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Performance of Multifaceted Plant Growth Promoting Rhizobacterial Strains as Bioinoculants in Aerobic Rice (Cultivar PMK 3)

B. Cibichakravarthy, D. Balachandar and K. Kumar*
Department of Agricultural Microbiology,
Tamil Nadu Agricultural University, Coimbatore - 641 003.

Plant growth promoting rhizobacterial isolates from the rhizosphere of two arid weed plants, *Prosopis juliflora* and *Parthenium hysterophorus* were evaluated for their multifunctional beneficial activities in aerobic rice under field condition. Four elite strains showing multifaceted beneficial activities including nitrogen fixation, mineral solubilization, phytohormone production with antagonistic activity against soil pathogens were inoculated to aerobic rice (cultivar PMK3) and compared with standard Azophos bioinoculant technology. The results of the field experiment revealed that the strain, PARZ11 from *Parthenium hysterophorus,* phylogenetically authenticated as *Azospirillum* sp., increased the plant growth and yield of aerobic rice compared to other isolates. The grain yield increase due to PARZ11 was on par with standard Azophos inoculant application and inoculating this strain enhanced the total bacterial-and diazotrophic counts in the rhizosphere of rice. The present study clearly demonstrated the ability of PGPR isolate PARZ11 as a suitable bioinoculant for aerobic rice. The added advantage of using this isolate was tolerance to abiotic stresses such as drought and salt.

Key words: PGPR, rhizobacteria, aerobic rice.

To sustain present food self-sufficiency and to meet future food requirements, India has to increase its rice productivity by 3 per cent per annum (Thiyagarajan and Selvaraju, 2001). Rice cultivation requires about 3000 - 5000 litres of water for producing one kg rice, depending on the different cultivation methods. Owing to increasing water scarcity, a shifting trend towards less-water demanding crops against rice is noticed in most part of India and this warrants alternate methods of rice cultivation that aims at higher crop productivity and better water economy (Yamah, 2002; Uphoff, 2005). Aerobic rice cultivation, where fields remain unsaturated throughout the season like an upland crops offers an opportunity to produce rice with less water (Bouman *et al.*, 2002). Hence, developing advanced nutrient management techniques, plant protection measures and rice breeds are essential for yield maximization of aerobic rice cultivation.

Plant Growth-Promoting Rhizobacteria (PGPR) are reported to influence the growth, yield and nutrient uptake by an array of mechanisms (Joseph *et al.,* 2007). The use of microorganisms with the aim of improving nutrient availability for plants is an important practice and necessary for agriculture (Lucy *et al.,* 2004). Some bacterial strains directly regulate plant physiology by mimicking synthesis of plant hormones, whereas others increase mineral- and nitrogen availability in the soil as a way to augment growth (Yasmin *et al.,* 2007). The isolates could exhibit more than two or three plant growth promoting traits, which may support the plant growth directly or indirectly or synergistically (Joseph *et al.,* 2007). The bacterial inoculations have a much better stimulatory effect on plant growth in nutrient-deficient soil than in nutrient-rich soil.

To aid the aerobic rice to adopt water-limited conditions and to enhance the drought-tolerance, inoculating PGPRs is an eco-friendly approach. In our earlier investigation, several multifaceted plant growth promoting rhizobacteria with higher nitrogen-fixing potential were characterized from rhizosphere of extreme drought resistant plants such as *Prosopis* and *Parthenium* (Cibichakravarthy *et al.,* 2011). In continuation of this, we hypothesized that these potential strains from *Prosopis* and *Parthenium* rhizospheres could be useful for efficient nutrient supply for aerobic rice cultivation. In the present study, we have investigated the field performance of four strains of PGPR from *Prosopis* and *Parthenium* rhizospheres as bioinoculant for aerobic rice.

Materials and Methods

Multifaceted PGPR isolates

Four multifaceted PGPR isolates *viz*., PARZ2, PARZ11, PRRZ6 and PRRP1, isolated from rhizosphere of *Prosopis* and *Parthenium* and authenticated by 16S rRNA gene sequencing were used for this study. The identity of 16S rRNA gene

*Corresponding author email: azollakumar@rediffmail.com

sequence was established by performing a similarity search against the GenBank database (http:// www.ncbi.nih.gov/BLAST) (Table 1). The multifaceted_plant growth promoting traits isolated from *Parthenium hysterophorus* and *Prosopis juliflora* obtained in this study have been submitted to GenBank database under accession numbers, HQ678655, HQ678664, HQ678675, HQ678677 respectively. The multifaceted plant growth promoting traits evaluated for the four isolates were summarized in Table 2 and their abiotic stress tolerances were presented in Table 3.

Field evaluation of PGPR isolates

All the four hyper-diazotrophic PGPR strains from rhizosphere soils of *Prosopis* and *Parthenium* were evaluated under field condition. The test strains were prepared as liquid inoculants (10^8 cfu per ml) and compared with carrier based existing Azophos bioinoculant, prepared using standard strains of *Azospirillum lipoferum* (Az204) and *Bacillus megaterium var. Phosphaticum* (Pb1). The trial was conducted at the Experimental fields of Wetlands, Tamil Nadu Agricultural University, Coimbatore during Kharif 2010-11 with rice variety PMK3 under aerobic conditions. The soil is deep clay loam with soil pH, electrical conductivity, organic carbon and soil available N, P and K were 7.3, 0.46 %, 0.64 % and 244 kg ha^{-1}, 17.2 kg ha^{-1} and 505 kg ha^{-1} respectively. The experiment was conducted in 12 sq.m plots in randomized block design with 3 replications. The data on the biometric observations of grain and straw yield were observed. The plant height and biomass were recorded at four stages of growth of rice. The rhizosphere soils collected at different stages of the crop were enumerated for total bacterial-, fungal- and diazotrophic bacterial populations as per the standard procedures (Weaver *et al.,* 1994). The productive tillers at flowering stage and grain and straw yield were recorded. All the data were statistically scrutinized by ANOVA and the significance among the treatments was discriminated by Duncan's Multiple Range Test.

Results and Discussion

Beneficial activities and biotic- and abiotic stress tolerance

The multifaceted PGPR isolated from rhizosphere of *Parthenium hysterophorus* and *Prosopis juliflora* are phylogenetically- and functionally diversified. The isolates PARZ2 and PRRP1, from rhizosphere of *Parthenium* and *Prosopis* respectively, belong to *Bacillus* sp. The *Parthenium* isolate PARZ11 and *Prosopis* isolate PRRZ6 were found to be *Azospirillum* sp. (Table 1). All these four strains exhibited multiple plant growth promoting activities and between the isolates, the PGPR activities varied quantitatively (Table 2). The highest nitrogenase activity was exhibited by isolate PARZ2 (74.95 n moles of ethylene mg^{-1} protein h^{-1}). All the 4 isolates showed production of IAA and the maximum amount of IAA was produced by *Azospirillum* sp. PARZ11 followed by *Bacillus subtilis* PRRZ6. Highest GA was produced by *Azospirillum* sp. PARZ11 and the least by *Bacillus subtilis* PRRP1. Similarly, siderophore production was maximum in *Bacillus* sp. PRRP1. *Azospirillum brasilense* PRRZ6 solubilized 470.90 mg of phosphorus ml^{-1} which was significantly higher than the other isolates. The isolate *Bacillus subtilis* PARZ2 was found to solubilize maximum amount of 182.71 mg of potassium l^{-1}. With regard to Zn solubilization, the best performance was put forth by *Azospirillum* sp. PARZ11 by solubilizing 482.47 mg l^{-1} of zinc. The antagonistic activity of all the diazotrophic isolates

Table 1. Molecular authentication of PGPR strains from the rhizosphere of *Parthenium hysterophorus* and *Prosopis juliflora* by 16S rRNA gene sequence homology

Multifaceted PGPR strains (GenBank Accession number)	Rhizosphere source	Sequence homology		
		Closest species[a]	GenBank accession number	Per cent homology[b]
PARZ2 (HQ678655)	*Parthenium hysterophorus*	*Bacillus subtilis*	FJ 263018	100
PARZ11(HQ678664)	*Parthenium hysterophorus*	*Azospirillum* sp.	AB114197	97
PRRZ6 (HQ678675)	*Prosopis juliflora*	*Azospirillum brasilense*	EF634029	98
PRRP1(HQ678677)	*Prosopis juliflora*	*Bacillus* sp.	GQ288417	99

[a]Species identified based on 16S rRNA gene sequence similarity; [b]Per cent similarity of the sequence in BLAST search

Table 2. Multifaceted plant growth promoting activities of PGPR isolates used in this study

PGP activities	PGPR isolates			
	PARZ2	PARZ11	PRRP6	PRRZ1
Nitrogen fixation (n moles of ethylene produced mg^{-1} protein h^{-1})	74.95	54.44	57.49	66.59
IAA production (mg l^{-1})	13.08	14.81	13.09	11.03
GA production (mg l^{-1})	24.18	27.63	25.28	18.21
Siderophore production (mg l^{-1})	-	-	-	39.32
P solubilization (mg l^{-1})	359.02	367.95	470.90	304.32
K solubilisation (mg l^{-1})	182.71	136.80	144.85	138.58
Zn solubilization (mg l^{-1})	342.61	482.47	368.84	276.72
Antagonistic activity against selected pathogens	*Mp, Sr, Lt, Cg, As*	*Mp, Sr, Lt*	*Mp, Sr, Lt*	*Mp, Sr, Lt*

Mp - Macrophomina phaseolina; Sr - Sclerotium rolfsii; Lt - Lasiodiplodia theobromae; Cg-Colletotrichum gloeosporioides; As - Alternaria solani.

was tested against 5 plant pathogenic fungi *viz.*, *Macrophomina phaseolina, Sclerotium rolfsii, Lasiodiplodia theobromae, Colletotrichum gloeosporioides* and *Alternaria solani.* Among the strains, *Bacillus subtilis* PARZ2 alone had high antagonistic activity against all the 5 plant pathogenic fungi tested and rest of them showed antifungal activity to 3 fungal pathogens each (Table 2). The multifaceted PGPR isolates were evaluated for their growth under different stress conditions and the results are reported in Table 3. From the results, it was concluded that the culture *Bacillus subtilis* PARZ2, was highly temperature tolerant exhibiting growth even at 55˚C and salt tolerance up to 20 % compared to other strains. Among the three moisture potentials tested, maximum survival of all isolates was recorded at a soil moisture potential of 0.03 MPa. Maximum heavy metal tolerance was recorded by PARZ2 and PRRZ1 followed by PRRP6 and PARZ11 showed the least tolerance.

Table 3. Abiotic stress tolerances of PGPR isolates

Abiotic stresses	PGPR isolates			
	PARZ2	PARZ11	PRRP6	PRRZ1
Temperature tolerance (ºC)	55	50	50	55
NaCl tolerance (%)	20	-	20	20
Drought tolerance	+	+	+	+
Heavy metal tolerance	Cd^{2+} (1.2 mg/l)	Pb^{2+} (0.3mg/l)	Pb^{2+} (0.3mg/l)	Pb^{2+} (0.7mg/l)

Field performance of diazotrophic isolates

The novel multifaceted PGPR strains isolated from rhizosphere soils of *Prosopis* and *Parthenium* were evaluated as bioinoculants in comparison with existing standard Azophos bioinoculant in aerobic rice. All the four isolates were applied individually with 75 per cent of recommended N and P fertilizers. The maximum plant height and biomass production at different stages of rice growth was recorded by standard Azophos bioinoculant and *Azospirillum* PARZ11 (strain from *Parthenium* rhizosphere) had higher influence than other strains and controls (Fig. 1). The yield parameters recorded also revealed that application of Azophos and *Azospirillum* PARZ11 recorded significantly higher tiller production as well as productive tillers per hill compared to other strains. The 100 per cent fertilizer control was on par with inoculants plus 75 per cent recommended

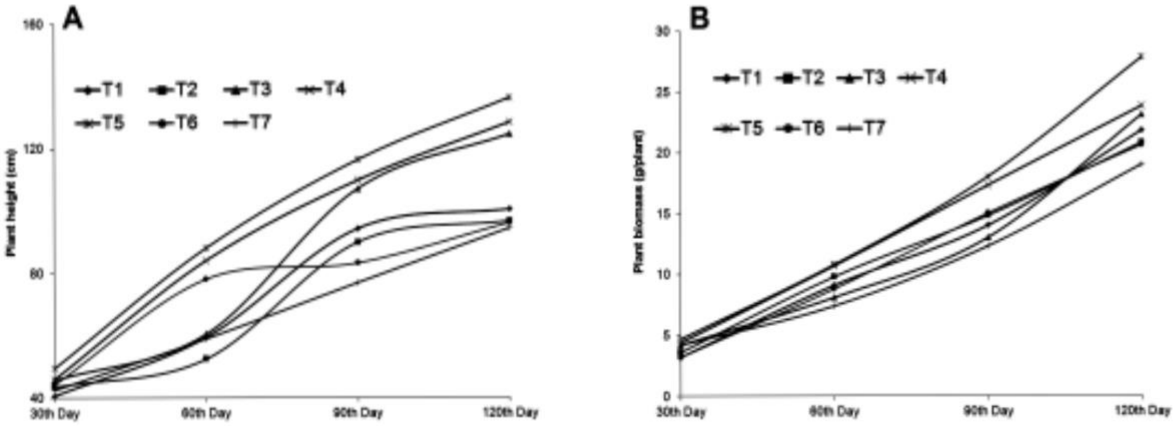

Fig. 1. Effect of multifaceted PGPR strains as bioinoculants on plant height (A) and biomass production (B) of aerobic rice (PMK3). The treatment details are presented in Table 4.

NP fertilizers, while the unfertilized control recorded the lowest tiller production (Table 4). Likewise, the grain and straw yield were maximum in the Azophos applied plot followed by *Azospirillum* PARZ11 inoculation. The yield increase due to the application of other three strains was on par with 100 per cent fertilized plots (Table 4). The unfertilized control plot recorded the lowest grain yield production.

In order to ascertain the impact of bioinoculants application on microbial activities in the rhizosphere of aerobic rice, soil samples were quantified for total culturable bacterial-, fungal- and diazotrophic bacterial counts. The microbial counts at active tillering stage revealed that all the microbial inoculants along with reduced dose (75 per cent) of chemical fertilizers recorded maximum microbial counts including free-living nitrogen fixers (Fig. 2). Among the different treatments, Azophos (T5), *Azospirillum* PARZ11 (T4) inoculation recorded statistically significant bacterial counts followed by *Azospirillum* PRRZ6. With reference to fungal population in the rhizosphere, there is not much significant difference noticed among the different strains evaluvated. The 100 per cent chemical fertilized- and unfertilized control rhizospheres recorded lowest microbial counts (Fig. 2).

Aerobic rice is the cultivation system in which suitable cultivar of rice grown under non-puddled and non-saturated- with well-drained soil condition. Unlike wetland system, aerobic rice can be either irrigated or rainfed, depending on the environmental condition. The irrigated system would bring moisture

Table 4. Performance of multifaceted PGPR strains as bioinoculants for aerobic rice (cultivar PMK3)

Treatments	Number of tillers	No.of productive tillers	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})
T1 - *Bacillus* PARZ2 + 75% NP + 100%K	13.3^{c}	$10.0^{b,c}$	3.4^{c}	$33.0^{a,b}$
T2 - *Bacillus* PRRP1 + 75% NP + 100%K	$18.3^{a,b}$	15.3^{a}	3.5^{c}	38.0^{a}
T3 - *Azospirillum* PRRZ6 + 75% NP + 100%K	$16.3^{b,c}$	$14.3^{a,b}$	3.5^{c}	$37.0^{a,b}$
T4 - *Azospirillum* PARZ11 + 75% NP + 100%K	$21.0^{a,b}$	17.3^{a}	3.7^{b}	39.0^{a}
T5 - Azophos + 75% NP +100%K	22.0^{a}	17.7^{a}	3.8^{a}	44.3^{a}
T6 - 100% NPK fertilizer	$18.3^{a,b}$	$13.3^{a,b,c}$	3.5^{c}	$36.0^{a,b}$
T7 - Unfertilized control	12.3^{c}	9.0^{c}	3.3^{d}	26.3^{b}

Values are mean of three replicates and values followed by same letter in each row are not significantly different from each other as determined by Duncan's Multiple Range Test ($p<0.05$).

condition of the root zone up to field capacity (Bouman and Lampayan, 2009). Rice cultivation under limited water usage and easy crop rotation are some of the advantages of the aerobic rice cultivation. Plant growth promoting rhizobacteria (PGPR) are the soil bacteria that are able to colonize the roots and enhance the plant growth through multifaceted beneficial activities (Zahir *et al.,* 2004).

PGPR promote the plant growth either by direct mechanisms including nitrogen fixation, production of phytohormones, enzymes and solubilization and mobilization of nutrients (Gray and Smith, 2005) or by indirect mechanisms including reduced pathogenecity through iron-sequestering and resistance induction (Van Loon*,* 2007). The rhizosphere of aerobic rice is unique in which

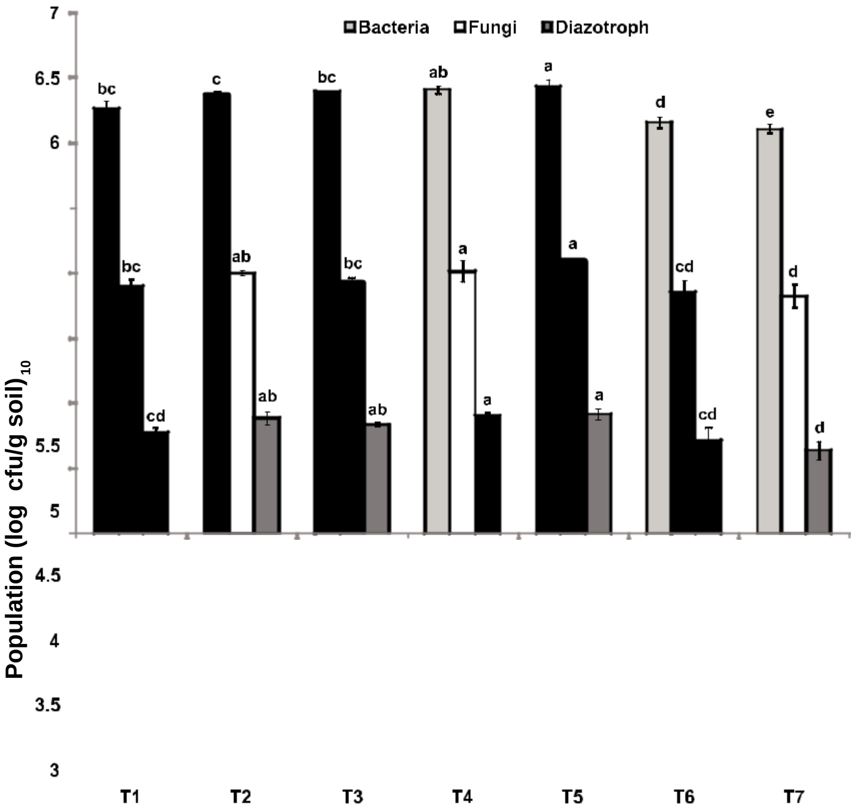

Fig. 2. Effect of multifaceted PGPR strains as bioinoculants on total bacterial-, fungal- and diazotrophic bacterial population in rhizosphere of aerobic rice (PMK3) at active tillering stage. The treatment details are presented in Table 4 and for each panel, different letters indicate significantly different at p<0.05 according to Duncan's Multiple Range Test.

interaction with microorganisms and soil would be more prominent than flooded rice. Hence in present investigation, an attempt was made to identify and evaluate suitable PGPR for aerobic rice. For this, the PGPR isolates from two weed plants, *Prosopis juliflora* and *Parthenium hysterophorus* which are grown in nutritionally-poor soil with environmental stress conditions were exploited. In our earlier study, we have demonstrated that the rhizosphere of these two exotic weed plants harbour diversified PGPRs capable of higher nitrogen fixation (Cibichakravarthy *et al.,* 2011). The culture independent metagenomic approach also revealed that the rhizosphere of these two plants was colonized by diversified eubacterial communities (Jothibasu *et al.,* 2011). Hence in the present study, we aimed to assess four selected PGPR isolates from *Prosopis juliflora* and

Parthenium hysterophorus rhizospheres to use them as bioinoculants for aerobic rice. Among the 20 isolates screened for multifaceted PGPR activities, four isolates showed promising activities (Table 2) which revealed that some of the PGPR were capable of secondary benefits apart from the targets (Avis *et al.,* 2008). Utilizing these isolates for bioinoculant technology would be more beneficial than single functional strains.

In the present study, we have compared the standard bioinoculant Azophos, mixed bioinoculant of *Azospirillum* and Phosphobacteria with four multifaceted PGPR isolates for their performance in aerobic rice. The results revealed that strain PARZ11, phylogenetically 99 per cent similar to *Azospirillum* sp. performed better than other isolates and the growth and yield increase in aerobic rice was on par with standard Azophos bioinoculant. This strain recorded significantly higher plant height, biomass and yield attributes of aerobic rice, on par with Azophos (Fig. 1 and Table 4). The present results are in accordance with the earlier works of Khorshidi *et al.* (2011), in which *Pseudomonas fluorescens* and *Azospirillum lipoferum* enhanced the growth and yield of rice. The positive interaction of this inoculant with roots of aerobic rice and multifaceted beneficial activities are the possible reasons for the better performance by PARZ11 than other isolates (De-Bashan *et al.,* 2008; Kennedy *et al.,* 2004). The additional benefit by the inoculants was enhanced microbial activities in the rhizosphere of aerobic rice. As discussed earlier, the strain PARZ11 improved the biological processes in the rhizosphere of rice and thereby increased the total bacterial- and diazotrophic populations (Fig. 2). In the present study, the new strain *Azospirillum* PARZ11 performed on par with Azophos application and the added advantages of the new strain is its antagonistic activity and abiotic stress tolerance. This strain controlled some of the soil borne pathogens (Table 2) and withstood high temperature and salt and could tolerate drought also (Table 3).

In conclusion, the strain, PARZ11 isolated from *Parthenium* rhizosphere was capable of exhibiting multifaceted beneficial activities. The 16S rRNA gene sequence homology showed 99 per cent similarity to *Azospirillum* sp.It also showed antagonistic property against soil pathogens and could withstand abiotic stresses. Inoculating this strain to aerobic rice (cultivar PMK3) enhanced the plant height and biomass and yield increase was as comparable with the standard Azophos bioinoculant.

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