RESEARCH ARTICLE

Antagonistic and Plant Growth Promotion Activities of Endophytic Bacteria Isolated from Deep Water Rice (*Oryza sativa* L. Cv. Ronga Bao) of Assam

Purnamsree Gogoi[1], Madhu Kamle[1] and Pradeep Kumar[2*]

DOI: 10.18811/ijpen.v10i02.06

Abstract

Ronga bao is a native variety of deepwater rice (DWR) that grows in the flood plains of the Brahmaputra valley in Assam. It is resistant to salt, alkalinity, drought, and floods. The goal of the current study was to examine bacterial endophytes that were isolated from Ronga bao and assessed for their ability to inhibit three rice pathogens: *Sarocladium oryzae*, *Rhizoctonia solani*, and *Bipolaris oryzae*, which cause sheath rot, brown spot, and sheath blight, respectively. The plant samples were collected from the Lakhimpur district of Assam. Then, endophytic bacteria were isolated from the roots, stem, and leaf regions of the plant. *In-vitro* antifungal screening was performed using the dual culture technique and cell-free culture filtrate (CFCF) of bacterial strains. Four root endophytes and only one stem endophyte showed antifungal activity against the phytopathogens. The plant growth-promoting activities were also examined for potential bacterial strains and recorded. Morphological, biochemical, and molecular characterization revealed that the isolated endophytic bacteria RB1 as *Lysinibacillus fusiformis* strain PK4, RB2 as *Enterobacter quasiroggenkampi* strain PK2, RB3 as *Shigella* sp., RB4 as *Pseudomonas fluorescens* strain PK3, and RB5 as *Klebsiella* sp. Potential isolates exhibiting antifungal and plant growth promotion activities can be further utilized for the development of biocontrol formulations for controlling multiple biotic stresses.

Keywords: Endophytic bacteria, phytopathogen, antagonism, plant growth promoting activity, 16S rRNA sequencing.

Highlights

- Isolation of endophytic bacteria from Ronga Bao, an indigenous deepwater rice variety of Assam.
- Antifungal screening conducted against rice pathogens *Rhizoctonia solani, Bipolaris oryzae*, and *Sarocladium oryzae*.
- Five potential bacteria were identified: *Lysinibacillus* sp., *Enterobacter* sp., *Shigella* sp., *Pseudomonas* sp., and *Klebsiella* sp.
- Isolates also demonstrated plant growth-promoting activities under *in-vitro* conditions.
- The 16S rRNA sequences have been deposited in the NCBI GenBank for final confirmation.

International Journal of Plant and Environment (2024); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

Rice (*Oryza sativa*) stands as a principal food crop for a significant portion of the world's population, contributing more than 20% of daily calorie intake (Ray *et al*., 2013). India, boasting the second-largest production scale globally at 42.9 million hectares, occupies 27.1% of the total rice-growing area, trailing only behind China (Singh *et al.,* 2012). Northeast India boasts diverse geographical regions with varied climatic conditions (Singh *et al.,* 2006). Assam, in particular, hosts numerous indigenous rice varieties, thriving in its favorable agro-climatic conditions (Sahai, 2016). Among these, Ronga bao, an indigenous deepwater rice (DWR) variety predominantly cultivated in Assam's upper districts along the Brahmaputra River, shines. Given the annual flooding crisis affecting over 23 districts, including the North Bank region of the Brahmaputra valley, DWR varieties have naturally become the choice crop due to their innate resistance to flood, drought, salinity, and alkalinity (Rohilla *et al*., 2019; Nahar *et al*., 2018; Gogoi *et al.,* 2020). The distinctive red kernels of Ronga bao are attributed to the accumulation of polyphenols and anthocyanins in the aleurone layers (Rohilla *et al*., 2019). Furthermore, DWR is esteemed for its richness in zinc, iron, vitamins, and minerals, coupled with its antioxidant properties and resilience against pests and diseases (Chowdhury *et al*., 2016; Mudoi and Das, 2019). Beyond its agricultural significance, Ronga bao holds deep cultural roots, with the rice grain prominently featured in Assam's popular cultural festival, Bihu, where it is cooked into a delectable dish known as "handoh guri."

Although several biotic factors affect rice production, diseases continue to be the primary challenge (Kumar *et al*., 2013). According to Etsami *et al.* (2019), bacteria, fungi, and nematodes are responsible for around 70% of rice illnesses. Sheath blight, sheath rot, and brown spot are common fungal diseases in Assam, which become additional risks to DWR

[1]Applied Microbiology Laboratory, Department of Forestry, North Eastern Regional Institute of Science and Technology, Nirjuli, Arunachal Pradesh, India.

[2]Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India.

***Corresponding author:** Pradeep Kumar, Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh, India., Email: pkbiotech@gmail.com

How to cite this article: Gogoi, P., Kamle, M., Kumar, P. (2024). Antagonistic and Plant Growth Promotion Activities of Endophytic Bacteria Isolated from Deep Water Rice (*Oryza sativa* L. Cv. Ronga Bao) of Assam. International Journal of Plant and Environment. 10(2), 190-201.

Submitted: 19/05/2024 **Accepted:** 29/05/2024 **Published:** 30/06/2024

verities (Islam *et al.,* 2004). *Sarocladium oryzae* and *Rhizoctonia solani* are the main causes behind sheath rot and sheath blight, respectively, which cause significant yield losses. Meanwhile, *Bipolaris oryzae* Subr wreaks havoc with brown spot disease, and Jain (=*Helminthosporium oryzae* Breda de Haan teleomorph= *Cochliobolus miyabeanus*) has been noted for its significant grain yield losses (Singh *et al*., 2014).

Optimizing rice disease management is essential to increase yield. Using beneficial microbes and biological control techniques are two viable alternatives to chemical fertilizers (Etesami *et al.,* 2019). Endophytic microbes, residing within plant tissues, emerge as eco-friendly allies, bolstering plant growth through various mechanisms, including hormone production, phosphate solubilization, siderophore production, and nutrient provision (Bandara *et al*., 2006). Moreover, they enhance nitrogen fixation and exhibit antimicrobial properties, curbing losses from pathogenic organisms (Verma *et al.,* 2001; Rahman and Saiga, 2005; Taktek *et al*., 2017).

According to Borah *et al*. (2021), there are roughly three million different plant species on earth. Although every plant contains certain beneficial microbes, very few species have had their endophytic mechanisms thoroughly studied. As a result, there is a relatively higher chance of discovering novel endophytic organisms from various plants in hotspot regions. Assam, nestled within the Indo-Burma biodiversity hotspot, serves as a secondary center for rice origin, offering fertile ground for such explorations. Thus, the current research endeavors to isolate and characterize endophytic bacteria from *Oryza sativa* L. cv. Ronga bao, along with evaluating their antifungal properties and plant growth-promoting activities

Methods And Materials

Collection of the plant sample and isolation of bacteria

The young and robust seedlings of Ronga bao were carefully gathered from a rice field in the Lakhimpur district of Assam, India. This collection took place during July/August, 2022; ensuring the prime condition of the specimens. Following collection, the samples were promptly transported to the laboratory for subsequent processing. The sample plants underwent a meticulous washing procedure under running tap water. Subsequently, the plants were carefully dissected into roots, stems, and leaves. Surface sterilization of these plant parts was then carried out using a combination of absolute alcohol and sodium hypochlorite solution. Following sterilization, the serial dilution method was employed for the isolation of bacteria. Pure cultures were successfully isolated and preserved at 4°C for future experimentation (Arora *et al*., 2014).

Rice phytopathogens and culture condition

Three fungal phytopathogens were selected for this study: *R. solani* (MTCC no. 9666), known to cause sheath blight disease; *Sarocladium oryzae* (MTCC no. 2046), which causes sheath rot and *B. oryzae* (ITCC no. 1319),the causal agent of brown spot disease of rice. Upon procuring disease-causing pathogens from the respective laboratory culture conditions, each fungal inoculum was promptly inoculated onto Potato Dextrose Agar (PDA) plates. In order to promote optimal mycelia growth, the plates were incubated at 28°C. *R. solani* displayed full mycelial development on PDA plates after two to three days of incubation period; while *B. oryzae* and *S. oryzae* required ten to fourteen days to develop their mycelial growth. After development and incubation, a 5 mm diameter mycelial disc was selected from each fungal culture, and it was then put on freshly prepared PDA plates and incubated at 28°C for an additional period of time. This was done three or five times according to the method described by Bibi *et al*. (2012), albeit with a few minor adjustments.

Invitro antifungal activity of isolated bacterial endophytes

A preliminary screening was performed for the selection of the antagonistic activity of bacteria. A 5 mm diameter of test fungi was inoculated at the center of potato dextrose agar plates. The isolated bacteria were then together inoculated against the fungal inoculum. The bacteria which inhibited the growth of fungi were selected for further testing (Agarwal *et al*., 2017).

Dual Culture Technique

Dual culture technique was performed for the antagonistic test of endophytic bacteria against rice pathogens. The growth inhibition (GI) was estimated using the following formula (Naik *et al*., 2006).

$$GI(\%) = \frac{Rc - Re}{Rc} \times 100$$

[Where, Rc = Radial growth of the fungal pathogen in control, Re = Radial growth of the fungal pathogen in the presence of endophytic bacteria strain (dual inoculation)]

Antifungal Activity of Cell-Free Culture Filtrate (CFCF)

Antagonistic tests of CFCF of bacterial strains were tested by agar well diffusion assay as described by Tagg and McGiven (1971). Zone of inhibition was recorded, and an inhibition percentage (%) was calculated using the following formula

$$\textbf{\textit{Inhibition}}\% = \frac{\text{Fungal growth in control} - \text{Fungal growth in culture filtrate}}{\text{Fungal growth in control}} \times \mathbf{100}$$

Evaluation for plant growth promotion (PGP) activities

Indole-3-acetic acid production test

Isolated endophytic bacteria were cultivated in tryptone broth. 0.1 mL of fresh bacterial cultures were inoculated onto the liquid medium, and it was then cultured for 48 hours at 35°C (Aneja, 2018). The supernatant was combined with 4 mL of salkowaski reagent and two drops of o-phosphoric acid for IAA estimation. The development of pink color indicated IAA production which was determined by measuring the absorbance at 535 nm. A standard curve was plotted in which IAA and Salkowaski reagents were dissolved in tryptone broth to quantify unknown IAA concentrations of samples (Etesami *et al*.,2014).

Gibberellic Acid (GA3) production test (Paleg and Coombe, 1967)

To detect Gibberellic acid, fresh bacterial cultures were added to the nutrient broth and incubated at 30°C for 72 hours. Around 5 ml of supernatant was taken in a test tube to which 0.4 mL of

potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. The supernatant was collected and 3 mL of 30% HCl was added and incubated at 20°C for 75 minutes. The control sample was treated with 5% HCl and the absorbance of the sample was measured at 254 nm in a UV-vis spectrophotometer (Bora et al., 2021).

Phosphate solubilization assay

The phosphate solubilization assay was accomplished in accordance with the guidelines provided by Dias *et al.,* 2008 with slight modifications. Briefly, on the pikovskaya medium, bacterial isolates were cultured. Colonies that could solubilize inorganic phosphate were seen to have halo zones surrounding them after fourteen days of culture. The following formula was used to determine solubilization efficiency.

$$Solubilisation\ efficiency(\%) = \frac{\text{Diameter of solubilisation halo}}{\text{Diameter of the colony}} \times 100$$

Zinc solubilization assay

The endophytic bacterial isolates were first inoculated on zinc solubilization agar plates. These plates were covered with aluminum foil and incubated in the dark at 28°C for 14 days (Ramesh et al., 2014). The colonies exhibiting clear halo zones were selected and solubilization efficiency was calculated using the following formula.

$$Solubilisation\ efficiency(\%) = \frac{\text{Diameter of solubilisation halo}}{\text{Diameter of the colony}} \times 100$$

Potassium solubilization assay

The endophytic bacteria were inoculated on Aleksandrow agar media and incubated for 3-4 days at 28 ± 2°C. The presence of halo zone around the bacterial colony was considered as an indicator of potassium solubilization. Solubilization efficiency was recorded using the following formula (Hu et al., 2006).

$$Solubilisation\ efficiency(\%) = \frac{\text{Diameter of solubilisation halo}}{\text{Diameter of the colony}} \times 100$$

Assessment for PGP activity in seed germination test and pot experiment

A pot experiment and a seed germination test were carried out to examine the impact of particular endophytic bacterial strains on plant growth (Naiwal *et al*., 2014; Sorty *et al*., 2016). The rice seeds (*Oryza sativa* L. cv. Joha) were surface sterilized by immersion in a 0.2% $HgCl_2$ solution for three minutes and exposed to 95% ethanol. After that, the seeds were washed five times in sterile, distilled water. The endophytic isolates underwent cultivation in broth at a temperature of 30 ± 2°C for 24 and 48 hours, respectively. Following the application of 1-mL of each culture treatment to the seeds, the treated seeds were dried. The seeds treated with a non-inoculated medium were used as the control. In order to assess seed germination, agar plates containing treatment and control seeds were incubated in the dark until the seeds germinated. For the pot experiment, pots were filled with autoclaved soil. Finally, the treated and control seeds were transferred to a pot to a depth of 5 mm. The experiment was performed in triplicate. After 15 days of observation, the plants were uprooted carefully and the length of root and shoot and fresh and dry weight of seedlings were measured. According to Mir *et al*., 2014, seed vigor index was calculated using the following formula:

$$\text{Vigor index} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination\%}$$

Morphological and biochemical identification of potent endophytic bacteria

Preliminary identification of each endophytic bacterial strain was done according to Bergey's manual of systematic bacteriology. Cell morphology of the potent strains was determined by the classical gram staining method (Bartholomew, 1962). KOH String test, an alternative to the gram staining test of bacteria (Arthi *et al.,* 2003) was also performed. Biochemical tests such as catalase test, amylase production test, MRVP test, lactose fermentation test, Urea agar test, gelatin hydrolysis test and casein hydrolysis test were performed according to methods described by Aneja, 2018.

Identification and phylogenetic analysis of endophytic bacteria

Extraction of genomic DNA

The isolated endophytic bacteria were cultured in LB broth at 28°C for 24 hours and then centrifuged at 14,000 rpm for 5 min. The genomic DNA was extracted from the obtained pellet by using HiPurA® 96 Bacterial Genomic DNA Purification Kit, Himedia according to the manufacturer's instructions. The purity of extracted bacterial genomic DNA was evaluated through 1% agarose gel electrophoresis following the method described by Koh *et al.,* (1998).

Identification and phylogenetic analysis of endophytic bacteria

The amplification of 16S rDNA was performed in a reaction with a final volume of 50 µL. This contained 1-µL (0.5–10 ng) of total DNA, 5 µL of 10x buffer, 4 µL (2.5 mM) of dNTPs mixture, 0.5 µL of Taq DNA polymerase, µL dNTPs (2.5 mM) mixture, and 0.75 µL (20 mM) of each primer, 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'- CGG TTA CCT TGT TAC GAC TT-3'). A negative control (PCR mix devoid of DNA) was incorporated in all PCR experiments. The parameters of the PCR reaction were as follows: 94°C for 5 minutes; 35 cycles of denaturation (60 s at 94°C), annealing (30 s at 55°C), and extension (60 s at 72°C) were performed; finally, a final extension was performed for 5 minutes at 72°C. The PCR products were then purified and sequenced (Barcode Biosciences, Bangalore, India).

Identification and phylogeny analysis

The obtained sequence data were analyzed using BLAST program in NCBI (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi) (Cole *et al*., 2009; Wang *et al*., 2007). We confirmed the experimental sequences' similarity to the reference sequences in the NCBI databases (Cole *et al*., 2009) and classified the selected potent isolates at the genus level. Using Clustal W, a multiple alignment program, homologous sequences were selected and aligned according to their maximum identity score. The phylogenetic tree of each powerful isolate was created based on 16S rRNA gene sequences using the neighbour joining

method in MEGA 11 software. Using 1,000 bootstrap replicates created from a random seed, the dependability of the trees was examined. In order to get the accession number, the produced nucleotide sequences for each bacterial isolate were submitted to Genbank

Statistical analysis

Each experiment was performed in triplicate. Means and standard error were calculated for all data sets and analysis of variance (ANOVA) was performed at $p \leq 0.05$ on Microsoft Excel 2021.

Results And Discussion

Isolation of bacteria and antifungal screening

To ascertain the presence of endophytic bacteria in Ronga bao, the roots, stems, and leaves of the plant have been utilized to separate the bacteria. From the sample plant, six bacteria related to the roots, four associated with the stem, and four associated with the leaves were isolated. Further, four bacteria associated with roots and one bacterium associated with stems were chosen from among the isolates based on their antifungal properties against the phytopathogens *R. solani, B. oryzae*, and *S. oryzae*. As the isolated bacteria did not exhibit hostility towards the test phytopathogens, they could not be selected. Table 1 shows the potent bacteria that were chosen, together with their host tissue and antagonistic activity against the test fungus.

Dual culture technique

Using the dual culture method on PDA plates, the five endophytic bacterial isolates were examined for their ability to impede the mycelial growth of test fungus in vitro. The fungal growth was inhibited by all five strains, as demonstrated by the results of the dual culture assay. Table 2 shows that the antagonistic effect of the bacterial isolates ranges from 23.99% to 58.53%. An inhibitory zone between 4 and 10 mm in diameter developed when the fungal hyphae of *R. solani* and, *B. oryzae* and *S. oryzae* could not enter the bacterial culture after 5 and 14 days of incubation (Fig. 1).

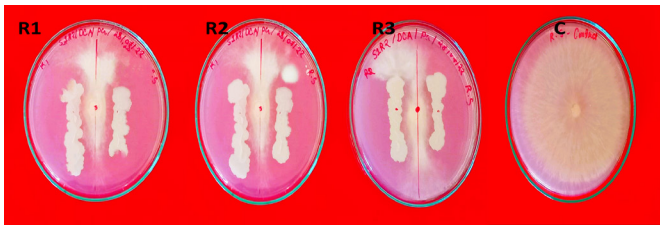

Fig. 1: Antifungal screening of potent endophytic bacteria against rice pathogens using the dual culture assay

Table 1: Selected endophytic bacteria based on their antifungal activity against rice phytopathogens

Isolates code	*Host tissue*	*R. solani*	*B. oryzae*	*S. oryzae*
RB1	Root	-	+	-
RB2	Root	+	-	-
RB3	Root	-	-	+
RB4	Root	+	-	-
RB5	Stem	-	+	-

('+' for antagonistic activity; '–'no antagonistic activity)

Antifungal activity of cell-free culture filtrate (CFCF)

When selected endophytic bacteria were tested for their capacity to produce antifungal compounds *in-vitro*, they demonstrated a strong antagonistic activity. The filtrates of bacterial strains were highly inhibitory to the growth of phytopathogens tested. The antifungal indices of bacterial culture filtrate ranges from 37.29 to 64.44% (Table 2). The highest inhibitory effect was observed with *R. solani* and the lowest with *S. oryzae* (Fig. 2).

In the same way, bacterial endophytes from various rice cultivars improved rice plant development and showed strong antagonistic effects against *R. solani in-vitro* (Ji *et al*., 2014). *Bacillus* sp. isolated from a rice variety showed antagonistic potential against *B. oryzae* indicated the significant reduction of intensity of brown spot disease of rice (Prabhukarthikayan *et al*., 2019). Saravanakumar *et al*. (2009) reported that *Pseudomonas* strains pf1, TDK1, and PY15 had superior efficacy in mitigating sheath rot disease in rice plants. The finding of Ajulo *et al*. (2024) also supported the result of the present investigation. They revealed that rice-associated endophytic bacteria reduced the growth of *B.oryzae* and suppressed brown spot severity by up to 90%. It is already stated that, members of the

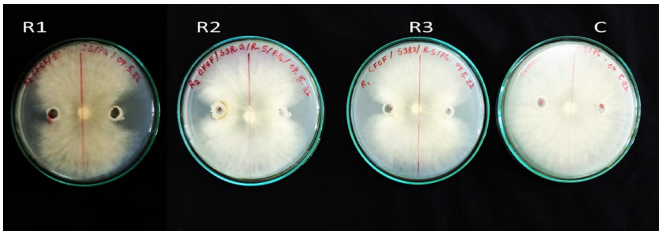

Fig. 2: Antifungal activity of cell-free culture filtrate (CFCF) of potent endophytic bacteria against rice pathogens

Table 2: *In vitro* antifungal activity (% inhibition) of potent endophytic bacteria against rice phytopathogens

Isolates code	*R. solani*		*B. oryzae*		*S. oryzae*	
	DCA	CFCF	DCA	CFCF	DCA	CFCF
RB1	-	-	23.99 ± 0.34	37.29 ± 1.37	-	-
RB2	28.14 ± 0.34	61.85 ± 1.28	-	-	-	-
RB3	-	-	-	-	49.60 ± 1.34	63.07 ± 1.54
RB4	40.36 ± 1.69	64.44 ± 1.11	-	-	-	-
RB5	-	-	58.53 ± 0	56.36 ± 1.38	-	-

(DCA= dual culture assay; CFCF= cell-free culture filtrate)
[Data are shown in Mean ± SD of three replicates, Significant (P≤0.05)]

family Enterobacteriaceae, including *Citrobacter, Enterobacter, Serratia* and *Klebsiella* are known to develop sustainable agricultural systems by reducing or shielding plants from disease (Jha *et al*., 2011). Several endophytic isolates into different genera including *Bacillus, Pseudomonas, Streptomyces, Exiguobacterium, Aeromonas, Chryseobacterium, Enterobacter*, and *Stenotrophomonas* demonstrated biocontrol activity against a variety of rice diseases, including bacterial leaf blight, leaf blast, brown spot, and sheath blight (Jasrotia *et al*., 2021).

Evaluation for plant growth promotion (PGP) activity

Screening of the potent bacterial isolates for PGP traits revealed that 36% were able to produce IAA, 29% were zinc solubilizers, 21% had phosphate solubilization activity and 14% were recognized as potassium solubilizers. Only one isolate RB2 exhibited all four activities, i.e., IAA production, P-solubilization, Zn- solubilization and K- solubilization.

Indole-3-acetic acid (IAA) production

The ability of the selected endophytic isolates to produce indole-3-acetic acid (IAA) contributes to the enhancement of plant growth. Tryptophan, found in plant roots and a precursor for the production of IAA utilized by endophytic bacteria, makes the IAA production test a popular method for characterizing bacteria connected with plants (Liaqat *et al.,* 2016). The production of IAA was examined in all five potent bacteria, and the results are presented in Table 3. According to Table 3, RB4 produced the highest amount of IAA (47.43 µg/ml), followed by RB1 (43.93 µg/ml) while RB3 produced the least (10.4 µg/ml). Phetcharat *et al.* (2011) conducted research work to identify and characterize *Pseudomonas* sp., *Azotobacter* sp., *Enterobacter* sp., and *Bacillus* sp. from the root and stem sections of the rice plant. The results showed significant IAA production. As a factor that promotes plant growth, endophytic bacteria isolated from various species have been identified in several previous studies to produce IAA (Rana et al., 2011, Jasim et al., 2014, Yaish et al., 2015, and Khan *et al*., 2020).

Gibberellic acid (GA3) production test

Gibberellins (GAs) are widely distributed plant hormones that trigger a variety of metabolic processes necessary for a plant's growth, including flower induction, anther development, fruit formation, senescence, stem elongation, and seed germination (Hedden & Kamiya, 1997). In the 1950s, Western scientists initially recognized gibberellins, now commonly known as gibberellic acid (GA3) (Shah *et al.,* 2023). In the present study, the amount of GA3 synthesized by potent endophytic bacteria was determined by comparing the standard curve of pure GA3. According to Table 3, the highest GA3 production was expressed by the isolate RB1 (3.745 mg/ml), followed by RB3 (3.645±0.303 mg/ml), whereas RB5 produced the lowest, 0.331 mg/ml. Borah *et al*. (2018) showed that rice-associated endophytic isolate *Microbacteriaceae* bacterium RS01-11 significantly produced GA3 (67.23 ± 1.83 µg/ml). Several bacterial species, including *Acinetobacter, Agrobacterium, Arthrobacter, Azotobacter, Bacillus, Flavobacterium, Micrococcus, Clostridium, Pseudomonas, Rhizobium,* and *Xanthomonas*, have been reported to synthesize gibberellic acid (Gutierrez-Manero *et al*., 2001). There have also been reports of endophytic *Pseudomonas* and *Bacillus* isolates from tropical legume crops secreting GA3 (Maheswari & Komalavalli, 2013).

Phosphates solubilization assay

Phosphorus (P) is one of the minerals that limits plant growth. It is available in the earth's soil as fertilizer but is typically inaccessible to plants due to bacterial immobilization mechanisms. On the other hand, plants can obtain phosphorus via endophytes that reside within them (Liaqat *et al*., 2016). According to Duangpaenga *et al*., 2013, root-colonizing endophytic bacteria can improve plant development by making more soil phosphorus available to vegetation. Both solubilization and mineralization of organic phosphates are possible through the action of bacteria linked to plants. There are a number of strategies to achieve phosphate solubilization, including hydrolysis and processes involving phosphatase enzymes. By reducing pH, forming Co_2, and reducing metals enzymatically, phosphatase releases both organic and inorganic acids (Matos *et al*., 2017). In the present study, endophytic bacterial isolates were screened qualitatively for their capacity to solubilize phosphate in the Pikovskaya medium. The colonies surrounding the isolates RB2, RB3, RB4, and RB5 formed a distinct zone, indicating a positive outcome. The highest solubilization efficiency (SE%) was exhibited by the strain RB4 (196.66 ± 5.77%), while the lowest was exhibited by RB2 (148 ± 11.86%) (Table 3). The result indicated the phosphate solubilization ability of the strains RB2, RB3, RB4 and RB5. Similarly, endophytic bacteria as phosphate solubilizers from a variety of crops have also been explored and reported (Walia *et al*., 2017).

Table 3: *In-vitro* plant growth promotion (PGP) activity of potent endophytic bacteria

Isolates code	*IAA production µg/ml*	*GA3 production mg/ml*	*P-solubilization assay SE (%)*	*Zn-solubilization assay SE (%)*	*K-solubilization assay SE (%)*
RB1	43.93 ± 0.305	3.745 ± 0.002	-	185.17 ± 6.41	-
RB2	12.5 ± 0.3	3.557 ± 0.015	148 ± 11.86	185 ± 37.74	195 ± 8.66
RB3	10.3 ± 0.1	3.645 ± 0.303	153 ± 5.77	226 ± 23.09	-
RB4	47.43 ± 0.37	2.789 ± 0.675	196.66 ± 5.77	-	255.55 ± 19.24
RB5	14.13 ± 0.15	0.331 ± 0.001	184.72 ± 16.84	-	-

[SE (%) = Solubilization efficiency %]
(Data are shown in Mean ± SD of three replicates, Significant (P≤0.05))

Table 4: Effect of selected endophytic bacteria on rice seedling growth in 24-hour and 48-hour culture treatment

Isolates code/ Treatment	24-hour culture treatment					48-hour culture treatment					
	Seed Germination %	Root length (cm)*	Shoot length (cm)*	Seed vigor index	Seedling fresh weight (mg)*	Seedling dry weight (mg)*	Seed Germination %	Root length (cm)*	Shoot length (cm)*	Seed vigor index	Seedling fresh weight (mg)*	Seedling dry weight (mg)*
RB1	60	2.83 ± 0.28	25.4 ± 0.1	1693	79.12 ± 5.49	39.23 ± 5.14	100	5 ± 0.1	29.66 ± 1.52	3466	82 ± 3	42.8 ± 2.36
RB2	100	3.03 ± 0.15	24.86 ± 0.15	2789	80.76 ± 1.28	20 ± 3.92	100	3.1 ± 0.55	25.33 ± 2.08	2843	68 ± 3.55	32.2 ± 3.20
RB3	80	2.13 ± 0.15	21.93 ± 0.11	1924.8	77 ± 1.39	18.23 ± 1.62	80	2.36 ± 0.11	22.03 ± 0.75	1951.8	61.4 ± 0.45	26.53 ± 3.70
RB4	100	4.06 ± 0.11	18.76 ± 0.25	2282	69.83 ± 2.24	28.76 ± 2.51	100	7.66 ± 0.20	23.06 ± 0.20	3072	55.7 ± 3.45	37.4 ± 2.26
RB5	80	2.6 ± 0.36	20.43 ± 0.51	1842.4	78.46 ± 3.33	23.23 ± 5.53	80	2.8 ± 0.1	20.73 ± 0.51	1822.4	52.7 ± 6.65	29.86 ± 2.13
Control	40	2.1 ± 0.26	17.66 ± 1.52	790.44	66 ± 1.63	13.13 ± 5.83	40	2.26 ± 0.25	15.06 ± 0.90	692.8	41.86 ± 1.91	15.63 ± 5.44

(Data are shown in Mean ± SD of three replicates, * significant at 5% level)

Zinc solubilization assay

Zinc (Zn), is an essential micronutrient for promoting plant growth as it is a component of several metabolic enzymes (Othman *et al*., 2018). Due to its limited mobility in plants, it suggests the need for a constant supply of available zinc for plants to grow and develop effectively (Saravanan *et al*., 2007). According to Impa & Johnson-Beebout (2012), flooded soil is said to have less zinc available for plant absorption than non-flooded soil. According to Fasim *et al*., 2002, endophytic bacteria have the ability to boost the amount of zinc that plants absorb by solubilizing it from both organic and inorganic pools of soil zinc. By measuring the diameter of the inhibitory zone using the plate technique, the effectiveness of the bacterial isolates' Zn solubilization was assessed in the current investigation. RB1, RB2 and RB3 strains exhibited zinc solubilization in ZnO supplemented medium by forming a clear halo zone around the colony. Zinc solubilizing efficiency was found to be significantly higher in the strain RB3 (226 ± 23.09%) when compared to RB1 (185.17 ± 6.41%) and RB2 (185 ± 37.74%) (Table 3). Yaish *et al*. (2015) investigated potential zinc-solubilizing endophytic bacteria belonging to the various genera of *Actinobacter, Bacillus, Enterobacter, Klebsiella*, and *Rhodococcus* from date palm seedling roots. According to Bhakat *et al*. (2021), *Burkholderia* sp. isolated from rice rhizospheric soil would be a suitable candidate to increase zinc solubility, which could slow the rate at which inorganic zinc is fed into agricultural soil.

Potassium solubilization assay

One of the most vital minerals for plants, potassium (K), is essential to the growth, metabolism, and development of plants. K is necessary to activate a number of enzymes that are involved in numerous plant processes, including starch hydrolysis, nitrogen fixation, photosynthesis, and others, in addition to strengthening the resilience of plants to abiotic and biotic

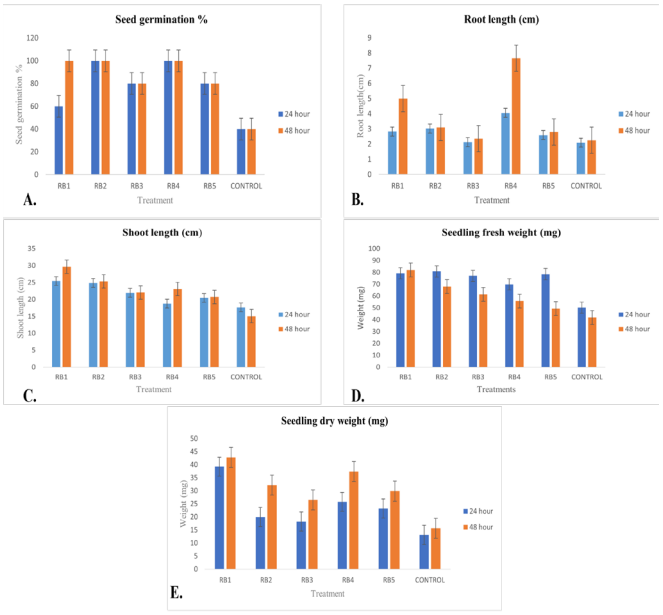

Fig. 3: Effect of selected endophytic bacteria on the growth of rice seedling (A. Seed germination, B. Root length, C. Shoot length, D. Seedling fresh weight, E. Seedling dry weight)

obstacles (Hussain *et al.,* 2016). Plants can only use 1 to 2% of the K that is present in the soil; the remaining K is bound to other minerals and is not available to plants. According to reports, certain beneficial bacteria have the ability to solubilize minerals, including K, and change their insoluble form into soluble forms that plants can absorb (Etesami *et al*., 2017). Therefore, pure isolates were screened for potassium solubilization efficiency. The isolate RB2 and RB4 were able to solubilize potassium on Aleksandrow medium by forming a significant clear halo zone around the colony with solubilization efficiency 195 ± 8.66% and 255.55 ± 19.24%, respectively (Table 3). Sev *et al*. (2016) isolated and evaluated K-solubilizing powerful bacterial isolates from some specific rice varieties for the promotion of plant growth. According to research by Nawaz *et al*. (2023), K-solubilizing endophytic bacteria can be used as bio-inoculants to boost rice crop growth and yield by improving cytosolic K+/Na+ ratio and K-assimilation during salt stress.

PGP activity in seed germination test and pot experiment

The current study revealed the impact of the five multi-functional PGP strains (RB1, RB2, RB3, RB4, and RB5) on different plant growth parameters and seed germination (Table 4; Fig. 3). When rice seeds were treated with the isolates RB2 and RB4, the tested strains increased the percentage of seed germination both in 24-hour and 48-hour culture treatment; this resulted in 100% germination in comparison to the control seeds 40%.

During 24-hour culture treatment, isolate RB4 had the highest increase in root length (4.06 ± 0.11 cm) and RB1 in shoot length (25.4 ± 0.1 cm) over the control and other treatments. Potent strain RB2 showed the highest seed vigor index (2789). Moreover, the maximum seedling fresh weight (80.76 ± 1.28 mg) and dry weight (39.23 ± 5.14 mg) were found by isolates RB2 and RB1, respectively.

The 48-hour culture treatment revealed that the isolate RB4 enhanced the root length (7.66 ± 0.20 cm) over the other treatment and the control. The highest increase in shoot length was recorded by RB1 (29.66 ± 1.52 cm). The findings also demonstrated that the endophytic strain RB4 enhanced the seed vigor index (3072), followed by RB1 (3466) when compared to other treatments and the control. The isolate RB1 had a higher effect on seedling fresh weight (82 ± 3 mg) and dry weight (42.8 ± 2.36 mg).

Endophytic isolates RB1, RB2 and RB4 demonstrated nearly superior results in every PGP parameter when compared to other treatment and control seeds. While the 48-hour culture treatment yielded a larger yield than the 24-hour culture treatment in the current investigation, the control seeds produced results deemed almost the same in both treatments. Increased vigor index and germination percentage are signs of better seedling establishment and crop improvement (Donald and Copeland, 1997). According to a study by Etesami *et al. (2014),* endophytes are effective at promoting rice seed germination. The seed germination index increased in response to the co-inoculants, *Microbacterium testaceum* and *Bacillus subtilis,* over the control in the Illongkari rice variety, showing a longer radicle (17.1%), plumule (45.7%), vigor index (702.75), and seed germination index (2611). The results of Hernández *et al.* (2023) support the current outcome. Based on improvements in the height, root length, fresh weight, and dry weight of the shoot and root, they identified three additional promising strains, viz., *Pantoea* sp. S5-1, *Pseudomonas* sp. S5-38, and *Pseudomonas* sp. S7-1, as potential bio-stimulators or bio-inoculants for Cuban rice crops. Numerous studies have noted a comparable improvement in seed germination rate and improved emergence of seedlings when both endophytic bacteria are injected into different crops, such as sorghum (Raju *et al*., 1999), and pearl millet (Raj *et al*., 2004). The production of IAA by endophytic bacteria might have contributed to root development, while GA promoted shoot growth. Along with phytohormone secretion, other growth-promoting characteristics of the isolates, such as P, K, and Zn solubilization activities, may have contributed to the increase in radicle and plumule length (Bahsan *et al*., 2004). According to this result, the endophytic strain RB1 boosted the seed vigor index when compared to other treatments and controls. The bacterial isolate's ability to solubilize P and K may have been the root cause of this. Van Brunt and Sultenfuss (1998) suggested that potassium and phosphorus as crucial macronutrients required for seed germination.

Table 5: Analysis of morphological and biochemical characteristics of potent endophytic bacteria

Isolates code	Morphological characteristics	Gram staining	KOH string test	Amylase production test	Gelatine hydrolysis test	Casein hydrolysis test	Methyl Red test	Vogues Proskauer test	Urea agar test	Catalase test	Lactose fermentation test	Probable organism
RB1	Thin and long rod-shaped bacteria	+	+	_	_	_	_	+	+	+	_	*Lysinibacillus* sp.
RB2	Rod-shaped bacteria	_	_	+	+	_	_	+	+	+	+	*Enterobacter* sp.
RB3	Rod-shaped bacteria	_	_	_	+	_	_	+	_	+	_	*Shigella* sp.
RB4	Rod-like bacteria occur in singles, pairs, or short chains.	_	_	_	+	+	_	+	+	+	_	*Pseudomonas* sp.
RB5	Rod-shaped bacteria	_	_	_	+	_	_	+	+	+	+	*Klebsiella* sp.

('+' for positive for the test; '-' for negative for the test)

Morphological and biochemical identification

Preliminary identification of bacterial endophytes was achieved through an analysis of their morphological, biochemical, and physiological traits. The findings showed that the bacterial species that were isolated from the Ronga bao's roots stem, and leaves were distinct. All of the endophytic bacterial isolates were identified morphologically as rod-shaped organisms with varying edges and elevations. Isolate RB1 was gram-positive when it came to gram staining, while the other four isolates were gram-negative. All of the isolates underwent various tests for biochemical identification, including lactose fermentation, urease, gelatinase, amylase and catalase. Bergey's manual of determinative bacteriology was utilized to define all the potent isolates based on gram staining, cell morphology, and biochemical testing (Table 5).

The strain RB1 showed a purple-stained rod-shaped colony in gram staining. Based on morphological and biochemical features and characteristics of *Lysinibacillus* sp. reported by Duan *et al.* (2013) and Shabanamol *et al.,* (2017) the initial identification of RB1 was assigned as *Lysinibacillus* sp. Khaskheli *et al.,* (2020) isolated root-associated *Lysinibacillus* sp. from *Oryza sativa* L. and evaluated their biocontrol property.

The strain RB2 exhibited pink-colored rod-shaped bacteria. By analyzing cell morphology and different positive and negative test biochemical parameters, strain RB2 was initially identified as *Enterobacter* sp. and the result is in confirmation with the finding of Panigrahi *et al*., (2020). Hardoim *et al.* (2013) isolated and characterized two novel species *Enterobacter oryzophilus* sp. Nov and *Enterobacter oryziphyticus* sp. nov from *Oryza sativa* L.

The study of cell morphology of the strain RB3 revealed gram-negative bacteria with rod-like form. By comparing the biochemical results with the report by Sharma *et al.,* (2010), the probable identification was *Shigella* sp. Akinsanya *et al*, (2015) identified *Shigella* sp. as an endophyte from Aloe vera. Lins *et al*., 2014 isolated and identified *Shigella flexneri* as an endophytic bacterium from cashew leaves.

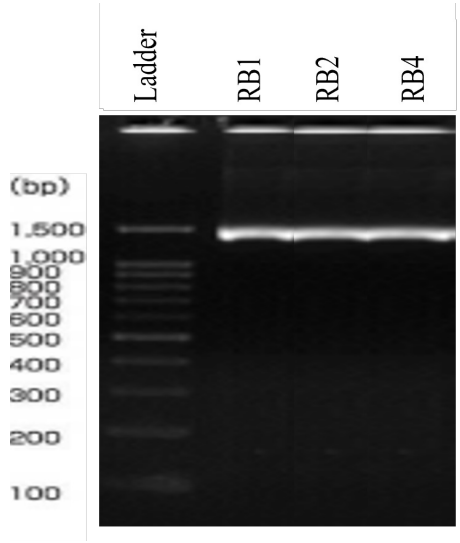

Fig. 4: Gel electrophoresis of 16S rRNA PCR amplified product for the most potent bacterial isolates RB1, RB2 and RB4 using 27F and 1492R primers

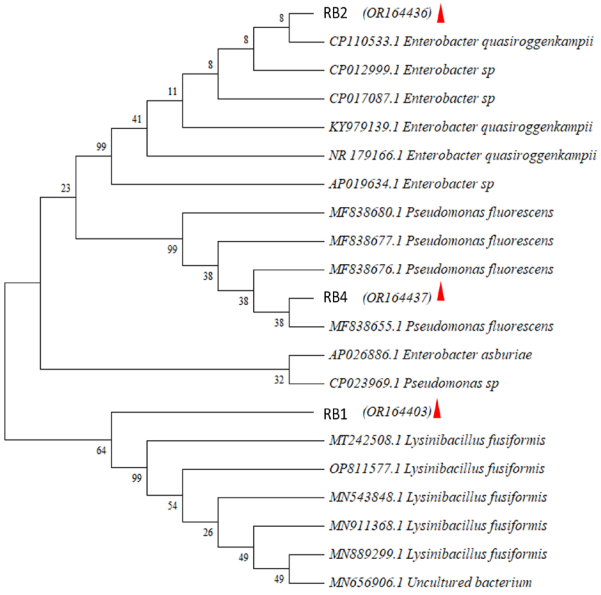

Fig. 5: Phylogenetic analysis of 16S rRNA sequences strain RB1, RB2 and RB4 along with the sequences from NCBI. The tree was constructed by neighbour joining method using MEGA11. The percentage of replicate trees in which the associated taxa clustered to gether in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site

Colonies of the strain RB4 showed pink-stained and rod-like bacteria. Compared with morphological and biochemical studies and the research work done by Kumar *et al.* (2016) the strain RB4 was initially assigned to *Pseudomonas sp.* Verma *et al*., 2018 isolated *Pseudomonas* sp. from the seedling of Rex rice (*Oryza sativa* L.) and evaluated their antifungal activity and PGP activity.

The strain RB5 showed gram-negative rod-shaped bacteria in the cell morphology study. Compared with the morphological and biological features of *Klebsiella* sp. (Hingole *et al*., 2016), the probable organism for the strain RB5 was *Klebsiella* sp. *Klebsiella* sp. is also reported to have an endophytic association with wheat, maize and rice plant etc., (Jasim *et al*., 2013).

Molecular characterization

Sequencing and phylogenetic analysis of 16S rRNA region provide a highly precise and adaptable method for classifying and identifying bacteria. Due to its widespread distribution among bacteria and the existence of species-specific variable regions, 16S rRNA-based identification is employed to identify bacteria (Ray *et al.,* 2017). Because phylogenesis is crucial for explaining the taxonomic classification of an organism based on its evolutionary history, the sequencing data is subsequently analyzed in phylogenetic tree form using the MEGA11 program (Ilmi *et al.,* 2018). In the present investigation, among the five isolates, only three bacterial isolates (RB1, RB2 and RB4) were subjected to molecular characterization based on their performance on antifungal screening and PGP activity.

The strains RB1, RB2, and RB4 genomic DNA were amplified using the 16S rRNA sequencing using primers 27F and 1492R.

After being resolved on an agarose gel, the 1500 bp PCR amplicon was observed (Fig. 4). NCBI BLAST alignments showed that the 16S rRNA sequence of strain RB1 and *Lysinibacillus fusiformis* (accession no. MT242508.1) had a shared sequence of homology sequence of 83.99% (coverage is 99%). The resultant sequences of RB1 were submitted to GenBank for the accession number (OR164403). Moreover, phylogenetic analysis using MEGA 11 software confirmed that the strain RB1 is closely related *to L. fusiformis* (Fig.5). Considering molecular sequence data for RB1, this strain was ultimately identified as gram-negative *Lysinibacillus fusiformis* strain *PK4* (OR164403)

NCBI BLAST alignments for strain RB2 showed that the 16S rRNA sequence of strain RB2 and *Enterobacter quasiroggenkampii* (accession no. CP110533.1) had a homology sequence of 99.79% (coverage is 100%). The sequences obtained by PCR amplification was submitted to GenBank and received the accession number-OR164436. Phylogenetic tree analysis provided that the strain RB2 and *E. quasiroggenkampii* belonged to the same cluster (Fig.5). Considering molecular sequence data for RB2, this strain was finally identified as *Enterobacter quasiroggenkampii* strain *PK2 (*OR164436)

The amplified sequences from the strain RB4 were submitted to GenBank (accession no. OR164437). According to BLAST analysis, it was found that RB4 had high similarity with *Pseudomonas fluorescens* (accession no. MF838680.1) with the homology sequence of 99.20% (coverage is 100%). Moreover, Strain RB4 and *P. fluorescens* (MF838655.1) were clustered in the same group in phylogenetic tree analysis (Fig.5). Therefore, strain RB4 is identified as *Pseudomonas fluorescens* strain *PK3* (OR164437).

Conclusion

The current study demonstrates the PGP and antifungal activities of a broad community of endophytic bacteria connected to rice plants. Taking into account all the information, this is the first report on the presence of endophytic bacteria from Ronga bao (*Oryza sativa* L.), specifically *Lysinibacillus fusiformis* strain PK4, *Enterobacter quasiroggenkampi* strain PK2, *Shigella* sp.*, Pseudomonas fluorescens* strain PK3, and *Klebsiella* sp. that showed great potential as biocontrol agents. After field investigations, it is also recommended that these bacterial strains may be used in plant disease management programs and modern agricultural techniques. The study will also draw attention to Assamese native rice types, which are not the subject of extensive study. It requires further research to find new bacteria in this plant.

Acknowledgments

The authors would like to acknowledge higher authorities of their respective departments, institutes and universities for their cooperation and support during studies.

Author contribution

PG is involved in the experiments, data compilations and writing of the article. PK and MK are involved in the overall idea, conception and design of the article and for giving crucial inputs and supervision from time to time. PK and MK is involved in the overall formatting and editing, critical revision and improvement of the article.

Conflicts of Interests

The authors declare no conflict of interest.

References

Agarwal, M., Dheeman, S., Dubey, R. C., Kumar, P., Maheshwari, D. K., & Bajpai, V. K. (2017). Differential antagonistic responses of *Bacillus pumilus* MSUA3 against *Rhizoctonia solani* and *Fusarium oxysporum* causing fungal diseases in Fagopyrum esculentum Moench. *Microbiological research*, *205*, 40–47. https://doi.org/10.1016/j.micres.2017.08.012

Ajulo, A. A., Bezerra, G. D. A., de Oliveira, R. S., Gonçalves, A. R., Moura, M. F. G. G., Cardoso, M. A. A., & de Filippi, M. C. C. (2024). Efficiency of bacterial isolates on suppressing brown spot in rice plants. *Journal of Phytopathology, 172*(2), e13294. https://doi.org/10.1111/jph.13294

Akinsanya, M. A., Goh, J. K., Lim, S. P., & Ting, A. S. Y. (2015). Diversity, antimicrobial and antioxidant activities of culturable bacterial endophyte communities in Aloe vera. *FEMS microbiology letters*, *362*(23), fnv184. https://doi.org/10.1093/femsle/fnv184Aneja,

K.R. (2018). Experiments in microbiology plant Pathology tissue culture and microbial Biotechnology. 5th edition. New Age International Publishers.

Arora, S., Patel, P. N., Vanza, M. J., & Rao, G. G. (2014) Isolation and characterization of endophytic bacteria colonizing halophyte and other salt tolerance plant species from coastal Gujarta. *African journal of microbial research*, *8*: 1779–1788. https://doi.org/10.5897/AJMR2013.5557

Arthi, K., Appalaraju, B., & Parvathi, S. (2003). Vancomycin sensitivity and KOH string test as an alternative to gram staining of bacteria. *Indian Journal of Medical Microbiology*, *21*(2), 121-123. https://doi.org/10.1016/S0255-0857(21)03135-2

Bashan, Y., Holguin, G., & De-Bashan, L. E. (2004). Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian journal of microbiology*, *50*(8), 521-577. https://doi.org/10.1139/w04-035

Bandara, W. M. M. S., Seneviratne, G., & Kulasooriya, S. A. (2006). Interactions among endophytic bacteria and fungi: effects and potentials. *Journal of biosciences*, 31(5): 645-650. https://doi.org/10.1007/BF02708417

Bartholomew, J. W. (1962). Variables influencing results, and the precise definition of steps in gram staining as a means of standardizing the results obtained. *Stain Technology*, 37(3): 139-155. https://doi.org/10.3109/10520296209117723

Bhakat, K., Chakraborty, A., & Islam, E. (2021). Characterization of zinc solubilization potential of arsenic tolerant *Burkholderia* spp. isolated from rice rhizospheric soil. *World Journal of Microbiology and Biotechnology*, *37*, 1-13. https://doi.org/10.1007/s11274-021-03003-8

Bhattacharyya, C., Banerjee, S., Acharya, U., Mitra, A., Mallick, I., Haldar, A., ... & Ghosh, A. (2020). Evaluation of plant growth promotion properties and induction of antioxidative defense mechanism by tea rhizobacteria of Darjeeling, India. *Scientific reports*, *10*(1), 15536. https://doi.org/10.1038/s41598-020-72439-z

Bibi, F., Yasir, M., Song, G. C., Lee, S. Y., & Chung, Y. R. (2012). Diversity and characterization of endophytic bacteria associated with tidal flat plants and their antagonistic effects on oomycetous plant pathogens. *Plant Pathology Journal*, *28*(1), 20-31. https://doi.org/10.5423/PPJ.OA.06.2011.0123

Borah, M., Das, S., Baruah, H., Boro, R. C., & Barooah, M. (2018). Diversity of culturable endophytic bacteria from wild and cultivated rice showed potential plant growth promoting activities. *BioRxiv,* 310797. https://doi.org/10.1101/310797

Borah, M., Das, S., Bora, S. S., Boro, R. C., & Barooah, M. (2021). Comparative assessment of multi-trait plant growth-promoting endophytes associated with cultivated and wild *Oryza* germplasm of Assam, India. *Archives of microbiology,* 203(5): 2007-2028. https://doi.org/10.1007/s00203-020-02153-x

Chowdhury, D., Sharma, K. K., & Maibangsa, S. (2016). Grain quality characteristics of red kernelled Bao rice of Assam. Monograph No. AAU/DR/17 (mono)129/2016. https://doi.org/10.20546/ijcmas.2020.911.060

Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., ... & Tiedje, J. M. (2009). The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic acids research*, *37*(suppl_1), D141-D145. https://doi.org/10.1093/nar/gkn879

Dias, A. C., Costa, F. E., Andreote, F. D., Lacava, P. T., Teixeira, M. A., Assumpção, L. C., ... & Melo, I. S. (2009). Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. *World Journal of Microbiology and Biotechnology*, *25*, 189-195. https://doi.org/10.1007/s11274-008-9878-0

Donald, M.B., Copeland, L.O. (1997), Seed Science and Technology Laboratory Manual, Scientific Publisher, Jodhpur.

Duan, Y. Q., He, S. T., Li, Q. Q., Wang, M. F., Wang, W. Y., Zhe, W., ... & Li, W. J. (2013). Lysinibacillus *tabacifolii sp. nov., a novel endophytic bacterium isolated from Nicotiana tabacum* leaves. *Journal of Microbiology*, *51*, 289-294. https://doi.org/10.1007/s12275-013-2338-z

Etesami, H., Mirsyed Hosseini, H., & Alikhani, H. A. (2014). In planta selection of plant growth promoting endophytic bacteria for rice (*Oryza sativa* L.). *Journal of soil science and plant nutrition*, *14*(2), 491-503. https://doi.org/10.4067/S0718-95162017000400012

Etesami, H. (2019). Plant growth promotion and suppression of fungal pathogens in rice (*Oryza sativa* L.) by plant growth-promoting bacteria. *Field crop: sustainable management by PGPR,* 351-383. https://doi.org/10.1007/978-3-030-18398-6_14.

Etesami, H., Emami, S., & Alikhani, H. A. (2017). Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects A review. *Journal of soil science and plant nutrition*, *17*(4), 897-911. https://doi.org/10.4067/S0718-95162017000400012

Fasim, F., Ahmed, N., Parsons, R., & Gadd, G. M. (2002). Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. *FEMS microbiology letters*, *213*(1), 1-6. https://doi.org/10.1016/S0378-1097(02)00712-8

Gogoi, N. K., Chowdhury, D., Saikia, P., & Sharma, K. K. (2020). Screening of Red-kernelled Deep Water Rice Germplasm of Assam, India for Disease and Pest Resistance. *Int. J. Curr. Microbiol. App. Sci*, *9*(11), 487-496. https://doi.org/10.20546/ijcmas.2020.911.059

Gutiérrez-Mañero, F. J., Ramos-Solano, B., Probanza, A. N., Mehouachi, J., R. Tadeo, F., & Talon, M. (2001). The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiologia Plantarum*, *111*(2), 206-211.https://doi.org/10.1034/j.1399-3054.2001.1110211.x

Hardoim, P. R., Nazir, R., Sessitsch, A., Elhottová, D., Korenblum, E., van Overbeek, L. S., & van Elsas, J. D. (2013). The new species *Enterobacter oryziphilus* sp. nov. and *Enterobacter oryzendophyticus* sp. nov. are key inhabitants of the endosphere of rice. *BMC microbiology*, *13*(1), 1-13. https://doi.org/10.1186/1471-2180-13-30

Hedden, P., & Kamiya, Y. (1997). Gibberellin biosynthesis: enzymes, genes and their regulation. *Annual review of plant biology*, *48*(1), 431-460. https://doi.org/10.1146/annurev.arplant.48.1.431

Hernández, I., Taulé, C., Pérez-Pérez, R., Battistoni, F., Fabiano, E., Villanueva-Guerrero, A., ... & Herrera, H. (2023). Endophytic Seed-Associated Bacteria as Plant Growth Promoters of Cuban Rice (*Oryza sativa* L.). *Microorganisms*, *11*(9), 2317. https://doi.org/10.3390/microorganisms11092317

Hingole, S. S., & Pathak, A. P. (2016). Isolation of halotolerant Plant growth promoting Klebsiella pneumoniae from Tuppa, Nanded, Maharashtra. *Int. J. Innov. Biol. Res*, *5*(6).

Hu, X., Chen, J., & Guo, J. (2006). Two phosphate-and potassium-solubilizing bacteria isolated from Tianmu mountain, Zhejiang, China. *World journal of microbiology and biotechnology,* 22(9): 983-990. https://doi.org/10.1007/s11274-006-9195-x

Hussain, Z., Khattak, R. A., Irshad, M., Mahmood, Q., & An, P. (2016). Effect of saline irrigation water on the leachability of salts, growth and chemical composition of wheat (*Triticum aestivum* L.) in saline-sodic soil supplemented with phosphorus and potassium. *Journal of soil science and plant nutrition*, *16*(3), 604-620. https://doi.org/10.4067/S0718-95162016000200024

Ilmi, N., Soelistya, D., Jekti, D., & Zulkifli, L. (2018). Molecular identification of endophytic bacteria from the stem's bark of moringa plant and their antibacterial activities. *IOSR Journal of Biotechnology and Biochemistry*, *4*(4), 21-30.

Impa, S. M., & Johnson-Beebout, S. E. (2012). Mitigating zinc deficiency and achieving high grain Zn in rice through integration of soil chemistry and plant physiology research. *Plant and Soil*, *361*, 3-41. https://doi.org/10.1007/s11104-012-1251-4

Islam, Z., Heong, K.L., Bell, M., Hazarika, L.K., Rajkhowa, D.J., Ali, S., Dutta, B.C. & Bhuyan, M. (2004). Current status of rice pests and their management in Assam, India-a discussion with extension agents. *International rice research notes,* 29: 95-97. https://doi.org/10.1017/S0263967X00023728

Jasim, B., John Jimtha, C., Jyothis, M., & Radhakrishnan, E. K. (2013). Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum. *Plant growth regulation*, *71*, 1-11. https://doi.org/10.1007/s10725-013-9775-7

Jasim, B., Joseph, A. A., John, C. J., Mathew, J., & Radhakrishnan, E. K. (2014). Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of *Zingiber officinale*. *3 Biotech*, *4*, 197-204. https://doi.org/10.1007/s13205-013-0128-6

Jasrotia, S., Salgotra, R. K., & Sharma, M. (2021). Efficacy of bioinoculants to control of bacterial and fungal diseases of rice (*Oryza sativa* L.) in northwestern Himalaya. *Brazilian Journal of Microbiology*, *52*, 687-704. https://doi.org/10.1007/s42770-021-00442-1

Jha, C. K., Aeron, A., Patel, B. V., Maheshwari, D. K., & Saraf, M. (2011). Enterobacter: Role in Plant Growth Promotion. In: Maheshwari, D. (eds) Bacteria in Agrobiology: Plant Growth Responses. Springer, Berlin, Heidelberg. 159-182. https://doi.org/10.1007/978-3-642-20332-9_8

Ji, S. H., Gururani, M. A., & Chun, S. C. (2014). Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbiological research*, *169*(1), 83-98. doi:10.1016/j.micres.2013.06.003

Khan, M. S., Gao, J., Zhang, M., Chen, X., Moe, T. S., Du, Y., ... & Zhang, X. (2020). Isolation and characterization of plant growth-promoting endophytic bacteria *Bacillus stratosphericus* LW-03 from *Lilium wardii*. *3 Biotech*, *10*, 1-15. https://doi.org/10.1007/s13205-020-02582-7

Khaskheli, M. A., Wu, L., Chen, G., Chen, L., Hussain, S., Song, D., ... & Feng, G. (2020). Isolation and characterization of root-associated bacterial endophytes and their biocontrol potential against major fungal phytopathogens of rice (*Oryza sativa* L.). *Pathogens*, *9*(3), 172. https://doi.org/10.3390/pathogens9030172

Koh, M. C., Lim, C. H., Chua, S. B., Chew, S. T., & Phang, S. T. W. (1998). Random amplified polymorphic DNA (RAPD) fingerprints for identification of red meat animal species. *Meat Science*, *48*(3-4), 275-285. https://doi.org/10.1016/S0309-1740(97)00127-9

Kumar, M. P., Gowda, D. S., Moudgal, R., Kumar, N. K., Gowda, K. P., & Vishwanath, K. (2013). Impact of fungicides on rice production in India. *Fungicides-showcases of integrated plant disease management from around the world*, 77-98.https://books.google.co.in/books?id=KuuPDwAAQBAJ

Kumar, A., Singh, R., Yadav, A., Giri, D. D., Singh, P. K., & Pandey, K. D. (2016). Isolation and characterization of bacterial endophytes of Curcuma longa L. *3 Biotech*, *6*, 1-8. https://doi.org/10.1007/s13205-016-0389-4

Liaqat, F., & Eltem, R. (2016). Identification and characterization of endophytic bacteria isolated from *in-vitro* cultures of peach and pear rootstocks. *3 Biotech*, *6*, 1-8. https://doi.org/10.1007/s13205-016-0398-3

Lins, M. D. C. R., Fontes, J. M., de Vasconcelos, N. M., da Silva Santos, D. M., Ferreira, O. E., de Azevedo, J. L., ... & de Souza Lima, G. M. (2014). Plant growth promoting potential of endophytic bacteria isolated from cashew leaves. *African journal of Biotechnology*, *13*(33). 10.5897/AJB2014.13835

Matos, A. D., Gomes, I. C., Nietsche, S., Xavier, A. A., Gomes, W. S., DOS SANTOS, J. A., & Pereira, M. C. (2017). Phosphate solubilization by endophytic bacteria isolated from banana trees. *Anais da Academia Brasileira de Ciências*, *89*, 2945-2954. https://doi.org/10.1590/0001-3765201720160268

Mir, M. I., Hameeda, B., Quadriya, H., Kumar, B. K., Ilyas, N., Kee Zuan, A. T., ... & Sayyed, R. Z. (2022). Multifarious indigenous diazotrophic rhizobacteria of rice (*Oryza sativa* L.) rhizosphere and their effect on plant growth promotion. *Frontiers in nutrition*, *8*, 781764. https://doi.org/10.3389/fnut.2021.781764

Mudoi, T., & Das, P. (2019). A study on phytochemicals and mineral content of indigenous red rice of Assam, India. *International journal of current microbiology and applied science,* 8(4): 1-12. https://doi.org/10.20546/ijcmas.2019.804.001

Nahar, S., Sahoo, L., & Tanti, B. (2018). Screening of drought tolerant rice through morpho-physiological and biochemical approaches. *Biocatalysis and Agricultural Biotechnology, 15, 150–159.* https://doi.org/10.1016/j.bcab.2018.06.002

Naik, B. S., Shashikala, J., & Krishnamurthy, Y. L. (2009). Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities *in-vitro*. *Microbial research,* 164(3): 290-296. https://doi.org/10.1016/j.micres.2006.08.003

Nawaz, A., Qamar, Z. U., Marghoob, M. U., Imtiaz, M., Imran, A., & Mubeen, F. (2023). Contribution of potassium solubilizing bacteria in improved potassium assimilation and cytosolic K+/Na+ ratio in rice (*Oryza sativa* L.) under saline-sodic conditions. *Frontiers in Microbiology*, *14*, 1196024. https://doi.org/10.3389/fmicb.2023.1196024

Othman, N. M. I., Othman, R., Saud, H. M., & Wahab, P. E. M. (2017). Effects of root colonization by zinc-solubilizing bacteria on rice plant (*Oryza sativa* MR219) growth. *Agriculture and Natural Resources*, *51*(6), 532-537. https://doi.org/10.1016/j.anres.2017.08.007

Paleg, L. G., & Coombe, B. G. (1967). Physiological effects of gibberellic acid. IX. Recovery of gibberellic acid following incubation with endosperm. *Plant physiology,* 42(3): 445-449, https://doi.org/10.1104/pp.42.3.445

Panigrahi, S., Mohanty, S., & Rath, C. C. (2020). Characterization of endophytic bacteria *Enterobacter cloacae* MG00145 isolated from *Ocimum sanctum* with Indole Acetic Acid (IAA) production and plant growth promoting capabilities against selected crops. *South African Journal of Botany*, *134*, 17-26. https://doi.org/10.1016/j.sajb.2020.06.024

Phetcharat, P., & Duangpaeng, A. (2012). Screening of endophytic bacteria from organic rice tissue for indole acetic acid production. *Procedia Engineering*, *32*, 177-183. https://doi.org/10.1016/j.proeng.2012.01.1254

Prabhukarthikeyan, S. R., Yadav, M. K., Anandan, A., Aravindan, S., Keerthana, U., Raghu, S., ... & Rath, P. C. (2019). Bio-protection of brown spot disease of rice and insight into the molecular basis of interaction between *Oryza sativa, Bipolaris oryzae* and *Bacillus amyloliquefaciens*. *Biological control*, *137*, 104018.

Raj, S. N., Shetty, N. P., & Shetty, H. S. (2004). Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. *International Journal of Pest Management*, *50*(1), 41-48. https://doi.org/10.1080/09670870310001626365

Raju, N. S., Niranjana, S. R., Janardhana, G. R., Prakash, H. S., Shetty, H. S., & Mathur, S. B. (1999). Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents. *Journal of the Science of Food and Agriculture*, *79*(2), 206-212. https://doi.org/10.1002/(SICI)1097-0010(199902)79:2<206::AID-JSFA167>3.0.CO;2-Y.

Rahman, M. H., & Saiga, S. (2005). Endophytic fungi *(Neotyphodium coenophialum*) affect the growth and mineral uptake, transport and efficiency ratios in tall fescue (*Festuca arundinacea). Plant and soil,* 272(1): 163-171. https://doi.org/10.1007/s11104-004-5443-4

Ramesh, A., Sharma, S. K., Sharma, M. P., Yadav, N., & Joshi, O. P. (2014). Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in vertisols of central India. *Applied soil ecology, 73*:87-96. https://doi.org/10.1016/j.apsoil.2013.08.011

Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., & Nain, L. (2011). Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Annals of microbiology*, *61*, 893-900. https://doi.org/10.1007/s13213-011-0211-z

Ray, D. K., Mueller, N. D., West, P. C., & Foley, J. A. (2013). Yield trends are insufficient to double global crop production by 2050. *PloS one*, *8*(6), e66428. https://doi.org/10.1371/journal.pone.0066428

Ray, S. S., Ali, M. N., Mukherjee, S., Chatterjee, G., & Banerjee, M. (2017). Elimination and molecular identification of endophytic bacterial contaminants during *in-vitro* propagation of *Bambusa balcooa*. *World Journal of Microbiology and Biotechnology, 33*, 1-9. https://doi.org/10.1007/s11274-017-2252-2](https://doi.org/10

Rohilla, M., Roy, P., Chowdhury, D., Sharma, K. K., Saikia, P., Sen, P., ... & Mondal, T. K. (2019). Bao Dhan of Assam. *Current Science*, *116*(5), 706-708. https://www.jstor.org/s/27137924

Sahai, S. (2016). Collection, characterization and conservation of indigenous rice varieties of assam and meghalaya gene campaign & north-east centre for rural livelihood research (NECR). https://sumansahai-blog.blogspot.com/2016/.

Saravanan, V. S., Kalaiarasan, P., Madhaiyan, M., & Thangaraju, M. (2007). Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn2+) and zinc chelates on root knot nematode *Meloidogyne incognita*. *Letters in applied microbiology*, *44*(3), 235-241. https://doi.org/10.1111/j.1472-765X.2006.02051.x

Saravanakumar, D., Lavanya, N., Muthumeena, K., Raguchander, T., & Samiyappan, R. (2009). Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. *Biocontrol*, *54*, 273-286. https://doi.org/10.1007/s10526-008-9166-9

Sev, T. M., Khai, A. A., Aung, A., & Yu, S. S. (2016). Evaluation of endophytic bacteria from some rice varieties for plant growth promoting activities. *J Sci Innov Res*, *5*(4), 144-148.

Shabanamol, S., Divya, K., George, T. K., Rishad, K. S., Sreekumar, T. S., & Jisha, M. S. (2018). Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic *Lysinibacillus sphaericus* isolated from rice (*Oryza sativa*). *Physiological and Molecular Plant Pathology*, *102*, 46-54.https://doi.org/10.1016/j.pmpp.2017.11.003

Shah, S. H., Islam, S., Mohammad, F., & Siddiqui, M. H. (2023). Gibberellic acid: A versatile regulator of plant growth, development and stress responses. *Journal of Plant Growth Regulation,* 42(12), 7352-7373. https://doi.org/10.1007/s00344-023-11035-7

Sharma, A., Singh, S. K., & Bajpai, D. (2010). Phenotypic and genotypic characterization of *Shigella* spp. with reference to its virulence genes and antibiogram analysis from river Narmada. *Microbiological Research*, *165*(1), 33-42. https://doi.org/10.1016/j.micres.2008.02.002

Singh, P. K., Mishra, M. N., Hore, D. K., & Verma, M. R. (2006). Genetic divergence in lowland rice of north eastern region of India. *Communication in biometry and crop science,* 1(1):35-40.

Singh, R. K, Mishra, R. P., Jaiswal, H. K., Kumar, V., Pandey, S. P., Rao, S. B., & Annapurna, K. (2006). Isolation and identification of natural endophytic rhizobia from rice (*Oryza sativa* L.) through rDNA PCR-RFLP and sequence analysis. *Current microbiology,* 52(5): 345-349. https://doi.org/10.1007/s00284-005-0282-3.

Singh, K. M., Jha, A., Meena, M., & Singh, R. (2012). Constraints of rainfed rice production in India: an overview. *SSRN electronic journal,* 71-84. https://dx.doi.org/10.2139/ssrn.2210401

Singha, M., Wu, B., & Zhang, M. (2016). An object-based paddy rice classification using multi-spectral data and crop phenology in Assam, northeast India. *Remote Sens,* 8(6):479. https://doi.org/10.3390/rs8060479

Tagg, J., & McGiven, A.R. (1971). Assay system for bacteriocins. *Applied microbiology,* 21(5): 943-947.

Taktek, S., St-Arnaud, M., Piché, Y., Fortin, J. A., & Antoun, H. (2017). Igneous phosphate rock solubilization by biofilm-forming mycorrhizobacteria and hyphobacteria associated with *Rhizoglomus irregulare* DAOM 197198. *Mycorrhiza*, *27*, 13-22. https://doi.org/10.1007/s00572-016-0726-z

Van Brunt, J., & Sultenfuss, J. H. (1998). Functions of Potassium in plants. *Better Crops*, *82*(3), 4-5.

Verma, S. K., Kingsley, K. L., Bergen, M. S., Kowalski, K. P., & White, J. F. (2018). Fungal disease prevention in seedlings of rice (*Oryza sativa*) and other grasses by growth-promoting seed-associated endophytic bacteria from invasive Phragmites australis. *Microorganisms*, *6*(1), 21. https://doi.org/10.3390/microorganisms6010021

Waghunde, R. R., Shelake, R. M., Pramanik, D., Suthar, H., Pandey, P., & Kim, J. Y. (2021). Application of cereal and vegetable endophytes in plant health management. In *Microbiomes and Plant Health* (pp. 29-51). Academic Press. https://doi.org/10.1016/B978-0-12-819715-8.00002-1.

Walia, A., Guleria, S., Chauhan, A., & Mehta, P. (2017). Endophytic bacteria: role in phosphate solubilization. *Endophytes: Crop Productivity and Protection: Volume 2*, 61-93.https://doi.org/10.1007/978-3-319-66544-3_4

Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and environmental microbiology*, *73*(16), 5261-5267. https://doi.org/10.1128/AEM.00062-07

Yaish, M. W., Antony, I., & Glick, B. R. (2015). Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (*Phoenix dactylifera* L.) and their potential role in salinity tolerance. *Antonie Van Leeuwenhoek*, *107*, 1519-1532. https://doi.org/10.1007/s10482-015-0445-z