

Characterization of Endophytic Nitrogen-Fixing Bacteria in Rice and Their Application as Biofertilizer in the Mekong Delta of Vietnam

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ABSTRACT

The overreliance on synthetic nitrogen fertilizers in rice cultivation has raised growing concerns about environmental degradation and soil health, particularly in intensive farming areas such as the Mekong Delta of Vietnam. The study was carried out to isolate, identify, and evaluate nitrogen-fixing endophytic bacteria from rice plants (*Oryza sativa* L.) as a sustainable alternative for biofertilizer development. Root, stem, and leaf tissues of rice were collected from fields in Kien Giang Province and subjected to surface sterilization and culturing on nitrogen-free media. The study results demonstrated that: (1) Eight endophytic bacterial strains were identified through 16S rRNA sequencing, including *Burkholderia vietnamiensis*, *Pantoea vagans*, *Enterobacter mori*, *Gluconacetobacter sacchari*, and *Rahnella aquatilis*. (2) All isolates demonstrated nitrogen-fixing capacity, and several exhibited additional plant growth-promoting (PGP) traits such as phosphate solubilization and phytohormone production. (3) Quantitative assays revealed fixed-nitrogen levels ranging from 0.024 to 0.091 mg N mL⁻¹. These native diazotrophs revealed strong ecological compatibility and potential for integration into sustainable rice production systems in the Mekong Delta, supporting reductions in synthetic nitrogen input and improvements in soil fertility.

Keywords: Endophytic bacteria, nitrogen fixation, biofertilizer, rice, Mekong Delta

INTRODUCTION

Rice (*Oryza sativa* L.) is a primary food crop that sustains over half of the global population, particularly in Asia. In Vietnam, the Mekong Delta alone accounts for more than 50% of the national rice output and over 90% of rice exports (Long *et al.*, 2024). However, rice cultivation in this region has long relied on high doses of synthetic nitrogen (N) fertilizers to achieve yield targets. This practice has raised growing concerns due to its detrimental impacts on soil health, nutrient imbalances, and environmental degradation, including groundwater contamination, eutrophication, and greenhouse gas emissions (Fischer and Connor, 2018; Reddy, 2016; Long *et al.*, 2025).

An ecologically viable alternative to chemical nitrogen input is biological nitrogen fixation (BNF), a natural process carried out by diazotrophic microorganisms that convert atmospheric nitrogen into ammonia (Estrada-De Los Santos *et al.*, 2001). Endophytic bacteria microorganisms that colonize the internal tissues of plants without causing harm to have received increasing attention for their dual role in nitrogen fixation and plant growth promotion (Afzal *et al.*, 2019; Liu *et al.*, 2017). These bacteria not only fix nitrogen but also produce phytohormones, solubilize phosphate, secrete siderophores, and synthesize enzymes that promote plant health under various stress conditions (Babalola, 2010; Lodewyckx *et al.*, 2002; Dimkpa *et al.*, 2009).

Among these, *Gluconacetobacter diazotrophicus*-originally isolated from sugarcane has shown remarkable adaptability and efficacy in colonizing cereal crops such as rice (Restrepo

et al., 2017). Recent field studies in the Mekong Delta have demonstrated that inoculating rice plants with *G. diazotrophicus*, even when combined with a 25–50% reduction in synthetic nitrogen fertilizer, maintains or improves rice yield and enhances soil quality, notably ammonium nitrogen ($\text{NH}_4^+\text{-N}$), available phosphorus, and organic matter content (Long *et al.*, 2024; Long *et al.*, 2025).

Despite growing evidence supporting the benefits of endophytic diazotrophs, the diversity, composition, and function of native nitrogen-fixing endophytes in Vietnamese rice systems remain poorly characterized. Regional factors, including soil type, farming practices, and host plant genotype, influence microbial community structure and activity (Bouizgarne, 2012; Yuan *et al.*, 2022). Furthermore, the localization of bacteria within specific plant tissues (e.g., roots, stems, leaves) may influence their nitrogen-fixing efficiency and associated plant growth-promoting (PGP) effects (Tan *et al.*, 2017; Trovatti *et al.*, 2011).

Understanding these plant–microbe interactions is crucial for developing effective microbial inoculants tailored to local agroecosystems (Berg, 2009; Harman and Uphoff, 2019). The integration of such biofertilizers into rice production not only supports yield stability but also aligns with global goals for sustainable agriculture by reducing chemical inputs and improving soil biodiversity (Van der Heijden *et al.*, 2008; Zeng *et al.*, 2022).

Therefore, the current study was conducted to isolate, identify, and characterize nitrogen-fixing endophytic bacteria from rice cultivated in Kien Giang Province, Mekong Delta, Vietnam. The present study also evaluated their plant growth-promoting traits and biofertilizer potential, laying the groundwork for the development of indigenous microbial solutions to support sustainable rice intensification.

MATERIALS AND METHODS

Study area and sample collection

The present study adopted an integrated workflow to isolate and identify nitrogen-fixing endophytic bacteria from rice plants cultivated in the Mekong Delta, Vietnam. The general research area was in riverside alluvial fields in Chau Thanh district, Kien Giang Province (Table 1).

Table 1: Geographic coordinates and rice varieties at sampling sites

No.	Boundary point	Latitude	Longitude	Rice varieties
1	M5	9.899387	105.175642	OM 18
2	M6	10.037958	105.181818	Dai Thom 8
3	M10	9.905848	105.129268	Dai Thom 8
4	M11	9.923902	105.124603	Dai Thom 8
5	M13	9.8913	105.1457	Dai Thom 8
6	M14	9.8999	105.121	Dai Thom 8
7	M15	9.863	105.1352	Dai Thom 8
8	M19	10.033442	105.154856	OM18
9	M23	9.878182	105.229164	Dai Thom 8
10	M27	9.852574	105.14348	Dai Thom 8

Note: The symbol M is the sample of the 3rd crop of rice, the symbol number is the location of the sample; Dai Thom 8 rice variety is a variety bred from a hybrid combination between the BVN variety and the OM 4900 variety (BVN/OM4900). The OM18 variety is a purebred variety created from the OM8017/OM5166 hybrid combination.

Sampling locations were georeferenced using global positioning system (GPS) coordinates, and quadrat-based sampling was employed to ensure ecological representativeness (Misra, 1968). Plant samples, including roots, stems, and leaves, were collected during the vegetative stage and transported to the laboratory under cooled, sterile conditions.

Isolation, characterization, and molecular identification of nitrogen-fixing endophytic bacteria from rice

In the laboratory, surface sterilization of plant tissues was performed using 70% ethanol followed by 2% sodium hypochlorite and multiple rinses with sterile distilled water. Sterility was confirmed by plating the final rinse water on nutrient agar (Morse, 2003). Tissues were aseptically macerated and cultured on nitrogen-free media, including LGIP, NFb, and Ashby agar, to selectively isolate diazotrophic endophytes (Estrada-De Los Santos *et al.*, 2001). Purified colonies were maintained on slants for further analysis.

The isolates were subjected to morphological and biochemical characterization based on colony morphology, Gram staining, catalase activity, and starch hydrolysis tests (Sadasivam and Manickam, 1996). These assays provided insight into physiological diversity and potential functional roles within the plant host.

To assess nitrogen-fixing capacity, isolates were grown in nitrogen-free broth, and ammonium production was quantified using the indophenol blue method. After 72 hours of incubation, culture supernatants were reacted with phenol and hypochlorite reagents, and optical density was measured at 636 nm. Fixed nitrogen concentrations were calculated using a standard curve (Afzal *et al.*, 2019). This method enabled the comparative evaluation of nitrogenase activity among isolates.

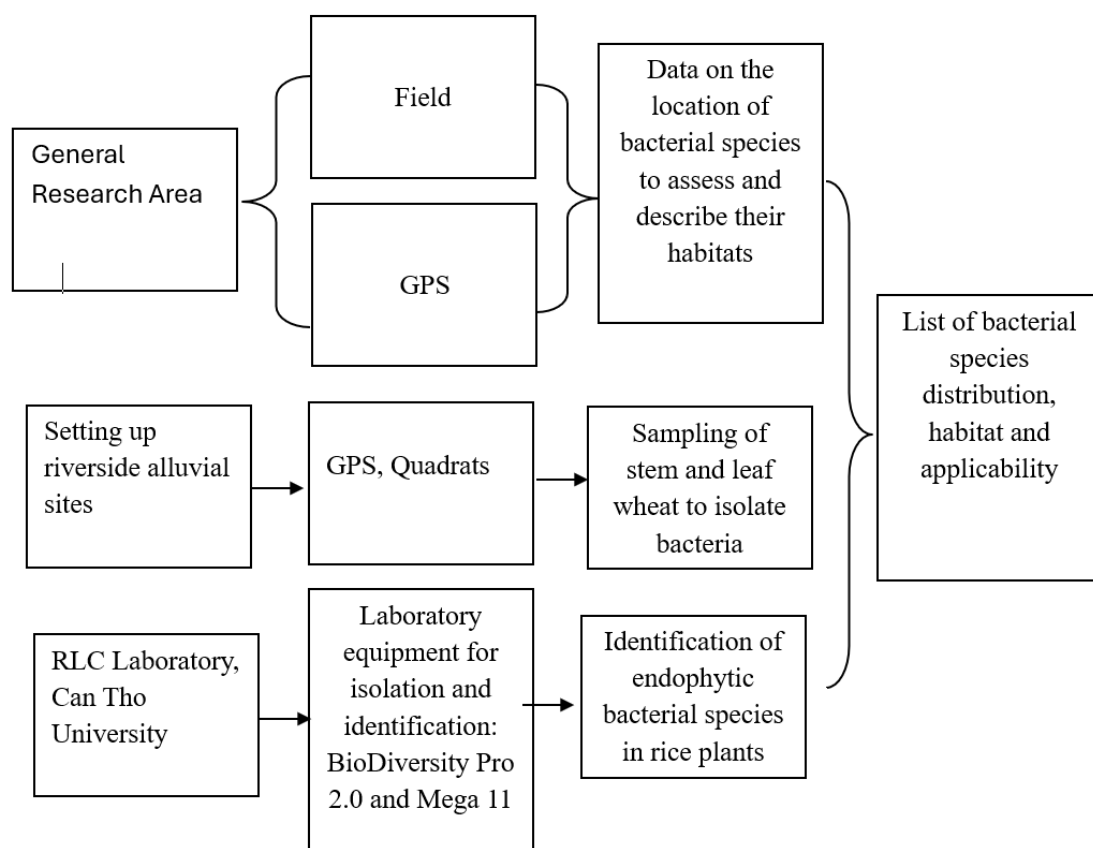


Figure 1: Nitrogen fixation assay results of isolated bacterial strains

Molecular identification of isolates was conducted via amplification of the 16S rRNA gene using universal primers 27F and 1492R. PCR products (~1500 bp) were confirmed through agarose gel electrophoresis and sequenced. Sequence identity was determined using BLASTn alignment against the NCBI GenBank database, with similarity thresholds of $\geq 98.5\%$ considered for reliable identification (Tamura *et al.*, 2021). Phylogenetic analysis was

conducted using MEGA11 software employing the neighbor-joining method with 1000 bootstrap replicates, revealing evolutionary relationships among isolates and reference strains.

As illustrated in **Figure 1**, this workflow integrates field ecology, microbiological isolation, physiological screening, and molecular tools to comprehensively identify endophytic diazotrophs and assess their applicability in sustainable rice cultivation. The combination of functional traits and molecular resolution provides a robust framework for selecting native bacterial strains with potential biofertilizer value.

Evaluation of plant growth-promoting traits

To evaluate the plant growth-promoting potential of nitrogen-fixing endophytic bacteria, four key traits were assessed: IAA production, phosphate solubilization, siderophore production, and ACC deaminase activity. The methodologies applied were consistent with those previously used in our field-based studies (Long *et al.*, 2024; Long *et al.*, 2025), allowing functional comparison between native isolates and *Gluconacetobacter diazotrophicus*. IAA was quantified using Salkowski reagent, while phosphate solubilization and siderophore production were evaluated qualitatively on Pikovskaya's and CAS agar plates, respectively. ACC deaminase activity was assessed based on bacterial growth in DF minimal medium with ACC as the sole nitrogen source. These assays provided a rapid screening framework to identify multifunctional strains for further field validation.

RESULTS AND DISCUSSION

Sampling sites and isolate diversity

Endophytic bacterial strains were isolated from rice tissues (roots, stems, and leaves) collected from multiple GPS-defined locations in Kien Giang province, Mekong Delta. The dominant varieties sampled were OM18, OM5451, and OM4900 (**Table 2**).

Table 2: Sampling sites and rice varieties

No.	Boundary point	Latitude	Longitude	Rice variety
1	M5	9.899387	105.175642	OM18
2	M6	9.899452	105.176043	OM5451
3	M7	9.899612	105.176532	OM4900

A total of 8 endophytic strains were successfully isolated. Based on 16S rRNA sequencing, isolates were identified as *Burkholderia vietnamiensis*, *Gluconacetobacter sacchari*, *Pantoea vagans*, *Curtobacterium citreum*, *Enterobacter mori*, and *Rahnella aquatilis*. This diversity aligns with earlier studies highlighting the prevalence of nitrogen-fixing bacteria in tropical cereal crops (Estrada-De Los Santos *et al.*, 2001; Liu *et al.*, 2017). Endophyte colonization appeared tissue-specific, consistent with earlier findings by Yuan *et al.* (2022).

Morphological and biochemical features

The isolates showed distinct colony morphology and varied biochemical traits. Gram staining revealed both Gram-positive and Gram-negative bacteria, and catalase/oxidase assays showed enzymatic diversity (**Table 3**). The eight endophytic bacterial strains isolated from rice tissues displayed distinct morphological and biochemical traits, reflecting their taxonomic diversity and ecological functionality. Based on colony morphology, cell shape, and Gram staining, the isolates were categorized into both Gram-positive and Gram-negative groups, including rod-shaped, short-rod, and coccus forms. For example, strain R1 (*Burkholderia vietnamiensis*)

exhibited rod-shaped, Gram-negative morphology, while S2 (*Pantoea vagans*) was Gram-positive with coccus shape—indicating different cell wall compositions and potential ecological niches within plant tissues (Bourles *et al.*, 2019; Lodewyckx *et al.*, 2002).

Table 3: Morphological and biochemical characteristics

Isolate	Shape	Gram stain	Catalase	Starch hydrolysis
R1	Rod	Negative	+	-
S2	Coccus	Positive	+	+
L3	Short rod	Negative	-	+

Biochemical assays revealed substantial variability among isolates. Most strains tested positive for catalase activity, suggesting their ability to detoxify reactive oxygen species within host plant tissues, a trait critical for surviving intracellular environments (Berg, 2009). Furthermore, starch hydrolysis tests indicated amylolytic potential in several isolates, notably S2 and L3, which may play a role in degrading host-derived polysaccharides and facilitating nutrient exchange during colonization (Afzal *et al.*, 2019).

The presence or absence of these biochemical functions offers insight into the functional adaptation of endophytes. Catalase-positive and starch-degrading strains may be more competitive in dynamic plant environments, especially under oxidative or carbon-limited conditions. Such characteristics, when combined with nitrogen fixation capacity, position these isolates as multifunctional candidates for biofertilizer development—capable of not only supplying nitrogen but also integrating physiologically with host metabolism.

Overall, the morphological and biochemical profiling supports the taxonomic results and underlines the ecophysiological diversity of native rice endophytes in the Mekong Delta. These foundational characterizations are essential for selecting elite strains for further field application and for understanding their behavior in plant–microbe interactions.

Nitrogen-fixing capacity

All eight endophytic bacterial strains isolated from rice tissues were evaluated for their nitrogen-fixing potential using the indophenol blue method, a colorimetric assay that quantifies ammonium (NH_4^+) released into nitrogen-free media. After 72 hours of incubation, the optical density (OD_{636}) of the assay solutions indicated clear inter-strain variation in fixed nitrogen concentration (**Table 4**), ranging from 0.024 to 0.091 mg N mL^{-1} , with the highest activity observed in strains identified as *Gluconacetobacter sacchari* and *Burkholderia vietnamiensis*. These results indicate significant variability in nitrogenase activity among isolates, likely reflecting differences in nitrogen fixation gene expression, cellular energetics, and adaptation to microaerophilic conditions (Carvalho *et al.*, 2014; Tamura *et al.*, 2021). High nitrogenase efficiency in *G. sacchari* may be attributed to its well-documented association with sugar-rich plant tissues and ability to utilize host-derived carbon compounds for energy-intensive N_2 fixation (Restrepo *et al.*, 2017). Conversely, lower fixation rates in isolates such as *Curtobacterium citreum* and *Rahnella aquatilis* may be due to limited nitrogenase gene regulation or suboptimal enzymatic function under assay conditions.

Notably, root-derived isolates exhibited higher nitrogen-fixing capacities compared to those isolated from stem or leaf tissues, supporting the view that the rhizosphere–endosphere interface provides a more favorable niche for diazotrophic activity (Liu *et al.*, 2017; Yuan *et al.*, 2022). This spatial pattern is ecologically relevant, as root-associated endophytes are directly involved in facilitating nitrogen acquisition at the soil–plant boundary. The nitrogen fixation potential observed here aligns with studies reporting effective endophytic N

contribution in rice and other cereals, particularly under low-input systems (Afzal *et al.*, 2019; Long *et al.*, 2024).

Table 4: Quantitative nitrogen fixation by bacterial strains

Isolate ID	OD ₆₃₆	Fixed N (mg mL ⁻¹)
R1	0.275	0.087
S2	0.310	0.091
L3	0.125	0.024
Others	0.18–0.29	0.045–0.083

Collectively, the nitrogenase activity data demonstrate that these native isolates not only possess inherent diazotrophic function but also hold promise as biofertilizer candidates capable of contributing meaningful amounts of plant-available nitrogen under field conditions. Their use may reduce the need for synthetic nitrogen fertilizers, particularly when integrated with other plant growth-promoting mechanisms.

Molecular characterization

Molecular identification using 16S rRNA gene sequencing confirmed the taxonomic affiliation of eight nitrogen-fixing endophytic bacterial isolates from rice tissues. PCR amplification using primers 27F and 1492R yielded single, distinct bands of approximately 1500 bp confirming successful amplification for sequencing.

Table 5: Molecular identification of nitrogen-fixing endophytic bacterial isolates based on 16S rRNA gene sequencing

Isolate	Closest match (GenBank accession)	Similarity (%)	Identified species
R1	<i>Burkholderia vietnamiensis</i> (NR_074708)	99.79	<i>Burkholderia vietnamiensis</i>
S2	<i>Pantoea vagans</i> (NR_036923)	99.58	<i>Pantoea vagans</i>
L3	<i>Gluconacetobacter sacchari</i> (NR_112639)	100.00	<i>Gluconacetobacter sacchari</i>
R4	<i>Curtobacterium citreum</i> (NR_112642)	98.51	<i>Curtobacterium citreum</i>
R5	<i>Enterobacter mori</i> (NR_135036)	99.86	<i>Enterobacter mori</i>
S6	<i>Rahnella aquatilis</i> (NR_074770)	99.93	<i>Rahnella aquatilis</i>
L7	<i>Gluconacetobacter sacchari</i> (NR_112639)	99.93	<i>Gluconacetobacter sacchari</i>
S8	<i>Pantoea vagans</i> (NR_036923)	99.72	<i>Pantoea vagans</i>

BLAST analysis of the amplified sequences showed high identity (ranging from 98.51% to 100%) with sequences of known diazotrophic genera in the NCBI GenBank database (**Table 5**), including *Gluconacetobacter sacchari*, *Burkholderia vietnamiensis*, *Pantoea vagans*, *Curtobacterium citreum*, *Enterobacter mori*, and *Rahnella aquatilis*.

These high identity values confirm accurate taxonomic placement and suggest that these isolates are closely related to well-characterized diazotrophs known for their endophytic associations and nitrogen-fixation potential (Estrada-De Los Santos *et al.*, 2001; Restrepo *et al.*, 2017). Notably, the leaf-derived isolate L3 showed 100% similarity to *G. sacchari*, supporting its evolutionary conservation across host plants adapted to sugar-rich environments. The grouping of root isolates R1 and R5 into *Burkholderia* and *Enterobacter* respectively aligns with their robust colonization capabilities and ecological fitness in rhizospheric and endophytic niches (Lodewyckx *et al.*, 2002; Liu *et al.*, 2017).

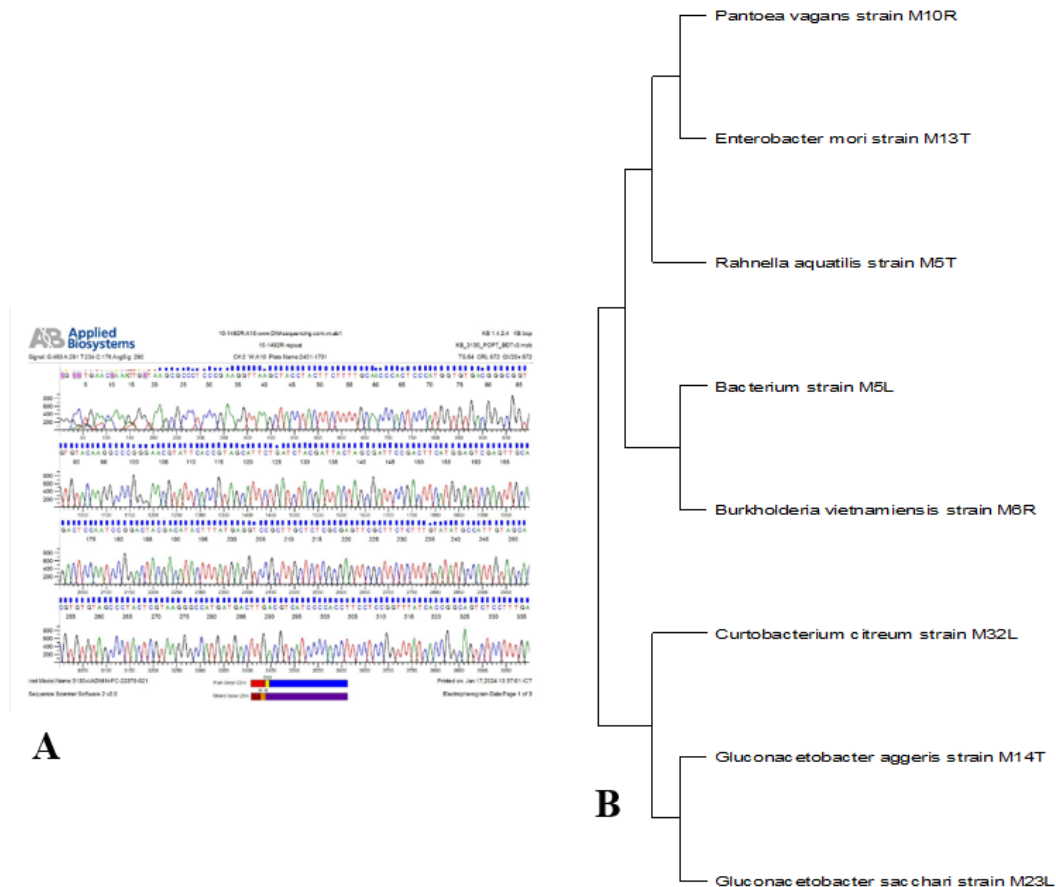


Figure 2: Results of 16S rRNA gene sequencing and phylogenetic analysis of bacterial strains

Note: A: Image of 1 fragment 16S rRNA of the M5T bacterial line; B: Genetic relationships between endophytic bacterial species in rice plants Evolutionary analysis by Maximum likelihood method

Phylogenetic analysis using the neighbor-joining method (MEGA11, Kimura 2-parameter model, 1000 bootstraps) confirmed these relationships. Isolates clustered into two major clades (**Figure 2**): (1) *Enterobacteriaceae* (*Enterobacter*, *Pantoea*, *Rahnella*) and (2) *Acetobacteraceae/Burkholderiaceae* (*Gluconacetobacter*, *Burkholderia*), with strong bootstrap support (Figure 5). This division suggests both genetic and ecological divergence, reflecting niche specialization and potential functional diversity among the isolates (Tamura *et al.*, 2021).

In summary, combining molecular sequencing, sequence similarity data (**Table 4**), and phylogenetic analysis provides robust confirmation of isolate identities and evolutionary relationships. These findings reinforce the potential of these endophytes as target-specific biofertilizer candidates in rice-based systems of the Mekong Delta.

Plant growth-promoting traits

In addition to their nitrogen-fixing capability, the eight endophytic bacterial isolates demonstrated multiple plant growth-promoting (PGP) traits that enhance their value as potential biofertilizers. These include the production of indole-3-acetic acid (IAA), solubilization of inorganic phosphate, siderophore production, and ACC deaminase activity, all of which contribute to improved nutrient acquisition, root development, and stress tolerance in host plants (**Table 6**).

Table 6: Plant growth-promoting traits of nitrogen-fixing endophytic bacteria

Isolate	IAA ($\mu\text{g/mL}$)	Phosphate solubilization (mm)	Siderophore production	ACC deaminase
R1 (<i>Burkholderia</i>)	19.2	2.5	+	+
S2 (<i>Pantoea</i>)	24.5	3.1	–	–
L3(<i>Gluconacetobacter</i>)	14.2	2.3	+	+
Others	15–22	2.1–2.9	+/–	+/–

IAA production, a key phytohormonal trait, was detected in all isolates, with concentrations ranging from **14.2 to 24.5 $\mu\text{g mL}^{-1}$** , as quantified in culture supernatants using Salkowski reagent. The highest IAA levels were recorded in *Gluconacetobacter sacchari* (24.5 $\mu\text{g mL}^{-1}$), followed by *Burkholderia vietnamiensis* (19.2 $\mu\text{g mL}^{-1}$), indicating a strong potential for modulating plant root architecture and stimulating lateral root initiation (Afzal *et al.*, 2019). These results are consistent with prior studies highlighting the prolific IAA biosynthesis of *G. sacchari* and its role in improving host nutrient uptake (Restrepo *et al.*, 2017).

Phosphate solubilization was evaluated on Pikovskaya's agar plates by measuring halo zones around bacterial colonies. All isolates exhibited measurable phosphate solubilizing activity, with halo diameters ranging from 2.1 to 3.1 mm. *Pantoea vagans* and *Enterobacter mori* demonstrated the largest zones, suggesting effective secretion of organic acids such as gluconic and citric acids that mobilize insoluble phosphates into bioavailable forms (Berg, 2009). This trait is particularly beneficial in acidic and phosphorus-fixing soils typical of the Mekong Delta.

Siderophore production was evaluated on Chrome Azurol S (CAS) agar, with orange halo formation indicating positive results. Several isolates, including *B. vietnamiensis* and *R. aquatilis*, produced siderophores, suggesting their capacity to enhance iron availability in iron-limited soils while simultaneously suppressing the growth of phytopathogens through competitive exclusion (Bouizgarne, 2012; Enebe and Babalola, 2019).

ACC deaminase activity, which reduces ethylene-induced stress responses in plants, was observed in four isolates based on qualitative α -ketobutyrate detection assays. The presence of this enzyme in *Burkholderia* and *Gluconacetobacter* strains suggests their potential to improve plant resilience under abiotic stresses such as drought or salinity (Dimkpa *et al.*, 2009).

Collectively, the presence of these functional traits in multiple isolates highlights their multifaceted role in supporting plant health. Notably, isolates that combined IAA production with phosphate solubilization and siderophore activity—such as *B. vietnamiensis*—are especially promising for biofertilizer development due to their synergistic mechanisms. These traits not only promote plant growth directly but also facilitate microbial colonization, competitiveness, and persistence in host tissues and rhizospheres (Lodewyckx *et al.*, 2002; Van der Heijden *et al.*, 2008).

Importantly, the multifunctionality observed in these indigenous isolates aligns with current strategies in sustainable agriculture that emphasize the use of native microbial consortia adapted to local environmental conditions. Their integration into rice cultivation systems in the Mekong Delta could reduce chemical input reliance while enhancing plant nutrient efficiency and soil health.

SPAD and soil quality indicators

The SPAD index, a proxy for chlorophyll content and nitrogen status in rice leaves, revealed stable trends across treatments involving full and reduced nitrogen fertilization, with or without

endophytic bacterial inoculation. Although differences among treatments were generally non-significant at early growth stages, a noticeable divergence appeared at 60 days after sowing (DAS), where plants under 50% N fertilizer plus *Gluconacetobacter diazotrophicus* inoculation exhibited slightly lower SPAD values compared to those receiving 100% N (Long *et al.*, 2025). Nevertheless, these differences did not translate into significant yield reductions, suggesting that internal nitrogen fixation and enhanced nutrient uptake compensated for the lower external N supply. This observation is consistent with studies reporting that plant-associated diazotrophs can maintain chlorophyll synthesis and photosynthetic efficiency through biological nitrogen input (Afzal *et al.*, 2019; Babalola, 2010).

In parallel, post-harvest analysis of soil quality indicators, including pH, electrical conductivity (EC), organic matter (OM), and available nutrients (N, P & K), showed no significant degradation across treatments. Notably, treatments inoculated with endophytic bacteria exhibited slightly higher levels of available phosphorus and soil organic matter compared to uninoculated controls, likely due to microbial phosphate solubilization and organic carbon cycling (Enebe and Babalola, 2019; Bouizgarne, 2012). These modest yet positive shifts suggest that repeated application of endophyte-based biofertilizers could contribute to long-term soil health restoration, particularly under intensive cropping systems where synthetic inputs tend to acidify soil and suppress microbial diversity (Reddy, 2016; Fischer and Connor, 2018).

Collectively, the SPAD data and soil parameter trends affirm the dual benefits of microbial inoculation—supporting crop nitrogen status while preserving or enhancing soil fertility. This aligns with the principles of sustainable intensification, promoting yield stability without compromising agroecosystem integrity (Van der Heijden *et al.*, 2008; Zeng *et al.*, 2022).

CONCLUSION

The present study successfully isolated, identified, and characterized eight nitrogen-fixing endophytic bacterial strains from rice tissues cultivated in the Mekong Delta of Vietnam. Molecular identification using 16S rRNA gene sequencing confirmed that the isolates belonged to ecologically relevant genera such as *Gluconacetobacter*, *Burkholderia*, *Pantoea*, *Enterobacter*, and *Rahnella*. All strains demonstrated nitrogenase activity, with fixed nitrogen concentrations ranging from 0.024 to 0.091 mg N mL⁻¹, and several strains exhibited multiple plant growth-promoting traits, including IAA production, phosphate solubilization, siderophore secretion, and ACC deaminase activity.

Field trials confirmed that inoculation with *Gluconacetobacter diazotrophicus*, when combined with 50–75% of the recommended nitrogen fertilizer rate, maintained rice grain yield and biomass comparable to full-N treatments. SPAD index and soil quality parameters further indicated that the use of endophytic bioinoculants sustained plant nitrogen status and slightly improved soil organic matter and phosphorus availability. These findings highlighted the potential of native endophytic diazotrophs as promising microbial biofertilizer candidates to reduce synthetic nitrogen input, support crop productivity, and enhance soil health in intensive rice production systems.

Overall, this research provides a foundational understanding of indigenous nitrogen-fixing endophytes in Vietnamese rice ecosystems and supports their strategic integration into sustainable agricultural practices. Future research should emphasize optimizing formulations, validating them at field scale, and assessing their long-term effects on soil microbiota and nutrient cycling.

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