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Rice bacterial endophyte *Bacillus* sp., and its potential for plant growth promotion

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ABSTRACT

In an environmentally benign way, plant growth-promoting endophytic bacteria hold great potential as a substitute for chemical fertilizers in terms of enhancing plant growth and output. In the current study, four endophytic bacteria (IIRR-F1, IIRR-F2, IIRR-F3 and IIRR-F4) were isolated from the rice roots and evaluated for a variety of PGP characteristics. Most of the isolates produced plant growth-promoting traits including indole acetic acid, phosphate solubilization, ammonia and siderophore production. It was concluded from the results that bacterial isolate IIRR-F2 showed most of the plant growth promoting traits. Under greenhouse circumstances, rice seeds treated with endophytic bacteria IIRR-F2 enhanced root length, shoot length, and dry weight in comparison to the control group. Based on morphological and biochemical characteristics, bacterial isolate IIRR-F2 was identified as Bacillus sp. These results strongly imply that the plant growth promoting endophytic bacteria identified in this work may be useful for the development of efficient bio-fertilizers/bio-inoculants to increase rice productivity and growth in a sustainable way.

Keywords: *Endophytic bacteria, Bacillus sp., Bio-inoculants, Sustainable agriculture, Rice*

1. Introduction

Plants host a diverse number of microorganisms—collectively known as the plant microbiome—that inhabit various tissues and organs, including seeds, roots, stems, leaves, flowers, and fruits [3]. These microbial populations exist both on the plant surface as epiphytes and within internal tissues as endophytes, where they significantly influence plant health and productivity [5]. Among them, bacterial endophytes establish symbiotic relationships with host plants without causing visible disease symptoms [6]. These microbes contribute to the host's physiological functions and developmental pathways, thereby playing an essential role in overall plant performance [10]. Bacterial endophytes are gaining global attention due to their dual capacity to promote plant growth and suppress disease through both direct and indirect mechanisms. Direct mechanisms involve traits that stimulate growth, such as the synthesis of phytohormones like indole-3-acetic acid (IAA), gibberellins, and cytokinins [10]. Furthermore, these microbes aid in nutrient acquisition by enhancing the bioavailability of essential minerals such as phosphorus, potassium, zinc, and iron [11–12]. Another important trait is the production of ACC deaminase, an enzyme that breaks down 1-aminocyclopropane-1-carboxylic acid (ACC)—a precursor of the plant stress hormone ethylene—into ammonia and α-ketobutyrate. This activity helps modulate ethylene levels, thereby reducing stress-induced growth inhibition [7, 9]. For instance, maize plants exhibited improved salt tolerance when inoculated with ACC deaminase-producing endophytes [4]. In terms of indirect mechanisms, endophytic bacteria contribute to plant defense by functioning as biocontrol agents. They suppress pathogens through the production of secondary metabolites, antimicrobial compounds, cell wall-degrading enzymes, and antibiotics. Additionally, they can induce systemic resistance in the host plant and compete with pathogens for space and nutrients [6, 16–17]. Research on these interactions is vital for advancing the use of beneficial microbes as bio-inoculants in sustainable agriculture and integrated pest management systems [3, 17–18].

Rice (*Oryza sativa* L.) is a staple crop, feeding more than half of the global population [1–2]. With global population figures projected to rise from 7.3 billion to 9.7 billion by 2064 (UN estimates), there is a growing demand for increased rice production. However, this must be achieved through environmentally responsible methods that minimize the use of synthetic agrochemicals. Traditional reliance on chemical inputs such as synthetic fertilizers, pesticides, and nutrient supplements has led to serious environmental and health concerns [1, 4, 15].

To address these issues, farming communities are increasingly encouraged to adopt bio-based alternatives. Biofertilizers and biopesticides—composed of non-pathogenic microorganisms—present a promising, eco-friendly solution. Their natural origin allows for sustainable crop production while safeguarding environmental and human health. Moreover, bioformulations offer a viable strategy for reducing the ecological footprint of modern agriculture [13]. In recent years, various endophytic bacterial species—including *Pseudomonas*, *Azospirillum*, *Bacillus*, *Klebsiella*, *Stenotrophomonas*, *Burkholderia*, *Serratia*, *Microbacterium*, *Enterobacter*, and *Alcaligenes faecalis*—have demonstrated potential as plant growth enhancers [21–22]. Empirical studies have shown that inoculating rice plants with these beneficial microbes can lead to improved growth, higher yields, and enhanced nutrient uptake [15, 19–20]. The present study aims to investigate the impact of endophytic bacterial inoculation on rice growth parameters under controlled pot conditions, contributing to the ongoing search for sustainable and efficient agricultural practices.

2. Materials and Methods

2.1 Sample Collection, Plant Material, and Isolation of Endophytic Bacteria

This study aimed to isolate endophytic bacterial strains from the roots of rice plants.

Root samples were collected from healthy rice plants (cultivar MTU1010) grown at the Indian Institute of Rice Research (IIRR-ICAR), Rajendranagar, Telangana, India. Whole plants, including their root systems, were carefully uprooted and transferred into sterile polythene bags. Samples were stored in a cool container and transported promptly to the laboratory for further processing. Upon arrival, excess soil was removed from the roots using running tap water. Surface sterilization was carried out by immersing the roots in 70% ethanol for 2 minutes, followed by treatment with 2.5% sodium hypochlorite solution for 5 minutes, to which 2–3 drops of Tween 20 were added as a surfactant [29–31]. The roots were then rinsed five times with sterile distilled water to remove any residual sterilizing agents. Sterilized root segments were homogenized using an autoclaved mortar and pestle in the presence of 2 mL phosphate saline buffer (PSB). The resulting suspension (1 mL) was serially diluted up to 10^{-7} using sterile distilled water. A 0.1 mL aliquot from each dilution was spread onto nutrient agar plates and incubated at 30 °C for 72 hours. Distinct colonies were selected based on morphological differences such as size, shape, and color, and subsequently purified through repeated streaking on fresh nutrient agar plates. Purified isolates were preserved in nutrient broth containing 30% glycerol and stored at –80 °C for further use.

2.2 Evaluation of Plant Growth-Promoting Traits of Endophytic Bacteria

The isolated endophytic strains were screened under in vitro conditions for several plant growth-promoting (PGP) traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore synthesis, and ammonia production.

2.2.1 Indole-3-Acetic Acid (IAA) Production

IAA production was assessed by culturing the bacterial isolates in nutrient broth supplemented with 5 mM L-Tryptophan (HiMedia). The cultures were incubated at 28 ± 2 °C for 48–72 hours. After incubation, Salkowski's reagent was added to each culture tube, and the tubes were kept in the dark for 30 minutes. The appearance of a pink or reddish coloration indicated a positive IAA response [23].

2.2.2 Phosphate Solubilization Assay

Phosphate solubilization potential was qualitatively evaluated by inoculating bacterial cultures onto National Botanical Research Institute's Phosphate (NBRIP) agar medium. Plates were incubated, and the development of a clear halo around the colonies was taken as a positive sign of phosphate solubilizing activity.

2.2.3 Siderophore and Ammonia Production

Siderophore production was determined using chrome azurol S (CAS) agar medium following the protocol described by [33]. Actively growing bacterial strains were spot-inoculated onto CAS plates and incubated at 30 °C for up to three days. The formation of yellow to orange halos around the colonies was interpreted as a positive siderophore response.

For ammonia production, isolates were inoculated into peptone water broth (5 mL) and incubated at 30 °C with shaking at 120 rpm for four days, as described by [34]. After incubation, 0.5 mL of Nessler's reagent was added to each culture. The development of a yellow to brown coloration was taken as a positive indication of ammonia synthesis.

2.3. Evaluation of selected potential bacterial endophyte on plant growth parameters of rice

Selected potential bacterial endophytes were further evaluated for their PGP potential in greenhouse conditions on rice (var. MTU1010). The rice seeds were surface sterilized using 95% ethanol for 1 minute and 0.2% $HgCl_2$ for 3 minutes. Afterward, they were washed three times with sterile distilled water. Bacterial culture that was actively growing was introduced into 100 ml of sterilized nutrient broth and placed in an incubator at a speed of 150 revolutions per minute and a temperature of 30±2ºC for duration of 48 hours. Subsequently, the mixture was subjected to centrifugation at a speed of 8,000 revolutions per minute for duration of 15 minutes. The pellet was rinsed twice using sterile saline solution and then utilized for seed bacterization. The rice seeds, which had been sterilized, were immersed in the corresponding suspension of cell pellets for the entire night. Following the process of seed bacterization, a total of 3 seeds were planted in plastic pots measuring 5 inches in diameter. The pots were filled with an optimal amount of sterile soil, reaching a level of three-fourths capacity. Subsequently, the pots were placed in the greenhouse and left to incubate for a period of 30 days. The control group consisted of seeds treated with distilled water. Two treatments (IIRR-F2, and un-inoculated control) were applied, each with three replications. Measurements of shoot length, root length, and dry weight (total biomass) were documented and compared to plants in the control group.

2.4. Statistical analysis

All experiments were performed twice by three replications each. Statistical analysis was done and mean, standard error and standard deviation were calculated.

3. Results

3.1. Isolation and PGP traits of isolated endophytic bacteria

In the present investigation, four morphologically distinct endophytic bacterial isolates—designated as IIRR-F1, IIRR-F2, IIRR-F3, and IIRR-F4—were successfully obtained from rice root samples (Table 1). These isolates were evaluated under in vitro conditions for key plant growth-promoting (PGP) characteristics, including the synthesis of indole-3-acetic acid (IAA), ammonia production, phosphate solubilization, and siderophore secretion.

Of the four isolates, IIRR-F1 and IIRR-F2 were capable of producing IAA. Notably, IIRR-F2 exhibited a strong positive reaction, evident by an intense pink coloration (+++), indicating a higher level of IAA production. In contrast, IIRR-F1 showed only a faint pink color (+), reflecting a relatively lower IAA output (Table 1).

Phosphate solubilization ability was detected in isolates IIRR-F2 and IIRR-F4, as demonstrated by the formation of clear solubilization halos on NBRIP medium (Figure 1B; Table 1). Regarding ammonia production, isolates IIRR-F2 and IIRR-F3 showed positive results. Among them, IIRR-F2 displayed strong ammonia production (+++), whereas IIRR-F3 exhibited a moderate level of activity (++) (Figure 1A; Table 1).

Siderophore production was observed exclusively in isolate IIRR-F2, confirmed by the appearance of a distinct orange halo surrounding the bacterial colony—an indicator of iron-chelating activity (Table 1).

Table 1. In vitro plant growth promoting traits of isolated endophytic bacteria

Isolate	IAA	P- Solubilization	Ammonia	Siderophore
IIRR-F1	+	-	-	-
IIRR-F2	+++	++	+++	+
IIRR-F3	-	-	++	-
IIRR-F4	-	+	-	-

3.2. Effects of potential endophytic bacterial isolate on growth parameters of rice

Among the isolated strains, IIRR-F2 (identified as Bacillus sp.) demonstrated all evaluated plant growth-promoting (PGP) attributes (Table 1) and was therefore selected for further assessment under greenhouse conditions to evaluate its effect on rice growth. The experimental results revealed that rice plants grown from seeds treated with IIRR-F2 showed significant improvement in growth parameters compared to untreated controls. Specifically, the inoculated plants exhibited a 42.5% increase in shoot length, a 48% increase in root length, and a 31% increase in dry biomass relative to the uninoculated control group (Figure 1C; Table 2).

Table 2. Effect of potential endophytic bacterial isolate (IIRR-F2) on the growth parameters of rice

Treatment	Root length (cm/plant)	Shoot length (cm/plant)	Plant biomass (mg/plant)
Control	8±0.3	16.1±0.1	17.3±0.3
IIRR-F2 (Bacillus sp.,)	15.5±0.4 (48%)	28±0.2 (42.5%)	25.2±0.7 (31%)

Values are the means of three replicates with ± SD

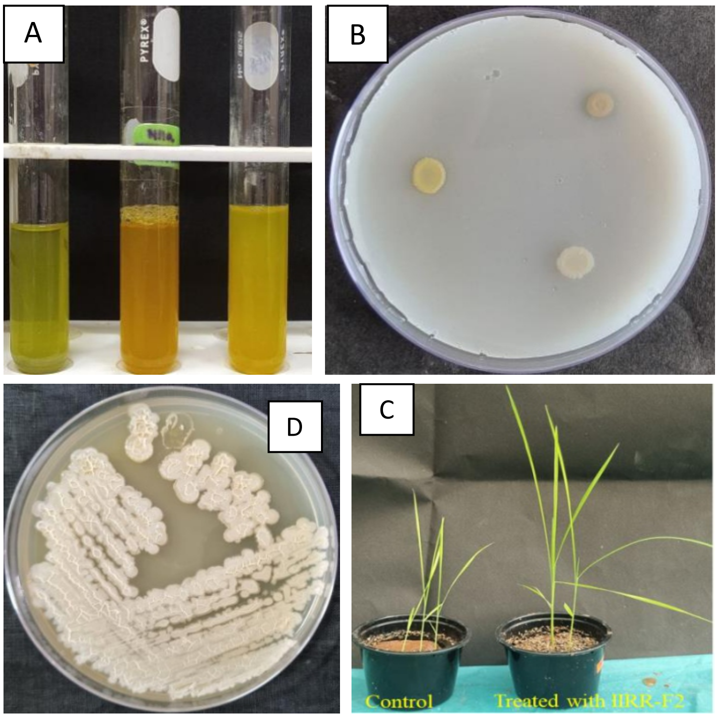

Fig.1. (A) Ammonia production by endophytic bacteria (B) Halo zone on NBRIP media indicating phosphate solubilization (C) Impact of endophytic bacterial isolate IIRR-F2 on growth parameters of rice (D) Purified culture of bacterial endophyte IIRR-F2 (Bacillus sp.).

4. Discussion

Plants thrive in a variety of ecological niches and constantly engage with numerous microbial partners. The recognition that plants and their associated microbial communities operate as a unified biological system when responding to environmental pressures has given rise to the concept of the holobiont. Among these associated microorganisms, endophytes are particularly notable for their ability to colonize internal plant tissues without causing visible disease symptoms or harm to the host. These symbiotic microbes are found across all plant species and can inhabit various plant parts, including leaves, stems, seeds, roots, and flowers. Certain endophytes confer multiple benefits to their host plants, such as enhancing growth, fixing atmospheric nitrogen, and offering protection against pathogens. While endophytic communities are mainly composed of bacteria and fungi, other less common groups like archaea, algae, protozoa, and nematodes may also be present and can significantly influence plant biology [24]. Bacterial endophytes, in particular, have demonstrated notable positive effects on plant health. They contribute to improved nutrient uptake—especially nitrogen—and synthesize phytohormones like indole-3-acetic acid (IAA) and cytokinins, which support plant development. Beyond promoting growth, endophytic bacteria play a key role in adjusting plant metabolic activities and hormone signaling pathways, thereby improving plant resilience to both biotic and abiotic stresses. Their presence within plant tissues also shields them from direct exposure to external environmental challenges such as drought, salinity, and other stressors, making them valuable allies in stress adaptation strategies [11–12, 26].

As global food demand increases, intensifying agriculture has become essential to ensure food security. However, the widespread use of synthetic fertilizers to boost crop yields and soil productivity has raised serious concerns due to its detrimental effects on ecosystems and public health. In this context, biofertilizers—composed of beneficial bacteria naturally associated with plant roots—offer a viable and sustainable alternative to chemical inputs. These microbial formulations hold promise not only for enhancing crop productivity in an eco-friendly manner but also for their potential integration into breeding programs and agricultural bio-economy initiatives aimed at achieving long-term food sustainability [21, 27].In this work, a total of 4 endophytic bacteria were recovered from the roots of rice. The bacterial cultures were screened for various plant growth promoting traits in vitro. This involved assessing features such as the production of indole acetic acid, solubilization of phosphate, production of ammonia (NH_3), generation of siderophores. In the present work, one potential bacterium namely IIRR-F2 exhibited most of the PGP traits which was identified based on morphological and biochemical characteristic as Bacillus sp. Indole-3-acetic acid (IAA) is the most prevalent and widely recognized kind of auxin in plants. It has a crucial function in processes such as cell division, growth, and specialization, as well as in promoting the germination of seeds and tubers, initiating the growth of lateral and adventitious roots, and facilitating the production of various metabolites [15]. Production of IAA is considered one of the main ways in which plant growth is promoted by PGPB. During the current study, Bacillus sp., strain IIRR-F2 exhibited the highest level of indole-3-acetic acid (IAA) synthesis (Table 1). Several studies have documented the synthesis of indole-3-acetic acid (IAA) by different strains of Bacillus [13; 28; 23].

A significant proportion of phosphorus (P) in soil exists in the form of insoluble inorganic compounds, making it inaccessible to plants. Phosphate solubilizing bacteria (PSB) are microorganisms that have the ability to solubilize phosphate. They can provide plants with a readily available form of phosphorus, which might potentially replace inorganic phosphatic fertilizers [13]. During the current study, Bacillus sp., strain IIRR-F2 exhibited the highest level of phosphate solubilization which is evident by the formation of halo zone on NBRIP media (Table 1). The findings of the previous study conducted by [30-31] align with the results of the current investigations, which showed that Bacillus sp., are capable of solubilizing inorganic phosphate. Similarly, [13] documented the ability of bacteria from the genera Bacillus, Stenotrophomonas, Enterobacter, and Pseudomonas, isolated from the rhizosphere of rice, to solubilize phosphate.

The findings of the current investigation shown that bacterial isolates namely IIRR-F2 (Bacillus sp.,) significantly enhanced the root length, shoot length, and dry weight in rice plants comparison to the control group (Table 2). The results of this study are consistent with the earlier studies conducted by [13; 32] which found that the growth parameters of rice increased when it was inoculated with PGP Bacillus sp. Similarly, [29] observed an augmentation in the growth characteristics of rice when it was inoculated with endophytic bacteria Bacillus subtilis. The increase in root length and shoot length of rice after being exposed to the aforementioned potent bacterial isolates may be attributed to the numerous plant growth regulator (PGR) characteristics exhibited by our bacterial inoculants.

5. Conclusion

At a global scale, the effects of continuous and heavy use of agrochemicals for improving agricultural productivity can cause serious damage on the soil fertility, life of living organisms and as well as their environment. Use plant growth-promoting endophytic bacteria as agricultural crop inoculums are costly reasonable and environmentally-friendly approach to increase crop production on a sustainable way. The present study concludes that one plant growth promoting endophytic bacteria IIRR-F2 (Bacillus sp.,) can be employed as bio-inoculants in the cultivation of rice in a sustainable manner. In future studies suitable PCR based genotypic techniques (16S rRNA sequence) can be employed to confirm its identity at strain level and the isolate can be subjected to field trials in order to improve the yield and available nutrients in the rice crop.

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