Research Article

Isolation and Characterization of Plant Growth Promoting Rhizobacteria for Growth Promotion of Rice (*Oryza sativa* L.) and Wheat (*Triticum aestivum*)

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Abstract: The use of rhizobacteria for plant growth enhancement is decades old. Still, in this era of the 21st century, biofertilizers have become the need of the day due to the health and environmental concerns associated with chemical fertilizers and pesticides. Rhizobacteria strains were isolated from the rhizosphere of rice and wheat. The selected bacterial strains were examined for nitrogen fixation, indole acetic acid (IAA) production, phosphorus solubilization, and antifungal activity on morphological, biochemical, and molecular levels. Production of IAA ranged from 6 µg/ml to 29.33 µg/ml while ethylene production (C₂H₄/hr) varied from 2 µmoles to 9.8 µmoles. Maximum Phosphorus Solubilization index (7), decrease in pH (4) and Solubilization % age (0.49) was observed in WM-2 (wheat microbe). Promising results were obtained concerning antifungal activity against *Rhizoctonia solani* and *Fusarium sp.* The effect of the potential PGPR strains on the germination of rice and wheat was significantly positive in Petri plates. In the case of rice, the highest shoot length (29.27 cm) was observed by inoculation with RPR-33 (Rice isolate), and the most increased root length (9.33 cm) was observed in the treatment inoculated with RPR-42. The highest shoot fresh weight (476.67 mg/plant) was recorded in the treatment inoculated with RPR-42. The maximum root weight was 170 mg/plant in the same treatment. For wheat, all recorded growth parameters were improved significantly by wheat microbe WM-5. All the PGPR isolates showed positive results for growth parameters of wheat and rice on inoculation. So, it is suggested that these PGPR isolates may be used as potential biofertilizers.

Keywords: PGPR, PSM, Nitrogen fixation, Phytohormones Production, Biofertilizers

Citation: Ayyaz, M.; Khan, Z.; Tabbasam, N.; Sultan, T.; Saeed, A.; Shan, M. Isolation and Characterization of Plant Growth Promoting Rhizobacteria for Growth Promotion of Rice (*Oryza sativa* L.) and Wheat (*Triticum aestivum*). *Pakistan Journal of Biochemistry and Biotechnology*, 2021, 2 (2), 177-194. <https://doi.org/10.52700/pjbb.v2i2.66>

Received: 17-10-2021
Accepted: 20-12-2021
Published: 31-12-2021

1. Introduction

Beneficial free-living soil bacteria in the rhizosphere, capable of colonizing plant roots that promote plant growth, were first defined by [1] and termed Plant Growth Promoting Rhizobacteria (PGPR). There are several ways in which different PGPR may directly or indirectly facilitate the proliferation of the plants. The mechanisms of PGPR-mediated enhancement of plant growth and yield of many crops are not yet fully understood. However, the possible explanations include (i) biological nitrogen fixation [2], (ii) solubilization of mineral phosphates and mineralization of other nutrients [3], (iii) ability to produce or change the concentration of plant hormones like indole acetic acid (IAA), gibberellic acid, cytokinin and ethylene [4]; (iv) antagonism against phytopathogenic microorganisms by producing siderophores, β -1,3-glucanase, chitinases, antibiotics, fluorescent pigment and cyanide [5].

IAA plays a key role in controlling many physiological processes in plants such as root proliferation, nitrogen fixation, cell division and shoot growth. Many plant-associated bacteria can produce IAA in culture. The production of IAA, GA and Zeatin has also been reported in *Bradyrhizobium japonicum* [6]. The bioavailability of soil inorganic

phosphorus in the rhizosphere varies considerably with plant species, soil nutritional status, presence of effective microorganisms and soil conditions. Rhizobium and phosphate solubilizing bacteria (PSB) are important to plant nutrition and perform a major role as PGPR in the bio fertilization of crops. It has been reported that inoculation of Phosphate solubilizing bacteria (PSB) increases the Phosphorous uptake by plants and crop yield. PGPR may promote plant growth by direct or indirect mechanisms acting as biological control agents [7]. Early studies on PGPR focused more on biological control of plant diseases than on growth promotion and involved bacteria like *Fluorescent pseudomonas* and *Bacillus subtilis* antagonistic to a range of soil-borne plant pathogens. The division of PGPR proposed into two classes: Biocontrol-PGPB (plant-growth-promoting-bacteria) and PGPB (plant-growth-promoting-bacteria) [8].

Significant increases in the growth and yield of agronomically essential crops in response to inoculation with PGPR have been reported. Multifaceted effects of plant growth-promoting rhizobacteria have been reported for plant growth and productivity enhancement, and disease reduction [9]. One of the major concerns in today's world is the pollution and contamination of soil by chemical fertilizers and pesticides. It has been reported that inorganic fertilizers and pesticides are contaminating our soils with heavy metals. Excessive use of synthetic fertilizers and pesticides has caused tremendous harm to the environment and indirectly affects the human population. In addition, continuous use of pesticides may lead to resistance in the pest, which is difficult to control. The use of synthetic chemical fertilizers leads to imperfectly synthesized protein in leaves, which is responsible for poor crops and, in turn, for pathological conditions in humans and animals fed with such deficient food [10]. By keeping the importance of biofertilizers for sustainable agriculture in view, the present research was conducted to better understand PGPR by characterization and inoculation for the growth enhancement of cereals, particularly rice and wheat.

2. Materials and Methods

The research experiments were carried out in the Soil Biology & Biochemistry Lab, Land Resources Research Institute (LRRI), National Agricultural Research Centre (NARC), Islamabad, in collaboration with Plant Genomics and Biotechnology Department, PARC institute of advance studies in agriculture (PIASA), Islamabad. This study was completed using the following procedures.

2.1. Isolation, Purification and Morphological Characterization of Rhizobacteria

The isolation was made using the serial dilution method. The serial dilution for 10^{-1} to 10^{-6} was made in already autoclaved capped tubes with 9 ml water. One-gram soil was dispersed in 10ml of sterile distilled water as described earlier [11]. An aliquot of 0.1ml from each dilution was taken with a micropipette and transferred on Luria-Bertini (LB) solid media in the Petri plates. The pure cultures were purified by single selection and further dilution on solid media again and again. The purified strains were cultured on solid media to observe the various colony morphological characteristics including colony color, colony margins, colony surface texture, colony shape, elevation and pigmentation associated with colonies. Slides of purified bacterial isolates were prepared for Gram staining reactions [12]. Pink-colored bacteria were Gram -ve while purple colored was Gram +ve. After gram staining, the slides were studied for cell morphology under a microscope.

2.2. Biochemical Characterization

PGPR strains were biochemically characterized using API strips (bioMérieux) according to the manufacturer's protocol.

2.2.1. Determination of Indole Acetic Acid (IAA) Production

Screening of isolates for IAA production was done as adopted [13]. Briefly, bacterial strains were inoculated in the 100ml LB broth media with 1mg/ml tryptophan. These cultures were incubated at $28 \pm 2^\circ\text{C}$ for one week on a stand bath. These cultures were centrifuged at 3000 rpm for 30 min, and 2ml of the supernatant was mixed with 2 drops orthophosphoric acid and 4ml of salkowski reagent (50 ml, 35% perchloric acid; 1ml 0.5% FeCl_3). Development of pink colour indicated IAA production. The optical density (O.D) was read as 530 nm using a spectrophotometer. The level of IAA produced was estimated by a standard IAA graph. IAA was also determined on HPLC using UV-detector and Tech sphere 5-ODS C-18 column.

2.2.2. Determination of Nitrogenase Activity and Amplification of *nifH* gene

Acetylene reduction assay (ARA) was conducted to determine the nitrogenase activity of PGPR isolates. PGPR isolates were grown in 20 ml McCartney vials containing semi-solid Nitrogen Free Medium (NFM) and incubated at 30°C for 48 hours. Each vial was sealed with rubber stoppers. One ml of acetylene was injected per vial. The vials were incubated at 30°C for 24 hours. One ml gas sample was used to determine ethylene production. Ethylene production was measured using a Gas Chromatograph equipped with a flame ionization detector and a Porapak Q column [14]. Nitrogenase activity was calculated as nmol per hour per tube. Moreover, *the nifH* gene was also PCR amplified by following forward and reverse *nifH* primers, respectively; PolF TGCGAYCCSAARGCBGACTC and PolR ATSGCCATCATYTCRCCGGA.

2.2.3. Determination of Phosphorus Solubilization

To determine Phosphorus Solubilization ability, the bacterial isolates were grown on Pikovskaya agar medium [15]. Each plate was inoculated with different bacterial strains with the help of a sterile toothpick. The plates were incubated at $28 \pm 2^\circ\text{C}$.

2.2.4. Determination of Solubilization Index

After incubation, the bacterial isolates' ability to solubilize insoluble phosphorus was studied by determining the Solubilization Index. Solubilization Index (SI) was measured using the following formula [16].

$$SI = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{colony diameter}}$$

2.2.5. Change in pH in Broth Cultures

Change in pH of the broth culture of PSB was determined with the help of a pH meter daily during seven days of incubation.

2.2.6. Phosphorus Solubilizing Capacity of Isolates in Broth Cultures

To determine Phosphorus solubilizing capacity, the broth or liquid Pikovskaya medium was prepared in 250 ml conical flasks. To each flask, 0.25 g insoluble phosphate in the form of Tri-calcium phosphate was added. The flasks were sterilized at 15 psi and 121°C for 15 minutes. After autoclaving, the flasks containing media were inoculated with bacterial isolates and allowed to grow for five days at 30°C . After five days, each culture was centrifuged at 15000 rpm for 30 minutes. The supernatant of each culture was collected in 100 ml volumetric flasks, and the volume was made up to 100 ml with distilled

water. The extract of each solution was prepared as described [17], and the available phosphorus in each broth culture was determined accordingly [18].

2.2.7. Biocontrol Activity

The inhibition of mycelium growth of *Rhizoctonia solani* and *Fusarium sp.* by the PGPR strains was tested on Potato Dextrose Agar (PDA) media in a dual plate assay. Suspension culture of PGPR strain (one ml from 108 cfu/ ml) was poured on the margin of rye media plates. A 6mm agar disc of respective fungus from fresh PDA cultures was placed at the other marginal side of the rye media plate for each bacterial isolates and incubated at 25±20 °C for 3-4 days. The radii of the fungal colony towards and away from the bacterial colony were noted. The percentage growth inhibition was calculated using the following calculation:

$$\% \text{ inhibition} = [(R - r) \times 100] / R$$

Where r is the radius of the fungal colony opposite the bacterial colony and R is the maximum radius of the fungal colony away from the bacterial colony.

2.3. Effect of PGPR on Germination and Growth of Rice and Wheat

2.3.1. Seed Germination Studies on Rice and Wheat (In Petri Plates)

It was the first evaluation study to see the effects of PGPR and PSB strain on germination and growth of rice and wheat. It was conducted in controlled conditions. In this study effect of eight strains of PGPR was observed on germination of Rice (385) and Wheat (NARC 2009) seeds. Eight treatments had four replicates and control without inoculation. The experiment was laid out in CRD experimental design. Treatments were as follow:

Detail of Treatments

T1	Control (without inoculation)
T2	WM-2
T3	WM-3
T4	WM-4
T5	WM-5
T6	WPR-61
T7	RPR-33
T8	RPR-41
T9	RPR-42

The seeds of wheat and rice were surface sterilized with mercuric chloride for 1 min, washed 3-4 times with sterile distilled water. After washing with water, the seeds were thoroughly soaked in the culture of all bacterial isolates WM-2, WM-3, WM-4, WM-5, WPR-61, RPR-33, RPR-41 and RPR-42 suspension separately for one hour to ensure uniform coating on the surface in aseptic conditions. Seeds soaked in sterilized distilled water only were taken as control. Five seeds were sown in each plate; then, the seeds were allowed to grow in Petri plates with autoclaved filter paper at 25 °C for 6 days in the growth cabinet. Germination was observed on the third day. During this period, autoclaved distilled water was added to Petri plates daily. The final data of germination was recorded after seven days of sowing. The root and shoot lengths of all treatments were recorded, and the vigour index of seedlings was also calculated using the formula;

Vigor index= (mean root length +mean shoot length) × % germination

2.4. Pot Experiment

The pot experiment was conducted to study the effect of PGPR on the growth of wheat and rice. The experiment was conducted in Complete Randomized Design (CRD) with three replicates. The treatments were the same as described in the Petri plate experiment. The seeds were surface sterilized and inoculated with PGPR strains. Sowing was done in the plastic pots containing 0.8 kg of autoclaved soil. The plants were harvested after thirty days, and growth parameters were recorded, i.e. root/shoot lengths, root/shoot fresh and dry weights.

2.5. Statistical Analysis

All the experiments were done in triplicate. The data obtained in the study were subjected to Analysis of Variance using MSTATC computer software [19], and means were compared by the least significant difference test [20].

3. Results and Discussion

3.1. Isolation and Morphological Characterization of PGPR Isolates

Out of eight bacterial isolates selected for further study, five were isolated from wheat (WM-2, WM-3, WM-4, WM-5 and WPR-61), and three (RPR-33, RPR-41 and RPR-42) were isolated from the rice rhizosphere (Table 1a and 1b). The isolates were purified with further sub-culturing on LB plates (Figure 1 and 2). All PGPR isolates were gram -ve bacteria whereas, isolate (RPR-33) was gram +ve. All isolates were examined microscopically, and different cell shapes were observed from rod to coccus (Figure 3 and 4).

Fig. 1. Wheat PGPR on LB plate

Fig. 2. Rice PGPR on LB plate

Fig. 3. Microscopic image of rod shape

Fig. 4. Microscopic image of cocci

Table 1a: Morphological and physiological characteristic of PGPR bacterial strains

PGPR Isolate	Source	P-Solubilizing	IAA	ARA
WM-2	Wheat rhizosphere	+ve	+ve	+ve
WM-3	Wheat rhizosphere	-ve	+ve	-ve
WM-4	Wheat rhizosphere	-ve	+ve	+ve
WM-5	Wheat rhizosphere	+ve	+ve	+ve
WPR-61	Wheat rhizosphere	+ve	+ve	-ve
RPR-33	Rice rhizosphere	+ve	+ve	+ve
RPR-41	Rice rhizosphere	+ve	+ve	+ve
RPR-42	Rice rhizosphere	+ve	+ve	+ve

Table 1b: Biochemical traits of PGPR strains

PGPR Isolate	Gram Staining	Cell Morphology	Colony Color on N.A	Form/shape of colony	Colony elevation and margin
WM-2	- ve	Spirilla	White gummy	circular	Convex and entire
WM-3	- ve	Coccus	Yellow shiny	circular	Pulvinate and entire
WM-4	-ve	Vibrio	Off-white	Rhizoid	Flat and erose
WM-5	- ve	Bacillus	White	filamentous	Raised and erose
WPR-61	- ve	Micrococcus	Off-white gummy	filamentous	Convex and undulate
RPR-33	+ve	Rod	Off-white	circular	Flat and entire
RPR-41	- ve	Coccus	Off-white	circular	Flat and entire
RPR-42	-ve	Rod	Off-white	circular	Flat and erose

3.2. Biochemical Tests

Basic biochemical tests of the isolates were conducted using API 20E (bioMérieux) according to the manufacturer's protocol (Figure 5). The results are given in Table 2.

Fig. 5. Determination of the biochemical activity of PGPR by API strips**Table 2:** Biochemical test results of PGPR through API 20 E strips (bioMérieux)

Test	Active Ingredients	W M 2	W M 3	W M 4	W M 5	W P R 61	R P R 33	R P R 41	R P R 42
OPGN	2-nitrophenyl-BD-glactopyranoside	-	+	+	+	-	+	+	+
ADH	L-arginine	+	+	-	-	+	-	+	+
LDC	L-lysine	+	-	-	-	-	-	-	+
ODC	L-ornithine	+	+	-	+	-	-	+	+
CIT	Trisodium citrate	+	+	-	+	-	-	+	+
H2S	Sodium thiosulfate	-	-	-	-	-	-	-	-
URE	Urea	+	+	-	-	-	-	-	+
TDA	L-tryptophane	-	-	-	-	-	-	-	-
IND	L-tryptophane	+	+	+	+	+	+	+	+
VP	Sodium pyruvate	-	-	-	-	-	-	-	-
GEL	Gelatin	-	-	+	+	+	+	+	+
GLU	D-glucose	+	+	+	-	-	+	+	+
MAN	D-mannitol	-	-	-	-	-	-	-	-
INO	Inositol	-	-	-	-	-	-	-	-
SOR	D-sorbitol	-	-	-	-	-	-	-	-
RHA	L-rhamnose	-	-	-	+	+	-	+	-
SAC	D-sucrose	-	-	+	-	-	+	-	-
MEL	D-melibiose	+	+	-	+	-	-	+	+
AMY	Amygdalin	-	-	+	-	-	+	+	-
ARA	L-arabinose	-	-	-	-	+	-	-	+

3.3. Characterization for PGP Activities

3.3.1. Quantification of Phytohormone (IAA) Production

Indole acetic acid is a phytohormone, which promotes germination and plants' growth, ultimately resulting in higher yield. IAA was determined in all PGPR isolates. All strains showed a significant quantity of indole acetic acid ranging from 29.33 µg/ml to 6 µg/ml (Table 3a). Among all isolates, WPR-61 showed the statistically highest quantity of IAA, 29.33 µg/ml. The minimum quantity was quantified in RPR-42, which was 6 µg/ml. It was observed that all of the wheat isolated showed a significant quantity of IAA, more than 20 µg/ml (Table 3b). Several reports have shown that PGPR produces specific plant growth hormones such as IAA, Gibberellic acid (GA), etc. Numerous soil microorganisms are actively involved in synthesizing auxins in pure culture and soil [21]. These results follow previous studies [22].

Table 3a: IAA Production ($\mu\text{g/ml}$) by PGPR isolates

PGPR Isolates	IAA Production
WM-2	17 d
WM-3	18.67 d
WM-4	20 cd
WM-5	23.67 b
WPR-61	29.33 a
RPR-33	22.67 bc
RPR-41	12 e
RPR-42	6 f

Table 3b: Effect of PGPR on growth parameters of wheat in a pot experiment

Treatments	Length (cm)		wt (mg/plant)			
	Shoot	Root	Shoot Fresh	Root Fresh	Shoot Dry	Root Dry
Control	11.33e	3.67f	200g	33.33g	43.33f	9e
WM-2	17cd	6.67c	360c	63.33d	73.33cd	12d
WM-3	19c	6.33cd	370bc	80c	76.67c	15.67c
WM-4	21.67b	6.67c	380b	86.67b	80bc	16c
WM-5	28.33a	8.67a	411.67a	93.33a	96.67a	20.33a
WPR-61	23.67b	7.67b	398.33a	90ab	86.67b	18.67b
RPR-33	16d	4.33f	230f	36.67f	60e	12d
RPR-41	16.67d	5.33e	273.33e	53.33e	63.33e	12.33d
RPR-42	16.33d	5.67de	306.67d	56.67e	66.67de	12.67d

^{3b} (LSD at $P < 0.05$)

3.3.2. Quantification of Phosphate Solubilization of PGPR Isolates

Phosphate solubilizing bacteria were characterized by a transparent hollow zone around the colony on Pikovskaya media. Tricalcium phosphate, used as an insoluble phosphorus source, was solubilized by the P-solubilizing bacteria. Out of eight isolates, six isolates were selected for phosphorus solubilization based on the diameter of the halo zone and the solubilization index.

3.3.3. Solubilization index

All of the isolates showed phosphorus solubilization except WM-3. The solubilization index ranges from 2.6 to 7, which is very significant. The PGPR isolates WM-2 (Figure 6) showed the highest solubilization index 7, followed by WPR-61 with 5.48. The lowest index was observed in the isolate WM-5, 2.6. Sometimes, different changes in the results may occur, as reported by several researchers [23]. Usually, the colony diameter increases with the days of incubation, as observed in WM-2 and WPR-61. But the results may be different, like observed in the isolates RPR-41, RPR-33 and RPR-42. The halo zone

diameter of the colonies increases with the days of incubation for seven days. Still, it may stop with time, as it has been reported that the solubilization ability may decrease with days of incubation [24]. We observed a similar effect in some of our isolates.

Figure 6. Solubilization Index measured for seven days of incubation

To check the effect of PGPR isolates on pH, the broth Pikovskaya media was inoculated with PGPR isolated and incubated for seven days. The pH was recorded daily for up to seven days. All of the isolates showed a significant decrease in pH from 6 to 4 (Figure 7). It was observed that the maximum reduction in pH was 4 in WM-2 broth culture, followed by WPR-61 with 4.8. Minimum pH decreases 6 was observed in WM-5. A similar decrease in pH has been observed in PSB liquid culture by [25].

Figure 7. Change in pH in PSM broth cultures during seven days of incubation

3.3.4. Phosphate Solubilization of Isolates in Broth Cultures

Phosphate solubilization of the PGPR isolated was also quantified in liquid Pikovskaya media. The available phosphorus was measured after five days of incubation. The phosphate solubilized by PGPR isolates was expressed as available P (%) in broth culture (Figure 8). The solubilization percentage of our isolates was within the range of 0.4 to 0.8%, supported by the values given previously [26]. Different PSB strains were isolated [27] and reported that the capacity of strains to solubilize P is 37 to 130 $\mu\text{g/ml}$.

Figure 8. Available P (%) in broth culture

3.3.5. Determination of Nitrogenase Activity and *nifH* Gene Amplification

The production of ethylene by the PGPR isolates ranges from 2 $\mu\text{moles C}_2\text{H}_4/\text{mg protein/hr}$ to 9.8 $\mu\text{moles C}_2\text{H}_4/\text{mg protein/hr}$. Maximum ethylene was produced by RPR-33; 9.8 $\mu\text{moles C}_2\text{H}_4/\text{mg protein/hr}$ following RPR-41; 8.4 $\mu\text{moles C}_2\text{H}_4/\text{mg protein/hr}$ (Table 4). Similar results were reported in diazotrophic bacteria isolated from the rhizosphere of rice plants. The nitrogenase activity based on ARA were ranged from 0.69 to 1.63 $\text{nmol C}_2\text{H}_4 \text{ mg protein}^{-1}\text{h}^{-1}$ [28]. Moreover, the bacterial strains which showed nitrogenase activity also had *nifH* gene amplifications about 350bp to 400bp (Figure. 9). Other researchers have reported the amplification of *nifH* gene from PGPRs isolated from the wheat rhizosphere [29].

Figure 9. Amplification of *nifH* gene. The lane M contains the ladder of 1kb (Fermentas). Lane C indicates negative control. Band intensity in lanes shows the quantity of the PCR product.

Table 4: Production of ethylene by PGPR isolates

PGPR Isolates	$\mu\text{moles C}_2\text{H}_4/\text{mg protein/hr}$
WM-2	6.4 c
WM-4	2 d
WM-5	2.1 d
RPR-33	9.8 a
RPR-41	8.4 b
RPR-42	4.4 cd

3.4. Biocontrol Aspects of PGPR

3.4.1. Petri Plate Assay

The maximum growth inhibition against *Rhizoctonia solani* was observed in WM-2; 50%. The ranking of the PGPR isolates against *Rhizoctonia solani* is WM-2> WPR-61> WM-4> WM-5 (Figure 10). In the case of the *Fusarium sp.* again, five of the PGPR isolates showed antifungal activity. The maximum growth inhibition was observed in WM-4; 55% following RPR-33; 50%. The order of PGPR isolates for the growth inhibition against *Fusarium sp.* is WM-4> RPR-33> WM-2> RPR-41> WPR-61. (Figure 11). PGPR strains, *P. fluorescens* PCL1751 *P. fluorescens* WCS365, have been used against *Fusarium sp.* and obtained promising results [30]. In another study, PGPR isolates were used against *Rhizoctonia solani* in wheat and concluded that most of the PGPR strains could suppress fungal growth [24].

Figure 10. Fungal (*Rhizoctonia solani*) growth inhibition by PGPR isolates

Figure 11. Fungal (*Fusarium sp.*) growth inhibition by PGPR isolates

3.5. Evaluation of Bacterial Isolates Under Controlled Conditions

3.5.1. Seed Germination and Vigour Index Studies on Rice (In Petri Plates)

All of the bacterial isolates have shown better results on germination and vigour of rice under axenic conditions. Enhanced germination in the inoculated treatments was observed as compared to the un-inoculated control. The final data was taken after seven days which revealed that the germination percentage is significantly high in the treatments inoculated with PGPR isolates (Figure 12). The highest germination was observed in the treatment inoculated with RPR-33 up to 90%, which is significantly increased compared to control (70%). The root length of control was 1.75 cm which was

increased up to 4 cm by the PGPR isolate RPR-41. The shoot length was 2.25 cm in control which was increased up to 5 cm by the PGPR isolate RPR-33. The Vigour index was significantly increased when compared to control (Figure 13). The highest vigour index was measured by RPR-41, which was statistically significant as compared to control.

Figure 12. Effect of PGPR isolates on germination of rice in petri plates (LSD

Figure 13. Effect of PGPR isolates on vigor of rice in petri plates after 7 days

3.5.2. Seed Germination and Vigour Index Studies on Wheat (In Petri Plates)

Like rice, all bacterial isolates showed better results on germination and vigour of wheat under axenic conditions. The germination in the inoculated treatments was observed earlier as compare to the un-inoculated control. Moreover, germination percentage is significantly high in the treatments inoculated with PGPR isolates (Figure 14). The germination observed in the treatment inoculated with WM-3 and WM-5 was 90%, and it was highest amongst all isolates evaluated. The root length of control was 1.66 cm which was increased up to 4.5 cm by the PGPR isolate WM-5, and the shoot length of control was 3.5 cm which was increased up to 8.33 cm by the PGPR isolate WM-5. In addition, like rice, the vigour index of wheat was also increased significantly (Figure 15). The highest vigour index 1155 was observed by WM-5, which was statistically significant compared to control; 321.67. It has been demonstrated, which supports our findings, that the PGPR isolates have a role in plant growth promotion in different crops [31]. The same results were observed by PGPR inoculation in maize [32].

Figure 14. Effect of PGPR isolates on Germination of wheat in petri plates after 7 days (LSD at $P < 0.05$)

Figure 15. Effect of PGPR isolates on vigor of wheat in petri plates after 7 days (LSD at $P < 0.05$)

3.6. Effect of PGPR on The Growth of Rice (In Pot Experiment)

We also evaluated the effect of PGPR isolates on the growth of rice in a pot experiment (Figure 16). The results revealed that the PGPR isolates increased the growth of rice significantly over un-inoculated control. The parameters of root/shoot length and root/shoot fresh and dry weight were recorded after thirty days of sowing in pots.

Figure 16. Pot experiment of rice after 30 days

3.6.1. Root/Shoot Length

It was observed that the root/shoot length of the thirty days old rice seedlings was increased significantly due to the inoculation of PGPR isolates (Figure 17). The maximum shoot length was observed by inoculation with RPR-33 (29.27 cm), which was statistically significant compared to control (18.33 cm). Shoot length due to the rest of the isolates ranges from 19.67 cm to 27.67 cm. The maximum root length was recorded in the treatment inoculated with RPR-42 (9.33 cm), significantly higher than control (3.33 cm). The increase in root length in the rest of the isolates ranges from 5 cm to 9 cm (Table 5).

Figure 17. Root/shoot lengths of rice after 30 days

3.6.2. Root/Shoot Fresh Weight

A significant increase in root/shoot fresh weight was recorded with the PGPR inoculation. The fresh shoot weight recorded varied from 283.33 to 476.67 mg/plant. Maximum shoot fresh weight was observed in the treatment inoculated with RPR-42 (476.67 mg/plant), followed by RPR-41 with 446 mg/plant. All the PGPR isolates have increased fresh root weight as compared to the un-inoculated control. Maximum root weight (170 mg/plant) was recorded in the treatment inoculated with the isolate RPR-42. Root fresh weight in the un-inoculated control was recorded as 83.33 mg/plant (Table 5).

Table 5: Effect of PGPR on growth parameters of Rice in pot experiment

Treatments	Length (cm)		wt (mg/plant)			
	Shoot	Root	Shoot Fresh	Root Fresh	Shoot Dry	Root Dry
Control	18.3d	4.7d	283.3g	83.33f	55.33g	17.33f
WM-2	19.7cd	5d	306.7f	93.33f	65f	18.67f
WM-3	22.3bcd	4.7d	316.7f	106.7e	66.67f	21.67e
WM-4	22.7bc	5.3d	350e	126.7d	68.33ef	25cd
WM-5	20.7cd	7.3c	376.7d	133.3cd	73.33d	26.67c
WPR-61	28.7a	8bc	360de	126.7d	71.67de	23.33d
RPR-33	29.7a	8.7ab	400c	140bc	81.67c	31.67b
RPR-41	27.7a	9a	446b	150b	86.67b	33.33b
RPR-42	25.7a	9.3a	476.7a	170a	96.67a	36.33a

⁵ (LSD at P<0.05)

3.6.3. Root/Shoot Dry Weight

The PGPR isolates have also increased the dry biomass of rice seedlings significantly. The maximum shoot dry weight was recorded as 96.67 mg/plant, RPR-42. The un-inoculated control has the least dry weight (55.33 mg/plant) of rice seedling compared to all of the treatments inoculated with PGPR isolates. Root dry weight of thirty-day-old seedlings was also measured, ranging from 17.33 mg/plant to 36.33 mg/plant. The maximum root dry weight was recorded in the treatment inoculated with RPR-42; 36.33 mg/plant (Table 5).

3.7. Effect of PGPR on Growth of Wheat (In Pot Experiment)

Like rice, a pot experiment was conducted to evaluate the PGPR isolates for the growth promotion of wheat. It was observed that all the isolates showed positive results on the growth of wheat (Table 6). The growth parameters: root/shoot length, root/shoot fresh weight and root/shoot dry weight were recorded after thirty days of sowing.

3.7.1. Root/Shoot Length

Root/shoot lengths of wheat plants were recorded after thirty days of sowing, and it was observed that all the isolates increased root/shoot lengths significantly over un-inoculated control. The shoot length of the wheat plants varied from 11.33 to 28.33 cm. A maximum shoot length of 28.33 cm was observed in the treatment inoculated with WM-5. WPR-61 was on the second number in increasing shoot length with 23.67 cm. Minimum shoot length was recorded in un-inoculated control; 11.33 cm. The root length of the wheat seedling varied from 3.67 to 8.67 cm. Minimum root length was observed in un-inoculated control; 3.67 cm. Maximum root length 8.67 cm was recorded in the treatment inoculated with WM-5, followed by WPR-61; 7.67 cm (Table 6).

Table 6: Effect of PGPR on growth parameters of wheat in a pot experiment

Treatments	Length (cm)		wt (mg/plant)			
	Shoot	Root	Shoot Fresh	Root Fresh	Shoot Dry	Root Dry
Control	11.33e	3.67f	200g	33.33g	43.33f	9e
WM-2	17cd	6.67c	360c	63.33d	73.33cd	12d
WM-3	19c	6.33cd	370bc	80c	76.67c	15.67c
WM-4	21.67b	6.67c	380b	86.67b	80bc	16c
WM-5	28.33a	8.67a	411.67a	93.33a	96.67a	20.33a
WPR-61	23.67b	7.67b	398.33a	90ab	86.67b	18.67b
RPR-33	16d	4.33f	230f	36.67f	60e	12d
RPR-41	16.67d	5.33e	273.33e	53.33e	63.33e	12.33d
RPR-42	16.33d	5.67de	306.67d	56.67e	66.67de	12.67d

^a (LSD at P<0.05)

3.7.2. Root/Shoot Fresh Weight

Root/shoot fresh weight was also significantly increased by inoculation with PGPR isolates. The shoot weight of thirty-day-old seedlings was recorded, varied from 200-411.67 mg/plant. Maximum shoot weight was recorded due to inoculation with WM-5; 411.67 mg/plant, which is statistically at a bar with the treatment inoculated with WPR-61; 398.33 mg/plant. The fresh root weight of thirty days old wheat seedlings ranges from 33.33-93.33 mg/plant. The isolate WM-5 also increased the maximum root weight; 93.33 mg/plant, followed by 90 mg/plant of WPR-61. The control was observed with 33.33 mg/plant, less than all the treatments inoculated with PGPR isolates (Table 6).

3.7.3. Root/Shoot Dry Weight

A significant increase in Root/shoot dry weight was observed in all the treatments inoculated with PGPR isolates over un-inoculated control. Shoot dry weight varied from 43.33 to 96.67 mg/plant. Maximum shoot dry weight was recorded in the treatment inoculated with WM-5; 96.67 mg/plant, followed by 86.67 mg/plant in the treatment inoculated with WPR-61. The PGPR isolates significantly increased root dry weight, which varied from 9-20.33 mg/plant. The PGPR isolate, WM-5, also increased maximum root dry weight (20.33 mg/plant). Minimum root dry weight was observed in the un-inoculated control; 9 mg/plant (Table 6).

A large body of evidence suggests that PGPR enhances growth, seed emergence and crop yield, and contributes to protecting plants against specific pathogens and pests [33, 34]. On the other hand, IAA-producing PGPR increases root growth and root length, resulting in greater root surface area, enabling the plant to access more nutrients from the soil. The results of the pot study of wheat and rice were supported by a previous study [35], which reported the effects of plant growth-promoting bacteria on wheat plants by evaluating shoot/ root fresh weights and shoot/ root dry weights. One native strain increased the shoot and root dry biomass by 23% and 45%, respectively. Other strains

increased the dry shoot biomass. Our results also strengthen the findings [36], who obtained about 24 percent increase in wheat shoot dry weight by PGPR inoculation compared to un-inoculated control. Our results are also supported by the findings [37], who reported a significant increase in plant height, root length, and dry matter production of shoot and root of rice seedlings after inoculation with PGPR. Furthermore, PGPR isolates remarkably increased the seed germination of rice.

Conclusions

PGPR strains can promote plant growth in different direct and indirect ways like phytohormone production, phosphate solubilization, nitrogen fixation and antifungal activities. All the PGPR isolates have increased wheat and rice plant growth, but most of them are crop-specific. Rice isolates have shown maximum growth enhancement in rice and wheat isolates in wheat. It has already been demonstrated that strains isolated from the same host plant were more efficient in the growth promotion of the that plants. After field evaluations, survival studies in the carrier and molecular analysis, the isolates would be commercialized as biofertilizers. Moreover, after the assessment of Zn and Fe solubilization ability of PGPR isolates, these isolates could be recommended for bio-fortification of rice and wheat as well.

Acknowledgments

We are thankful to Mrs. Shahida N. Khokhar, Dr. Muhammad Zakria. Mr. Muhammad Shahbaz and Dr. Muhammad Aslam (NARC) for their support and guidelines.

Conflicts of Interest/Competing Interests

Authors declare that they have no conflict of interest.

Ethics Approval

This chapter does not contain any studies with human participants or animals performed by any of the authors.

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