Journal of Tropical Biodiversity and Biotechnology
Volume 10, Issue 02 (2025): jtbb14798
DOI: 10.22146/jtbb.14798

Research Article

Identification and Characterisation of Endophytic Bacteria in Rice Bean (*Vigna umbellata*)

Marjohn C. Niño[1,2]*, Jerome H. Ruiz[1,3], Maria Lima D. Pascual[1]
1)College of Agriculture, Food Science, Agribusiness and Development Communication, Cebu Technological University Barili Campus Cagay, Barili, Cebu, 6036, Philippines
2)Current Address: College of Agriculture, Northern Iloilo State University Barotac Viejo Campus, Puerto Princesa, Barotac Viejo, Iloilo, 5011, Philippines
3)Current Address: College of Technology, Siquijor State College, Larena, Siquijor, 6226, Philippines
* Corresponding author, email: mjnino@nisu.edu.ph

Keywords:
Endophytic bacteria
Indigenous crop
Plant growth-promotion
Rice bean
16S rDNA
Submitted:
16 July 2024
Accepted:
02 December 2024
Published:
21 April 2025
Editors:
Miftahul Ilmi
Liya Audinah

ABSTRACT
Rice bean (*Vigna umbellata*) is an underutilised legume with unexplored endophytic microbiome. Identification and characterisation of these endophytes are critical in understanding their roles in plant's growth, health, and productivity. In this study, twelve morphologically and biochemically distinct bacterial endophytes were isolated from rice bean roots. Sequence analysis of the 16S rDNA of the isolates revealed that members of Proteobacteria, including *Stenotrophomonas* sp., *Shinella* sp., *Roseomonas* sp., *Pantoea dispersa*, and *Serratia marcescens* dominated the root tissues of rice bean. The remaining isolates were found to be members of Actinobacteria, Bacteroidetes, and Firmicutes. The *in vitro* assays showed the potential abilities of the endophytic *Stenoprophomonas* sp., *Shinella* sp., *Microbacterium gilvum*, *Serratia marcescens*, and *Bacillus qingshengii* in indole acetic acid production, exopolysaccharide production, and phosphate solubilisation. Overall findings suggest diverse and potentially multifunctional endophytes in rice bean that can be incorporated into agricultural practices and crop improvement programs.

How to cite:
Niño, M.C., Ruiz, J.H. & Pascual, M.L.D., 2025. Identification and Characterisation of Endophytic Bacteria in Rice Bean (*Vigna umbellata*). *Journal of Tropical Biodiversity and Biotechnology*, 10(2), jtbb14798. doi: 10.22146/jtbb.14798

INTRODUCTION

Rice bean (*Vigna umbellata*) is an annual, diploid legume member of family Fabaceae (Verma et al. 2022) that produces yellow flowers and small edible beans encased in pods. This fast-growing plant is a multipurpose legume that is more commonly cultivated in the South, Southeast, and East Asia, which are considered as its place of origin (Pattanayak et al. 2019). It is mainly grown for its protein- and micronutrient-packed seeds (Pattanayak et al. 2018) and also utilised for fodder and green manure (Dhillon & Tanwar 2018). However, when it comes to global production rice bean is nowhere close to its relative species, mung bean and cowpea, which are the most important domesticated *Vigna* species (Verma et al. 2022). In some countries in Southeast Asia, including The Philippines, this legume is traditionally cultivated in remote areas for the farmers' sustenance and livelihood. As a legume, rice bean is suitable in mixed-cropping systems as a rich nitrogen source, which could aid in improving soil fertility.

Unlike other notable *Vigna* species the potential of rice bean is only recognised in recent years; thus, scientific knowledge about this crop is still evolving as recent and ongoing studies uncover and offer new insights into its significance. It is characteristically known for its high biomass and grain yield potential and possess tolerance to abiotic and resistance to pest and disease, traits that are essential to thrive in harsh conditions (Guan et al. 2022). The intricate relationship between host plants' notable agronomic and resilience traits and their association with complex micro-ecosystem such as endophytes are increasingly recognised.

Plants possess a complex microbiome with diverse bacteria that colonise various organs and tissues and promote plant growth and fitness. Endophytes are either transmitted through seeds demonstrating plant growth promotion activities once the seeds have germinated or are recruited from soil (Verma & White 2018). These beneficial microorganisms have been demonstrated to play an important role in (i) facilitating nutrient-transfer symbioses for the uptake and utilization of nutrients, (ii) promoting plants growth, development, and production, (iii) alleviating oxidative stress, (iv) providing protection in plants from diseases and pests, and (iv) inhibiting growth of weeds (White at al. 2019).

The advancement in molecular techniques has made it possible to uncover complex microbial communities residing in the tissues of legumes and other host plants. Some of the current techniques employed that propel the field of plant microbiomes include DNA genome sequencing to determine the genes encoded by these plant growth-promoting bacteria (PGPB); transcriptomics, proteomics, and metabolomics that are used to understand the plant gene expression in association with PGPB; PGPB genome editing; and development of encapsulated microbial inoculants (Gamalero et al. 2022). Accumulating evidence revealed the diverse microbial endophytes contributing to the productivity and resilience of plants, which include members of several phyla such as Actinobacteria, Acidobacteria, Bacteroidetes, Deinococcus–thermus, Firmicutes, Proteobacteria, and Verrucomicrobia (Rana et al. 2020). Proteobacteria are the most dominant, well investigated group followed by Firmicutes and Actinobacteria, with *Bacillus*, *Pseudomonas*, *Burkholderia*, *Rhizobium*, and *Klebsiella*, as the dominant genera in most leguminous and non-leguminous plants (Rana et al. 2020).

However, the microbial community associated with rice bean is unexplored, which limits our understanding of the intricate relationships between these microorganisms and their host. Therefore, identifying its culturable endophytic bacterial community is essential for gaining valuable insights into the endophytic bacterial taxa with functional characteristics vital to the growth and development of plants. This information is vital in developing

strategies such as integration of endophytic inoculants into agricultural practices and crop improvement programs for enhancing crop resilience and productivity.

MATERIALS AND METHODS

Plant Materials and Sampling

Healthy seeds of rice bean accessions obtained from the germplasm collection of the Center for Studies in Biotechnology, Cebu Technological University were surface-sterilised and allowed to germinate in sterile petri plates lined with moistened filter paper. About ten sprouts per treatment in three replications were sown in the experimental pot at the experimental nursery area of Cebu Technological University Barili Campus under natural day-night conditions. The soil medium used was collected from the areas where accessions were sampled. All soil samples were mixed equally to create a composite soil sample.

Endophytic Bacteria Isolation

Root samples of rice bean at vegetative stage that were collected in triplicates were surface-sterilised using a 50 ml solution containing 2 % sodium hypochlorite (NaClO) and Phosphate Buffer "Saline" (PBS – 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4, 0.20 g of KCI, 8.00 g of NaCl, pH 7.4) for 25 min. Subsequently, the samples were washed with 50 ml 2 % sodium thiosulfate (Miché & Balandreau 2001) three times for 10 min. The roots were rinsed eight times with sterile distilled water before 1-hour rehydration in 100 mL sterile deionised water at room temperature. The efficiency of the sterilisation process was confirmed by plating 100 µL of the final rinse on nutrient agar (NA) and was incubated for six days at 28 °C. The root samples were weighed and macerated in 1 mL PBS buffer. The suspensions were used for counting and isolation from the dilution (1: 10, v v^{-1}) in PBS to 10^{-3} dilution as described by Lopez et al. (2016); subsequent dilutions, in three replica vials for each dilution, were inoculated on trypticase soy agar (TSA).

Morpho-cultural and Biochemical Characterisation

Serial dilutions of extracted suspensions were performed up to threefold and 100 µL of the last dilutions were spread on Nutrient Agar (NA) plates using a sterile spreader. The cultures were incubated for 24 hours at room temperature. Colonies were characterised by margin, colour, elevation, texture, and shape. The isolates were then differentiated based on the structural property of their cell wall using the Gram Stains kit (Himedia, Maharashta, India).

The starch hydrolysis test was performed using the iodine test described in Dunican and Seeley (1962), while the catalase activity of the strains was evaluated using the H_2O_2 solution drop method for bubble production (Zhu et al. 2022). Moreover, carbohydrate fermentation and CO_2 production assay using different sugars including glucose, maltose, and sucrose, was done following the protocol described in Reiner (2012). Yellow cultures indicate positive fermentation results while gas production (visible air bubbles in the inverted Durham tube) indicates positive CO_2 production (Reiner 2012). In addition, the antibiotic sensitivity assay using disc diffusion assay (Sarker et al. 2014) was carried out for amoxicillin, chloramphenicol, cephalexin, and doxycycline.

In vitro assay for plant growth promotion property

Screening for IAA Production

All strains were screened for Indole-3-acetic acid (IAA) production as described by Loper and Schroth (1986). Absorbance was measured at 530 nm after 30 min at room temperature. The concentration of IAA from each cul-

ture medium was calculated against the pure IAA standard curve (where: $y = 0.0098x + 0.0245$; $R^2 = 0.9972$).

Screening for Phosphate-solubilising Activity

The phosphate-solubilizing activity was determined qualitatively following the protocol of Nautiyal (1999) with modification. A clear halo formed around the colonies indicates phosphate-solubilising activity.

Screening for Exopolysaccharides (EPS) Production

Exopolysaccharides (EPS) production was tested as described previously in Zlosnik et al. (2008). Production of EPS was monitored visually according to the morphology of the colony and the viscous aspect.

PCR and Sequencing

A pure culture of isolates was grown on NB for 24 hrs. After incubation, the samples were centrifuged for 15 min at 1100 rpm to harvest the pellet. The supernatant liquid was discarded while the pellets were added with 100 µL sterilized deionized water. The total genomic DNA was extracted using an InstaGene™ Matrix DNA extraction kit (Bio-Rad Laboratories). Amplicons of the 16S rRNA region of each of the isolates were generated using polymerase chain reaction (PCR) with the use of the universal primer pairs 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). A Single-pass sequencing was carried out on ABI 3730XL (Applied Biosystems, Carlsbad, CA; Macrogen, Inc., South Korea) using the primers 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′).

Analysis and deposition of 16S rDNA sequences

The sequence consensus for each isolate was inferred with Genious Prime 2024 (version 11.0.20.1) software and queried against the non-redundant nucleotide database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast/) using Basic Local Alignment Search Tool (BLAST). Nucleotide sequence hits with at least 99 % similarity were identified for each isolate and downloaded for further analysis to verify identity. Generated 16S rDNA sequences from this study were deposited in GenBank, NCBI database.

RESULTS AND DISCUSSION

A total of 12 cultivable, distinct bacteria based on morpho-cultural characteristics, (Supplementary Table 1) were isolated from root samples of rice bean. Characteristics of bacterial endophytes based on Gram classification, starch hydrolysis, catalase activity, carbohydrate fermentation (glucose, maltose, and sucrose), CO_2 formation, and antibiotic sensitivity reaction are summarised in Table 1. Gram staining test revealed that 58.3 % of the isolates are Gram-negative and 41.7 % are Gram-positive. All isolates except VU-26 hydrolyzed starch, indicating that most produce exoenzyme amylase that breaks down starch into simpler sugar forms such as monosaccharides. Catalase production was also confirmed in 58.33 % of the isolates. Catalase facilitates cellular detoxification by catalysing the decomposition of hydrogen peroxide into water and hydrogen and has been implicated in regulating cellular signaling pathways for growth and apoptosis (Rasheed 2024). The antibiotic sensitivity assay also showed that all isolates were susceptible to cephalexin and doxycycline (Table 1), which are widely used broad-spectrum antibiotics (Wang et al. 2020). In addition, six isolates (50 %) are susceptible to all four antibiotics.

A bonitur scale was generated to identify bacterial isolates with plant-growth promotion potential (El-Sayed et al. 2014), as shown in Table 2. Re-

sults show that all strains are capable of producing IAA, with the highest level exhibited by VU-02 followed by VU-03, VU-15, VU-18, and VU-30. The remaining strains produced IAA with levels below <50 µg mL^{-1}. Auxin IAA is one of the most essential phytohormones that regulate growth and developmental processes in plants (Egamberdieva et al. 2017). Microbes colonising the rhizosphere of plants can produce this substance, which plants can use for cell division, differentiation, and enhancement of the host's uptake of minerals and nutrients from the soil (Shokri & Emtiazi 2010).

Likewise, the ability of beneficial bacteria to break down fixed and insoluble soil phosphorus into usable form that can be absorbed by plants is essential for growth and metabolism, resulting in an improved yield (Pan & Cai 2023). In the current study, VU-18 and VU-30 produced substantial phosphorous solubilisation abilities, while minimal activities were detected for VU-20, VU-24, VU-26, and VU-30. In plants, phosphorus uptake will increase in root and stem development, improve seed formation, and increase crop maturity and nitrogen fixation (Billah et al. 2019). In addition, phosphorus is critical in various stages of the photosynthetic process, including ATP synthesis (Khan et al. 2021).

Moreover, two isolates, including VU-02 and VU-03 produced substantial levels of Exopolysaccharides (EPS). EPS is a microbial polysaccharide synthesised by bacteria. It is involved in cell communication and nodule development in legumes and is thus crucial in nitrogen fixation (Liu et al. 2017). In addition, EPS produced by endophytic bacteria can alleviate salinity stress as it can bind with Na thus decreasing the quantity of Na ions available for plant uptake (Kumar et al. 2020).

A homology search of the 16S rDNA sequences using the Basic Alignment Search Tool (BLAST) in NCBI revealed the identity of the isolates, which were then deposited in GenBank, NCBI database and were given accession numbers OR342355 to OR342366. The phylogenetic tree showed that half (50 %) of the isolates belong to Phylum Proteobacteria with six members, followed by Firmicutes with three members (25 %), Actinobacteria with two members (16.67 %), and Bacteroidetes (8.33 %) (Figure 1). Despite the low number of endophytes analyzed, the affinity of isolates demonstrates significant diversity.

The homology search revealed that the phylum Proteobacteria, which dominated the roots of rice bean is composed of *Stenotrophomonas* sp. (OR342355) (100 % homology), *Shinella* sp. (OR342356) (99.50 %), *Roseomonas* sp. (OR342357) (100 %), two strains of *Pantoea dispersa* (both 99.86 %) (OR342362 and OR342364), and *Serratia marcescens* (OR342365) (99.93 %). The three members of Firmicutes include *Bacillus altitudinis* (OR342358) (99.93 %), *Bacillus qingshengii* (OR342366) (99.87 %), *and Bacillus* sp. K2DN333 (OR342363) (99.93 %). The Actinobacteria include *Microbacterium gilvum* (OR342359) (99.79 %) and *Microbacterium nanhaiense* (OR342360) (99.65 %), and the only member of Bacteroidetes isolated in this study is *Sphingobacterium mucilaginosum* (OR342361) (99.18 %) (Table 3).

Overall, *Stenotrophomonas* sp. (OR342355) has the highest assessment value (5) marked with the highest IAA level produced among isolates and a substantial level of EPS production, followed by *Shinella* sp. (OR342356), *Microbacterium nanhaiense* (OR342360), and *Serratia marcescens* (OR342365). This finding is consistent with previous report on the PGP and stress tolerance activities of *Stenotrophomonas* sp. isolated from field-grown poplar, which was found to produce IAA and detected to have active genes for auxin biosynthesis pathways and spermidine pathway (Ulrich et al. 2021). Likewise, inoculation with *Shinella* sp. has been reported to improve the growth of sugarcane, marked by an increase in dry weight (Taulé et al. 2016). Moreover, *Serratia marcescens* was found to participate in nitrogen cycling via denitrification-

Table 1. Biochemical characteristics of endophytic bacteria in rice bean

Bacterial Code	Isolate	Gram reaction	Starch hydrolysis	Catalase activity	CO_2 Formation	Carbohydrate Fermentation			Antibiotic sensitivity (30µg disc^{-1})			
						Glucose	Maltose	Sucrose	Amoxicillin	Chloramphenicol	Cephalexin	*Doxycycline*
VU-02		-	+	-	-	-	-	-	R	R	S	S
VU-03		-	+	-	-	+	+	+	S	S	S	S
VU-06		-	+	-	-	-	-	-	R	R	S	S
VU-12		+	+	+	-	-	-	-	S	S	S	S
VU-15		+	+	-	+	+	+	+	S	R	S	S
VU-18		+	+	-	+	-	+	-	R	S	S	S
VU-20		-	+	+	+	-	-	+	S	S	S	S
VU-24		-	+	+	-	-	+	-	S	S	S	S
VU-26		+	-	+	-	-	-	-	I	I	S	S
VU-29		-	+	+	-	+	+	+	I	S	S	S
VU-30		-	+	+	+	+	+	+	S	S	S	S
VU-31		+	+	+	-	-	-	-	S	S	S	S

(+) = positive reaction; (-) = negative reaction
(R) = Resistant; (I) = Intermediate Resistant; (S) = Susceptible

Table 2. Plant-growth promoting activity of endophytic bacterial isolates from root tissues of rice bean.

Accession	PGP Test			Total assessment point (9)[d]	Rank
	IAA production [a]	Phosphate solubilizing[b]	EPS production[c]		
VU-02	3	0	2	5	1
VU-03	2	0	2	4	2
VU-06	1	0	1	2	7
VU-12	1	0	1	2	7
VU-15	2	0	1	3	5
VU-18	2	2	0	4	2
VU-20	1	1	0	2	7
VU-24	1	1	0	2	7
VU-26	1	1	0	2	7
VU-29	1	0	1	2	7
VU-30	2	1	1	4	2
VU-31	1	2	0	3	5

[d]*Bonitur scale points (9):*

[a]*IAA production (0 = no production; 1 = <50 µg mL^{-1} IAA; 2 = 50-100 µg mL^{-1} IAA; 3 = >100 µg mL^{-1} IAA)*

[b]*Phosphate solubilizing (0 = no halo or lack of activity; 1 = small halo (up to 2 mm)/ little activity; 2 = medium halo (2–4 mm)/ medium activity; 3 = large halo (>4 mm)/high activity)*

[c]*Exopolysaccharides production (0 = no EPS production; 1 = low EPS production; 2 = average EPS production; 3 = high EPS production*

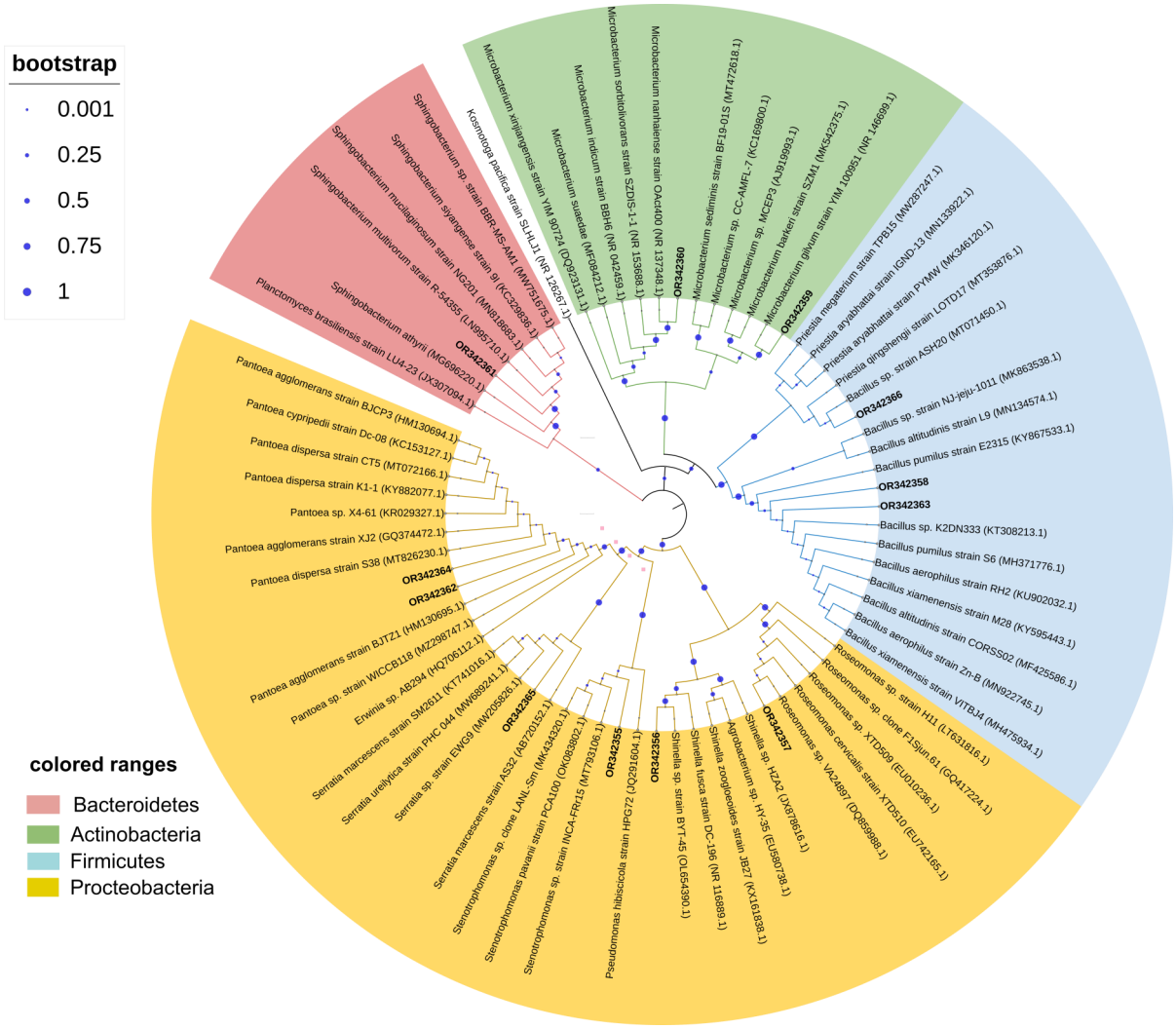

Figure 1. Phylogenetic tree constructed from 16S rRNA sequences using the maximum likelihood method based on the Tamura-Nei model (Tamura & Nei 1993) in MEGA 11 (Tamura et al. 2021) with 1000 bootstrap replicates. The analysis involved 72 nucleotide sequences, including the outgroup, *Kosmotoga pacifica* strain SLHLJ1(NR 126267.1). The phylogenetic tree was visualized in Interactive Tree of Life version 6.8.1 (Letunic & Bork 2021). GenBank accession numbers of nucleotide sequences are shown along with the name of the bacterial species.

Table 3. The nearest phylogenetic relative of bacterial isolates based on the 16S RNA sequencing.

Isolate's Accession Code	Nearest Phylogenetic Neighbor	Homology (%)	E - value	Family	Phylum
VU-02 (OR342355)	*Stenotrophomonas* sp. strain INCA-FRr15 (MT793106.1)	100	0	*Xanthomonadaceae*	Proteobacteria
VU-03 (OR342356)	*Shinella* sp. strain BYT-45 (OL654390.1)	99.50	0	*Brucellaceae*	Proteobacteria
VU-06 (OR342357)	*Roseomonas* sp. VA24897/2006 (DQ859988.1)	100	0	*Acetobacteraceae*	Proteobacteria
VU-12 (OR342358)	*Bacillus altitudinis* strain CORSS02 (MF425586.1)	99.93	0	*Bacillaceae*	Firmicutes
VU-15 (OR342359)	*Microbacterium gilvum* strain YIM 100951 (NR_146699.1)	99.79	0	*Microbacteriaceae*	Actinobacteria
VU-18 (OR342360)	*Microbacterium nanhaiense* strain OAct400 (NR_137348.1)	99.65	0	*Microbacteriaceae*	Actinobacteria
VU-20 (OR342361)	*Sphingobacterium mucilaginosum* strain NG201 (MN818683.1)	99.18	0	*Sphingobacteriaceae*	Bacteroidetes
VU-24 (OR342362)	*Pantoea dispersa* strain S38 ITI (MT826230.1)	99.86	0	*Enterobacteriaceae*	Proteobacteria
VU-26 (OR342363)	*Bacillus* sp. K2DN333 (KT308213.1)	99.93	0	*Bacillaceae*	Firmicutes
VU-29 (OR342364)	*Pantoea dispersa* strain K1 -1 (KY882077.1)	99.86	0	*Enterobacteriaceae*	Proteobacteria
VU-30 (OR342365)	*Serratia marcescens* strain AS32 (AB720152.1)	99.93	0	*Enterobacteriaceae*	Proteobacteria
VU-31 (OR342366)	*Bacillus qingshengii* strain LOTD17 (MT353876.1)	99.87	0	*Bacillaceae*	Firmicutes

DNRA-nitrification pathway, which is essential to sustain plant growth and development (Hamada & Soliman 2023). However, no studies have yet explored the potential of *Microbacterium nanhaiense* as a plant growth-promoting endophyte in plants.

CONCLUSIONS

Overall findings of this study revealed the presence of cultivable bacterial endophytes in rice bean with potential plant growth-promotion activities. However, the evaluation for potential PGP properties in this study is limited to a few assays. To confirm these initial findings, additional relevant PGP screenings, including assessments of abiotic stress tolerance, biotic resistance, and influence on morphological and yield traits of crops, must be explored. Furthermore, in-depth studies uncovering the mechanisms of such roles in host plants and other crops are necessary to fully understand the extent of their beneficial effects and their potential utilisation for crop improvement.

AUTHOR CONTRIBUTION

M.C.N. designed the research, analysed some datasets, wrote some parts of the manuscript, and supervised all the processes; J.H.R. performed the experiments, analysed some datasets, and wrote some parts of the manuscript; and M.L.D.P. aided in designing the research, supervised all the processes, and proofread the manuscript.

ACKNOWLEDGMENTS

This work was supported by Cebu Technological University.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest. The authors are responsible for the content and article writing.

REFERENCES

Billah, M. et al., 2019. Phosphorus and phosphate solubilizing bacteria: keys for sustainable agriculture. *Geomicrobiology Journal*, 36(10), pp.904-916. doi: 10.1080/01490451.2019.1654043.

Dhillon, P.K. & Tanwar, B., 2018. Rice bean: A healthy and cost-effective alternative for crop and food diversity. *Food Security*, 10, pp.525-525. doi.org/10.1007/s12571-018-0803-6.

Dunican, L.K. & Seeley, H.W., 1962. Starch hydrolysis by *Streptococcus equinus*. *Journal of Bacteriology*, 83(2), pp.264-269. doi: 10.1128/jb.83.2.264-269.1962.

Egamberdieva, D. et al., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, 8, 2104. doi: 10.3389/fmicb.2017.02104.

El-Sayed, W.S. et al., 2014. *In vitro* antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Frontiers in Microbiology*, 5, 651. doi: 10.3389/fmicb.2014.00651.

Gamalero, E., Bona, E. & Glick, B.R., 2022. Current Techniques to Study Beneficial Plant-Microbe Interactions. *Microorganisms*, 10(7), 1380. doi: 10.3390/microorganisms10071380.

Guan, J. et al., 2022. Genomic analyses of rice bean landraces reveal adaptation and yield related loci to accelerate breeding. *Nature Communications*, 13, 5707. doi.org/10.1038/s41467-022-33515-2.

Hamada, M.A. & Soliman, E.R.S., 2023. Characterization and genomics identification of key genes involved in denitrification-DNRA-nitrification pathway of plant growth-promoting rhizobacteria *(Serratia marcescens* OK482790). *BMC Microbiology*, 23, 210. doi.org/10.1186/s12866-023-02941-7.

Khan, M.A. et al., 2021. Rhizospheric *Bacillus* spp. Rescues Plant Growth Under Salinity Stress *via* Regulating Gene Expression, Endogenous Hormones, and Antioxidant System of *Oryza sativa* L. *Frontiers in Plant Science*, 12, 665590. doi: 10.3389/fpls.2021.665590.

Kumar, A. et al., 2020. Plant growth-promoting bacteria: biological tools for the mitigation of salinity stress in plants. *Frontiers in Microbiology*, 11, 1216. doi: 10.3389/fmicb.2020.01216.

Letunic, I. & Bork, P., 2021. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 49 (1), pp.293-296. doi: 10.1093/nar/gkab301.

Liu, J. et al., 2017. Recent advances in endophytic exopolysaccharides: production, structural characterization, physiological role and biological activity. *Carbohydrate Polymers*, 157, pp.1113-1124. doi: 10.1016/j.carbpol.2016.10.084

Loper, J.E. & Schroth, M.N., 1986. Influence of bacterial sources of indole-3-acetic acid on root elongation of sugar beet. *Phytopathology*, 76(4), pp.386-389. doi: 10.1094/Phyto-76-386

Lopez, K.B.A. et al., 2016. Culturable endophytic bacterial communities associated with field-grown soybean. *Journal of Applied Microbiology*, 120(3), pp.740-755. doi: 10.1111/jam.13046.

Miché, L. & Balandreau, J., 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated *Burkholderia vietnamiensis*. *Applied and Environmental Microbiology*, 67(7), pp.3046-3052. doi: 10.1128/AEM.67.7.3046-3052.2001.

Nautiyal, C.S., 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, 170, pp. 265-270. doi: 10.1111/j.1574-6968.1999.tb13383.x.

Pan, L. & Cai, B., 2023. Phosphate-Solubilizing Bacteria: Advances in Their Physiology, Molecular Mechanisms and Microbial Community Effects. *Microorganisms*, 11(12), 2904. doi.org/10.3390/microorganisms11122904.

Pattanayak, A. et al., 2018. Diversity analysis of rice bean (*Vigna umbellata* (Thunb.) Ohwi and Ohashi) collections from North Eastern India using morpho-agronomic traits. *Scientia Horticulturae*, 242, pp.170–180. doi.org/10.1016/j.scienta.2018.08.003.

Pattanayak, A. et al., 2019. Rice bean: a lesser known pulse with well-recognized potential. *Planta*, 250(3), pp.873–890. doi.org/10.1007/s00425-019-03196-1

Rana, K.L. et al., 2020. Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie Van Leeuwenhoek*, 113(8), pp.1075-1107. doi: 10.1007/s10482-020-01429-y.

Rasheed, Z., 2024. Therapeutic potentials of catalase: Mechanisms, applications, and future perspectives. *International Journal of Health Sciences (Qassim)*, 18(2), pp.1-6.

Reiner, K., 2012. Carbohydrate Fermentation Protocol. *American Society for Microbiology*.

Sarker, M.M.R. et al., 2014. Studies of the impact of occupational exposure of pharmaceutical workers on the development of antimicrobial drug resistance. *Journal of Occupational Health*, 56, pp.260-270. doi: 10.1539/joh.14-0012-OA.

Shokri, D. & Emtiazi, G., 2010. Indole-3-acetic acid (IAA) production in symbiotic and non-symbiotic nitrogen-fixing bacteria and its optimization by Taguchi design. *Current Microbiology*, 61(3), pp.217-225. doi: 10.1007/s00284-010-9600-y.

Tamura, K. & Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, pp.512-526. doi: 10.1093/oxfordjournals.molbev.a040023.

Tamura, K., Stecher, G. & Kumar, S., 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7), pp.3022-3027. doi: 10.1093/molbev/msab120.

Taulé, C. et al., 2016. Endophytic colonization of sugarcane (*Saccharum officinarum*) by the novel diazotrophs *Shinella* sp. UYSO24 and *Enterobacter* sp. UYSO10. *Plant Soil*, 403, pp.403–418. doi: 10.1007/s11104-016-2813-5.

Ulrich, K. et al., 2021. Genomic Analysis of the Endophytic *Stenotrophomonas* Strain 169 Reveals Features Related to Plant-Growth Promotion and Stress Tolerance. *Frontiers in Microbiology*, 12, 687463. doi: 10.3389/fmicb.2021.687463.

Verma, S.K. et al., 2022. Vignette of Vigna domestication: From archives to genomics. *Frontiers in Genetics*, 13, 960200. doi: 10.3389/fgene.2022.960200.

Verma, S.K. & White, J.F., 2018. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). *Journal of Applied Microbiology*, 124, pp.764–778. doi: 10.1111/jam.13673.

Wang, B. et al., 2020. Unravelling kinetic and microbial responses of enriched nitrifying sludge under long-term exposure of cephalexin and sulfadiazine. *Water Research*, 173, 115592. doi: 10.1016/j.watres.2020.115592.

White, J.F. et al., 2019. Review: Endophytic microbes and their potential applications in crop management. *Pest Management Science*, 75(10), pp.2558-2565. doi: 10.1002/ps.5527. doi: 10.1002/ps.5527.

Zhu, W. et al., 2022. Isolation and characterization of tick-borne *Roseomonas haemaphysalidis* sp. nov. and rodent-borne *Roseomonas marmotae* sp. nov. *Journal of Microbiology*, 60(2), pp.137-146. doi: 10.1007/s12275-022-1428-1.

Zlosnik, J.E. et al., 2008. Differential mucoid exopolysaccharide production by members of the *Burkholderia cepacia* complex. *Journal of Clinical Microbiology*, 46(4), pp.1470-1473. doi: 10.1128/JCM.02273-07.

APPENDICES

Supplementary Table 1. Morphological characterization of isolates based on colony.

Accession	Morpho-cultural characteristics				
	Margin	**Color**	**Elevation**	**Texture**	**Form**
OR342355	undulate	white	flat	dry	round
OR342356	entire	white	convex	dry	punctiform
OR342357	entire	pink	convex	slimy	round
OR342358	filamentous	white	flat	slimy	rhizoid
OR342359	lobate	yellow green	flat	slimy	rhizoid
OR342360	lobate	yellow	umbonate	slimy	rhizoid
OR342361	entire	milky	flat	slimy	round
OR342362	erose	white	flat	dry	irregular
OR342363	entire	white	umbonate	dry	round
OR342364	erose	white	flat	slimy	rhizoid
OR342365	entire	red	convex	slimy	round
OR342366	entire	white	umbonate	dry	round