According to Walpola and Yoon (2012), different types of bacteria produce different kinds and amounts of organic acids.

Indole-3-Acetic Acid (IAA) is a plant growth stimulant that also contributes to promoting plant growth by increasing biomass, root length, and number of roots, as well as yield (Keswani et al. 2020; Çakmakçı et al. 2021). In the present research, results showed that the average amount of IAA concentration in bacterial strains ranged from 0.011-0.028 µg/mL. The finding is lower than in a study by Riddech et al. (2022), who revealed that all rhizospheric isolates had the ability to produce the IAA, which ranged from 2.175 ± 0.25 µg/mL to 8.397 ± 0.46 µg/mL. In endophytic bacteria, in contrast, IAA content fluctuated from 3.043 ± 0.15 µg/mL to 4.242 ± 0.35 µg/mL. Besides, the highest IAA level recorded in the rhizospheric bacterial isolates MSR7, LGR5, and AVR1 was 8.397 ± 0.46 µg/mL, 7.8914 ± 1.62 µg/mL, and 6.8687 ± 0.49 µg/mL, respectively. In endophytic bacteria, isolates MGE4 and MSE5 were the highest IAA-producing isolates, with IAA concentrations of 4.242 ± 0.35 µg/mL and 4.0909 ± 0.59 µg/mL, respectively. In Vietnam, Huy and Hiệp (2018) found that all 116 strains of salt-tolerant bacteria collected from rice-shrimp soils were able to synthesize IAA. Of these, IAA could be synthesized by two isolates PL2 and PL9 at concentrations of 45.31 µg/mL, and 46.46 µg/mL, respectively. Tam et al. (2020) showed that fourty-one isolates from sugarcane farmed in Tay Ninh province can produce IAA ranging from 1.9 to 12.8 mg/L. In maize (*Zea mays*) farmed on acrisols of the Southeast of Vietnam, Thanh et al. (2016) found that fifty-five bacterial isolates could produce IAA in the presence and absence of tryptophan in the range of 0.14 to 12.51 and 0.24 to 9.99 mg/L, respectively. The difference in IAA content between bacterial isolates may be due to the presence of tryptophan in the bacterial culture medium (Brandi et al. 2011).

In addition to the ability of rhizosphere bacteria to fix nitrogen, they also have other functions, such as solubilizing phosphorus and producing plant growth stimulants, such as IAA and gibberellin to increase crop productivity (Saeed et al. 2021; de Andrade et al. 2023; Cẩm et al. 2023). In this study, 3 bacterial strains of BĐ2.2, BĐ2.3.3, and BĐ2.3.1 had the capacity to fix nitrogen and solubilize phosphate. This finding is in line with Thanh and Tram (2018), who showed that a total of 118 isolates were isolated from black pepper plants cultivated in Binh Phuoc province of Vietnam, and all isolates had the capacity to fix nitrogen and solubilize phosphate. In the finding, Vy et al. (2023) found that endophytes associated with *Sesbania sesban* obtained in Long An Province had the characteristics of promoting plant growth through nitrogen fixation and phosphate solubilization. The results of present investigation revealed that three bacterial strains (BĐ2.3.2, BĐ1.3, and BĐ2.4) had the abilities of nitrogen fixation, phosphate solubilization, and IAA production. This observation is similar to those of Phượng et al. (2020), who illustrated that endophytic bacteria from the rice root possess multiple plant growth promoting characters, such as ammonia production, IAA synthesis, siderophore production, and phosphate solubilization. In India, similarly, Sherpa et al. (2021) found that rhizospheric bacteria derived from paddy fields indicated the production of IAA, potassium, and phosphate solubilization and had the ability to fix nitrogen as well. In China, Zhang et al. (2021) reported that most of the isolated endophytic bacteria originated from the seeds of wild rice (*O. rufipogon*) were able to produce IAA and siderophores and solubilize phosphate. They also demonstrated ACC deaminase activity and fixing nitrogen. Thu et al. (2022) reported that strain LP1-R4, from *Polyscias fruticosa* in An Giang Province, Vietnam, had the abilities of nitrogen fixation, phosphate solubilization, and IAA synthesis with concentrations of 31.6 ± 0.39, 18.8 ± 0.78, and 6.65 ± 0.26 mg/L, respectively. To our knowledge, this is the first report of isolation of rhizospheric bacteria from *O. rufipogon* with the characteristics of nitrogen fixation, phosphorus solubilization, and IAA production in the Mekong Delta, Vietnam.

In conclusion, the results of this investigation demonstrate the isolated bacteria (strains BĐ1.2, BĐ1.3 and BĐ2.3.1) were capable of nitrogen fixation, phosphorus solubilization, and IAA synthesis from the rhizospheric soil of wild rice in the Mekong Delta. In particular, strain BĐ1.3 was identified as *Stenotrophomonas* sp. (91.74% similarity) and had three simultaneous activities of nitrogen fixation, phosphate solubilization, and IAA synthesis. The findings show the potential application of bacterial strains in biosafety rice cultivation.

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