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Characterization of cultural beneficial bacterial diversity for plant growth promoting attributes associated with rice rhizosphere

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Abstract

Aim : The study was undertaken to isolate, characterize and molecularly identify plant growth promoting rhizobacteria (PGPR) associated with rice rhizosphere occurring in Gangetic plains of the country.

Methodology : One hundred forty three plant growth promoting rhizobacteria were isolated on various culture media and characterized culturally and morphologically through Scanning Electron Microscope. These rhizobacterial isolates were further screened for plant growth promoting activities like HCN production, siderophore production and phosphate solubilisation. The 16S rDNA sequencing was done to decipher phylogenetic relationship among 19 selected PGPRs. Besides, these were also tested for anti-oxidative enzyme production to mitigate deleterious effects of various stresses on plant growth and metabolism.

Results : Nineteen potential plant growth promoting rhizobacteria were characterized for plant growth promoting attributes and were identified at molecular level. Rice is well colonized by bacteria mainly *Bacillus* spp., *Pseudomonas* spp., *Burkholderia* spp., and *Acinetobacter* spp., Rice seeds treated with microbial consortium of MAU 143 + MRT 84 recorded in increased antioxidant production—SOD activity (0.02 U mg⁻¹ f.w.t.), POX activity (0.16 U mg⁻¹ f.w.t.), POP activity (0.06 U mg⁻¹ f.w.t.) and TPC assay (1.17 µg gallic acid mg⁻¹ extract) which might have stimulated the growth of radical (2.2 cm) and plumule (1.6 cm) over control. These three PGPRs viz., MAU 143, MRT 84 and MRT 92 strain showed great potential to improve antioxidant and total polyphenol production and seed vigour of rice seedlings. The identified rhizobacterial strains may be used for plant growth promotion and improvement in nutrients mobilization and oxidative stresses in rice production.

Interpretation : The study suggested that plant growth promoting rhizobacteria isolated from rice rhizosphere have greater potential to improve soil nutrients status while provide resistance against infection of pathogenic microbes due to production of anti-oxidative enzymes and phenolic compounds in rice seedlings.

Key words: 16S rDNA, PGPRs, Rhizosphere, Rice, Seed vigour

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Introduction

Rice is an important cereal crops next to wheat and is grown worldwide. About 90% of rice is being cultivated and produced in Asian countries. In India, rice is grown mainly in Indo-Gangetic plain which is most populated area of the country. Consumers prefer to add various products in their dietary activities and their demand is increasing day by day. To fulfill needs of the consumers, non judicious application of chemical fertilizers is used which pose negative effects on human health and environment (Kyriaki *et al.*, 2017). So, there is an urgent need to use substitutes without or with less harmful side effects. Recently, beneficial microorganisms and their products (bio-fertilizers and bio-agents) are being a matter of great interest to reduce the harsh use of chemicals (Souza *et al.*, 2015, Kim *et al.*, 2016). In this context, it is worth while to explore strains of beneficial rice root associated bacteria which have plant growth promoting attributes, thereby reducing the insect-pests and diseases (Pham *et al.*, 2017, Elbeltagy *et al.*, 2000).

A diverse group of microorganisms dwell in surrounding or within plant, causing harmful, neutral or beneficial effects on plant growth and development (Kumar *et al.*, 2012, Adesemoye *et al.*, 2009; Altomare *et al.*, 1999). Root associated microorganisms can solubilise and mineralize even fix atmospheric nitrogen to make it available to crop (Ji *et al.*, 2014, Fatnassi *et al.*, 2015, Das *et al.*, 2003). Some beneficial microbes suppress plant diseases through competition, predation or antagonism mechanism against plant pathogens or through induction of plant defence systems (Glick, 1995). It has been reported that rhizosphere supports the development and activity of a huge and diversified microbial community (Adediran *et al.*, 2016). The plant growth-promoting rhizobacteria (PGPR) colonize roots of monocots and also dicots, and enhance plant growth by direct and indirect mechanisms (Huang *et al.*, 2016).

These plant growth promoting bacteria can increase nutrients uptake through phosphate solubilisation and mineralization (Oteino *et al.*, 2015), nitrogen fixation (Figueiredo *et al.*, 2008) and siderophore production (Sayyed *et al.*, 2005), produce phytohormones, such as indole-3-acetic acid (Karnwal, 2012; Khamna *et al.*, 2012; Ahmed and Hashnain 2014) biological control of pathogens mainly caused by synthesis of siderophores, antibiosis and induced resistance in crops against a broad spectrum of insect-pests and diseases (Islam *et al.*, 2015, Beneduzi, 2012, Priscilla *et al.*, 2017). Therefore, keeping the importance of beneficial microbe's in agricultural research, an attempt was made to identify and characterize cultural beneficial bacterial diversity for plant growth promoting attributes associated with rice.

Materials and Methods

Soil sampling and isolation: One hundred fifty soil samples were collected from rice fields of six rice growing regions of Gangetic plain (Lucknow, Kanpur, Meerut, Gorakhpur, Mau and

Varanasi) by adopting V-shaped method. They were pooled to minimize sampling error. During sampling, samples were kept in ice bucket, brought to laboratory and stored in cold room for isolation of beneficial bacteria. Samples were carefully processed before making serial dilutions. The larger particles were removed using 2 mm sieve. Ten gram soil of each sample was mixed separately in 90 ml sterilized distilled water in a conical flask. Series of dilutions (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6}) were made by transferring 1ml aliquot from the flask into 9 ml sterile distilled water in each test tube. A 0.1ml suspension was spread onto culture plate containing pre-solidified Nutrient agar, Jensen agar, Pikovshaya agar, Burk, s medium, NFB medium, Malate Medium and YEMA medium. The inoculated plates were incubated at $30 \pm 1^\circ\text{C}$ for 48 hrs. Representative colonies of bacteria were separately picked at random and sub-cultured on nutrient agar media for purification. Purified cultures were preserved in 30% glycerol at -20°C and used during the course of study.

Characterization for plant growth promoting traits: The rhizobacterial isolates were characterized for following plant growth promoting traits.

HCN production assay: Hydrogen cyanide production from glycine was tested following the procedure of Lorck, 1948. Nutrient agar supplemented with glycine (4.4 g^{-1}) was prepared and autoclaved. A loop full of 24 hr-old-broth culture of the isolates was inoculated in the test tubes. Sterile filter paper strips soaked in 0.5% (w/v) picric acid were fixed to the underside of the cap. The test tubes were then sealed with parafilm and incubated for 5-7 days at 30°C . An uninoculated plate and an inoculated plate without picric acid impregnated papers were used as control. A change in the colour of filter strips from yellow to brown or reddish-brown was regarded as indication of cyanogenic production.

Siderophore production assay: Siderophore production was checked on solid CAS universal blue agar plates following the procedure of Schwyn and Neilands (1987). Actively growing cultures were spot inoculated on the above agar plate and incubated at 30°C for 3 to 4 days. Formation of yellow-orange halo around the colony indicated production and release of siderophores on agar plate.

Phosphate solubilisation assay: The qualitative analysis of phosphate solubilisation of rhizobacterial isolate was measured on Pikovskaya's medium (1948). The medium was spot inoculated and incubated at $28 \pm 1^\circ\text{C}$ for 5 days. The qualitative measure of the zone formed around the colonies was considered for phosphate solubilisation activity.

Molecular identification: Out of 143 rhizobacterial isolates, nineteen plant growth promoting rhizobacterial isolates (Table 1) were cultured in 50 ml of nutrient broth overnight at 30°C . A 1.5 kb fragment of 16S rRNA gene was amplified from each of the isolates using PCR primers 9bfm (5' GAGTTTGATYHT GGCT CA G-3', Mühling *et al.*, 2008 and 1512uR (5'-ACGGHTACCTT GTT ACGACTT-3' (Weisburg *et al.*, 1991). DNA was prepared by

incubating a 50 µl aqueous suspension of cells of rhizobacterial isolates at 98°C for 15 min followed by centrifugation (5 min at 12000 g) to remove fragments of the lysed cells. The 1 µl of the supernatant was added to PCR mix to serve as DNA template. PCR reaction was carried out in a 50 µl volume containing 1.5 mmol l⁻¹ MgCl₂, 200 µmol l⁻¹ dNTPs, 1 U *Taq* DNA polymerase (Qualigen) and 500 nmol l⁻¹ each of the primers. PCR cycle included an initial denaturation step of 4 min at 96°C, followed by 30 cycles (94°C for 60 s, 52°C for 60 s, 72°C for 45 s) and a final extension step at 72°C for 10 min. The PCRs yielded single amplified gene only specific products by agarose gel electrophoresis. PCR products were purified (Axygen PCR product purification kit) and sequencing was done by Sanger method (Sanger *et al.*, 1977).

Antioxidant study of rhizobacterial isolates on rice seeds:

The rhizobacterial isolates were grown in broth nutrient agar medium for 48 hours and their cell density (10⁶-10⁹ CFU ml⁻¹) was optimized by using spectrophotometer (optical density of 0.5 recorded at wavelength of 535 nm). It was achieved by diluting with sterile distilled water. Rice seeds were surface sterilized by treating it with 70% ethanol for 10-20 sec followed by washing with sterile distilled water three times in order to remove excess amount of chemicals. The rice seeds were then air dried and mixed with 2% carboxymethyl cellulose (CMC) solution. Test tubes containing coarse sand were sterilized by autoclaving at 121°C for 20 min at 15psi and the sand was moistened by adding sterile Hoagland solution. Rice kernels were placed onto test tube separately and covered with aluminium film. Test tubes were incubated at room temperature for 15 days and regular watering was done with sterile distilled water.

Superoxide dismutase assay: Superoxide dismutase activity was estimated by the method of Giannopolitis and Ries (1977). The assay was performed the freshly harvested rice seedlings grown under *in vitro* conditions. For study, composition of reaction mixture was prepared by adding 50 mM phosphate buffer (pH 7.8), 0.1 µM EDTA, 13 mM methionine, 75 µM NBT, 2 µM riboflavin and 100 µl of enzyme extract. The riboflavin was added at the last step and tubes were illuminated with fluorescent tubes of 20 W after proper shaking. After incubation period of 15 min, light was switched off and the tubes were kept in dark condition. The absorbance of the samples was recorded at 560 nm using spectrophotometer. One unit of SOD activity (U) is defined as the amount of enzyme required to inhibit photo reduction of NBT by 50 % and is expressed as U mg⁻¹ of fresh weight.

Peroxidase and polyphenoloxidase activity assay: The peroxidase and polyphenoloxidase activity were estimated as described by Kar and Mishra, 1975 with some modification. The aerial tissues (100 mg) were homogenized with phosphate buffer pH 6.8 (0.1 M) and centrifuged at 2°C for 15 min at 17,000×g in a refrigerated centrifuge. The clear supernatant was analyzed for enzyme activity. The assay mixture for the POD activity comprised 0.1 M phosphate buffer (pH 6.8), 50 µl pyrogallol, 50 µl H₂O₂ and 0.1 ml enzyme extract. The mixture was incubated for 5

min at 25°C after which the reaction was stopped by adding 0.5 mL 5% (v/v) H₂SO₄. The amount of purpurogallin formed was determined by the absorbance at 420 nm. The same assay mixture as that of POD without H₂O₂ was used to assay the activity of polyphenoloxidase. One unit of POD and polyphenol oxidase was defined as an increase of 0.1 U of absorbance.

Total polyphenol: Total polyphenol content was determined by Folin–Ciocalteu colorimetric method as described by Kumazawa *et al.* (2004). Aerial parts (100 mg) were ground with 80% ethanol, and extracts (0.5 ml) were mixed with 0.5 ml of Folin–Ciocalteu reagent and 0.5 ml Na₂CO₃, and the absorbance was measured at 760 nm after 1 hr incubation at room temperature. Total polyphenol content was expressed as extract. (µg gallic acid mg⁻¹).

Statistical analyses: The experiments were performed in replicate under *in-vitro* conditions. The results were expressed in term of Mean, P-value, CV (%), SE (d) and LSD by using SAS software.

Results and Discussion

In this study, 143 rhizobacterial isolates were isolated on various culture media adopting serial dilution plate method. The rhizobacterial isolates were screened for plant growth promoting activities. In HCN production assay, 35 out of 143 rhizobacterial isolates were observed with positive results. Some workers have reported that all strains of plant growth promoting bacteria had potential to produce hydrogen cyanide. These strains were isolated from rice rhizosphere. The PGPR strains were *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Acinetobacter* sp., and *Micrococcus* sp., isolated from rice rhizosphere had potential to produce hydrogen cyanide (Timmusk *et al.*, 2011; Kumar *et al.*, 2012; Haas and Défago, 2005). In siderophore production assay, formation of yellow-orange halo zone around the colony indicated production and release of siderophores on the agar plate by 25 rhizobacterial isolates. It is well known that iron is the basic requirement for all organisms and assist in biological processes, such as photosynthesis, respiration, chlorophyll biosynthesis (Kobayashi and Nishizawa, 2012) and biological nitrogen fixation (Dixon and Kahn, 2004). In anaerobic (flooded) and acidic soils, high concentrations of ferrous ions generated through reduction of ferric ions might lead to iron toxicity due to excessive Fe uptake. Under aerobic conditions, iron solubility is low and it reflects the predominance of ions typically observed as oxyhydroxide polymers.

This limits iron ferric supply for different forms of life, particularly in calcareous soils. However, microorganisms have developed active strategies for iron uptake. Bacteria can overcome the nutritional iron limitation by using chelator agents called siderophores. Siderophores is defined as low-molecular-mass molecules (< 1000 Da) with high specificity and affinity for chelating or binding ferric ions, followed by transportation and deposition iron within bacterial cells (Souza *et al.*, 2015). Studies

Fig. 1 : Phylogenetic tree formed by aligning multiple sequences of 16S rDNA using phylogeny.

Fig. 2 : Antioxidative enzyme production assay on rhizobacterial strain treated rice seeds.

indicate that siderophore is largely produced by bacterial strains associated with plant root. This characteristic was the most common trait found in bacterial isolates associated with sunflower (Ambrosini *et al.*, 2012) and rice (Chaiham *et al.*, 2009). In the present investigation, 22 rhizobacterial isolates were found positive in phosphate solubilisation. During the last three-four decades, importance of phosphate solubilizing microorganisms in agriculture has increased significantly (Richardson *et al.*, 2001; Rodriguez and Fraga, 1999) and several strains of bacterial

species have been identified for their phosphate-solubilizing capabilities (Glick, 1995, He *et al.*, 1997) which belongs to *Pseudomonas* and *Bacillus* bacteria (Illmer and Schinner, 1992). Out of 143 isolated bacteria strains, nineteen potential rhizobacterial strains having preferably good PGP activities were selected for genetic diversity analysis based on nucleotide sequence variation (Table 1). The 16S rDNA sequencing was carried out by Sanger method. It is observed that large numbers of PGP bacteria are Gram positive which mainly to belong *Bacillus*

Fig. 3 : Total phenol content production assay on rhizobacterial strain treated rice seeds.

Table 1: Soil samples collected from different rice cultivating regions of Gangetic plain

Sample	Location	Sample	Strain	Bacterial genera identified	Gen Bank Acc. No.
1.	Gorakhpur region	Soil	GR 1	<i>Bacillus cereus</i>	Mg457715
2.	Gorakhpur region	Soil	GR 6	<i>Bacillus cereus</i>	Mg457716
3.	Gorakhpur region	Soil	GR 23	<i>Bacillus flexus</i>	Mg457732
4.	Lucknow region	Soil	LUCK 24	<i>Bacillus megaterium</i>	Mg457727
5.	Lucknow region	Soil	LUCK 31	<i>Bacillus megaterium</i>	Mg461556
6.	Lucknow region	Soil	LUCK 38	<i>Pseudomonas gessardii</i>	Mk183001
7.	Lucknow region	Soil	LUCK 45	<i>Pseudomonas aeruginosa</i>	Mk183004
8.	Lucknow region	Soil	LUCK 50	<i>Bacillus cereus</i>	Mg457728
9.	Kanpur region	Soil	KAN 69	<i>Bacillus cereus</i>	Mg520329
10.	Meerut region	Soil	MRT 84	<i>Bacillus endophyticus</i>	Mg461557
11.	Meerut region	Soil	MRT 92	<i>Bacillus velezensis</i>	Mg457729
12.	Mau region	Soil	MAU 104	<i>Bacillus subtilis</i>	Mg457730
13.	Mau region	Soil	MAU 107	<i>Pseudomonas aeruginosa</i>	Mk183006
14.	Mau region	Soil	MAU 114	<i>Burkholderia gladioli</i>	Mk183027
15.	Mau region	Soil	MAU 123	<i>Acinetobacter calcoaceticus</i>	Mk183026
16.	Mau region	Soil	MAU 132	<i>Acinetobacter calcoaceticus</i>	Mk183010
17.	Mau region	Soil	MAU 136	<i>Bacillus cereus</i>	Mg457733
18.	Mau region	Soil	MAU 139	<i>Burkholderia gladioli</i>	Mk183011
19.	Mau region	Soil	MAU 143	<i>Bacillus endophyticus</i>	Mg457732

spp., *Pseudomonas* spp., *Burkholderia* sp., and *Acinetobacter* spp., respectively (Fig. 1).

Phylogenetic analysis have shown that most of the Gram positive PGPR, are either low-or high-G+C strains (Beneduzi et al., 2012). It shows that rice rhizosphere is major habitat for *Bacillus* group of plant growth promoting rhizobacteria. Similarly, association of diverse group of bacterial genera (*Enterobacter*, *Burkholderia*, *Enterobacter*, *Grimontella*, *Klebsiella*, *Stenotrophomonas*, *Rhizobium*, *Herbaspirillum* and *Citrobacter*)

with rhizosphere has been reported earlier (Ashrafuzzaman et al., 2009; Damam et al., 2016). Plant growth promoting bacteria can colonize in the roots and promote plant growth and development and also minimize the effect of insect-pest and plant disease. PGPR like *Bacillus* (Idriss et al., 2002, *Enterobacter* (Gupta et al., 1998) and *Corynebacterium* (El-Banana and Winkelmann, 1988) have been reported to benefit plants by enhancing plant growth and improving plant health through various direct and indirect mechanisms. Therefore, these are commonly used as bio-inoculants for improving the growth and yield of agricultural

Table 2 : Effect of rhizobacterial strain on antioxidant enzyme production and total polyphenol content in rice seedlings

Rhizobacterial strain	SOD assay (U mg ⁻¹ f.w.t.)	POX Assay (U mg ⁻¹ f.w.t.)	Polyphenol oxidase assay (U mg ⁻¹ f.w.t.)	Total polyphenol content assay (µg gallic acid mg ⁻¹ extract)
GR 1	0.02	0.04	0.04	3.61
GR 6	0.01	0.08	0.01	3.61
GR 23	0.01	0.06	0.01	3.61
LUCK 24	0.02	0.09	0.01	3.84
LUCK 31	0.02	0.08	0.02	3.40
LUCK 38	0.02	0.10	0.03	3.07
LUCK 45	0.04	0.12	0.02	3.44
LUCK 50	0.03	0.03	0.03	3.61
KAN 69	0.03	0.14	0.01	3.61
MRT 84	0.04	0.22	0.06	3.81
MRT 92	0.04	0.21	0.06	3.70
MAU 104	0.02	0.11	0.02	3.61
MAU 107	0.03	0.04	0.03	3.61
MAU 114	0.04	0.05	0.01	3.61
MAU 123	0.03	0.10	0.01	3.61
MAU 132	0.03	0.05	0.02	3.61
MAU 136	0.04	0.11	0.02	3.61
MAU139	0.02	0.12	0.02	3.61
MAU 143	0.05	0.20	0.06	3.80
Control	0.02	0.06	0.01	2.84
Mean	0.03	0.10	0.03	3.56
p-Value	<.0001	<.0001	<.0001	<.0001
CV (%)	3.12	0.35	1.52	0.01
SE (d)	0.001	0.000	0.000	0.000
LSD at 1%	0.0019	0.0008	0.0009	0.0006

Table 3 : Effect of consortium of rhizobacterial strains on seedling growth in relation to antioxidant enzyme production and total polyphenol content

Rhizobacterial strain	SOD assay (U mg ⁻¹ of f.w.t.)	POX assay (U mg ⁻¹ of f.w.t.)	Polyphenol oxidase assay (U mg ⁻¹ of f.w.t.)	Total polyphenol content assay (µg gallic acid mg ⁻¹ extract)	Radicle length (cm)	Plumule length (cm)
MAU 143 + MRT 84	0.04	0.22	0.07	4.01	3.80	2.90
MAU 143 + MRT 84 + MRT 92	0.04	0.18	0.10	3.91	3.80	2.50
MRT 84 + MRT 92	0.03	0.21	0.06	3.91	3.60	2.80
Control	0.02	0.06	0.01	2.84	1.60	1.30
Mean	0.03	0.17	0.06	3.67	3.20	2.38
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV (%)	0.00	0.00	0.00	0.06	0.00	0.00
SE (d)	0.000	0.000	0.000	0.002	0.000	0.000
LSD at 1%	0	0	0	0.0061	0	0

crops and offer an attractive way to replace chemical fertilizers, pesticides and supplements (Stefan *et al.*, 2008; Ashrafuzzaman *et al.*, 2009; Saharan and Nehra, 2011; Sang, *et al.*, 2014). PGPR associated with rice root system produce enzymes and secrete organic acids which results in phosphate solubilization from insoluble complexes, making it available for plant uptake (Richardson *et al.*, 2009). Variation in phosphate solubilisation

was assessed by Gram staining behaviour of bacteria. Gram negative bacteria were more potent to solubilise tri calcium phosphate as also observed by Hilda *et al.* (2000).

During stress condition, within the plants reactive oxygen species (ROS) are generated which are deleterious to growth and metabolism of the plants. Hydrogen peroxide (H₂O₂), superoxide

ions (O_2^-), singlet oxygen (1O_2) and hydroxyl radical (OH^-) are common reactive oxygen species produced in plant cells under various stresses. These are highly reactive and cause damage to bio molecules such as lipids, proteins and nucleic acid (Apel and Hirt, 2004). Anti-oxidative assay was performed to mitigate oxidative stress in relation to enhanced anti-oxidative enzymes (–SOD assay, POD assay, POP assay and TPC assay following treating with rice seeds with potential rhizobacterial isolates. SOD activity was higher in treatments with MAU 143 ($0.03\text{ U mg}^{-1}\text{ f.w.t.}$) followed by MRT 84 ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$), MRT 92 ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$), MAU 136 ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$), MAU 114 ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$) and LUCK 45 ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$) as compared to control.

Peroxidase activity was estimated higher with MRT ($0.16\text{ U mg}^{-1}\text{ f.w.t.}$) followed by MRT ($0.15\text{ U mg}^{-1}\text{ f.w.t.}$) and MAU 143 ($0.14\text{ U mg}^{-1}\text{ f.w.t.}$) as compared to control. In polyphenol oxidase assay, these strain treated rice seedlings were identified non-significantly different but were higher ($0.05\text{ U mg}^{-1}\text{ f.w.t.}$) compared with control. The total polyphenol content production was recorded higher production with MRT ($0.97\text{ }\mu\text{g gallic acid mg}^{-1}\text{ extract}$) and MAU 143 ($0.96\text{ }\mu\text{g gallic acid mg}^{-1}\text{ extract}$) and followed by MRT ($0.86\text{ }\mu\text{g gallic acid mg}^{-1}\text{ extract}$) as compared to control (Table 2; Fig. 2 and 3).

Further, best performing rhizobacterial isolates in antioxidant production assay, microbial consortium was prepared. Rice seeds treated with microbial consortium of MAU 143 + MRT 84 recorded increased antioxidant production– SOD activity ($0.02\text{ U mg}^{-1}\text{ f.w.t.}$), POX activity ($0.16\text{ U mg}^{-1}\text{ f.w.t.}$), POP activity ($0.06\text{ U mg}^{-1}\text{ f.w.t.}$) and TPC assay ($1.17\text{ }\mu\text{g gallic acid mg}^{-1}\text{ extract}$) which might have stimulated the growth of radical and plumule over control. Addition of rhizobacterial strain MRT 92 to this consortium resulted in increased enhanced activity of only POP ($0.09\text{ U mg}^{-1}\text{ f.w.t.}$) without changing other antioxidant and growth parameters. In other treatment, the response of consortium of rhizobacterial strain MRT 84 and MRT 92 recorded increased response in TPC activity only ($1.07\text{ }\mu\text{g gallic acid mg}^{-1}\text{ extract}$) while for parameters it was similar to consortium of rhizobacterial strain MAU 143 + MRT 84 over control (Table 3).

This indicates that consortium of rhizobacterial strain had more or less similar impact on antioxidant and polyphenol production and seed vigour. Thus, microbial consortium of rhizobacterial strain isolated from rice rhizosphere had positive impact for rice plant growth and oxidative stresses. Chun Juan et al. (2012) reported that the application of PGPR strain, *B. cereus* AR156 on tomato (*Lycopersicon esculentum*) under abiotic stress, showed enhanced SOD, POX and CAT activities in plants, probably indicating enhance defence activation when treated with PGPR strain.

The study suggested that plant growth promoting rhizobacteria associated with rice crop have great potential to colonize, mobilize plant nutrients and protect from soil born plant pathogens. These three PGPRs viz., MAU 143, MRT 84 and MRT

92 showed great potential to improve nutrient availability, increased rice seed vigour index and anti-oxidative enzyme and poly phenol production. The identified PGPRs may be used developing microbial inoculants for rice crop cultivation in the Gangetic region.

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References

- Adediran, G.A., B.T. Ngwenya, J.F. Mosselmans, K.V. Heal and B.A. Harvie: Mixed planting with a leguminous plant out performs bacteria in promoting growth of a metal remediating plant through histidine synthesis. *Int. J. Phytore.*, **18**, 720–729 (2016).
- Adesemoye, A.O. and E.O. Ugoji: Evaluating *Pseudomonas aeruginosa* as plant growth-promoting rhizobacteria in West Africa. *Arch. Phytopath. Plant Protec.*, **42**, 188–200 (2009).
- Ahmed, A. and S. Hasnain: Auxins as one of the factors of plant growth improvement by plant growth promoting rhizobacteria. *Polish. J. Microbiol.*, **63**, 261–266 (2014).
- Altomare, C., W.A. Norvell, T. Bjorkman and G.E. Harman: Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai. *Appl. Environ. Microbiol.*, **65**, 2926–2933 (1999).
- Ambrosini, A., A. Beneduazi, T. Stefanski, F.G. Pinheiro, L.K. Vargag and L.M.P. Passaglia: Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). *Plant and Soil*, **356**, 245–264 (2012).
- Apel, K. and H. Hirt: Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annual Revie. Plant Biol.*, **55**, 373–399 (2004).
- Ashrafuzzaman, M., F.A. Hossen, M.R. Ismail, M.A. Hoque, M.Z. Islam, S.M. Shahidullah and S. Meon: Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afri. J. Biotechnol.*, **8**, 1247–1252 (2009).
- Beneduzi, A., A. Ambrosini and L.M.P. Passaglia: Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. *Genet. Mol. Biol.*, **35**, 1044–1051 (2012).
- Chaiham, M., S. Chunhaleuchananon and S. Lumyong: Screening siderophore producing bacteria as potential biocontrol agent for fungal rice pathogens in Thailand. *World J. Microbiol. Biotechnol.*, **25**, 1919–1928 (2009).
- ChunJuan, W., G. YaHui, W. Chao, L. Hong Xia, N. Dong Dong, W. Yun Peng and G. Jian Hua: Enhancement of tomato (*Lycopersicon esculentum*) tolerance to drought stress by plant-growth-promoting rhizobacterium (PGPR) *Bacillus cereus* AR156. *J. Agri. Biotech.*, **20**, 1097–1105 (2012).
- Damam, M., K. Kaloori, B. Gaddam and R. Kausar: Plant growth promoting substances (phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal plants. *Int. J. Pharm. Scie. Rev. Res.*, **37**, 130–136 (2016).
- Das, K., V. Katiyar and R. Goel: 'P' solubilization potential of plant growth promoting *Pseudomonas* mutants at low temperature. *Microbio. Results*, **158**, 359–362 (2003).
- Dixon, R. and D. Kahn: Genetic regulation of biological nitrogen fixation. *Nat. Rev. Microbio.*, **2**, 621–631 (2004).
- Ei-Banana, N. and G. Winkelmann: Pyrrolnitrin from *Burkholderia cepacia*: Antibiotic activity against fungi and novel activities against streptomycetes. *J. Appl. Microbiol.*, **85**, 69–78 (1988).

- Elbeltagy, A., K. Nishioka, H. Suzuki, T. Sato, Y.I. Sato, H. Morisaki, H. Mitsui and K. Minamisawa: Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci. Plant Nutri.*, **46**, 617–629 (2000).
- Fatnassi, I.C., M. Chiboub, O. Saadani, M. Jebara and S.H. Jebara: Phytostabilization of moderate copper contaminated soils using co-inoculation of *Vicia faba* with plant growth promoting bacteria. *J. Basic Microbio.*, **55**, 303–311 (2015).
- Figueiredo, M.V.B., C.R. Martinez, H.A. Burity and C.P. Chanway: Plant growth promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). *World J. Microbiol. Biotechnol.*, **24**, 1187–1193 (2008).
- Giannopolitis, C.N. and S.K. Ries: Superoxide dismutases. I. Occurrence in higher plants. *Plant Physiol.*, **59**, 309–314 (1997).
- Glick, B.R.: The enhancement of plant growth by free-living bacteria. *Can. J. Microbio.*, **41**, 109–117 (1995).
- Gupta, A., A.K. Saxena, M. Gopal and K.V.B.R. Tilak: Effect of plant growth promoting rhizobacteria on competitive ability of introduced *Bradyrhizobium* sp. (Vigna) for nodulation. *Microbiol. Res.*, **153**, 113–117 (1998).
- Haas, D. and G. Défago: Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbio.*, **3**, 307–319 (2005).
- He, Z.L., J. Wu, A.G. O'Donnell and J.K. Syers: Seasonal responses in microbial biomass carbon, phosphorus and sulphur in soils under pasture. *Biol. Ferti. Soils*, **24**, 421–428 (1997).
- Hilda, R., T. Gonzalez and G. Selman: Expression of a mineral phosphate solubilizing gene from *Erwinia herbicola* in two rhizobacterial strains. *J. Biotechnol.*, **84**, 155–161 (2000).
- Huang, J., Z. Liu, S. Li, B. Xu, Y. Gong, Y. Yang and H. Sun: Isolation and engineering of plant growth promoting rhizobacteria *Pseudomonas aeruginosa* for enhanced cadmium bioremediation. *J. Gen. App. Microbio.*, **62**, 258–265 (2016).
- Idriss, E.E., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner and H. Bochow: Extracellular phytase activity of *Bacillus amyloquelaciens* FZB45 contributes to its plant growth promoting effect. *Microbiol.*, **148**, 2097–2109 (2002).
- Illmer, P.A. and F. Schinner: Solubilization of inorganic phosphates by microorganisms isolated from forest soil. *Soil Biol. Biochem.*, **24**, 389–395 (1992).
- Islam, S., A.M. Akanda, A. Prova, M.T. Islam and M.M. Hossain: Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Microbio.*, **6**, 1360 (2015) doi: 10.3389/fmicb.2015.01360
- Ji, S.H., M.A. Gururani and S.C. Chun: Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbio. Res.*, **169**, 83–98 (2014).
- Kar, M. and D. Mishra: Inorganic pyrophosphatase activity during rice leaf senescence. *Can. J. Bot.*, **53**, 503–510 (1975).
- Karnwal, A.: Production of indole acetic acid by fluorescent *Pseudomonas* in the presence of L-tryptophan and rice root exudates. *J. Pl. Pathol.*, **19**, 61–63 (2009).
- Khamna, S., A. Yokota and J.F. Peberdy: Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *Eur. Asian J. Biosci.*, **4**, 23–31 (2010).
- Kim, S.I., J.S. Kwak, J.T. Song and H.S. Seo: Long-term effect of *niclosamide* on inhibition of bacterial leaf blight in rice. *J. Pl. Protec. Res.*, **56**, 323–327 (2016).
- Kobayashi, T., R.N. Itai, M.S. Aung, T. Senoura, H. Nakanishi and N.K. Nishizawa: The rice transcription factor IDEF1 directly binds to iron and other divalent metals for sensing cellular iron status. *Plant J.*, **69**, 81–91 (2012).
- Kumar, P., R.C. Dubey and D.K. Maheshwari: *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbio. Res.*, **167**, 493–499 (2012).
- Kumar, A., A. Kumar, S. Devi, S. Patil, C. Payal and S. Negi: Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activities: An *in-vitro* study. *Rec. Res. Sci. Technol.*, **4**, 1–5 (2012).
- Kumazawa, S., T. Hamasaka and T. Nakayama: Antioxidant activity of propolis of various geographic origins. *Food Chemistry*, **84**, 329–339 (2004).
- Kyriaki, R. and K. Phoebe: Environmental effects on public health: An economic perspective. *Int. J. Environ. Res. Public Hlth.*, **6**, 2160–2178 (2009).
- Lorck, H.: Production of hydrocyanic acid by bacteria. *Physiol. Plant*, **1**, 142–146 (1948).
- Muhling, M., J.A. Woolven-Allen, J.C. Murrell and I. Joint: Improved group-specific PCR primers for DGGE analysis of the genetic diversity of complex microbial communities. *Int. Socie. Micro. Eco. J.*, **2**, 379–392 (2008).
- Oteino, N., R.D. Lally, S. Kiwanuka, A. Lloyd, D. Ryan and K.J. Germaine: Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front. Microbiol.*, **6**, 1–9 (2015).
- Pham, V.T., H. Rediers, M.G. Ghequire, H.H. Nguyen, R. De Mot, J. Vanderleyden and S. Spaepen: The plant growth-promoting effect of the nitrogen-fixing endophyte *Pseudomonas stutzeri* A15. *Archi. Microbio.*, **199**, 513–517 (2017).
- Pikovskaya, R.I.: Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Microbiologiya*, **17**, 362–370 (1948).
- Priscilla, R.D., G. Gabriela, M. Lenise, R.M. Clarissa de, S.B. Maria, F. P. Enilson, L. Saccol de, S. Claudio and A.V. Paula: Beneficial rhizobacteria immobilized in nano-fibers for potential application as soybean seed bio-inoculants. *PLoS ONE* (2017) <https://doi.org/10.1371/journal.pone.0176930> (2017).
- Richardson, A., J.M. Barea, A. McNeill and C. Prigent-Combaret: Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil*, **321**, 305–339 (2009).
- Richardson, A.E., P.A. Hadobas, J.E. Hayes, C.P. O'Hara and R.J. Simpson: Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil microorganisms. *Plant Soil*, **229**, 47–56 (2001).
- Rodriguez, H. and R. Fraga: Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol. Adv.*, **17**, 319–339 (1999).
- Saharan, B.S. and V. Nehra: Plant growth promoting rhizobacteria: A critical review. *Life Sci. Medi. Res.*, **21**, 1–30 (2011).
- Sang, H.J., M.A. Gururani and S. Chulchin: Isolation and characterization of plant growth promoting endophytic diazotrophic bacteria from Korean rice cultivars. *Microbio. Res.*, **169**, 183–198 (2014).
- Sanger, F., S. Nicklen and A.R. Coulson: DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 5463–5467 (1977).
- Sayyed, R.Z., M.D. Badgujar, H.M. Sonawane, M.M. Mhaske and S.B. Chincholkar: Production of microbial iron chelators (siderophores) by fluorescent *Pseudomonads*. *Indian J. Biotechnol.*, **4**, 484–490 (2005).
- Schwyn, B. and J.B. Neilands: Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.*, **160**, 47–56 (1987).
- Souza, R., A. Ambrosini and L.M. Passaglia: Plant growth promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.*, **38**, 401–419 (2015).
- Stefan, M., M. Mihasan and S. Dunca: Plant growth promoting rhizobacteria can inhibit the *in-vitro* germination of *Glycine max* L. seeds. *Scientific Annals of University "Alexandru Ioan Cuza" Iasi, Sect. Genet. Mol. Biol.*, **3**, 105–110 (2008).
- Timmusk, S., V. Paalme and T. Pavicek: Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. *PLoS ONE*, **6**, e17968 (2011).
- Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane: 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.*, **173**, 697–703 (1973).