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Research Paper

Native Multiphasic Plant Growth Promoting Rhizobacteria Consortia for Growth and Yield of Rice

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A B S T R A C T

An investigation was taken up to isolate, characterize and evaluate the effect of native plant growth promoting rhizobacteria (PGPR) based consortia on growth and yield of rice plants. Different functional groups of PGPR were enumerated from rhizosphere of rice crop in Wayanad district by using selective media. Nitrogen fixers and solubilizers of phosphate, potassium and zinc were isolated by using Jensen's agar, Pikovskaya's agar, Aleksandrov agar and mineral salts medium (MSM) amended with 1% ZnO respectively. In the preliminary screening 32 nitrogen fixers, 16 phosphate solubilizers, four K solubilizers and six zinc solubilizers were selected for further screening based on their plant growth promoting activities such as production of IAA, NH_3, HCN and siderophore. Promising isolates of each group were subjected to further quantification of nitrogen fixation and solubilization of nutrient (phosphate, K and Zn) *in vitro*. Ten most promising isolates were selected from different functional groups and they were subjected to molecular characterization based on 16S rRNA gene sequencing. Sequences of ten promising isolates were deposited in the Genebank of NCBI and accession numbers obtained. Three new consortia were developed by using native PGPRs after confirmation of their compatibility. Each consortium consisted of five PGPR isolates; two nitrogen fixers and one phosphate, potassium and zinc solubilizer each. Three native PGPR- based consortia were evaluated on growth and yield of rice plant in pot experiment. Considering all the parameters best native PGPR based consortium was selected and it consisted native five PGPRs from rice rhizosphere viz. *Bacillus* sp. AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* PkZnS3.

***Key words*:** PGPR, Nitrogen fixers, Phosphate solubilizers, Potassium solubilizers, Zinc solubilizers, Consortia

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that colonize the plant roots and exert beneficial effects on plant growth and yield, through direct and indirect mechanisms. Most of the rhizosphere bacteria play a crucial role in plant nutrition through fixation of nitrogen and solubilization of nutrients such as phosphate, potassium and zinc. Discovery of the promising native PGPR from plant rhizosphere and applying them as biofertilizers is a new trend of field of agricultural research in environmentally safe and sustainable crop production system [1].

Rice is one of the most important staple foods for more than half of the world's population. With the increase of world's population, the global rise in rice consumption has increased. Green revolution in India resulted in high-yielding rice varieties, which demanded extensive application of chemical fertilizers such as nitrogen (N), phosphorus (P) and potassium [2]. Therefore, it is a great challenge to grow rice as sustainable and environmentally safe manner for ensuring food and nutritional security of the growing population. Use of PGPR as biofertilizer offers an alternative technology to reduce quantity of chemical fertilizer usage in rice cultivation, by improving soil nutrition.

During the last two decades, the use of PGPR based biofertilizers has tremendously increased. Application of commercially available *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* formulations as single inoculants or as consortia has been reported to improve growth and yield of many crops including rice, wheat and many horticultural crops [3-4]. However, PGPR strains vary widely with crop and soil types and their growth performances and PGP activities depend on complex interaction of soil, plant and microbes. Many researchers studied native PGPR isolates on plant growth and yield and reported their colonization and plant growth improvement are comparatively higher. Therefore, exploitation of native PGPR from rice rhizosphere with multiple PGP characters are important in development of an effective consortium.

Effect of individual microbe and microbial consortium viz., *Azospirillum lipoferum* Az-024, *Bacillus megaterium* var. *phosphoticum* and *Pseudomonas fluorescens* Pf-1 on rice plant growth under hydroponic conditions and revealed that the consortium improved colonization and enhanced crop growth [5]. The rhizobacterial consortium (UKA-24: *Rhizobium radiobacter*, UKA-72: *Bacillus pumilus*, UKA-27: *Stenotrophomonas maltophila* and AKA-1: *Pseudomonas putida*) was more effective than single inoculant on growth

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promotion and nutrient uptake of Basmati rice cultivar Pusa Sugandha 4 [6]. The present study was aimed to evaluate the effects of native rhizobacterial consortia with different functions such as nitrogen fixation, solubilization of phosphate, potassium and zinc on rice growth and yield in Wayanad district of Kerala. Wayanad is a plateau situated at a height between 700 meters and 2100 meters above the mean sea level nested among the mountains of the Western Ghats on the Eastern portion of North Kerala. Climatic conditions vary from sub-tropical to temperate.

MATERIALS AND METHODS

Isolation of native rhizosphere bacteria

The experiment was conducted at Department of Agricultural Microbiology, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur. rhizosphere soil samples were collected from two months old rice plants in ten different rice growing locations in Wayanad district. Selected locations were Thanivayal, Edakkal, Kolagappara, Nellara, Kuppamudi, Ambalavayal, Marathat, Ambukuthi, Malavayal and Pottankoli. Rhizosphere soils were taken with intact root system and placed in polythene bags and stored at 4^0C in refrigerator. Serial dilution followed by spread plating were done for all the samples for the isolation of PGPR from rice rhizosphere. Selective media viz. Jensen's agar, Pikovskaya's agar, Aleksandrov agar and mineral salts medium (MSM) amended with 1% ZnO were used for isolating nitrogen fixers, phosphate solubilizers, K solubilizers and Zn solubilizers respectively. Serial dilution technique was performed up to 10^{-4} dilution and 0.1ml aliquot of this dilution was spread on the plates of respective media and incubated 3 days at room temperature. All samples were processed in triplicates. Different morphotypes on Jensen's agar were selected and purified for further studies. For isolating phosphate, potassium and zinc solubilizers, colonies which produced clear halo zones were selected and purified on respective media for further studies. These isolates were designated after the location from where isolated and their function such as nitrogen fixation (NF), phosphate solubilization (PS), potassium solubilization (KS) and zinc solubilization (ZnS).

In vitro evaluation of plant growth promoting activities

Predominant isolates of nitrogen fixers, phosphate, potassium, and zinc solubilizers were screened for plant growth promoting activities (PGP) such as production of IAA [7], NH_3 [8], HCN [9] and siderophore [10] by adopting standard procedure. IAA production of isolates indicated development of pink colour in the solution qualitatively and the quantity of IAA produced was determined by spectrophotometry and expressed in $\mu g\ ml^{-1}$ by referring to a standard graph of IAA.

Quantification of nitrogen fixation

Nitrogen fixers which showed multiphasic PGP activities were further subjected to the quantification of nitrogen fixation by micro-Kjeldahl method of [11] and [12]. Sterilized Jensen's N-free broth was inoculated with selected nitrogen fixing isolates and incubated for fourteen days at room temperature. After incubation, 10 ml aliquot from the respective treatment was drawn and digested with 10 ml concentrated sulfuric acid. In distillation, 40% NaOH and 4% boric acid with mixed indicator was used for trapped NH_3. After distillation it was titrated against 0.01N HCl. Total fixed nitrogen was expressed as mg N fixed per gram of sucrose utilized.

Solubilization efficiency of phosphate, potassium and zinc solubilizers

The ability of the bacteria to solubilize phosphate, potassium and zinc was measured by qualitative and quantitative methods. For qualitative analysis, selected isolates were inoculated to the center of Pikovskaya's agar, Aleksandrov agar and Mineral salt medium for phosphate, K and Zn solubilizers respectively and incubated at room temperature. Diameter of solubilization zone and colony were measured after 7 days and solubilization efficiency of phosphate, K and Zn were calculated by using formula:

$$\text{Solubilization efficiency (\%)} = \frac{\text{Diameter of halo zone}}{\text{Diameter of colony}} \times 100$$

Phosphate solubilizing isolates were also inoculated to the 50ml Pikovskaya's broth containing flasks and incubated at room temperature for 14 days. Quantity of released P in broth was measured by using Mo-blue method described by [13]. Quantification of potassium was done by inoculating selected isolates in 50 ml Aleksandrov broth and estimating the solubilized potassium in the broth measured by using flame photometer [14], after 14 days of incubation. Quantity of Zn solubilized by selected isolates was carried out as per [15]. Selected zinc solubilizers were inoculated in 50 ml mineral salts broth amended with ZnO and solubilized zinc was measured by using atomic absorption spectrophotometer, after 14 days incubation.

Molecular characterization of selected isolates

Most efficient isolates of each functional groups which had multiphasic PGP activities were identified by 16S rRNA gene sequencing. Amplification of 16S rRNA gene was carried out by colony PCR [16] by using universal primers 8F and 1522R [17] and Mastermix (Takara, Japan) in Eppendorf Mastercycler. The PCR products were purified and sequenced at Agri Genome labs Pvt. Ltd, Kochi with primer 8F and 1522R, using Sanger's method. The BLAST (Basic Local Alignment Search Tool) programme of NCBI (National Centre for Biotechnology Information) was used for sequence analysis and nucleotide homology alignment of each isolates. The sequences of all the isolates were deposited in the GenBank of the NCBI by using BankIt tool and accession numbers were obtained.

Compatibility test and consortia development

Ten most promising isolates were subjected to compatibility studies by using cross streaking followed by dual culture method. After confirming their co-culture nature, three consortia were developed and each consortium consisted of two nitrogen fixers, one isolate each with phosphate, potassium and zinc solubilization potential.

Evaluation of PGPR based consortia on rice growth and yield under pot culture

Native PGPR based consortia were evaluated on rice under pot culture conditions, with reference commercial biofertilizer PGPR Mix-1, as positive control. The traditional variety '*Valichoori*', popular in Wayanad among tribal farmers was used as the test variety. The pot culture experiment was conducted at the Regional Agricultural Research Station (RARS), Ambalavayal. Germinated seeds

were treated with carrier-based consortium, at the rate of 200 g for 10 kg seeds. Earthen pots filled with 10 kg soil collected from the rice fields were used. Five nursery trays were maintained as uninoculated, PGPR Mix-1 treated and consortium 1, 2 and 3 treated separately. After 21 days, seedlings were dipped in 10% biofertilizer for 10 minutes and transplanted in pots, at the rate of 3 plants /pot. Treatments consisted of combined application of biofertilizer with two levels of inorganic fertilizer (50% and 75% of t recommended dose of N, P and K). The treatments were T_1: Consortia 1 + (50% RDF of N, P & K), T_2: Consortia 2 + (50% RDF of N, P & K), T_3: Consortia 3 + (50% RDF of N, P & K), T_4: PGPR Mix- 1 + (50% RDF of N, P & K), T_5: Consortia 1 + (75% RDF of N, P & K), T_6: Consortia 2 + (75% RDF of N, P & K), T_7: Consortia 3 + (75% RDF of N, P & K), T_8: PGPR Mix-1 + (75% RDF of N, P & K), T_9: Organic Package of Practices (KAU, 2017), T_{10}: RDF (100% RDF of N, P and K only), T_{11}: Package of Practices (KAU, 2017) and T_{12}: Control (No inoculum, no fertilizers). T_9 (Organic Package of Practices) included farm yard manure at 5t ha^{-1} and package of practice [18] was 100% RDF with compost at 5 t ha^{-1}.

Observations on growth parameters (plant height, number of tillers/plants, number of leaves/tillers) and yield attributes (number of panicles/plants, filled grains/panicle average grain weight/plants) were recorded.

RESULTS AND DISCUSSION

Isolation of rhizobacteria belonging to each functional group

Based on the growth of isolates on Jensen's nitrogen free agar, thirty-two nitrogen fixers were selected. Thirty-two phosphate solubilizers, four potassium solubilizers and six zinc solubilizers were selected, from the respective selective media, based on the presence of solubilization zone.

In vitro evaluation of plant growth promoting activities

Plant growth promoting activities of each functional group were evaluated *in vitro* and results revealed that many isolates of nitrogen fixers and phosphate solubilizers exhibited multiple plant growth promoting characters (Table 1). All promising nitrogen fixers produced higher level of IAA, medium level of ammonia and were positive for siderophore production. Quantification of IAA production revealed that isolate $KgNF_1$ had highest level of IAA production (34.83 µg ml^{-1}). Selected phosphate solubilizers produced medium level of IAA and low level of siderophore. None of the nitrogen fixers and phosphate solubilizers produced HCN. All potassium and zinc solubilizers were positive for ammonia production and negative for all other tested PGP characters (production of IAA, HCN and siderophore). [19] isolated bacteria from maize rhizosphere and reported significant level of nitrogen fixing ability with ammonia and siderophore production *in vitro*. [20] reported two phosphate solubilizing strains JY17 and JY22 with multiple PGP traits, including nitrogen fixation and production of IAA, siderophores.

Table 1 PGP activities of promising isolates under various functional groups

Isolate	Production of IAA (µg ml^{-1})	Production of NH_3	Production of HCN	Production of siderophore
		Nitrogen fixers		
$KgNF_1$	34.83	++	-	+
$AkNF_3$	23.16	++	-	++
$PkNF_4$	20.66	++	-	+
		Phosphate solubilizers		
$PkPS_1$	4.66	+	-	+
$AkPS_4$	4.66	+	-	+
$AvPS_1$	8.16	++	-	+
		Potassium solubilizers		
$MvKS_1$	-	+	-	-
$MvKS_3$	-	+	-	-
		Zinc solubilizers		
$PkZnS_3$	-	+	-	-
$ThZnS_1$	-	+	-	-

NF: nitrogen fixers, PS: phosphate solubilizers, KS: potassium solubilzers, ZnS: zinc solubilizers, Kg: Kolagappara, Ak: Ambukuthi, Pk: Pottankoli, Av: Ambalavayal, Mv: Malavayal, Th: Thanivayal

Estimation of nitrogen fixation and solubilization of phosphate, potassium and zinc

Twenty N-fixers with multi-phasic PGP activities were further subjected to quantification of nitrogen fixation by micro-Kjeldahl method and the amount of nitrogen fixed varied from 1.86 to 9.33 µg ml^{-1}. Higher amount of N fixed was 9.33 mg N fixed g^{-1} of sucrose in the case of $AkNF_3$ and $PkNF_4$ followed by $KgNF_1$ (8.86 mg N fixed g^{-1} of sucrose). They were selected as promising isolates from nitrogen fixers for consortial development. Significantly higher amount of fixed nitrogen by *Enterobacter* sp. and *Microbacterium arborescens* (9.32 and 8.89 mg N g^{-1} of carbon oxidized) respectively with higher amount of IAA production [21]. Our results are in agreement with these results, as nitrogen fixers with high level of IAA production could be identified.

Solubilization of phosphate was indicated by the presence of a clear zone around the colony on Pikovskaya's agar and PSE was calculated using formula described under Materials and Methods. Solubilization was highest in $PkPS_1$. Maximum amount of P solubilized in broth was observed in the isolate $PkPS_1$ (134.88 µg ml^{-1}). This was followed by $AkPS_4$ (121.33 µg ml^{-1}) and $AvPS_1$ (113.83 µg ml^{-1}). These three isolates were selected as most promising isolates among phosphate solubilizers. [22] isolated inorganic phosphate-solubilizing bacteria from agricultural field and reported four *Bacillus megaterium* isolates Y95, Y99, Y924 and Y1412 which released 134.49 µg ml^{-1}, 159.48 µg ml^{-1},136.83 µg ml^{-1} and 138.68 µg ml^{-1} soluble P respectively when cultured in Pikovskaya's broth.

Potassium solubilization by the four selected isolates was determined both qualitatively and quantitatively. Per cent solubilisation of K was ighest in $MvKS_1$ (142.69). Quantification of K solubilization by the isolates in Aleksandrov broth revealed that the isolate $MvKS_1$ (4.19 µg

ml^{-1}) solubilized higher amount of K, followed by MvKS3 (3.01 µg ml^{-1}). Both these isolates were selected as most promising isolates from potassium solubilizers. [23] screened KSB strains in Aleksandrov broth supplemented with K-feldspar powder as a sole source of K and reported that K solubilization ranged from 0.07 to 1.75 mg l^{-1}.

Qualitative estimation of zinc solubilization efficiency was measured based on solubilisation zone produced on mineral salt medium with 1% ZnO. Highest solubilization efficiency was recorded in $PkZnS_3$ (143.98 per cent) followed by $ThZnS_2$ (121.78 per cent). In quantitative assay also, highest amount of zinc released to the broth was recorded by the isolate $PkZnS_3$ (18.57 µg ml^{-1}) and followed by $ThZnS_2$ (17.43 µg ml^{-1}). These two isolates were selected as promising zinc solubilizers for consortial development. [24] reported that solubilizing ability of isolate ZSB-S-2 (*Pseudomonas* sp.) was 13.40 mg kg^{-1} of zinc in broth assay. The details of ten most promising isolates selected based on their quantification of nitrogen fixation, solubilization of phosphate, potassium and zinc (Table 2).

Table 2 Efficiency of nitrogen fixation and solubilization of phosphate, potassium and zinc by selected isolates

Nitrogen fixers	N fixed (mg g^{-1} of sucrose)	Phosphate solubilizers	Phosphate solubilization		Potassium solubilizers	K solubilization		Zinc solubilizers	Zinc solubilization	
			PSE %	Quantity of P solubilized (µg ml^{-1})		KSE %	Quantity of K solubilized (µg ml^{-1})		ZnSE %	Quantity of Zn solubilized (µg ml^{-1})
$KgNF_1$	8.86	$PkPS_1$	127.7	134.88	$MvKS_1$	142.69	4.19	$PkZnS_3$	143.98	18.57
$AkNF_3$	9.33	$AkPS_4$	85.1	121.33	$MvKS_3$	111.18	3.01	$ThZnS_1$	121.78	17.43
$PkNF_4$	9.33	$AvPS_1$	122.5	113.33	-	-	-	-	-	-

NF: nitrogen fixers, PS: phosphate solubilizers, KS: potassium solubilzers, ZnS: zinc solubilizers, Kg: Kolagappara, Ak: Ambukuthi, Pk: Pottankoli, Av: Ambalavayal, Mv: Malavayal, Th: Thanivayal

Molecular characterization of promising isolates

Sequences of PCR amplified 16S rRNA gene from ten isolates were obtained and analysed using BLASTn, to find out the similarity with sequences available in NCBI databank (Table 3). The accession sharing maximum homology with the query sequences was considered for identification of isolate. These sequences were deposited in GeneBank of NCBI (Table 3).

Table 3 Details of isolates selected for consortial formulations

Isolate	Accession number in GenBank	Identity based on 16S rRNA gene sequence
$KgNF_1$	MW288152	*Pseudomonas putida* strain KgNF1
$AkNF_3$	MW288141	*Bacillus* sp. strain AkNF3
$PkNF_4$	MW269608	*Pseudomonas* sp. strain PkNF4
$AvPS_1$	MW290516	*Achromobacter* sp. strain AvPS1
$AkPS_4$	MW291534	*Acinetobacter schindleri* strain AkPS4
$PkPS_1$	MW290515	*Bacillus megaterium* strain PkPS1
$MvKS_1$	MW295415	*Microbacterium* sp. strain MvKS1
$MvKS_3$	MW295416	*Acinetobacter calcoaceticus strain* MvKS3
$ThZnS_2$	MW284891	*Achromobacter marplatensis* strain ThZnS2
$PkZnS_3$	MW295426	*Cytobacillus kochii* strain PkZnS3

The N-fixing isolates $AkNF_3$, $PkNF_4$ and $KgNF_1$ were identified as *Bacillus* sp., *Pseudomonas* sp. and *Pseudomonas putida* based on 16S rRNA gene sequencing. Nitrogen fixation ability of *Bacillus* sp. has been reviewed by many authors. [25] tested 19 different *Bacillus* strains and reported that 16 isolates had *nifH* gene. [26] isolated 98 indigenous PGPR from different rice cultivars in Afghanistan soils and results revealed that the isolate *Pseudomonas putida* AF137exhibited highest nitrogenase activity (647.4 nmol ethylene h^{-1}).

In the present study, $PkPS_1$ which solubilized highest quantity of phosphate was identified as *Bacillus megaterium*. Isolate $AkPS_4$, which fixed 121.33 µg ml^{-1} phosphorus in liquid medium was identified as *Acinetobacter schindleri* and AvPS1 as *Achromobacter* sp. Several researchers have reviewed phosphate solubilization by *B. megaterium*. [27] reported that phosphate solubilization potential of *Acinetobacter* sp. WR922 was relatively high *in vitro*. [28] studied PGP characters of free living diazotrophs and reported that isolate *Achromobacter* PNF_{11} had the ability to solubilize phosphate and produce IAA and siderophore.

Two promising potassium solubilizers were identified as *Microbacterium* sp. and *Acinetobacter calcoaceticus*, based on 16S rRNA gene sequencing. *Microbacterium foliorum* isolated from tobacco rhizosphere in China solubilized potassium [29]. [30] isolated several bacteria from salt pan and one of the promising bacterial strains that exhibited potassium solubilizing potential under *in vitro* conditions was identified as *Acinetobacter soli*.

Efficient zinc solubilizing isolates $PkZnS_3$ and $ThZnS_2$ were identified as *Cytobacillus kochii* and *Achromobacter marplatensis* respectively, based on 16S rRNA gene sequencing. The genus *Cytobacillus* is closely related to *Bacillus*. Many authors have reported zinc solubilization ability of *Bacillus* sp.

Compatibility test and consortial development

Compatibility test among ten selected isolates showed that there was no antagonism among the isolates. Three PGPR based consortial formulations were prepared by using the ten potential isolates from different functional groups. Every consortium included five isolates, consisting of two nitrogen fixers, one P solubilizer, one K solubilizer and one zinc solubilizer. Consortium 1 consisted *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1and *Achromobacter marplatensis* ThZnS2. Consortium 2 included *Bacillus sp.* AkNF3,

Pseudomonas putida KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3 and *Cytobacillus kochii* kZnS3. Consortium 3 was prepared by using *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1 and *Cytobacillus kochii* PkZnS3.

Effect of PGPR based consortia on rice growth and yield in the pot experiment

Growth and yield parameters of rice crop are depicted in (Table 4). Plant height and number of leaves/tillers were not significantly different among the treatments. Number of tillers/pots in treatments with integrated application of native PGPR consortia with either 50% and 75% RDF was on par with 100% RDF applied treatments (T_{10} and T_{11}). Number of panicles/pot of native PGPR based consortia with 75% RDF was on par with T_{11} (PoP, KAU). Observations on the number of grains/panicles showed that values of integrated application of biofertilizer with 75% RDF was statistically on par with T_{10} and T_{11}. Per cent filled grains was significantly low in T_{12} (control). Treatments T_{11}, T_{10} and T_6 were statistically on par with respect to grain yield /pot.

Table 4 Effect of PGPR on the growth and yield of rice under pot culture

Treatments	Plant height (cm)	No. of tillers per pot	No. of leaves per tiller	No. of panicles per pot	No. of grains per panicle	Filled grain (%)	Grain yield per pot (g)
T_1: Consortium 1 + 50% RDF	78.66	11.00 ^{abc}	5.33	6.00 ^{c}	83.00 ^{cd}	87.93 ^{bc}	13.88 ^{bc}
T_2: Consortium 2 + 50% RDF	78.33	11.66 ^{ab}	5.00	6.60^{abc}	86.00 ^{bcd}	89.53 ^{ab}	14.61 ^{bc}
T_3: Consortium 3 + 50% RDF	76.00	10.33 ^{abc}	5.33	6.30 ^{bc}	83.33 ^{cd}	89.01 ^{abc}	14.18 ^{bc}
T_4: PGPR Mix 1 + 50% RDF	82.66	8.33 ^{cd}	5.16	3.60^{d}	84.33 ^{bcd}	89.13 ^{ab}	14.40 ^{bc}
T_5: Consortium 1 + 75% RDF	86.33	12.33 ^{ab}	5.83	6.60^{abc}	96.00 ^{ab}	90.24 ^{ab}	14.44 ^{bc}
T_6: Consortium 2 + 75% RDF	87.66	13.00 ^{a}	6.33	8.00 ^{ab}	99.00 ^{a}	92.27 ^{ab}	16.47 ^{ab}
T_7: Consortium 3 + 75% RDF	81.66	12.66 ^{ab}	5.83	7.00 ^{abc}	96.66 ^{ab}	91.58 ^{ab}	15.09 ^{bc}
T_8: PGPR Mix-1 + 75% RDF	83.00	9.66 ^{bcd}	5.66	5.60 ^{c}	93.00 ^{abc}	91.61 ^{ab}	14.74 ^{bc}
T_9: Organic POP	71.66	9.66 ^{bcd}	3.16	6.30 ^{bc}	89.33 ^{abcd}	88.04 ^{bc}	16.18 ^{b}
T_{10}: 100% RDF	80.66	12.00 ^{ab}	5.66	6.30 ^{bc}	95.00 ^{abc}	91.53 ^{ab}	18.97 ^{a}
T_{11}: POP, KAU	89.00	13.00 ^{a}	6.50	8.3 ^{a}	99.33 ^{a}	92.30 ^{a}	19.05 ^{a}
T_{12}: Control	64.33	6.00 ^{d}	4.80	3.3 ^{d}	80.00 ^{d}	85.12 ^{c}	12.710 ^{c}

Consortium 1: *Bacillus* sp. AkNF3, *Pseudomonas* sp. PkNF4, *Achromobacter* sp. AvPS1, *Microbacterium* sp. MvKS1, *Achromobacter marplatensis* ThZnS2

Consortium 2: *Bacillus sp.* AkNF3, *Pseudomonas putida* KgNF1, *Bacillus megaterium* PkPS1, *Acinetobacter calcoaceticus* MvKS3, *Cytobacillus kochii* kZnS3

Consortium 3: *Pseudomonas* sp. PkNF4, *Pseudomonas putida* KgNF1, *Acinetobacter schindleri* AkPS4, *Microbacterium* sp. MvKS1, *Cytobacillus kochii* PkZnS3

Considering all the parameters, it was concluded that consortium 2 consisting of five native PGPRs isolated from rice rhizosphere, viz. *Bacillus* sp. strain AkNF3, *Pseudomonas putida* strain KgNF1, *Bacillus megaterium* strain PkPS1, *Acinetobacter calcoaceticus* strain MvKS3 and *Cytobacillus kochii* PkZnS3, along with 75% RDF was able to produce similar growth and yield of 100% recommended dosage of N, P and K fertilizer. The results also revealed that native PGPR are better suited because of their adaptability to soil conditions, than introduced PGPR. Hence the study also emphasizes on the exploitation of native flora with multiple plant growth promoting traits for improving the growth and yield of rice, for a sustainable and environmentally safe method of cultivation.

PGPR strains (*Pseudomonas fluorescens* + *Bacillus subtilis* + *Azospirillum brasiliens*) along with 75% of nitrogen fertilizer were able to improve rice promising lines GZ9461 – 4 – 2 – 3- 1 and reducing inorganic fertilizer by 25% [31]. The present study also suggested that 25% of N, P, K fertilizer could be substituted with inoculation of native PGPR as consortia, which could enhance plant growth and yield without reduction of yield.

CONCLUSIONS

Isolation of promising native PGPR strains with multiple PGP characters and integration of a possible combination in the consortium to plants is vital for the promotion of plant growth and yield. In the present study, we isolated ten potential PGPR which exhibited different plant growth promoting traits such as nitrogen fixation, solubilization of phosphate, potassium and zinc. The results suggested that application of native PGPR in a consortium with synergistic bacteria having multiple plant growth promoting traits could be used for reduction of N, P and K fertilizer in rice cultivation in a sustainable manner.

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