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Isolation and Characterization of Plant Growth-Promoting Endophytic Diazotrophic Bacteria from Sri Lankan Rice Cultivars and Rapid Screening for their Effect on Plant Growth Promotion

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Abstract*-The present study was conducted to isolate and identify endophytic diazotrophic bacteria in two Sri Lankan rice (Oryza Sativa L.) varieties; Suwandel and Bg 358 and to evaluate their potential to promote rice plant growth. A total of 15 putative endophytic diazotrophic bacterial isolates were obtained from shoots and roots of Suwandel and Bg 358 rice varieties out of which 7 isolates were selected based on their ability to produce IAA and phosphate solubilization. According to the morphological characters and biochemical tests, these bacteria were identified belong to genera Bacillus (IN003, IN006, and IN007), Klebsiella (IN008 and IN018), Pantoea (IN009), and Enterobacter (IN015). All selected bacterial isolates produced IAA (7.1µmgl^{-1} to 30.9 µ mgl^{-1}) in the tryptophan supplemented medium. Five out of seven bacterial isolates (IN006, IN007, IN008, IN015, and IN018) were able to solubilize inorganic phosphate on Pikovskaya's agar medium. Rice seeds (Suwandel variety) treated with these endophytic diazotrophic bacteria with plant growth-promoting ability showed significantly enhanced shoot length, root length, shoot fresh weight, shoot dry weight and root fresh weight compared to the uninoculated control. Plant inoculation experiment indicated that Enterobacter sp. (IN015) was most effective in rice plant growth promotion among seven bacterial isolates tested. These results strongly suggest that endophytic diazotrophic bacteria characterized in this study could be successfully used to promote rice plant growth.*

Keywords*— *Endophytic diazotrophic bacteria, IAA production, Phosphate solubilization, Plant growth promotion, Rice.

I. INTRODUCTION

Rice (*Oryza sativa*) is the staple crop for more than half of the world's population [26] including Sri Lanka. With the increasing world population, the demand for rice is expected to increase and therefore there is an immense pressure for higher production to feed the largely growing world population. One of the major problems associated with the rice production is the use of a massive amount of chemical fertilizers which cause negative impacts on both human health and environment. Therefore, there is an urgent need to identify the less harmful substitutes to chemical fertilizers to increase rice production. Natural endophytic bacteria associated with the plants have become a promising alternative to chemical fertilizer due to their plant growth promoting abilities [36].

Endophytic bacteria lives inside the plant tissues without causing any harmful effect to the host plant [34] and endophytic bacteria involved in the biological nitrogen fixation process are known as endophytic diazotrophs [15]. These endophytes are most commonly originated from the rhizosphere and enter into the plant through the cracks at the point of lateral root emergence and root tips and then systematically spread throughout the plant colonizing various tissues including seeds, stem, roots, and leaves of the plant [26]. A diverse range of endophytic diazotrophic bacteria have been isolated from the surface sterilized stem, leaves, seeds, and roots of rice plant using nitrogen-free medium and most of these isolated bacteria belong to the genus *Pantoea*, *Klebsiella*, *Azospirillum*, *Enterobacter*, *Herbaspirillum*, *Serratia*, and *Bacillus* [5,6,8,13,25,41]. Thee endophytic bacteria can promote the growth, and yield of plants when applied to seed or crops due to their plant growth-promoting properties. The widely recognized mechanisms of plant

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growth promotion are the production of phytohormones, diazotrophic fixation of nitrogen, and solubilization of phosphate [26]. In addition to that these bacteria also have ability to produce siderophore to chelate various metals, including Fe, Zn, and Cu [1,4] and also suppress pathogens by producing inhibitory compounds [24,42].

Bacterial endophytes have been reported to produce various phytohormones including Indole-3-acetic acid (IAA), cytokinin and gibberellins [19].Among these IAA is one of the most vital hormones, mainly due to its function in lateral and adventitious root formation [11] and root elongation [18] According to the [39], bacterial species belong to genera *Pseudomonas*, *Pantoea*, *Bacillus*, *Klebisella*, *Enterobacter*, and *Serrartia* have ability to produce IAA. Earlier studies have found that endophytic bacteria with the ability to produce IAA can enhance the growth of plants by increasing plant height and biomass [37, 38]. Most of the soil phosphorous is in the form of insoluble phosphate and cannot be utilized by the plant and phosphorous deficiency in plants result in an inhibited stem and root development, poor flowering, lack of seed formation [16]. Endophytic bacteria can solubilize inorganic phosphate in soil by secreting organic acids and this in turn help to increase plant growth [17]. Endophytic bacteria have a potential to use as an inoculant to promote plant growth due to these plant growth-promoting properties. Previous study indicated that *Pantoea agglomerans* YS19 can enhanced the biomass of the rice seedlings after application which can be attributed to their nitrogen-fixing ability and phytohormone production [9]. A significant increase in the dry weight of leaf and root of rice plants has been recorded by rice plants inoculated with *Bacillus subtilis* [16].

Few studies have been conducted so far to identify the beneficial endophytic bacteria in Sri Lankan rice varieties. Therefore, the present study was carried out to isolate and identify endophytic diazotrophic bacteria with plant growth-promoting properties from roots and shoots of two Sri Lankan rice (*Oryza sativa* L.) varieties; Suwandel and Bg 358. Bacterial isolates were characterized on the basis of morphological and biochemical features. Furthermore, the effect of plant growth-promoting endophytic diazotrophic bacterial inoculation on the growth of rice seedlings was also evaluated to identify their potential to promote rice plant growth when applied to the plant.

II. MATERIALS AND METHODOLOGY

2.1 Collection of plant materials

The two rice (*Oryza Sativa* L.) varieties, Suwandel and Bg 358 were randomly collected in their heading stage from the soil pots in a greenhouse of Department of Botany, University of Kelaniya, Sri Lanka. The stock seeds of rice were provided by the Rice Research and Development Institute (RRDI), Bathalagoda, Sri Lanka.

2.2 Isolation of endophytic diazotrophic bacteria

The endophytic bacteria from roots and shoots of two rice (*Oryza Sativa* L.) cultivars were isolated using nitrogen-free semi-solid media (Nfb). Plant tissue samples (0.5 g) were washed with running tap water and cut into 2-3 cm long sections with a sterile blade. Plant tissues were surface sterilized with 70% ethanol for 3min, subsequently with fresh NaOCl (v/v) for 5 min and finally with 70% ethanol for 30 s. Tissue samples were then washed five times with sterile distilled water. To confirm the success of the sterilization process, aliquots of 0.1 ml of distilled water from the final rinse were plated on nutrient agar (NA) plates and examined for contaminants after incubation at room temperature (29ºC) for 3 days. No contaminants were found indicating that the surface sterilization procedure was effective. Surface sterilized roots and shoots samples were homogenized separately in a sterilized mortar with 9.5 ml of 4% sucrose solution. Serial dilutions (10^{-4}, 10^{-6} and 10^{-8}) were prepared using a homogenous solution of tissue samples. Aliquots of 0.1 ml of these serial dilutions were used to inoculate into vials containing 5ml of the semi-solid N-free medium (Nfb) (Malic acid (5g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1g),$CaCl_2$ (0.02 g), 0.5% bromothymol blue in 0.2 N KOH (2ml), 1.64% Fe-EDTA solution(4 ml),vitamin solution (1 ml), micronutrient solution (2 ml), agar (1.9g) per liter, pH 6.8. The vitamin solution contained in 100 ml: biotin (10mg), pyridoxol-HCl (20mg) and the micronutrient solution consist of: $CuSO_4$ (0.4 g); $ZnSO_4.7H_2O$ (0.12 g); H_2BO_3 (1.4 g); $Na_2MoO_4.2H_2O$ (1.0 g) and $MnSO_4.H_2O$ (1.5 g) per liter) [21] and incubated for 4 to 6 days at 30 ºC. The growth of bacteria was observed by pellicle formation near the surface of the tube (qualitative evidence of bacterial ability to fix atmospheric nitrogen). The vials showing bacterial growth were used to inoculate plates of the same solid media with additional 20 mg l^{-1} of yeast extract [21]. After incubation at 30 ºC for 5 to 6 days, the single well separated and morphologically different bacterial colonies growing on the plates were randomly picked and transferred into fresh N-free solid media by streak plate method for purification. The transfer procedure mentioned above was carried out 3–4 times to isolate single colonies. The purified colonies were transferred into agar slants and stored in refrigerator at 4 ºC for further studies. For long-term storage at -20 ºC, the isolates were preserved in the 20% glycerol.

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2.3 Screening for plant growth-promoting characteristics of isolated bacteria

2.3.1 Quantitative analysis of Indole-3-Acetic Acid (IAA) production

The quantification of IAA production by bacterial isolates was determined by Salkowski's colorimetric test. The bacterial isolates were inoculated into the tubes containing Nfb medium supplemented with 0.5 gl^{-1} tryptophan. The bacterial cultures were harvested after 48 h of incubation at 30°C and centrifuged at 15000 rpm for 15 min. The supernatant (2 ml) was mixed with 3 ml of Salkowski's reagent and incubated 30 min in darkness at room temperature (29°C) for color development. IAA production was observed as the development of the light red color and the absorbance was measured at 530 nm using a spectrophotometer [30]. The concentration of IAA produced by each bacterial isolate was determined by using a standard curve prepared from commercial IAA solutions (0, 10, 20, 30,40,50,60,70,80,90 and 100 ppm). The test was repeated twice with three replicates for each and mean was calculated.

2.3.2 Plate assay for mineral phosphate-solubilizing ability

The ability of bacterial isolates to solubilize inorganic phosphate was tested on Pikovskaya's agar medium (Glucose (10 g), $Ca_3(PO_4)_2$ (5 g), $(NH_4)_2SO_4$ (0.5 g) and yeast extract(0.5 g), $MgSO_4.7H_20$ (0.2 g), NaCl (0.1 g), traces of $FeSO_4$(per liter), pH 7). Bacterial isolates were spot inoculated into the medium containing plates and incubated for 7 days at 30 °C [33]. The presence of clear zone around the bacterial colony was considered as an indicator for positive mineral phosphate solubilization.

2.4 Morphological and Biochemical characterization of bacteria

Endophytic diazotrophic bacteria with plant growth-promoting ability were identified according to Bergey's Manual of Systematic Bacteriology [2, 29]. The cultural characteristics of bacteria were determined on nutrient agar (NA) plates after 48 h of incubation. The cell shape was determined by microscope following gram staining. The Gram reaction was performed using 3% KOH [40]. Motility was examined on cultures grown in semi-solid nutrient agar (NA) medium. Bacterial isolates grown on nutrient agar (NA) were tested for the presence of catalase and oxidase within 24 h. Methyl Red (MR) and Voges-Proskauer (VP) tests were performed by inoculating MR-VP broth in a screw-capped tube, incubating for 24-48 h at 37°C and then 5 drops of methyl red solution was added for MR test and 5% (w/v) α–naphthol in absolute ethanol and 40% (w/v) KOH was added for VP test. The red color formation was taken as positive for MR and VP test. Lysine decarboxylase test was performed by inoculating lysine decarboxylase broth with bacterial cultures and incubated at 37°C for 24 h. The bacterial isolates that convert the color of the medium from purple to yellow after 24 h of incubation and ability to change back to purple from yellow after next 24 h of incubation were recorded as positive for the test. Starch hydrolysis was tested by inoculating bacterial cultures on starch agar plates. After incubation at 37°C for 2-4 days, plates were flooded with Gram's iodine solution. Bacterial isolates with the ability to produce clear zones around the colonies were taken as positive for the test. Gelatin hydrolysis was tested by inoculating Frazier's agar plates with bacterial cultures. After incubation at 37°C for 2 to 14 days plates, were flooded with 1% HCl solution. Gelatin hydrolyzing bacteria were identified by the clear zones around their colonies while non-hydrolyzing bacteria formed an opaque precipitate with HCl reagent. For citrate test, Simmons' citrate agar slants were inoculated with bacteria and incubated at 37°C for overnight. The bacterial isolates positive for the test. The bacterial isolates that positive for the test were changed the color of the media from green to blue. H_2S test was performed by inoculating tubes containing Kliger's iron agar with bacterial cultures by stabbing with inoculating needle and incubated at 37°C for 48 h. After incubation positive result was indicated by black precipitate formed at the lower portion of the tube. Nitrate reduction test was carried out by inoculating screwed capped tubes containing peptone water with 0.02% KNO_3 and 1ml of Sulfanilic acid. After incubation for 2-4 days at 37°C, 1 ml of Napthyamine was added. A red color developing within 5 min was taken as the positive reaction, while the absence of color indicated negative results. Negative results were confirmed using Zn dust. For the urease test, urea broth was inoculated and incubated at 37 °C for 48 h. The color change from yellow to pink was considered positive for the test. Acid production from D-glucose and lactose was tested by inoculating peptone broth containing tubes with bacterial cultures and incubated for 2-5 days at 37°C. The change of color from red to yellow was taken as positive for the test. Based on the results of specific biochemical tests all bacterial isolates were partially identified.

2.5 Preparation of bacterial culture for seed inoculation

Seven endophytic diazotrophic bacteria (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) with plant growth-promoting ability were used to test their effect of inoculation on different growth parameters of rice. The Suwandel rice seeds were surface sterilized with 70% (v/v) ethanol for 30 sec and shaken in 1% (w/v) NaOCl

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solution for 5 min. Seeds were then washed three times with sterilized distilled water with shaking (15 min each). To ensure the sterilization efficiency, seeds were subjected to sterility check on nutrient agar media. For preparing bacterial cultures, each bacterial strain (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) was cultured in 100 ml conical flasks containing 50 ml of nitrogen-free broth with 0.1% NH_4Cl on a rotary shaker at 100 rpm for 38 h at 32°C. Bacterial cells were collected via centrifugation at 8000 rpm for 10 min and the bacterial cell pellets were suspended in 10 ml of 66 mM phosphate buffer (pH 7.0). The surface sterilized seeds of rice cultivars were inoculated by soaking seeds in the respective bacterial culture for 6 h at 32°C. The treated seeds were spread on a sterilized petri dish and air dried in a sterilized environment for overnight at room temperature (29°C). The bacterial cell culture was standardized to 10^7CFU/ml via serial dilution before inoculation.

2.6 Screening the effect of bacterial seed treatment on growth of rice seedlings

To study the effect of rice seedling growth promotion by plant growth-promoting endophytic diazotrophic bacteria, 40 rice seeds inoculated with respective bacterial culture were placed in 7 sterilized transparent plastic containers (10 X 12 cm) containing moistened paper towel. Then the containers were sealed and incubated at room temperature (29°C). As a control treatment seeds inoculated with un-inoculated sterilized broth were established. Seeds were sprayed with distilled water to maintain moisture required for the germination. After 3 days, 40 germinated seedlings (inoculated) were transferred into hydroponic seed tray containing 800 ml full strength Hogland's nutrient solution (without N and P) along with 1 g of tricalcium phosphate while 40 germinated seedlings (un-inoculated) were transferred into hydroponic seed tray containing 800 ml of full strength Hogland's solution (with N and P) [23]. After 12 days, 10 rice plants were taken randomly and shoot lengths and root lengths were measured. The shoots and roots were separated, and fresh weights were measured. For determining dry weight shoots and roots of rice plants were kept in an oven at 60°C for 72 h to obtain a constant weight. The experiment was planned as a completely randomized design with 3 replications for each isolate.

III. RESULTS AND DISCUSSION

3.1 Isolation and Identification of isolated endophytic diazotrophic bacteria

Total of 15 putative endophytic diazotrophic bacterial isolates were obtained from shoots and roots of Suwandel and Bg 358 rice varieties. In this study screening for nitrogen fixing ability was done on N-free medium and all the isolates showed growth on the medium. However, diazotrophic capacity of isolated bacteria should be further confirmed by the presence of *nif H* gene or acetylene reduction assay. Seven diazotrophic bacterial isolates (IN003, IN006, IN007, IN008, IN009, IN015, and IN018) were selected for further studies based on their ability to produce IAA and phosphate solubilization. Among this 7 bacterial isolates, 4 isolates (IN003, IN006, INS008, and IN015) were obtained from Suwandel and 3isolates (IN007, IN009, and IN018) were obtained from Bg 358. The colony morphology of seven bacterial isolates was tested on nutrient agar (NA) medium and characteristics including shape, color, elevation, margin, and texture were studied (Table 1). Most of the colonies were circular to irregular and whitish or cream in color while yellow color is also observed. The margins of the colonies of isolated bacteria were found to be entire, undulate and irregular. The surface characteristics of bacterial isolates were found to be smooth and glistening. The cells were rod-shaped. Out of seven bacterial isolates, 4 were gram-negative while 3 isolates were gram-positive in reaction. All the isolates were motile except IN008 and IN018.Bacterial isolates were identified via different biochemical tests (Table 2) according to Bergey's Manual of Systematic Bacteriology [2, 29]. On the basis of morphological, and biochemical characteristics, 07 bacterial isolates were identified that belong to four different genera; *Bacillus* (IN003, IN006, and IN007), *Klebsiella* (IN008 and IN018), *Pantoea* (IN009), and *Enterobacter* (IN015). These bacteria were commonly isolated from various tissues of the rice plant. However, identification of bacterial strain to species level requires further molecular characterization. Therefore, the molecular characterization targeting 16S rDNA region needed to be done to confirm these results.

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Table.1.: Morphological characteristics of 07 plant growth-promoting endophytic diazotrophic bacteria on nitrogen-free(Nfb) media

Bacterial Strains	Colony characteristics on Nfb media						Cell shape	Gram's nature
	Size(mm)	Color	Shape	Elevation	Margin	Texture		
IN003	1-3	Cream	Circular	Flat	Entire	Smooth	Rods	+
IN006	2-4	Cream	Circular	Raised	Undulate	Smooth	Rods	+
IN007	1-2	White	Irregular	Raised	Undulate	Smooth	Rods	+
IN008	2-3	Light cream	Irregular	Convex	Entire	Smooth	Straight rods	-
IN009	2-3	Pale yellow	Circular	Convex	Entire	Smooth	Straight rods	-
IN015	2-3	White	Circular	Flat	Irregular	Glistening	Straight rods	-
IN018	3-4	Cream	Circular	Slightly raised	Entire	Glistening	Straight rods	-

(-): Positive; (+): Negative

Table 2: Biochemical characteristics of 07 plant growth-promoting endophytic diazotrophic bacteria

Bacterial strain	Motility	Catalase test	Oxidase test	MR Test	VP test	Lysine Decarboxylase test	Starch hydrolysis	Gelatin hydrolysis	Simmons Citrate Utilization	H_2S production	Nitrate Reduction	Urease Test	Acid from D- glucose	Acid from D-Lactose	Identified bacterial species
INS003	+	+	+	-	+	-	+	-	+	+	+	-	+	-	*Bacillus sp.*
INS006	+	+	-	+	-	-	+	-	+	-	-	-	+	-	*Bacillus megaterium*
INS007	+	+	-	+	-	-	+	+	-	-	-	-	+	-	*Bacillus pumilus*
INS008	-	+	-	-	-	+	-	-	+	-	+	+	-	-	*Klebsiella sp.*
INS009	+	+	-	-	-	-	-	+	+	-	+	-	-	+	*Pantoea sp.*
INS015	+	-	+	-	+	-	-	+	-	-	+	-	+	-	*Enterobacter sp.*
INS018	-	+	-	-	+	+	-	+	+	-	+	+	+	-	*Klebsiella sp.*

(-): Positive reaction; (+): Negative reaction

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3.2 Characterization of plant growth-promoting ability of putative endophytic diazotrophic bacteria

3.2.1 Production of IAA

All 7 diazotrophic endophytic bacterial isolates produced reddish pink color after addition of Salkowski's reagent indicating their ability to produce IAA through tryptophan. In the presence of tryptophan, isolated bacteria produce IAA with concentration ranging from 7.1 µg $ml^{-1}$to 30.9 µgml^{-1}(Table 3). The highest amount of IAA was produced by isolate IN015 (*Enterobacter* sp.) while lowest IAA production was recorded by isolate IN009 (*Pantoea* sp.). IAA produced by these bacteria has a favorable effect on plant growth promotion. The results of this study indicated that the amount of IAA produced by different bacterial species can be varied. Similar results had been reported by the [23]. This can be due to the several factors that affect the level of IAA production in bacteria such as the location of auxin biosynthesis genes in the genome, IAA biosynthetic pathways, culture conditions, growth stage of the bacteria and substrate availability [28].

Table.3: Concentration of IAA produced by 7 endophytic diazotrophic bacterial isolates

Bacterial strain	Concentration of [a] IAA(µg/ml)
[b]*Serratia marcescens*	20.2±0.78*
IN003	10.8±0.36
IN006	9.8±0.42
IN007	20.8±0.92
IN008	15.2±0.91
IN009	7.1±0.26
IN015	30.9±0.33
IN018	12.2±0.18

[a]IAA production was estimated on Nfb medium supplemented with tryptophan with absorbance at 540 nm.

[b]*Serratia marcescens* was used as positive control for IAA producer.

* Values are the Mean ± SE The experiment was repeated twice with three replicates for each isolate

3.2.2 Phosphate solubilization ability

Phosphate is one of the most important nutrients required for rice plant growth and development. In the soil, phosphate usually forms insoluble complexes, unavailable to plant. Therefore, the efficiency of uptake and use of phosphorous after fertilizer application is low [33]. Phosphate-solubilizing bacteria can increase the phosphorous availability to the plant by converting inorganic phosphorous into more available form [26]. These bacteria solubilize inorganic phosphate by producing organic acids which decrease the pH of the culture media [30, 34]. Among 7 bacterial isolates tested, only 5 bacterial isolates (IN006, IN007, IN008, IN015, and IN018) were showed inorganic phosphate-solubilizing ability by forming halo zones on Pikovskaya's agar plates after 7 days of incubation (Table 4). These bacterial isolates were identified as *Bacillus megaterium* (IN006), *Bacillus pumilus* (IN007) and genus *Klebsiella* (IN018 and IN008) and *Enterobacter* (IN015). Previous studies also indicated the phosphate-solubilizing ability of bacteria belong to genera *Bacillus*, *Enterobacter*, and *Klebsiella* [7, 10, 14, 16]. Moreover, all these five isolates which solubilized phosphate were also IAA producers.

Table.4: Phosphate solubilizing ability of 07 endophytic diazotrophic bacterial isolates

Bacterial Strain	Phosphate Solubilization
Pseudomonas aeruginosa[*]	+
IN003	-
IN006	+
IN007	+
IN008	+
IN009	-
IN015	+
IN018	+

The bacterial strains able to solubilize inorganic phosphate on Pikovskaya's agar (+); the bacterial strains unable to solubilize inorganic phosphate on Pikovskaya's agar **Pseudomonas aeruginosa* was used as a positive control

3.3 Plant growth-promoting parameters

Most of the bacterial isolates have a significant effect on the shoot length, root length, shoot fresh weight, shoot dry weight and root fresh weight of the rice seedlings compared to the un-inoculated control (Table 4; Fig 1 A, B). All the bacterial isolates significantly ($p \leq 0.05$) enhanced the shoot length of the rice seedlings compared to untreated control and maximum shoot length (23.33±0.34 cm) was observed in seedlings treated with *Enterobacter* sp. (IN015). However, inoculation with *Bacillus megaterium* (IN006) significantly ($p \leq 0.05$) reduced the root length compared to un-inoculated control whereas inoculation with *Enterobacter* sp. (IN015) resulted in longest root length (8.76±0.05cm) (Table 4; Fig 1 A). Inoculation with all bacterial isolates enhanced shoot fresh weight and dry weight, but there was no significant difference in the shoot dry weights between

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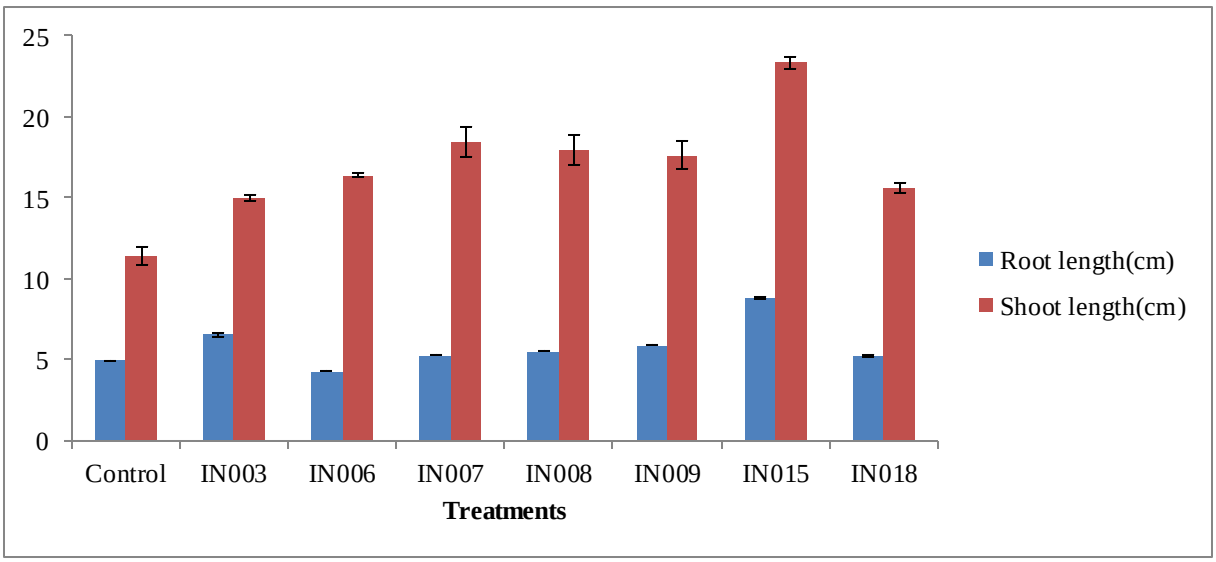
the plants treated with bacterial isolates except *Enterobacter* sp.(IN015) (Table 4; Fig 1 B). Treatment with all bacterial isolates increased the root fresh weight and dry weight of inoculated plants and significantly ($p \leq 0.05$) high root fresh weight and dry weight were recorded in plants inoculated with *Enterobacter* sp. (IN015) compared to the control and other bacterial isolates. The results of this study indicated that among all the other bacterial isolates, *Enterobacter* sp. (IN015) was more effective in rice plant growth promotion. Previous studies have also reported that certain *Enterobacter* sp. as effective plant growth promoters since they possess multiple plant growth-promoting activities [3, 20, 30]. Rice seedlings growth promotion could be attributed to the multiple plant growth-promoting properties of inoculated bacteria such as IAA production, nitrogen fixation, and phosphate solubilization. In the hydroponic test system, inoculated plants were not supplied with N or soluble phosphorous which are major nutrients required for rice plant growth. Therefore, N and P requirement of the plant could be supplied only by the inoculated bacteria with their ability to fix nitrogen and solubilize inorganic phosphate. All seven bacterial isolates used in the plant inoculation experiment have the ability to produce IAA which is an important mechanism of plant growth promotion. IAA promotes the growth of the plant by increasing the number and size of adventitious and lateral roots and thereby facilitates the nutrient uptake by the plant [22].

Table 5: Effect of bacterial isolates on growth parameters of rice seedlings

Treatments	Shoot Length (cm)	Root Length (cm)	Shoot fresh weight (mg/plant)	Root fresh weight (mg/plant)	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)
Control	11.37±0.52^{d}	4.92±0.03^{f}	0.34±0.01 ^{f}	0.27±0.02 ^{d}	0.20±0.01^{c}	0.14±0.03 ^{c}
IN003	14.97±0.15^{c}	6.51±0.10^{b}	0.58±0.02 ^{e}	0.33±0.01^{cd}	0.49±0.03^{b}	0.19±0.01^{bc}
IN006	16.40±0.15^{bc}	4.26±0.04^{g}	0.66±0.01 ^{d}	0.39±0.02^{bc}	0.47±0.02^{b}	0.18±0.01^{bc}
IN007	18.43±0.93^{bc}	5.25±0.04^{e}	0.79±0.01 ^{b}	0.40±0.01^{bc}	0.59±0.05^{b}	0.22±0.01^{bc}
IN008	17.93±0.95^{bc}	5.50±0.04^{d}	0.65±0.01 ^{d}	0.42±0.02^{bc}	0.54±0.03^{b}	0.22±0.03^{bc}
IN009	17.63±0.87^{bc}	5.87±0.02^{c}	0.76±0.01^{bc}	0.39±0.02^{bc}	0.59±0.01^{b}	0.18±0.01^{bc}
IN015	23.33±0.34^{a}	8.76±0.05^{a}	0.99±0,02 ^{a}	0.57±0.04 ^{a}	0.81±0.01^{a}	0.37±0.03^{a}
IN018	14.90±0.35^{bc}	5.20±0.04^{e}	0.72±0.01 ^{c}	0.45±0.01 ^{b}	0.62±0.04^{b}	0.24±0.02^{b}

Values followed by the same letter are not significantly different as determined by Tukey's mean comparison test ($p \leq 0.05$). The statistics were performed separately for the data in each column (means of three replicates, ± standard error).

A



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B

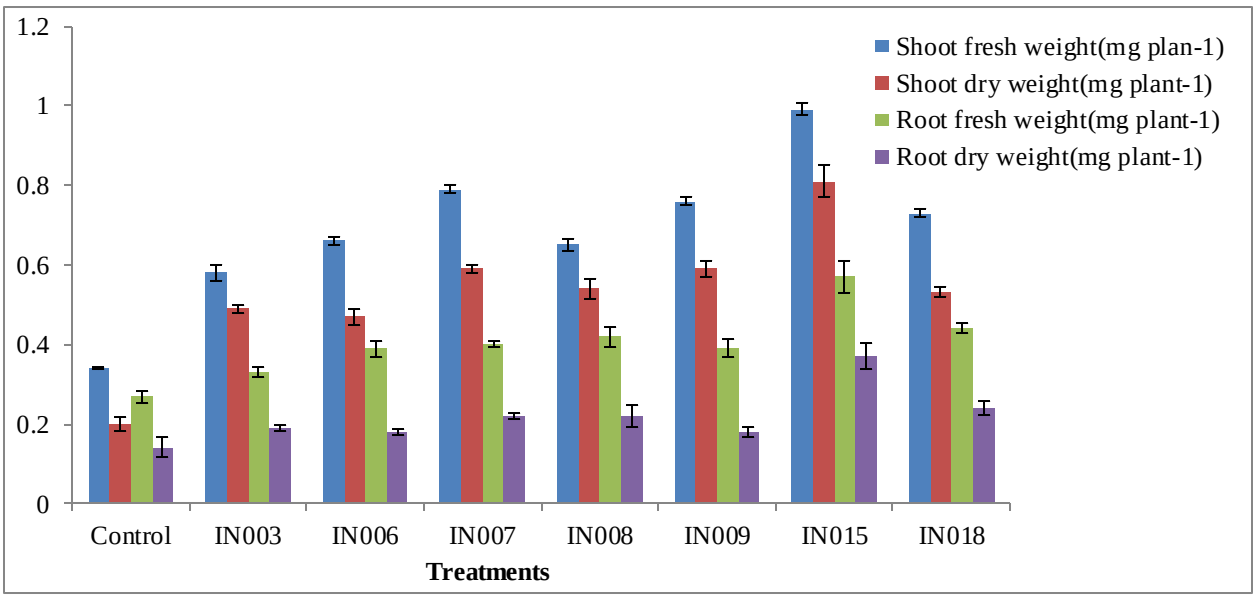

Fig 1: Effect of Plant growth-promoting endophytic diazotrophic bacteria inoculation on shoot and root length (A) and shoot and root fresh weight and dry weight (B) of rice seedling (Suwandel variety) growing in hydroponics. Error bars are SE from three replicates per same treatment.

IV. CONCLUSION

The current study indicated that endophytic bacteria isolated from the shoots and roots of Suwandel and Bg 358 has beneficial effects on plant growth promotion through IAA production, phosphate solubilization, and nitrogen fixation. Seven bacterial isolates belong to different species produced IAA and fix nitrogen in the nitrogen-free media while 5 out of 7 bacterial isolates showed phosphate solubilizing activity. Rapid screening of their ability to promote rice plant growth was carried out using a hydroponic system and the results of this study clearly demonstrated the positive effect of these bacterial inoculations on rice plant growth. However, plant inoculation experiment was carried out under laboratory conditions. Therefore, results obtained in this study may not reproduce exactly under the field conditions. Further studies under field conditions are recommended to identify the real potential of these bacteria to promote rice plant growth. Multiple plant growth-promoting abilities and positive effect on plant growth parameters in the plant inoculation experiment suggest that the bacteria isolated in this study have the potential to develop as biofertilizer to promote rice plant growth. *Enterobacter* sp. (IN015) showed most effective in rice plant growth promotion compared to other bacterial isolates. However, more research is needed to understand the interaction between endophytic bacteria and rice plant to fully exploit their potential as biofertilizers.

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